doi.org/10.36721/PJPS.2023.36.5.REG.1467-1481.1

Phytochemical profiling and anti-inflammatory, analgesic activity of *Ficus religiosa* L. and molecular docking study against iNOS, TNF-α enzyme

Sarfaraz Ahmad[1], Mohammad Daud Ali[2], Abir Muhsin Alotibi[3], Sarah Mohammed Aljedaie[4], Khalid Saad Alotaibi[5], Nawazish Alam[1], Md Sajid Ali[6], Nafis Haider[7], Md Sarfaraz Alam[8], Sarfaraj Hussain[9,10] and Md Salahuddin Ansari[11]*

[1]Department of Clinical Pharmacy, College of Pharmacy, Jazan University, Jazan, Kingdom of Saudi Arabia
[2]Department of Pharmacy, Mohammed Al-Mana College for Medical Sciences, Dammam, Kingdom of Saudi Arabia
[3]Comprehensive Specialized Clinics at Security Forces Hospital, Arar, Kingdom of Saudi Arabia
[4]Jamjoom Pharma, Ar Rayan Street, Riyadh, Kingdom of Saudi Arabia
[5]Innova Pharmacy, Afif, Kingdom of Saudi Arabia
[6]Department of Pharmaceutics, College of Pharmacy, Jazan University, Kingdom of Saudi Arabia
[7]Department of Basic Medical Sciences, Prince Sultan Military College of Health Sciences, Dhahran, Kingdom of Saudi Arabia
[8] HIMT College of Pharmacy, Greater Noida, Uttar Pradesh, India
[9]Department of Pharmacognosy, Faculty of Pharmacy, Misurata University, Misurata, Libya
[10]Lord Buddha Koshi Pharmacy College, Bihar, India
[11]Department of Pharmacy Practice, College of Pharmacy, Shaqra University, Aldawadmi, Kingdom of Saudi Arabia

Abstract: *Ficus religiosa* L., a member of the Moraceae family, is a medicinal plant having a number of pharmacological properties. The anti-inflammatory and analgesic actions of an ethanolic extract of *F. religiosa* bark FRE (at 100 and 200mg/kg dosages) and the biomarker component quercetin QC (at 5 and 10mg/kg doses) were investigated. The estimate of quercetin was carried by using an HPTLC analysis of FRE. Additionally, qualitative and quantitative screening for key important phytocomponents was done using dried, ground plant stem barks. By using molecular docking, the molecular interaction profile with several anti-inflammatory drug targets was examined. Both the FRE as well as QC showed a substantial decline in paw volume when compared with the relevant control groups ($p<0.01$ & $p<0.001$). Following the administration of acetic acid to mice, the FRE and QC both demonstrate a substantial lengthening of the paw licking or leaping towards Eddy's hot plate as well as a decrease in the number of writhes ($p<0.01$ & $p<0.001$). This study supports the use of these herbs in conventional medicine to treat pain and inflammation by through similar mechanism as compound quercetin (QC).

Keywords:*Ficus religiosa,* inflammation, pain, quercetin, carrageenan, acetic acid, molecular docking, COX enzymes.

INTRODUCTION

Inflammation and pain have become major research topics for scientists worldwide due to their connections to practically every illness that may affect people or animals. Pain is an unpleasant experience that the brain controls using sensory neurons and emotional memory revealed by realistic and practicable bodily injury (Paliwal *et al.,* 2017). As a prolongation of cellular damage resulting from many etiologies, such as infections and mechanical and thermal trauma, accompanied by regional edema and severe tissue destruction (Kumar and Elavarasi, 2016; Oyeleke *et al*., 2018). The pain is therefore more than just an externally experienced symptom because it can also produce a perception of the location, intensity and duration of the pain. It also consists of numerous episodes, including vasodilatation, plasma extravasations, cellular migration and the release of numerous pain mediators (Moreno-Quirós *et al*., 2017). An extensive range of pathogenic actions are highlighted by inflammation. An adaptive reaction was triggered by tissue stress and degradation and it showed para-inflammation. This mechanism, which frequently depends on tissue-dwelling macrophages, stands between a generalised inflammatory reaction and the fundamental homeostatic reflex. In every instance, para-inflammation is appropriate for the ongoing inflammatory components of the current human illness (Huang *et al*., 2022). Arachidonic acid mediates COX-1 and COX-2 and increases prostaglandins. NSAIDs are insufficient to control their release (Phuyal *et al*., 2019). Nonsteroidal anti-inflammatory medicines (NSAIDs) are commonly used to relieve pain and inflammation, but they have been linked to a number of undesirable side effects, including GI irritation and ulceration. Synthetic medications are also sometimes used to treat fever and discomfort, which can harm the liver and kidneys (Muhammad *et al*., 2017). In healthy persons, liver damage with these medications is not a common side effect, but patients with cirrhotic liver illnesses experience bleeding irregularities and renal failure brought on by a reduction in prostaglandin-

**Corresponding author:* e-mail: msdpharma@gmail.com

mediated blood flow to the kidney (Wang *et al*., 20). Because of 22 comparably similar pattern of effectiveness and protection to those of NSAIDs and conventional analgesics, naturally isolated pharmacological moieties have received increasing attention. This is due to the fact that they have comparatively less side effects (Dhir, 2019). Tribal people's use of plants and herbs is today a reliable source for numerous notable medications (Mushtaq *et al*., 2019). These herbal sources currently provide a number of therapeutic agents (Leite *et al*., 2016). Medicinal plants have traditionally claimed their significance for the collection of novel pharmacological moieties as a source of therapeutically promising chemicals (Nooreen, *et al*., 2019). Due to the decreased safety and efficacy as well as the specificity of commercially accessible drugs, the scientific tendency towards phytotherapeutic roots has been revived in modern times (Bindu *et al*., 2020). Molecular docking is another in silico technique that illustrates the adaptable and potential binding poses of ligands to proteins. This ability to mimic ligand-receptor atomic binding can provide an important perspective on complex and experimentally viable interactions to elucidate the ligand-receptor mechanism (Morgan and Anghelescu, 2020).

Ficus religiosa (L.) is a huge perennial tree that is commonly planted along roadsides. This tree is widely planted in temple sites, particularly in Southeast Asia. Bodhi tree is the popular name for *F. religiosa* (Moraceae), which is revered by both Buddhists and Hindus as a sacred tree (Hashmi *et al*., 2018). Because of the many advantages it offers, this tree is known by many names, including the Tree of Life, the Bodhi Tree, the Wisdom Tree and the Sacred Tree *F. religiosa* is regarded as an herb. The medicinal potential of six tree components bark leaves seeds, fruits, roots and latex is examined. Since the wood is so porous, it cannot be used for medicinal purposes. The therapeutic effects of plants extend to every component. They can be utilized in a variety of ways and combining them with other therapeutic herbs produces the finest effects (Murugesu *et al*., 2021). Their bark is used to treat a variety of illnesses in a few other Asian nations, including skin conditions brought on by the application of kumkum (bindhi), epilepsy, ulcers, inflammation, infectious infections and acetylcholinesterase inhibitory action (Kalpana, *et al*., 2009). The leaves provide evidence of their anti-venom efficacy and menstrual cycle regulation. Brushes are made from *F. religiosa's* delicate branches (Shim *et al*., 2022). This plant's fruit powder can be used to cure its latex, which is used as a tonic (Miransari *et al*., 2021). Diarrhea and dysentery are treated with the bark and leaves. Diabetes, diarrhoea, leucorrhoea, menorrhagia and mental illnesses are all treated with the bark because it is astringent, cooling, hemostatic and laxative, as well as vaginal and other urogenital illnesses. The powdered fruit treats asthma and the latex treats warts (Li *et al*., 2020). In order to treat skin conditions like eczema, leprosy and rheumatism, medicated oil prepared from the root bark is administered topically. These trees' fruit, leaves and bark extracts contain a variety of bioactive substances, including tannin, saponin, flavonoids, terpenoids and alkaloids, etc. that exhibit a wide range of pharmacological effects, including anti-inflammatory, antihelminthic, antioxidant, antibacterial, anticancer, antipyretic, antidiabetic, immunomodulatory and wound healing capabilities (Kala *et al*., 2006; Li *et al*., 2022; Govindan and Francis, 2015; Tripathi *et al*., 2015), can be employed as potential therapeutic agents (Riaz *et al*., 2022). The plant is said to include 28-isofucosterol, α-amyrin, β-amyrin, β-sitosteryl-D-glucoside, Vitamin K, noctacosanol, methyl oleanolate, lanosterol, stigmasterol, lupeol, campestrol and stigmasterol. Bergapten and bergaptol have been isolated from the bark (Rathod *et al*., 2018). Tyrosine and asparagine have been extracted from several fruits and they have a variety of pharmacological effects, including those that are anti-inflammatory, antihelminthic, antioxidant, antibacterial, anticancer, antipyretic, anti-diabetic, immunomodulatory and wound healing. (Rathee *et al*., 2015; Palshetkar *et al*., 2020; Dube *et al*., 2019) and can also be used as potential therapeutic agents (Sharma *et al*., 2015). *F. religiosa* methanol extract has recently been demonstrated to have neurotrophic benefits as well as acetyl-cholinesterase inhibitory activities (Pandey *et al*., 2021). It also has anti-inflammatory properties (Jung *et al*., 2008) and several studies have focused primarily on its antitumor, antibacterial (Hembram *et al*., 2018), anthelmintic activity (Hari *et al*., 2011), antifungal activity (Zhou *et al*., 2021; Yeung *et al*., 2018), kidney and urinary disorders (Khanom *et al*., 2000; Pang *et al*., 2019), kidney and urinary (Alam *et al*., 2021).

