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Evaluation of Invitro Antioxidant and Anti-Inflammatory Potentials of Phytochemicals Identified from Leaf Extracts of Hibiscus Lobatus Kuntze.

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KEYWORDS

Rheumatic, investigated, antioxidant, inflammatory, Phytochemical.

ABSTRACT:

Hibiscus lobatus Kuntze. is traditionally, used to sunburn, rheumatic arthritis and healing of diabetic wounds. This study investigated the antioxidant and anti-inflammatory potentials of leaf extracts; Phytochemical screening of leaf ethanol extract (LEE) was rich in phenolics (57.96 mg GAE/g) and flavonoids (25.33 mg QE/g). HRLCMS profiling of LEE showed the antioxidant compounds namely, genistin, kaempferol 3-rhamnoside 7-xyloside, and 6-deoxyfagomine. LEE demonstrated potent antioxidant activity by quenching the free radicals with IC_{50} values of 204.43 µg/mL (DPPH) and 188.84 µg/mL (ABTS) and showed high reducing power in the FRAP assay. *In vitro* anti-inflammatory activity assessed by protein denaturation, albumin denaturation, and RBC membrane stabilization revealed the lowest IC_{50} values 331.46 µg/mL, 293.42 µg/mL, and 315.73 µg/mL respectively. These findings suggested the strong antioxidant and anti-inflammatory activities of *Hibiscus lobatus* leaves and could serve as a therapeutic source to cure sun burn and inflammatory disorders.

Introduction

Phytochemicals are biologically active secondary metabolites known for their therapeutic potential, particularly in combating oxidative stress and inflammation [1] (Malhotra et al., 2023). Historically, the use of medicinal plants dates to the dawn of human civilization and forms the foundation of many modern medicines. Globally, about 75–80% of the population still depends on plant-based remedies for their primary healthcare needs, a practice further encouraged by the World Health Organization. *Hibiscus lobatus* is an annual herb belonging to the family Malvaceae, found seasonally in the dry tropical biome of the Western Ghats in Karnataka, India. Despite its significant use in traditional medicine, scientific research on this species remains limited. Local traditional healers in the forest regions of Sagara and Tirthahalli taluks have long utilized whole-plant extracts of *H. lobatus* for the treatment of sunburn, skin peeling, rheumatic arthritis and wound healing processes. This study seeks to bridge the gap between traditional knowledge and modern science by investigating the phytochemical constituents and pharmacological potential of *H. lobatus*, with a particular focus on its antioxidant and anti-inflammatory activities.

Oxidative stress, caused by an imbalance between free radicals and antioxidants, is implicated in the pathogenesis of various chronic diseases including atherosclerosis, stroke, diabetes, cancer, and neurodegenerative disorders. Antioxidants play a crucial role in neutralizing these free radicals and preventing cellular damage. Among plant-based antioxidants, flavonoids and phenolic compounds are widely studied for their free radical scavenging, anti-inflammatory, and anti-carcinogenic properties [2] (Priya et al., 2011). Due to the production of secondary metabolites with clinically curative effects, medicinal plants play an important role in the development of new and potent drugs [3] (Fatima et al., 2018)

Inflammation is a biological response aimed at eliminating harmful stimuli and initiating tissue repair. It involves the release of chemical mediators from injured tissues and immune cells, leading to vasodilation and increased capillary permeability[4] (Chandra et al., 2012). Numerous researchers have worked incessantly on medicinal plant extracts to explore their anti-inflammatory activity via in vitro and in vitro studies and on the identification and characterization of the key natural bio-active compounds responsible for the anti-inflammatory activity [5]. (Gandhi et al., 2022). While non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used to manage inflammation, their prolonged use is associated with adverse effects such as gastric ulcers. This has driven the search for safer, natural alternatives with anti-inflammatory potential [6](Gunathilake et al., 2018).