In silico techniques can significantly help in the development of natural product-based medicines by assisting experimentalists during the hit discovery, hit-to-lead and lead optimisation phases. They have been shown to be particularly useful not only in discovering bioactive natural chemicals but also in prioritizing plant materials for testing, allowing synthetic chemists to focus their efforts on the most promising molecules (Chen and Kirchmair, 2020). Molecular docking is one of the computational techniques used to help researchers discover and evaluate new drug candidates. It has been widely utilized to highlight ligand molecules' chemical interactions and has shown to be a valuable tool throughout the drug research and development process (Azam *et al*., 2022; Ahmaed *et al*., 2012). In order to investigate the anti-inflammatory and anti-nociceptive properties of *F. religiosa* bark, the current work screened its chemical components in order to determine if it may interact with macromolecular pharmacological targets related to inflammation and nociception. For the purpose of measuring quercetin in the bark of *F. religiosa,* we

have created an entire high-performance thin-layer chromatography (HPTLC) technique and procedure.

MATERIALS AND METHODS

Chemicals and drugs

All of the pharmaceuticals and chemicals were bought from Sigma-Aldrich Chemical in St. Louis, Missouri and were of standard grade. A free sample of the standard medications Indomethacin, Diclofenac and Tramadol was provided by Ranbaxy Labs in New Delhi.Natural Remedies Pvt. Ltd. in Bangalore, India provides quercetin (purity: 99% w/w), while E. Merck in Darmstadt, Germany supplies silica gel G pre-coated TLC plates for thin-layer chromatography. From SISCO Research Lab in Mumbai, India, other chemicals, solvents and kits are ordered. Prior to use in the experimental assays, the test compounds were freshly made.

Plant material collection and authenticity

In January 2016, the plant *Ficus religiosa* fresh bark was gathered from a field in the Azamgarh district of Uttar Pradesh. Prof. (Dr) Mohammed Arif, a plant taxonomist at the Department of Pharmacognosy & Phytochemistry, Faculty of Pharmacy, Integral University, Lucknow, Uttar Pradesh, India, verified the authenticity of the plant specimens. J01/FR/2017 voucher specimen was deposited in the Pharmacy Herbarium, Faculty of Pharmacy, Integral University, Lucknow, Uttar Pradesh, India.

Preparation of plant crude extracts

Fresh stem bark from the *F. religiosa* plant weighing 1.5 kg was washed to eliminate dust and allowed to air dry in the shade for three weeks before being ground into a coarse powder and stored in a plastic sealed container with adequate labelling to prevent contamination until it was utilised for the research activities. The extraction was prepared using the 95% ethanol as a solvent. Using the Soxhlet technique, 500g of coarsely powdered pharmaceuticals were extracted over the course of 8 hours at a temperature of 45ºC. 19.3g of dark brown material was obtained after the extract was filtered using a No. 42 Whatman filter and thickened to generate viscous consistency using a rotary evaporator at 50ºC.

Phytochemical screening

According to established procedures, the presence of a group of bioactive components, including alkaloids, glycosides, tannins, saponins, terpenoids, flavonoids and sterols, in the ethanolic extract of *F. religiosa* was assessed independently (Das *et al*., 2020).

Total phenolic content analysis

Using the Folin-Ciocalteu reagent, the extract's total phenolic content was calculated. Distilled water was used to dissolve the extractFollowing that, 500µl of the Folin-Ciocalteu reagent, 400µl of sodium carbonates and 5µl of distilled water were combined with 100µl of the sample. After 30 minutes of letting this solution settle at room temperature, its 760nm absorbance was measured. Total phenolic content was concluded using gallic acid as standard (Kumar *et al*., 2021).

Total flavonoid content estimation

500µl of the aqueous extract were combined with 1.5ml of ethanol, 100ml of 10% aluminium nitrate, 100ml of 1 M potassium acetate and 2.8ml of water. A spectrophotometer was used to test the solution's absorbance after it had been left at room temperature for 40 minutes. Total flavonoid content was recorded according to a standard established curve with quercetin (Pengkumsri*et al*., 2019).

Crude saponin content

In a conical flask with 100ml of 20% aqueous ethanol, add 20g of medication powder. The solution was heated at 55°C for 4 hours while being constantly stirred. After filtering the mixture, 200ml of 20% ethanol was used to extract the marc. The solvent was then removed from the 40ml of extract after mixing the two extracts. In a separating funnel, the concentrate was extracted using 20ml of diethyl ether. The ether layer was discarded and the aqueous layer was recovered.

In order to purify the aqueous extracts, 60ml of n-butanol were added. Additionally, it was cleaned twice with 10ml of 5% aqueous NaCl. The solution was dried and the saponin content was calculated as a percentage (Siddiqui *et al*., 2017).

Tannin content determination

By dissolving 100mg of precisely weighed tannic acid in water, a stock solution of 1mg/ml tannin acid was created. Amounts of 1-10ml were divided into aliquots and each test tube received 0.5ml of the Folin-Denis reagent and 1ml of sodium carbonate solution. Each tube contained 10 millilitres of distilled water. Each tube's contents were well mixed, left undisturbed for approximately 30 minutes, then read at 760nm against a blank reagent (Shahidi *et al*., 2015).

Determination of alkaloid content

Dimethyl sulphoxide (DMSO) was used to dissolve the plant extract (1mg) and then 1ml of 2 N HCl was added and filtered. This solution was transferred to a separating funnel and 5ml of the phosphate buffer and 5 ml of the bromocresol green solution were then added. The mixture was vigorously agitated with 1, 2, 3 and 4ml chloroform before being collected in a 10-ml volumetric flask and chloroform-diluted to the required volume. In the same way as previously stated, a series of reference standard solutions of atropine (20, 40, 60, 80 and 100g/ml) were created. Using a UV/Vis spectrophotometer, the absorbance of the test and standard solutions was

measured against the reagent blank at 470nm. The total alkaloid content was expressed as mg of AE/g of extract (Arroyo-Manzanares *et al*., 2021).

High-performance thin layer chromatography (HPTLC) fingerprinting for quantification of quercetin

Chromatography was carried out as previously described (Hussain *et al*., 2016, 2019, 2020, 2012a, 2012b, 2012c, 2011, 2017) using 20cm × 10cm aluminum Lichrosphere HPTLC plates that had silica gel 60F254 precoated in 200µm l layers (E. Merck, Darmstadt, Germany). A CAMAG Linomat-V Automatic applicator with a 100-µL syringe and the ethanolic extract (dissolved in pure ethanol) was used to spot ten microliters of the substance in the shape of a band (fig. 1). 160nL s-1 was the constant application rate. A 20cm × 10cm twin-trough glass chamber (Camag) that had been previously saturated with mobile phase for 15 minutes at ambient temperature (25±2°C) and relative humidity 60±5% was used for the linear ascending development using chloroform: methanol (9:1, v/v) as the mobile phase. 20mL of mobile phase was utilized, with a development distance of 8cm and a development time of 10min. To find compact bands The plates were preheated (at 75°C for 5 minutes) and air dried at room temperature. Densitometric analysis was performed at 280nm in reflectance mode (Version 1.2.0) using a Camag TLC scanner III and WinCATS software. The scanning speed was 20 mm s-1 and the slit diameters were 5 mm 0.45 mm.

Animals

Adult Swiss albino mice of both sexes (25-30g) were selected for analgesic action, whereas healthy Wistar albino rats of both sexes weighing 140 to 160g had been chosen for anti-inflammatory activity. They were housed in sterile, spotless polypropylene cages at room temperature (21±2°C) with a 12-hour dark/light cycle and provided unlimited access to commercial pellet food and water. After being randomly allocated to separate groups, the mice were isolated for a week for environmental and handling acclimatisation prior to the commencement of the trials. The experimental procedure was authorised by the Institutional Ethical Committee of Misurata University's Faculty of Pharmacy in Misurata, Libya and the studies followed their guidelines (Phar-08/2017).

Safety profile study

In order to determine the LD_{50}, acute toxicity research was carried out using the design (Annexure 2d) of CPCSEA, OECD guideline No. 423. Six groups with six mice each were formed from Swiss albino mice of either sex. The following doses of ethanol extract from *F. religiosa* were administered to mice in one dose: 500, 1000, 1500, 2000 and 2500mg/kg. Throughout 24 hours, the animals were intermittently observed for signs of toxicity and mortality and then every day for 14 days (Hussain *et al*., 2017).

Administration of drugs

All of the experimental animals used an ethanolic extract of *F. religiosa* (FRE) at doses of 100 and 200mg/kg and a marker compound quercetin (QC) at doses of 5 and 10mg/kg body weight. As a standard anti-inflammatory medicine, indomethacin 5mg/kg was employed. Tramadol 0.1ml (40mg/kg s.c.) and Diclofenac 5mg/kg were utilized as pain-inhibiting drugs in the hot plate method and acetic acid-influenced writhing in mice, respectively. All of the test and reference drugs were prepared into an emulsion using 0.3% carboxy methyl cellulose (CMC), in order to reach the required dosage on an animal's body weight basis (mg/kg). A ball-ended feeding needle was then used to administer this mixture orally. Following dosing, the animals were given unrestricted access to food and water (Wahab *et al*., 2022).

Carrageenan-induced rat hind paw edema

Six groups of thirty Wistar albino rats (n=6) were created. As a control group, animals in Group I were given a dosage of 0.3% CMC. Group II received a dose of the common drug indomethacin of 5mg/kg. FRE was administered to Groups III and IV at doses of 100 and 200mg/kg, while the standard chemical QC was administered at doses of 5 and 10mg/kg to Groups V and VI. Acute inflammation was brought on in all six groups by giving the rats' right hind paws a sub-plantar injection of 0.1ml of newly made carrageenan suspension diluted in normal saline. The animals were pre-medicated with tests and standard drugs 30 minutes before the carrageenan injection (Hussain *et al*., 2022a; 2022b). At first, second, third, fourth and fifth-hour intervals, the volume of the injected paws and contralateral paws were measured using a plethysmometer. The percent inhibition was calculated according to the following formula: % inhibition=100 (1-Vt/Vc), Where 'Vc' represents edema volume in control and 'Vt' represents edema volume in the group treated with tested drugs Shahzad *et al*., 2020).

Analgesic activity

Both chemical (acetic acid-induced writhing response) and thermal approaches (hot plate reaction time) were used to investigate the analgesic activity of the ethanolic extract of *F. religiosa* and the common biomarker phytochemical quercetin.

Hot plate test

Eddy's hot plate was used to perform the pain-relieving (analgesic) test, which was kept at a temperature of 55±1°C. All mice had their baseline response times to thermal heat verified before being given a vehicle (0.3% CMC p.o.), an ethanolic extract of *F. religiosa* (FRE) at doses of 100 and 200mg/kg, a common marker compound called quercetin (QC) at doses of 5 and 10mg/kg b.wt and a standard drug called tramadol at a dose of 10mg/kg s.c. The mice in each group were put one at a time on the hot plate that was kept at 55°C after the test and routine drug

administration took place for an hour. Reaction time was measured as the number of seconds needed for paw licking or bouncing. The removal phase is maintained for 30 seconds to avoid the paw's damage. The accompanying calculation was used to determine the pain inhibition percentage (PIP) (Hussain *et al*., 2022a, b).

Pain inhibition percentage (PIP)=$((T_1-T_0)/T_0)$ x 100

T_1 is post-drug latency and T_0 is pre-drug latency time.

Writhing test - Acetic acid-induced

Using the acetic acid-induced writhing response technique, the analgesic efficacy of the ethanolic extract of *F. religiosa* and common biomarker chemicals were assessed. The animals were divided into five groups (n=6), with group I receiving a vehicle (0.3% CMC p.o.) as pre-medication, group II and III receiving FRE at a dose (100 and 200mg/kg), group IV and V receiving the standard biomarker quercetin (QC) (5 and 10mg/kg) and group VI receiving the standard drug diclofenac (5mg/kg). All animal groups received an intraperitoneal injection of acetic acid (1% v/v) 1ml/kg body weight 1 hour after receiving the test and reference medications. After injecting acetic acid for 30 minutes, the number of writhes was counted to determine the amount of writhing. The writhe is signified by abdominal constriction and full extension of the hind limb (Hussain *et al*., 2022b; Sarris, 2018).

ADMET analysis

Using Chemdraw Ultra 12.0, the chemical structures of all the test compounds quercetin were sketched and then converted to mol files (fig. 1). Afterward, these files were imported into the Maestro 9.0 workspace, where ligand preparation was performed. Once the ligands were prepared, they were subjected to Qikprop tool for absorption, distribution, metabolism, excretion and toxicology (ADMET) profiling (Hussain *et al*., 2022a). Data obtained from ADMET prediction have been enlisted in table 3.

Molecular docking

Molecular docking methods are frequently employed to compute the binding affinities of a range of ligands (Anand *et al*., 2016; Kumar *et al*., 2023; Baliyanv *et al*., 2022). Using the Maestro 10.1 programme (Schrodinger Inc., USA), a molecular docking study of the substance under study, quercetin, was carried out on various target proteins retrieved from the protein data bank (PDB Ids: 1EQG; 3LN1; 2AZ5; 4NOS; 5C1M) to establish various types of interactions between the test compound and each of the target proteins. The protein preparation wizard tool was initially used to prepare the study's target proteins. After pre-processing, all unwanted residues, including water molecules, were eliminated. Then, energy reduction and optimisation of hydrogen bonds were applied to it. The binding site was identified with the help of small ligand molecules co-crystallized within the protein and defined as a grid box using the receptor grid generation tool in Glide. The test compound's 3D-structure was downloaded as an SDF file from the PubChem database and its energy was reduced using Maestro's LigPrep module. At pH 7.0 2.0, all conceivable ionisation states were produced and minimised. The Ligand molecule thus obtained was subjected to the qikprop tool for ADME/T prediction and also docked into the active binding site of each target protein in standard precision mode (SP) using Glide. Different chemical interactions (hydrogen bonds, pi-pi interactions and hydrophobic contacts) were seen when test compounds were docked into the active region of the target protein and these interactions were thought to be the cause of the compounds' observed activity.

Ethical approval

The experimental procedures were approved by the Institutional Ethical Committee of the Faculty of Pharmacy at Misurata University, Misurata, Libya and the experiments were carried out in compliance with their guidelines (Phar-08/2017).

STATISTICAL ANALYSIS

Graph Pad Prism V2.01 (Graph Pad Software, Inc., San Diego, California, USA) was used to conduct a one-way analysis of variance and Dunnett's post-hoc test. The data

Table 1: Phytochemical Screening of *F. religiosa*

S.No.	Constituents	*F. religiosa*
1	Alkaloids	+++
2	Carbohydrates	++
3	Glycosides(Cardiac and Anthraquinone glycosides)	-
4	Phenolic compounds and tannins	+++
5	Flavonoids	+++
6	Terpenoids	++
7	Saponins	++
8	Sterols	++
9	Proteins	+++
10	Resins	-

(+++)=Prominent; (++)=Medium; (+)=very less present; (-)=Absent.

were presented as the mean standard error of the mean and statistical significance was determined by P<0.05 and P<0.01.

RESULTS

Phytochemical screening

As demonstrated in table 1, the ethanolic extract of the stem bark of *F. religiosa* consisted of volatile oils, carbohydrates, tannins, glycosides, flavonoids, phenolics and glycosides, according to a preliminary phytochemical investigation.

Quantitative estimation of Phytoconstituents

By using a UV spectrophotometric approach, the total phenolic, flavonoid, tannin, saponin and alkaloid contents of an ethanolic extract of the stem bark of *F. religiosa* were identified. Table 2, displays the findings of quantitative estimation of various classes of phytoconstituents in *F. religiosa* extract. The values reflect the mean and standard deviation of three separate determinations.

Chromatography

At 366nm, UV densitometric analysis was carried out using HPTLC-UV in the reflectance mode. At RF 0.56±0.06 for quercetin, compact, precise, symmetrical bands with great resolution were obtained (figs. 1, 2, 3). In the same solvent solution used for standards, ethanolic extract of *F. religiosa* sample produced well-resolved quercetin at Rf 0.56 (table 3). The chemicals were discovered to absorb in a variable spectrum range, thus the plates were observed at two separate wavelengths, 254 and 366nm (fig. 2 and 3). The identities of the bands of compounds 1 through 10 in the sample extracts were confirmed by superimposing their UV absorption spectra with those of the standards at 280nm (table 3).

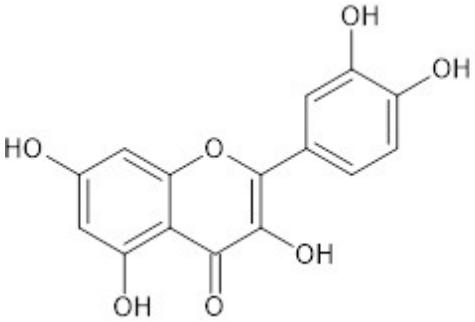

Fig. 1:Chemical structure of Quercetin

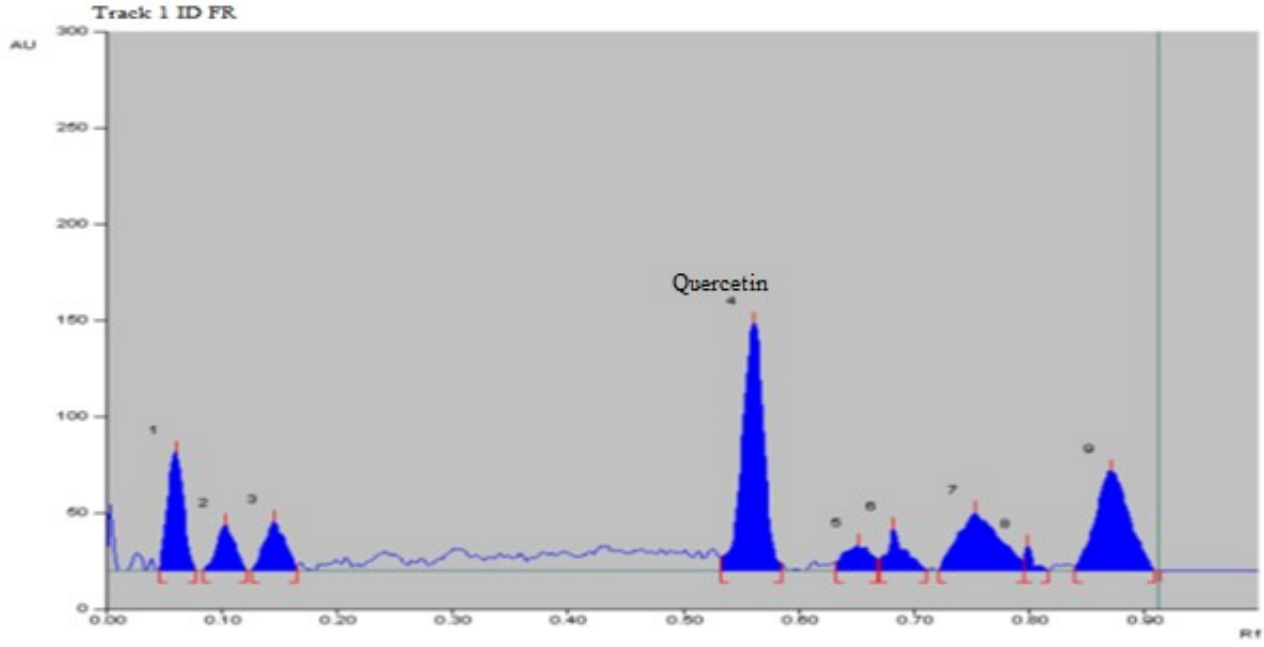

Fig. 2: HPTLC chromatogram of ethanolic extract of *F. religiosa*leaves scanned at 280nm [peak 1-10; quercetin ($R_{F=}$0.56)].