Oxidative stress and inflammation are closely linked, often forming a vicious cycle in chronic diseases. Excessive production of reactive oxygen and nitrogen species (ROS and RNS) can damage cellular components and accelerate aging processes. Plant-derived polyphenols have demonstrated the ability to neutralize these free radicals, thereby protecting cells from oxidative damage and degenerative changes [7] (Pandey et al., 2013). Plant-based phytochemicals are known for their potential sources of anti-inflammatory agents. Various studies reported the potential disease-healing efficiency of medicinal plants' crude extracts, pure compounds, and their metal/metal oxide nanoparticles (M/MONPs) against inflammation. [8] (Gonfa et al., 2023). Polyphenols, a diverse group of secondary metabolites found in plants, are among the most significant micronutrients associated with health benefits. High-resolution mass spectrometry (HR-LCMS) has become an essential tool for the analysis of polyphenols, enabling the identification, characterization, and authentication of natural extracts. The high accuracy of HR-LCMS facilitates the detection of molecular structures and elemental compositions, making it an ideal platform for phytochemical profiling [9] (Lucci et al., 2015). This investigation reporting the phytochemical profiling of *H lobatus* through HR-LCMS analysis and validate the ethnomedicinal potential by *in vitro* antioxidant and anti-inflammatory activities.

Plant material and location

Fig. 1. (a) Whole plant of *Hibiscus lobatus* in its natural habitat; (b) Flowering stage of *H. lobatus*; (c) Shade-dried leaf used for extraction; (d) Ethanol extract obtained through sequential Soxhlet extraction.

Study Location

The study plant *Hibiscus lobatus* was collected from the moist and dry deciduous forests of the Shankaraghatta hill range, located at 13°44'27.816"N latitude and 75°37'26.04"E longitude. This region falls within Central Western Ghats.

Materials and Methods

Sequential soxhlet extraction and phytochemical evaluation

The leaves of *H. lobatus* were thoroughly washed with deionized water, shade-dried, and powdered using a mechanical blender. For extraction, 1 kg of powdered material (leaves, stems, and roots) was subjected to sequential Soxhlet extraction using solvents of increasing polarity petroleum ether (40°–60°C),

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chloroform (50°–70°C), and ethanol (all from Merck, Mumbai, India). Each extraction was carried out for 48 hours in multiple batches to ensure maximum yield. Between each extraction step, the residue (marc) was completely dried before proceeding with the next solvent. The resulting extracts were filtered through Whatman No.1 filter paper and concentrated under reduced pressure using a rotary evaporator (Buchi, Switzerland). Final traces of solvent were removed by evaporation on a water bath, and the dried extracts were stored in desiccators. The percentage yield of each extract was recorded.

Qualitative phytochemical screening

Preliminary phytochemical screening was conducted on the sequential extracts to detect the presence of major phytochemical classes using standard procedures described by Brain and Turner (1975) [10] and Evans (1996)[11].

Quantitative phytochemical analysis

Determination of total flavonoid content (TFC)

Total flavonoid content was estimated following the modified method of Zhishen et al. (1999)[12]. Different concentrations of each extract (petroleum ether, chloroform, ethanol, and aqueous) were mixed with 6 µL of 5% sodium nitrite and incubated at room temperature for 5 minutes. This was followed by the addition of 6 µL of 10% Aluminum Chloride and 40 µL of 1 M Sodium hydroxide. After 5 minutes, 48 µL of distilled water was added. The absorbance was measured at 510 nm using a spectrophotometer. The TFC was expressed as quercetin equivalents (QE, µg/mg of dry mass).

Determination of Total Phenolic Content (TPC)

Total phenolic content was determined using the method described by Chang et al. (2002)[13]. Each extract (500 µg/ml) was mixed with 50 µL of Folin–Ciocalteu reagent (diluted 1:10, v/v) and incubated for 5 minutes. Subsequently, 40 µL of 7.5% sodium carbonate solution was added and incubated at 50°C for 10 minutes. Absorbance was recorded at 750 nm. TPC was calculated as gallic acid equivalents (GAE, µg/mg of dry mass).

HRLC-MS analysis of Leaf ethanol extract.