Table 2: Quantitative estimation of different class phytconstituents in *F. religiosa*

Parameters	*F. religiosa* (µg/ ml)
Phenolic contents	103.28 ±1.22
Flavonoidal contents	101.20±3.77
Tannins content	101.11±0.72
% Content w/w	
Alkaloidal contents	2.26±0.011
Saponin contents	0.88±0.003

Table 3: R_F, linear regression data for the calibration curve and sensitivity parameter for quercetin

Parameter	Quercetin
R_F	0.56
Linearity range (ng band^{-1})	100-1000
Regression equation	Y=0.0047X+0
Correlation coefficient (r^2)	0.99862
Slope±sd	0.0047±0.0008
Intercept±sd	nil
Standard error of slope	0.0064
Standard error of intercept	0.0079
LOD	38
LOQ	114

*not available (na)

Table 4: Quercetin contents estimated in ethanolic extract of ***F. religiosa*** by developed method

	Quercetin*	
	Content (ng spot^{-1})	RSD
Ethanolic extract of *F. religiosa*	27.0	1.06

*Volume applied in each replicate was ten microlitres

Table 5: Quercetin ADME/T study and the various parameters

S. No.	ADME/T properties	Quercetin (PubChem ID: 5280343)
1.	Molecular Weight	302
2.	Rotational bonds	5
3.	Dipole	6.59
4.	H-bond acceptor	5.25
5.	H-bond donor	4
6.	QPlogPo/w	0.39
7.	QPlogHERG	-4.98
8.	QPPCaco (nm/s)	21.06
9.	QPlogBB	-2.31
10.	QPPMDCK (nm/s)	7.62
11.	QPlogKhsa	0.35
12.	Rule of Five	0
13.	% Human Oral Absorption	52.91

Table 6: The docking scores with different targets

S. No.	PDB ID	Docking Score (KJ/mol)	Binding Energy ΔG (KJ/mol)
1.	1EQG	-7.60	-6.48
2.	3LN1	-8.26	-33.93
3.	2AZ5	-6.70	-103.38
4.	4NOS	-6.54	0.06
5.	5C1M	-6.52	-

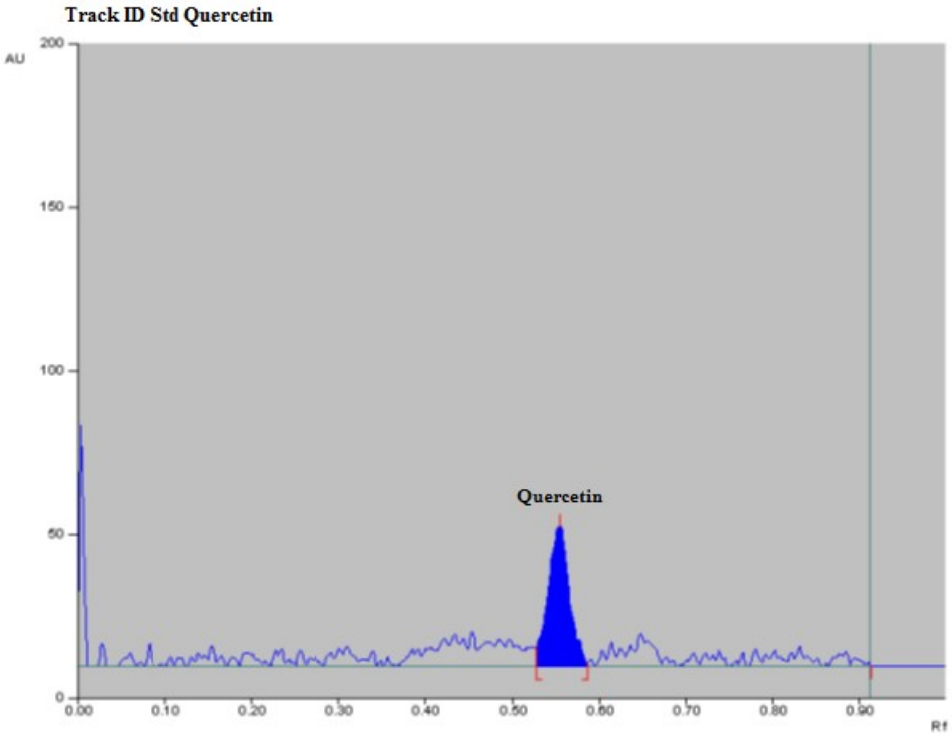

Fig. 3: Chromatogram of standard drug Quercetin determined in by using Chloroform: Methanol (9:1, v/v) as solvent system scanned at 280nm Quecetin (R_F=0.56).

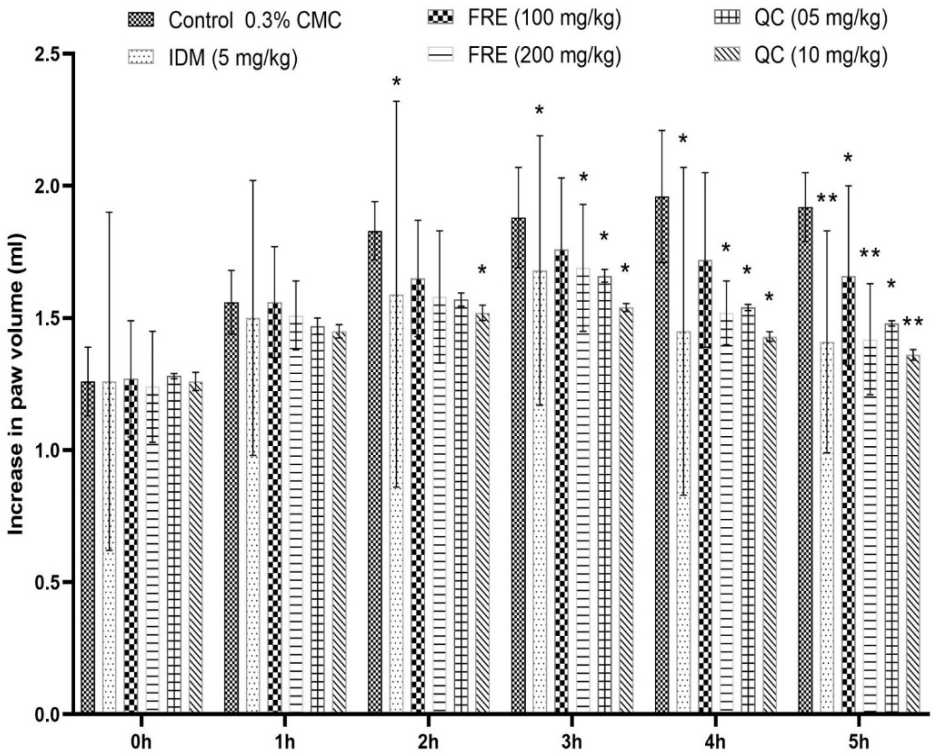

Fig. 4: Effect of ethanolic extract of *F. religiosa* (FRE 100 & 200mg/kg/b.wt.), marker compound QC (5 & 10mg/kg. b.wt.) and Indomethacin (IDM) on carrageenan induced rat paw edema. Each values are expressed in Mean±S.E.M. one way ANOVA followed by Dunnett's test. P: *p< 0.05 & **p< 0.01compare to respective control group.

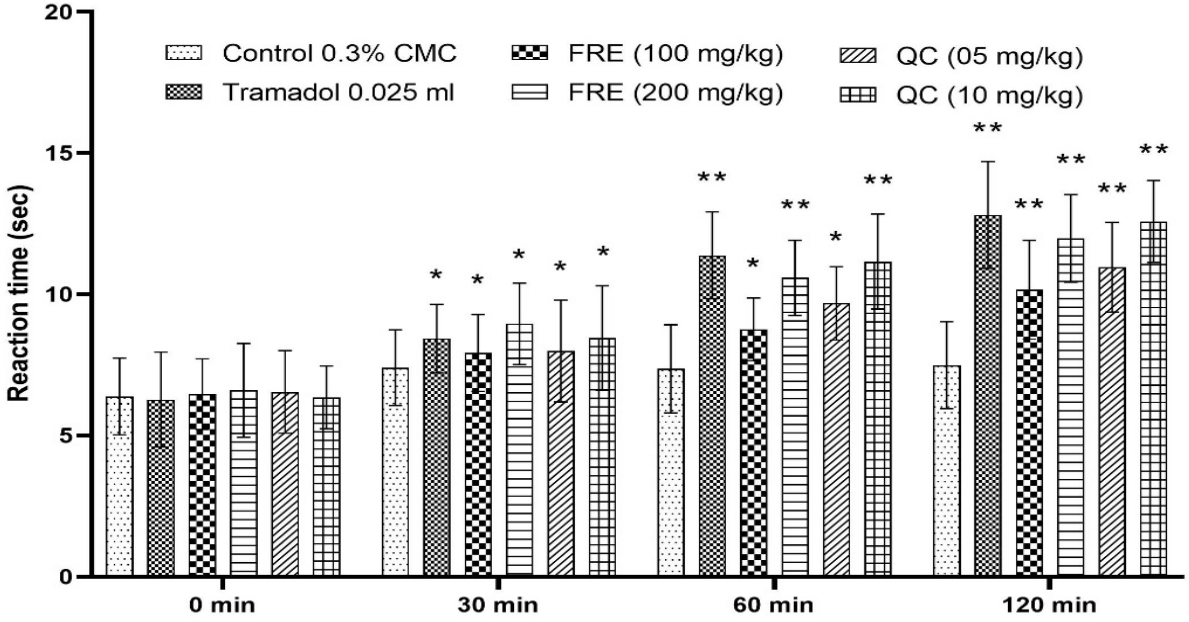

Fig. 5: Effect of ethanolic extract of *F. religiosa* (FRE 100 & 200mg/kg/b.wt.), marker compound QC (5 & 10mg/kg. b.wt.) and Tramadol (Standard) on reaction time of mice exposed to hot plate. Each values are expressed in Mean ± S.E.M. one way ANOVA followed by Dunnett's test. P: *p< 0.05 & **p< 0.01compare to respective control group.

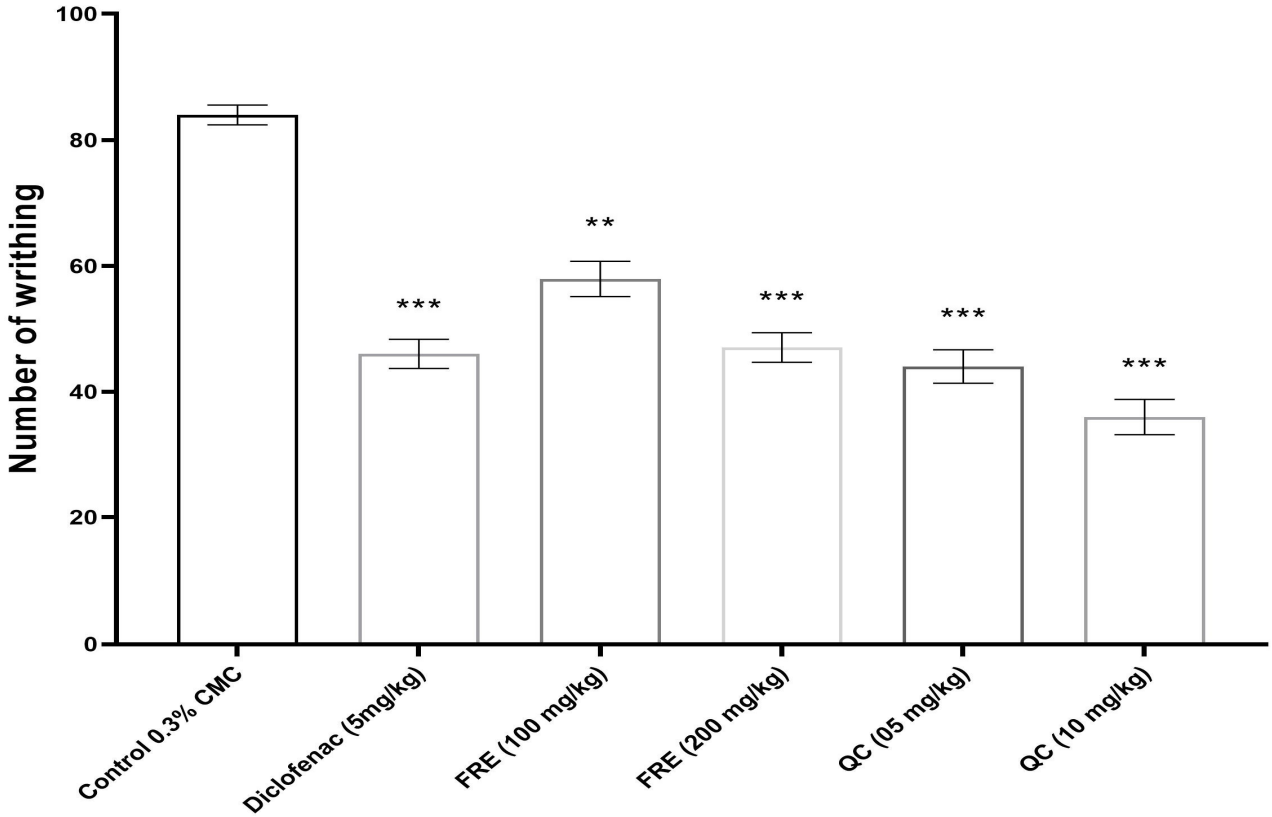

Fig. 6: Acetic acid induced wrihthing test: ethanolic extract of *F. religiosa* (FRE 100 & 200 mg/kg/b.wt.), marker compound QC (5 & 10 mg/kg. b.wt.). Each value is expressed in Mean § S.E.M. one way ANOVA followed by Dunnett's test. P: **p< 0.01 and ***p< 0.001 compare to control group.

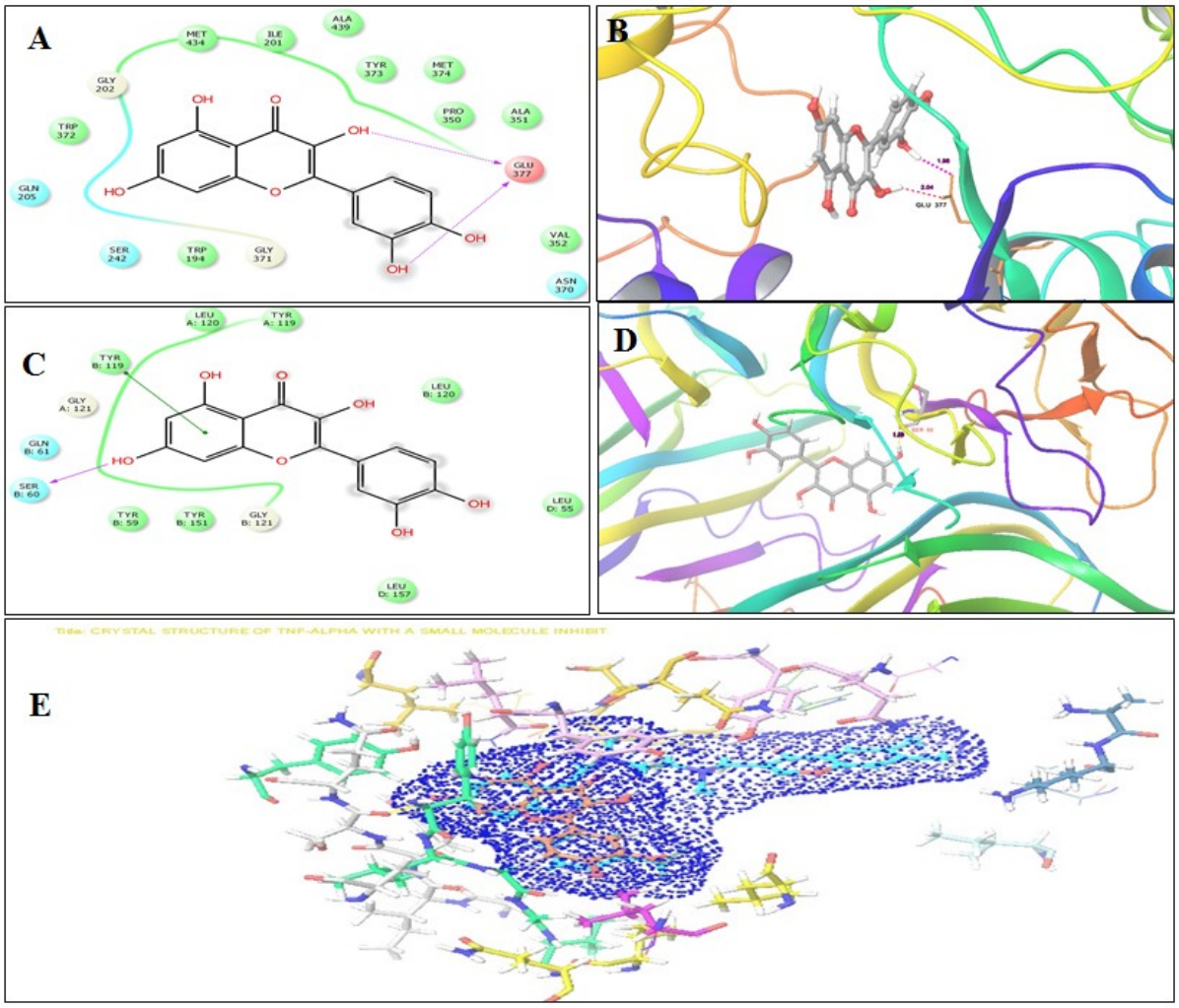

Fig. 7(A-E).; A: 4NOS QUER 2D-2D ligand-receptor interaction diagram depicting different types of chemical interactions between various receptor residues at the binding site of human inducible nitric oxide synthase and quercetin.; B: 4NOS QUER 3D- 3D ligand-receptor interaction diagram depicting hydrogen bond and bond length between various receptor residues at the binding site of human inducible nitric oxide synthase and quercetin.; C: QUER 2AZ5-2D-2D ligand-receptor interaction diagram depicting different types of chemical interactions between various receptor residues at the binding site of TNF-α and quercetin.; D- 4NOS QUER 3D- 3D ligand-receptor interaction diagram depicting hydrogen bond and bond length between various receptor residues at the binding site of TNF-α and quercetin.; E- 4NOS QUER 3D- 3D ligand-receptor interaction diagram depicting overlap between co-crystallized ligand and quercetin and various receptor residues at the binding site of TNF-α. Quercetin is shown in brown colour while co-crystallized ligand appears in torquise colour.

Safety profile study

The safety assessments of the bioactive constituents under investigation commonly present challenges for the development of drugs derived from natural products (Omokhua-Uyi and Van Staden, 2021). The ethanolic extract of F. religiosa (FRE) was tested on mice to establish its acute toxicity. Drug-related toxicity or fatalities were not brought on by the FRE. Up to 2.5g/kg orally, the mice tolerated the FRE well and behaved normally. There was no sign of accommodation or vocalisation; instead, all animals displayed their usual vigilance and responded to touch, pain and scent in the expected ways.