A high-resolution liquid chromatograph mass spectrometer (HR-LCMS) G6550A (Agilent technologies) instrument was used to examine the bioactive components from the sequential solvent extracts of *Hibiscus lobatus* leaves extracts. The method used for chromatography was 30 mins ± ESI 10032014_MSMS.m. HR-LC-MS analysis was performed at the Sophisticated Analytical Instrument Facility (SAIF), Indian Institute of Technology, Mumbai, India. A needle wash was used to inject 5 µl of RPE, REE and RME into an Agilent ultra-high performance liquid chromatography system that was fitted with a Hypersil Gold column (C18 100 × 2.1 mm-3 MICRON). The elution solvent was 0.1% formic acid in water (A) and acetonitrile (B) at a flow rate of 0.3 ml/minute. The gradient system changed its composition from 95% A: 5% B to 5% A: 95% B in 50 minutes, returned to the original composition in 10 minutes, and then retained that composition for 5 minutes. The following MS source parameters were used: capillary voltage of 3,500 V, nebulizer pressure of 35 psi, gas temperature of 250°C, and drying gas flow of 13 l/minute. The method used for chromatography was 30 mins ± ESI 10032014_MSMS.m. HR-LC-MS analysis was performed at the Sophisticated Analytical Instrument Facility (SAIF), Indian Institute of Technology, Mumbai, India. The MS analysis was conducted using the positive and negative ionization modes of the ESI. The compounds were identified by comparison with their retention time (RT), mass, abundance area and m/z cloud best match with stored Metlin Library available with IIT, Bombay. The mass spectrometric analysis was done using Agilent Mass Hunter software.

***In vitro* antioxidant activity**

DPPH radical scavenging Assay

The free radical scavenging activity of the extracts was evaluated using the DPPH method described by Braca et al., (2003)[14]. Different concentrations (100, 200, and 300 µg/mL) of each extract were added to 100 µL of 0.004% DPPH in ethanol. The mixture was incubated in the dark at room temperature for 30 minutes. Absorbance was recorded at 517 nm. BHT was used as a standard. The percentage inhibition was calculated using:

% of inhibition = [(Acontrol - Atest)/Acontrol] x 100

Where Acontrol is the absorbance of the control and Atest is the absorbance of the extract reaction. IC50

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value was calculated using the formula: IC50 = [(ΣC/ΣI) × 50], where ΣC is the sum of extracts and pure compounds concentrations used to test and ΣI is the sum of the percentage of inhibition at different concentrations.

ABTS radical scavenging assay

The ABTS radical scavenging activity was determined as per Qabaha et al. (2023)[15]. $ABTS^{\bullet+}$ was generated by mixing 7 mM ABTS with 2.45 mM potassium persulfate (1:1) and incubating the mixture in the dark at 27°C for 16 hours. The resulting solution was diluted to an absorbance of 0.700 at 734 nm. Plant extracts at concentrations of 50, 100, 150, and 200 µg/mL were mixed with 160 µL of the $ABTS^{\bullet+}$ solution, and the absorbance was measured after 30 minutes. The scavenging activity was calculated as Percent inhibition of absorbance at 734 nm was calculated using the formula, ABTS•+ scavenging effect (%) = ((AB – AA)/AB) ×100 (2), where, AB is absorbance of ABTS radical + methanol; AA is absorbance of ABTS radical + sample extract/standard

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was measured following the method of Klein et al. (1981)[16]. Extracts (100–500 µg/mL) were reacted with iron-EDTA, EDTA (15 µL, 0.018%), DMSO (30 µL, 0.85%), and ascorbic acid (15 µL, 0.22%). The mixture was heated at 80–90°C for 15 minutes, after which the reaction was stopped using 30 µL of 17.5% TCA. Nash reagent (100 µL) was added and incubated for 15 minutes at room temperature. Absorbance was recorded at 412 nm. Scavenging activity was calculated by the formula, % of inhibition = 1 - [Asample/Ablank] x 100, where A sample is the extract containing reaction mixture absorbance; Ablank is the blank absorbance. The IC50 value was calculated using the formula: IC50 = [(ΣC/ΣI) x 50], where ΣC is the sum of test samples concentrations used to test and ΣI is the sum of the percentage of inhibition at different concentrations.

Metal chelating activity

The metal chelating ability of the extracts was assessed following the method by Ebrahimzadeh et al. (2010)[17]. Extracts (100–500 µg/mL) were incubated with 10 µL of 2 mM ferrous chloride and 40 µL of 5 mM ferrozine. After 10 minutes of incubation in the dark at room temperature, absorbance was measured at 562 nm. Ascorbic acid was used as the standard. Chelating activity was calculated by using the following formula, % inhibition = t (Acontrol - Atest)/Acontrol] x 100, where Acontrol is the control reaction absorbance and A test is the test sample reaction absorbance. IC50 value was calculated using the formula: IC50 = [(ΣC/ΣI) x 50], where ΣC is the sum of extracts/isolated constituents concentration used to test and ΣI is the sum of the percentage of inhibition at different concentrations.

***Invitro* Total anti-oxidant and reductive power assay**

Phosphomolybdate assay system was used to determine the total antioxidant activity of the different extracts using the method by Umamaheswari et al., (2008)[18] reagent solution; sulphuric acid (0.6 M), sodium phosphate (28 mM) and ammonium molybdate (4 mM) was mixed with 100 µl of each sample and incubated at 90°C in a water bath for 90 min. After cooling to room temperature; absorbance was recorded at 765 nm against reagent blank. Total antioxidant capacity of the ascorbic acid was also estimated for reference.

The reducing power of various *Hibiscus lobatus* leaf extracts was determined following the method of Singhal et al (2011)[19]., with slight modifications. Different concentrations of the extracts (10–50 µg/mL) were mixed with 50 µL phosphate buffer (20 mM, pH 6.6) and 50 µL potassium ferrocyanide [1% $K_3Fe(CN)_6$]. The reaction mixture was incubated at 50°C for 30 minutes. Subsequently, 50 µL of 10% trichloroacetic acid was added, followed by 10 µL of 0.1% ferric chloride. The final mixture was incubated at room temperature for 10 minutes, and the absorbance was recorded at 700 nm. An increase in absorbance indicated higher reducing power. Ascorbic acid served as the reference standard, and phosphate buffer (pH 6.6) was used as the blank.

The total antioxidant capacity and total reductive power was determined by using following formula:

Total anti-oxidant capacity (%) = ([control absorbance-sample absorbance]/control absorbance) *100.

***In vitro* anti-inflammatory activity**

Inhibition of albumin denaturation

The anti-inflammatory activity of *H. lobatus* extracts were assessed by the inhibition of albumin denaturation method, as described by Mizushima et al., (1968)[20] and

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Sakat et al., (2014)[21] with minor modifications. The reaction mixture contained test extracts and 1% aqueous bovine serum albumin. The pH was adjusted with 1N HCl. Samples were incubated at 37°C for 20 minutes and then heated to 51°C for another 20 minutes. After cooling, the turbidity was measured at 660 nm. The percentage inhibition of protein denaturation was calculated using the formula.

Percentage inhibition = (Abs Control –Abs Sample) X 100/ Abs control.

Human red blood cell (HRBC) membrane stabilization assay

The membrane stabilization potential of *H. lobatus* leaf extract was evaluated using the Human Red Blood Cell (HRBC) membrane stabilization method Parvin et al., (2015)[22]. Blood was collected from healthy volunteers and mixed with an equal volume of Alsever's solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid, and 0.42% sodium chloride in water). The mixture was centrifuged at 300 rpm, and the packed cells were washed with isotonic saline (0.85%, pH 7.2). A 10% v/v HRBC suspension was prepared in isosaline. The assay mixture consisted of the test extract, 1 mL phosphate buffer (0.15 M, pH 7.4), 2 mL hypotonic solution (0.36% NaCl), and 0.5 mL of HRBC suspension. Diclofenac was used as a standard drug. For control, distilled water was used instead of the hypotonic solution. All samples were incubated at 37°C for 30 minutes and centrifuged. The hemoglobin content in the supernatant was measured at 560 nm.