Carrageenan-induced rat paw edema

On a carrageenan-disrupted rat paw, the anti-inflammatory effects of ethanolic extracts of *F. religiosa* (FRE) at dosages of 100 and 200mg/kg, standard marker chemical quercetin (QC) at doses of 5 and 10mg/kg b.wt and indomethacin at a dose of 5mg/kg are shown in (fig. 4). All evaluated medications started to have anti-inflammatory effects 60 minutes after carrageenan challenge. Rat paw edoema peaked at 4 hours after the carrageenan challenge, while animals receiving indomethacin showed a significantly ($P<0.05$) lower paw volume at 2 hours. In rats stimulated by carrageenan, treatment with indomethacin and quercetin (5 and

10mg/kg b.wt), FRE at 100 and 200 and showed a substantial decrease (P0.05) of paw volume. The effects of QC at portions of 5 and 10mg/kg b.wt and 200mg/kg of FRE were shown to be more effective in reducing the size of the swollen paw than a greater quantity of indomethacin (5mg/kg). Numerous pathophysiologies of different clinical problems, such as joint inflammation, malignant development, gout and vascular illnesses, are linked to aggravation. Many medicinal plants are used in the range of conventional clinical frameworks for the assistance of warning indications of pain and irritability. In this study, the ethanolic extract of F. religiosa (FRE) at dosages of 100 and 200mg/kg and marker component quercetin (QC) at doses of 5 and 10mg/kg body weight both shown relaxing pain-relieving activity. Carrageenan is used as a phlogistic agent and boosted the mixture of prostaglandins and bradykinins at various times. All of the studied medications reduced the amount of paw edoema from 1 hour to 5 hours and prolonged the anti-inflammatory action after 3 hours. This analysis unequivocally demonstrates that the effects of all examined medicines may support the spiking of prostaglandins. Interestingly, from 1 hour to 5 hours after carrageenan-induced paw edoema, both dosage levels of QC showed a similar pattern of reduction. Prostaglandins, platelet-activating factors (PAF) and other inflammatory mediators are what start the inflammation that carrageenan causes. Histamine, 5-HT and kinin support the first stage (0-1 hr), whereas the second stage (3-5 hr) is accompanied by the production of prostaglandin and bradykinin. Despite the fact that the calming effect at 10mg/kg was greater to that at 05mg/kg. The findings in carrageenan-induced paw edoema, where both doses of the marker chemical quercetin significantly altered the inflammatory response brought on by the carrageenan, were directly related to this outcome.

Hot plate test

The reaction time of the animals to the heat source was considerably (P<0.01) accelerated by QC at both dosages (5 and 10mg/kg), FRE at dose 200mg/kg and tramadol (fig. 5). In a hot plate test using the marker chemical Quercetin at a dose of (5, 10mg/kg p.o.) FRE 200mg/kg and Tramadol (10mg/kg s.c.) showed a pain inhibition percentage (PIP) of 74.29%, 80.73%, 71.73% and 81.36%.

Acetic acid-induced writhing methods

The acetic acid-induced writhing test in mice was used to evaluate the analgesic effect of ethanolic extract of *F.religiosa* (FRE) at dosages of 100 and 200mg/kg, marker component quercetin (QC) (5 and 10mg/kg b.wt) and diclofenac (5mg/kg). The amount of abdominal constriction and pull out of the hind limbs induced by the injection of acetic acid was greatly reduced (P0.001) by QC (5 and 10mg/kg b.wt), FRE 200mg/kg and diclofenac (fig. 6). In contrast to the usual medicine Diclofenac (5mg/kg), the percentage of lessening the amount of abdominal constriction in QC 5 and 10mg/kg and FRE at 200mg/kg was 44.85%, 36.27% and 47.23%. Prostaglandins and phlogistic mediators like PGE2 and PGE2a are released intraperitoneally and their levels were elevated in the peritoneal fluid of acetic acid-induced mice. Prostaglandins E (majorly involved in the contraction of digestive smooth muscles) act on their own EP1, EP2 and EP3 receptors. Therefore, it is possible that the concentrates' pain-relieving effects are applied, maybe by preventing the combination of prostaglandins.

ADME/T Study

The compound under study i.e. Quercetin was subjected to an ADME/T study and the various parameters obtained are enlisted in table 3.

Molecular Docking Studies

To determine the test substance quercetin's capacity to attach to the target proteins, molecular docking was used. The docking scores with different targets have been enlisted in table 4. All of the target proteins were able to bind with quercetin. The participation of numerous chemical bonds with various residues of the target proteins is suggested by 2D interaction diagrams, including hydrogen bonds, hydrophobic interactions, van der wall forces, etc (figs. 7-A to E).

DISCUSSION

Plants and plant-derived products are used for their flavours, aromas and therapeutic properties. There are several advantages of using plants and plant phytoconstituents instead of pharmaceuticals. It has been shown that the plant's phytoconstituents and extracts exhibit biological properties that include anti-diabetic, anti-hyperlipidemic, free-radical-scavenging and anti-inflammatory actions. Free radicals typically contribute greatly to the development of metabolic diseases, which have an effect on quality of life. A balanced environment for living a good and healthy life is provided by nature, which is a system in equilibrium(Sreelekshmi *et al*., 2007; Rauf *et al*., 2021). *Ficus religiosa* L. (Moraceae) has been extensively used in traditional medicine for a number of disorders involving the respiratory, reproductive, digestive and central neurological systems. Central India, Bengal and the sub-Himalayan area are its native habitats. Through cultivation, it has been widely dispersed throughout the world (Lu *et al*., 2021). The *F. religiosa* tree grows up as an epiphyte before suffocating its host with its deep, sprawling roots to become an independent tree. The bark is thin or membranous and flat or slightly curved; crustose lichen patches are frequently present. The exfoliated bark has a greyish or ashy appearance and is flaked off in uneven, spherical flakes that are 2-2.5 cm thick. The hue of the central regions of the bark is brownish or light reddish brown. The inner part is

composed of layers of light yellowish or orange-brown coloured granular tissue. The bark has no smell and astringent flavor (Singh *et al*., 2021; Shakib *et al*., 2019). Phytoconstituents of phenols, tannins, steroids, alkaloids and flavonoids, as well as vitamin K, n-octacosanol, methyl oleanolate, lanosterol, stigmasterol and lumpen-3-one, have been found in the stem bark of *F. religiosa* (Yang, *et al*., 2002). Traditional medical systems like Ayurveda, Unani and others have suggested that *F. religiosa* has therapeutic benefits. The respiratory system (asthma, cough, etc.), gastrointestinal tract (vomiting, ulcers, stomatitis, constipation, liver diseases, etc.), endocrine system (diabetes, etc.), central nervous system (epilepsy, migraine, etc.) and endocrinal system (diabetes, etc.) have all been treated with it. (Chickenpox, gonorrhoea, scabies, leprosy, elephantiasis, TB, etc.). Ongoing studies are being conducted to confirm its earlier medicinal applicability. For its possible health advantages, quercetin, an aglycone form of flavonoid glycosides derived from plants, has been used as a dietary supplement. Cardiovascular protection, anticancer, antitumor, anti-ulcer, anti-allergy, antiviral, anti-inflammatory activity, anti-diabetic, gastroprotective benefits, antihypertensive, immunomodulatory and anti-infective properties are only a few of the positive effects (Eftekhari *et al*., 2017; Morovati *et al*., 2022). Quercetin can protect against free radicals induced by environmental factors like smoking. Erythrocyte membranes have been reported to be harmed by free radicals from cigarette tar. Quercetin and its conjugate metabolites were also demonstrated to protect erythrocytes from the damage that smoking causes to their membrane structure (Eftekhari *et al*., 2017).

The marker compound quercetin and ethanolic extract of *F. religiosa* demonstrate anti-inflammatory activity. At 100 and 200mg/kg, *F. religiosa* ethanolic extract also shown efficacy. From the first hour to the fourth hour, paw volume was reduced by both extract and marker chemical. A physiologically active phytoconstituent is quercetin. It was discovered that the HPTLC method for estimating quercetin was cost-effective, quick, precise and took less time. It may be utilised as an analytical tool for estimating flavonoids in herbal medications and formulations. Quercetin was subjected to an ADME/T study and based on the results it was identified as a drug-like compound based on Lipinski's rule of five and other parameters. A molecular docking study suggests that Quercetin was able to dock with all the target proteins under study with good docking scores. Docking scores of quercetin with Human Inducible Nitric Oxide Synthase (4NOS) and Tumor Necrosis Factor-α (2AZ5) were found to be more than respective co-crystallized ligands suggesting this to be a noble candidate for targeting these proteins. Docking with 4NOS exhibits two hydrogen bonds with one residue GLU377 of the binding site besides several hydrophobic interactions with residues like TRP194, TRP372, MET434, ILE201, ALA439, TYR373, MET374, PRO350, ALA351 and VAL352. Though Quercetin occupies free space nearby the co-crystallized inhibitor exhibits better interactions with the binding site of the protein which might be responsible for a higher docking score.Similarly, docking of Quercetin with 2AZ5 resulted in the formation of one hydrogen bond with SER60 residue and pi-pi interaction with TYR119 besides other hydrophobic interactions. The molecule occupies the same binding pocket as the co-crystallized inhibitor except for the side-extending chain bearing benzpyranone moiety (Muhammad *et al*., 2017).

CONCLUSION

The relevance of medicinal plants in local traditional remedy has worldwide impact. The world is blessed with an abundant supply of therapeutic plants. Rural residents, particularly those in distant areas of developing nations with scant health facilities, depend heavily on medicinal plants for a variety of purposes. The results of this investigation demonstrated that the marker compound quercetin and the ethanolic extract of *F. religiosa* can function as potential natural sources of anti-inflammatory medicines in a variety of *in vitro* situations. The stem bark of *F. religiosa* contained flavonoids and phenolic principles which can be used to treat oxidative stress and a variety of ailments. Since flavanoids and phenolic compounds have been well established for their anti-oxidant properties. The leaf extract outperformed the roots because of the high quantity of flavonoids and phenolics in it. Even the correlation study supported the leaf ethanol extract's advantageous impacts. The research might thus be used to the development of herbal medications for the treatment of ailments like inflammation brought on by oxidative stress. Our research provides empirical and scientific justification for the traditional analgesic and anti-inflammatory properties of this plant. In our opinion, is one of the most intriguing ones for complementary and alternative medicine.

ACKNOWLEDGMENTS

The facilities required for this research were provided on the university grounds and the authors are grateful to the Honourable President of Misurata University in Misurata, Libya. We thank Prof. (Dr.) Mohammed Arif, a plant taxonomist at the Faculty of Pharmacy, Integral University, Lucknow, Uttar Pradesh, India, for his assistance in verifying the authenticity of plant material.

REFERENCES

Ahmed M, Azam F, Gbaj AE, Zetrini A Abodlal S, Rghigh A, Elmahdi A, Hamza E, Salama A and Bensaber MMS (2016). Ester prodrugs of ketoprofen: Synthesis, *in vitro* Stability, *in vivo* biological

evaluation and *in silico* comparative docking studies against COX-1 and COX-2. *Curr. Drug Discov. Technol*., **13**(1): 41-57.

Alam MM, Emon NU, Alam S, Rudra S, Akhter N, Mamun MMR and Ganguly A (2021). Assessment of pharmacological activities of *Lygodium microphyllum* Cav. leaves in the management of pain, inflammation, pyrexia, diarrhea and helminths: *In vivo*, *in vitro* and *in silico* approaches. *Biomed. Pharmacother*., **139**: 111644.

Anand David AV, Arulmoli R and Parasuraman S (2016). Overviews of biological importance of quercetin: A bioactive flavonoid. *Phcog. Rev.,* **10**(20): 84-9.

Arroyo-Manzanares N, Rodríguez-Estévez V, García-Campaña AM, Castellón-Rendón E and Gámiz-Gracia L (2021). Determination of principal ergot alkaloids in swine feeding. *J. Sci. Food. Agric*., **101**(12): 5214-5224.

Azam F, Taban IM, Eid EEM, Iqbal M, Alam O, Khan S, Mahmood D, Anwar MJ, Khalilullah H and Khan MU (2022). An in-silico analysis of ivermectin interaction with potential SARS-CoV-2 targets and host nuclear importin α. *J. Biomol. Struct. Dyn.,* **40**(6): 2851-2864.

Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey RP and Chang CM (2022). Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of *Ficus religiosa*. *Molecules*, **27**(4): 1326.

Bindu S, Mazumder S and Bandyopadhyay U (2020). Non-steroidal anti-inflammatory drugs (NSAIDs) and organ damage: A current perspective. *Biochem. Pharmacol*., **180**: 114-147.

Chen Y and Kirchmair J (2020). Cheminformatics in natural product-based drug discovery. *Mol. Inform.,***39**(12): 171.

Das K, Khan SM, Sounder J, Mohan U and Prasad VS (2020). Phytochemical screening and establishment of the antidiabetic potential of aqueous leafextract of the endangered plant *Decalepis nervosa* in rats with alloxan-induced diabetes. *Turk. J. Pharm. Sci.,***17**(3): 319-328.

Dhir A (2019). An update of cyclooxygenase (COX)-inhibitors in epilepsy disorders. *Expert Opin. Investig. Drugs,* **28**(2): 191-205.

Dube AN, Shahbazker SN and Nasim M (2019). Evaluation of anti-microbial activity of *Ziziphus. mauritiana* (Ber), *Ocimum sanctum* (Tulsi) and *Ficus religiosa* (Peepal) on *Staphylococcus aureus* strains isolated from environment of various ICUs. *Int. J. Health Sci. Res*., **9**(1): 28-35.

Eftekhari E, Ahmadian V, Panahi-Azar H, Hosseini M, Tabibiazar and Maleki Dizaj S (2017). Hepatoprotective and free radical scavenging actions of quercetin nanoparticles on aflatoxin B1-induced liver damage: *In vitro*/*in vivo* studies. *Artif. CellsNanomed Biotechnol.*, **46**(2):411-420.

Govindan V and Francis GS (2015). Qualitative and quantitative determination of secondary metabolites and antioxidant potential of *Ficus benghalensis* Linn seed. *Int. J. Pharm. Pharm. Sci*., **7**(7): 118-124.

Hari BV, Kumar PS and Devi DR (2011). Comparative In-Vitro anthelmintic activity of the latex of *Ficus religiosa, Ficus elastica* and *Ficus bengalensis*. *J. Phytol*., **3**(3): 26-30.

Hashmi MA, Khan A, Farooq U and Khan S (2018). Alkaloids as cyclooxygenase inhibitors in anticancer drug discovery. *Curr. Protein Pept. Sci*., **19**(3): 292-301.

Hembram KC, Kumar R, Kandha L, Parhi PK, Kundu CN and Bindhani BK (2018). Therapeutic prospective of plant-induced silver nanoparticles: Application as antimicrobial and anticancer agent. *Artif.Cells Nanomed. Biotechnol*., **46**(3):S38-S51.

Huang Z, Xiao L, Xiao Y and Chen C (2022). The modulatory role of growth hormone in inflammation and macrophage activation. *Endocrinol.,* 1; **163**(8):bqac088.

Hussain MS, Azam F, Ahamed KFHN, Ravichandiran V and Alkskas I (2016). Anti-endotoxin effects of terpenoids fraction from *Hygrophila auriculata* in lipopolysaccharide-induced septic shock in rats. *Pharm. Biol.,***54**(4): 628-636.

Hussain MS, Azam F, Eldarrat HA, Ali M and Saeed M (2022c). Structural, functional, molecular and biological evaluation of novel triterpenoids isolated from *Helichrysum stoechas* (L.) Moench. collected from Mediterranean Sea bank, Misurata, Libya. *Arabian J. Chem*., **15**(6): 103818.

Hussain MS, Azam F, Eldarrat HA, Alkskas I, Mayoof JA, Dammona JM, Ismail H Ali M, Arif M and Haque A (2020). Anti-inflammatory, analgesic and molecular docking studies of lanostanoic acid 3-O-α-D-glycopyranoside isolated from *Helichrysum stoechas*. *Arab. J. Chem.,* **13**(12): 9196-9206.

Hussain MS, Azam F, Mezogi J, Enwiz F, Arif M and Saeed M (2022b). A simple validated HPTLC method for analysis of flavonoid and structural analysis of novel terpenoid glycoside from ethanolic extract of *Carya illinoinensis* bark with potential anti-inflammatory activity. *South African J. Bot*., **147**: 596-07.

Hussain MS, Fareed S and Ali M (2011), Preliminary phytochemical and pharmacognostical screening of the Ayurvedic drug *Hygrophila auriculata* (K. Schum) Heine. *Pharmacogn. J.,***3**(23): 28-40.

Hussain MS, Fareed S, Saba A, Rahman MA, Ahmad IZ and Saeed M (2012c). Current approaches toward production of secondary plant metabolites. *J. Pharm. Bioallied Sci.,***4**(1): 10.

Hussain MS, Ismail H, Fareed S and Ali M (2019). New aliphatic ester constituents from *Hygrophila auriculata* (K. Schum) Heine from the basin area of Koshi River. *Ori. Phar. Exp. Med.,* **19**(3): 251-258.

Jung HW, Son HY, Minh CV, Kim YH and Park YK (2008). Methanol extract of *Ficus* leaf inhibits the production of nitric oxide and proinflammatory cytokines in LPS-stimulated microglia via the MAPK pathway. *Phytotherapy Res*., **22**(8): 1064-1069.

Kala CP, Dhyani PP and Sajwan BS (2006). Developing the medicinal plants sector in northern India: challenges and opportunities. *J. Ethnobiol. Ethnomed*., **2**(32): 32-46.

Kalpana G and Rishi RB (2009). Ethnomedicinal knowledge and healthcare practices among the Tharus of Nawal parasi district in central Nepal. *For. Ecol. Manage*., **257**(10): 2066-2072.

Kanjikar AP, Hugar AL and Londonkar RLN (2018). Characterization of phyto-nanoparticles from Ficus krishnae for their antibacterial and anticancer activities. *Drug Dev. Ind. Pharm.,* **44**(3): 377-384.

Khanom F, Kayahara H and Tadasa K (2000). Superoxide-scavenging and prolyl endopeptidase inhibitory activities of Bangladeshi indigenous medicinal plants. *Bioscience, Biotechnol. Biochem*., **64**(4): 837-840.

Kumar D, Ladaniya MS, Gurjar M, Kumar S, Mendke S (2021). Quantification of flavonoids, phenols and antioxidant potential from dropped *Citrus reticulate* blanco fruits influenced by drying techniques. *Molecules,* **26**(14): 4159.

Kumar KH and Elavarasi P (2016). Definition of pain and classification of pain disorders. *J. Evid. Based Integr. Med*., **3**(3): 87-90.

Kumar S, Arif M, Kamal M, Jawaid T, Khan MM, Mukhtar B, Khan A, Ahmed S, Al-Sanad SM and Al-Khamees OA (2023). Ex Vivo Antiplatelet and thrombolytic activity of bioactive fractions from the new-fangled stem buds of *Ficus religiosa* L. with Simultaneous GC-MS Examination. *Molecules*, **28**(9): 3918. .

Leite JSM, Cruzat VF, Krause M and De Bittencourt PIH (2016). Physiological regulation of the heat shock response by glutamine: Implications for chronic low grade inflammatory diseases in age-related conditions. *Nutrire*, **41**(1): 17.