The percentage of HRBC membrane stabilization or protection was calculated using the following

formula. % protection= 100-(O.D of drug treated sample/O.D of control)*100

Proteinase Inhibitory Activity

The proteinase inhibitory activity of *H. lobatus* leaf extract was evaluated following the protocol of Sakat et al., as modified by Gunathilake et al. (2018)[6]. The reaction mixture (2 mL) contained 0.06 mg trypsin, 1 mL of 20 mM Tris-HCl buffer (pH 7.4), and 1 mL test sample (comprising 0.02 mL extract and 0.98 mL methanol). The mixture was incubated at 37°C for 5 minutes, after which 1 mL of 0.8% casein solution was added. Incubation was continued for an additional 20 minutes. The reaction was terminated by adding 2 mL of 70% perchloric acid, and the mixture was centrifuged. The absorbance of the supernatant was recorded at 210 nm using buffer as a blank. Phosphate buffer was used as the control.

Results and Discussion.

Table 1: Qualitative and Quantitative analysis of *Hibiscus lobatus* leaf extracts.

Tests	**Qualitative analysis**			**Quantitative estimation**		
Extracts	LPE	LCE	LEE	LPE	LCE	LEE
Alkaloids	-	-	-	237.62± 4.4µg/g	244.66± 4.2 µg/g	301.66 ± 5.2 µg/g
Flavonoids	+	+	+			
Tannins	-	-	+			
Saponins	-	-	+	15.4± 1.2 µg/g	14.8± 1.4 µg/g	16.5±1.2 µg/g
Phenols	+	+	+			
Cardiac Glycosides	+	+	-			
Terpenoids	-	-				

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Qualitative analysis of *Hibiscus lobatus* leaf extracts showed that the Leaf ethanol extract (LEE) contained the widest range of phytochemicals, including flavonoids, tannins, saponins, phenols, and terpenoids. Both leaf chloroform extract (LCE) and leaf petroleum ether extract (LPE) showed the presence of phenols and cardiac glycosides, while alkaloids were absent in all extracts. The higher phytochemical diversity in LEE highlights the effectiveness of ethanol in extracting a broad spectrum of bioactive compounds with potential pharmacological relevance.

Among the three extracts of *Hibiscus lobatus* LEE showed the highest total flavonoid (301.66 ± 5.2 µg/g) and phenolic content (16.5 ± 1.2 µg/g), followed by LCE and LPE Shown in Table 1. The enhanced levels of flavonoids and phenols in LEE correlate with its rich phytochemical profile observed in the qualitative estimation suggesting its potential for strong antioxidant and pharmacological activities.

HR-LCMS profiling of the leaf ethanol extract.

The HR-LCMS chromatogram of the leaf ethanol extract of *Hibiscus lobatus* revealed the presence of 87 distinct compounds (Fig 2). Among these, 11 significant phytocompounds were identified, exhibiting highest DB Hits with notable pharmacological properties are shown in Table 2 **.**

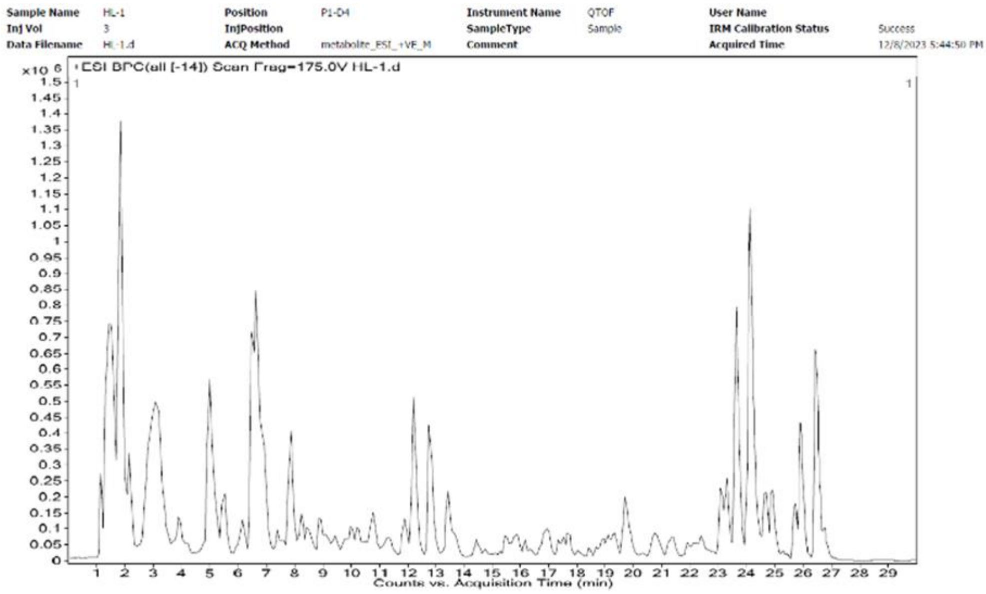
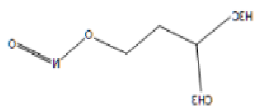