Li KY, Liang JJ, Peng YF, Ling XF, Cai YT and Yi RH (2022). Leaf spots on bodhi tree (*Ficus religiosa)* caused by *Diaporthe tulliensis*. *Plant Dis.,* **106**(10): 20.

Li Y, Kong D, Fu Y, Sussman MR and Wu H (2020). The effect of developmental and environmental factors on secondary metabolites in medicinal plants. *Plant Physiol. Biochem.,* **148**: 80-89.

Lu W, Shi Y, Wang R, Su D, Tang M, Liu Y and Li Z (2021). Antioxidant activity and healthy benefits of natural pigments in fruits: A review. *Int. J. Mol. Sci*., **22**(9): 4945.

Miransari M, Mahdavi S and Smith D (2021). The biological approaches of altering the growth and biochemical properties of medicinal plants under salinity stress. *Appl. Microbiol. Biotechnol.,* **105**(19): 7201-7213.

Moreno-Quirós CV, Sánchez-Medina A, Vázquez-Hernández M, Reyes AGH and García-Rodríguez RV (2017). Antioxidant, anti-inflammatory and anti-nociceptive potential of *Ternstroemia sylvatica* Schltdl. & Cham. *Asian Pac. J. Trop. Med.,***10**(11): 1047-1053.

Morgan KJ and Anghelescu DL (2020). Opioid therapy for pediatric cancer pain, opioid therapy in infants, children and adolescents. *Springer*, **9**(3): 251-281.

Morovati MR, Ghanbari-Movahed M, Barton EM, Farzaei MH and Bishayee A (2022). A systematic review on potential anticancer activities of *Ficus carica* L. with focus on cellular and molecular mechanisms. *Phytomedicine*, **105**: 154333.

Muhammad S, Aleem AA, Muhammad N, Khalid M, Khan A, Farooq U, Ahmad I, Khan FA and Bukhari SM (2017). Investigation of antipyretic activity of *Viola serpens* on Breweris yeast induced pyrexia in mice. *Acta Pol. Pharm*., **74**(6): 1797-1802.

Muhammad S, Aleem AA, Muhammad N, Khalid M, Khan A, Farooq U, Ahmad I, Khan FA and Bukhari SM (2017). Investigation of antipyretic activity of *Viola serpens* on breweris yeast induced pyrexia in mice. *Acta Pol. Pharm*., **74**(6): 1797-1802.

Murugesu S, Selamat J and Perumal V (2021). Phytochemistry, pharmacological properties and recent applications of *Ficus benghalensis* and *Ficus religiosa*. *Plants,* **10**(12): 2749

Mushtaq, MN, Ghimire S, Akhtar MS, Adhikari A, Auger C and Schini-Kerth VB (2019). Tambulin is a major active compound of a methanolic extract of fruits of *Zanthoxylum armatum* DC causing endothelium-independent relaxations in porcine coronary artery rings via the cyclic AMP and cyclic GMP relaxing pathways. *Phytomedicine*, **53**: 163-170.

Nooreen Z, Kumar A, Bawankule DU, Tandon S, Ali M, Xuan TD and Ahmad A (2019). New chemical constituents from the fruits of *Zanthoxylumarmatum* and its *in vitro* anti-inflammatory profile. *Nat. Prod. Res.,***33**(5):665-672.

Oyeleke SA, Ajayi AM, Umukoro S, Aderibigbe A and Ademowo OG (2018). Anti-inflammatory activity of *Theobroma cacao* L. stem bark ethanol extract and its fractions in experimental models. *J. Ethnopharmacol*., **222**(10): 239-248.

Paliwal SK, Sati B, Faujdar S and Sharma S (2017). Studies on analgesic, anti-inflammatory activities of stem and roots of *Inula cuspidata* CB Clarke. *J. Tradit. Complement. Med*., **7**(4): 532-537.

Palshetkar A, Pathare N, Jadhav N, Pawar M, Wadhwani A, Kulkarni S and Singh KK (2020). *In vitro* anti-HIV activity of some Indian medicinal plant extracts. *BMC Complement. Med. Ther*., **20**(1): 1-11.

Pandey DK, Kaur P, Kumar V, Banik RM, Malik T and Dey A (2021). Screening the elite chemotypes of *Gloriosa superba* L. in India for the production of

anticancer colchicine: Simultaneous microwave-assisted extraction and HPTLC studies. *BMC Plant Biol*., 5, **21**(1):77.

Pang GM, Li FX, Yan Y, Zhang Y, Kong LL, Zhu P, Wang KF, Zhang F, Liu B and Lu C (2019). Herbal medicine in the treatment of patients with type 2 diabetes mellitus. *Chin. Med. J.* (Engl). **132**(1): 78-85.

Pengkumsri N, Kaewdoo K, Leeprechanon W and Sundaram Sivamaruthi B (2019). Influence of extraction methods on total phenolic content and antioxidant properties of some of the commonly used plants in Thailand. *Pak. J. Biol. Sci*., **22**(3): 117-126.

Phuyal N, Jha PK, Raturi PP and Rajbhandary S (2019). *Zanthoxylum armatum* DC: Current knowledge, gaps and opportunities in Nepal. *J. Ethnopharmacol*., **229**(30): 326-341.

Rathee D, Rathee S, Rathee P, Deep A, Anandjiwala S and Rathee D (2015). HPTLC densitometric quantification of stigmasterol and lupeol from *Ficus religiosa*. *Arab. J. Chem*., **8**(3): 366-371.

Rathod VD, Digambar NW, Pillai S, Bhangale, JO and Bhangale PJ (2018). Anti-arthritic activity of ethanolic extract of *Ficus religiosa* leaves in FCA induced arthritis in rats. *World J. Pharm. Res*., **7**(2): 778-78.

Rauf A, Akram M, Semwal P, Mujawah AAH, Muhammad N, Riaz Z, Munir N, Piotrovsky D, Vdovina I, Bouyahya A, Adetunji CO, Shariati MA, Almarhoon ZM, Mabkhot YN and Khan H (2021). Antispasmodic Potential of Medicinal Plants: A Comprehensive Review. *Oxid. Med. Cell Longev.,* **11**: 4889719.

Riaz A, Nosheen S and Mughal TA (2022). Fabrication of biogenic silver nanoparticles from *Ficus religiosa* bark extract and their application for chromium removal. *Microsc. Res. Tech*., **85**(11): 3618-3622.

Sarris J (2018). Herbal medicines in the treatment of psychiatric disorders: 10-year updated review. *Phytother. Res.,* **32**(7): 1147-1162.

Shahidi F and Ambigaipalan P (2015). Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects - A review. *J. Funct. Foods*, **18**: 820-897.

Shahzad F anderson D, Najafzadeh M (2020). The antiviral, anti-inflammatory effects of natural medicinal herbs and mushrooms and sars-cov-2 infection. *Nutrients*, 25; **12**(9): 2573.

Shakib Z, Shahraki N, Razavi BM and Hosseinzadeh H (2019). *Aloe vera* as an herbal medicine in the treatment of metabolic syndrome: A review. *Phytother. Res.,* **33**(10): 2649-2660.

Sharma H, Yunus G, Agrawal R, Kalra M, Verma S and Bhattar S (2016). Antifungal efficacy of three medicinal plants *Glycyrrhiza glabra, Ficus religiosa* and Plantago major against oral *Candida albicans*: A comparative analysis. *Indian J. Dent. Res*., **27**(4): 433-436.

Shim KH, Sharma N and An SSA (2022). Mechanistic insights into the neuroprotective potential of sacred *Ficus* Trees. *Nutrients,***14**(22): 4731.

Siddiqui N, Rauf A, Latif A and Mahmood Z (2017). Spectrophotometric determination of the total phenolic content, spectral and fluorescence study of the herbal Unani drug Gul-e-Zoofa (*Nepeta bracteata* Benth). *J. Taibah Univ. Med. Sci.,* **12**(4): 360-363.

Singh P, Arif Y, Bajguz A and Hayat S (2021). The role of quercetin in plants. *Plant Physiol. Biochem.,* **166**: 10-19.

Tripathi R, Kumar A, Kumar S, Prakash S and Singh AK (2015). *Ficus benghalensis* Linn.: A tribal medicine with vast commercial potential. *Indian J. Agric. Allied Sci*., **1**(3): 95-102.

Virani NV, Chandola HM, Vyas SN and Jadeja DB (2010). Clinical study on erectile dysfunction in diabetic and non-diabetic subjects and its management with *Ficus relegiosa* Linn. *Ayu.*, **31**(3): 272-9.

Wahab S, Alsayari SA, Muhsinah AB, Almaghaslah D, Haque A, Khalid M, Alnasser SM, Azam F and Hussain MS (2022a). The structural, biological and in-silico profiling of novel capryloyl tetra-glucoside and aliphatic ester constituents from the *Abutilon indicum* offers new perspectives on the treatment of pain and inflammation. *Plants,* **11**(19): 2583.

Wang R, Tao L, Lu Q, Hao F, Zhao S, Ma Y, Han L and Bai C (2022). The analgesic activities of total alkaloids of the ethnic medicine *Cynanchum komarovii* Al. Iljinski. *J. Ethnopharmacol*., **285**: 114861.

Yang C, Bahar E, Adhikari SP, Kim SJ, Kim H and Yoon H (2019). Precise modeling of the protective effects of quercetin against mycotoxin via system identification with neural networks. *Int. J. Mol. Sci*., **20**(7): 1725.

Yeung KS, Hernandez M, Mao JJ, Haviland I and Gubili J (2018). Herbal medicine for depression and anxiety: A systematic review with assessment of potential psycho-oncologic relevance. *Phytother. Res.,* **32**(5): 865-891.

Zhou L, Liu J, Bu LL, Liao DF, Cheng SW and Zheng XL (2021). Curcumin acetylsalicylate extends the lifespan of *Caenorhabditis elegans*. *Molecules,* **26**(21): 6609.