Fig 2: HR-LCMS profiling of LEE showing positive ion mode chromatogram

Table 2: Phytoconstituents of LEE, identified from positive ion mode of HR-LCMS study

LEE (+)	**Formula**	**Score**	**RT**	**Mass (DB)**	**DB Hits**	**Structure**
Isoamyl nitrite	$C_5 H_{11} N O_2$	84.98	1.354	117.07	9	

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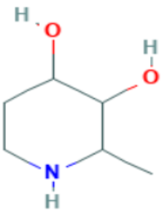
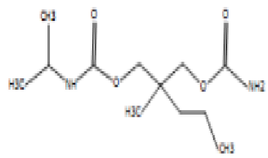
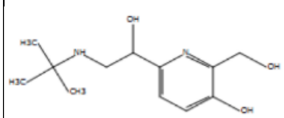
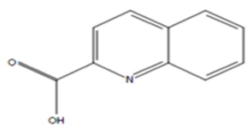
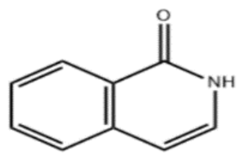
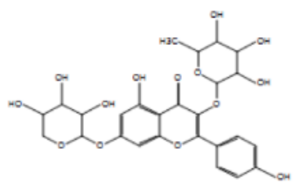
Oxacyclotetradecan-2-one	$C_{13}H_{24}O_2$	66.34	1.412	212.17	10	
6-Deoxyfagomine	$C_6H_{13}NO_2$	77.88	1.42	131.09	10	
Carisoprodol	$C_{12}H_{24}N_2O_4$	75.39	1.482	260.17	10	
Pirbuterol	$C_{12}H_{20}N_2O_3$	89.7	2.048	240.1459	10	
Quinaldic acid	$C_{10}H_7NO_2$	88.83	2.129	173.0464	10	
Isocarbostyril	C_9H_7NO	83.65	4.937	145.052	10	
Kaempferol 3-rhamnoside 7-xyloside	$C_{26}H_{28}O_{14}$	92.5	6.44	564.146	10	

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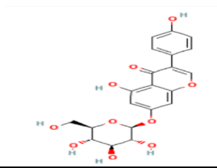
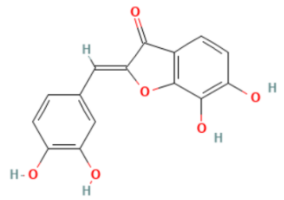
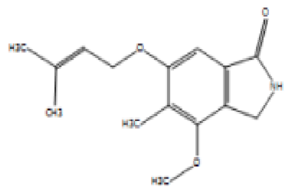
Genistin	$C_{21}H_{20}O_{10}$	82.64	7.404	432.10	10	
Maritimetin	$C_{15}H_{10}O_6$	83.29	8.278	286.04	10	
Zinnimidine	$C_{15}H_{19}NO_3$	96.66	9.482	261.13	10	

The HR-LCMS chromatogram of negative ion mode spectra is shown Fig 3, The results indicated the presence of 20 distinct compounds and among these, 3 significant phytocompounds were identified shown in Table 3, exhibiting notable DB hits, abundant value, and pharmacological properties.

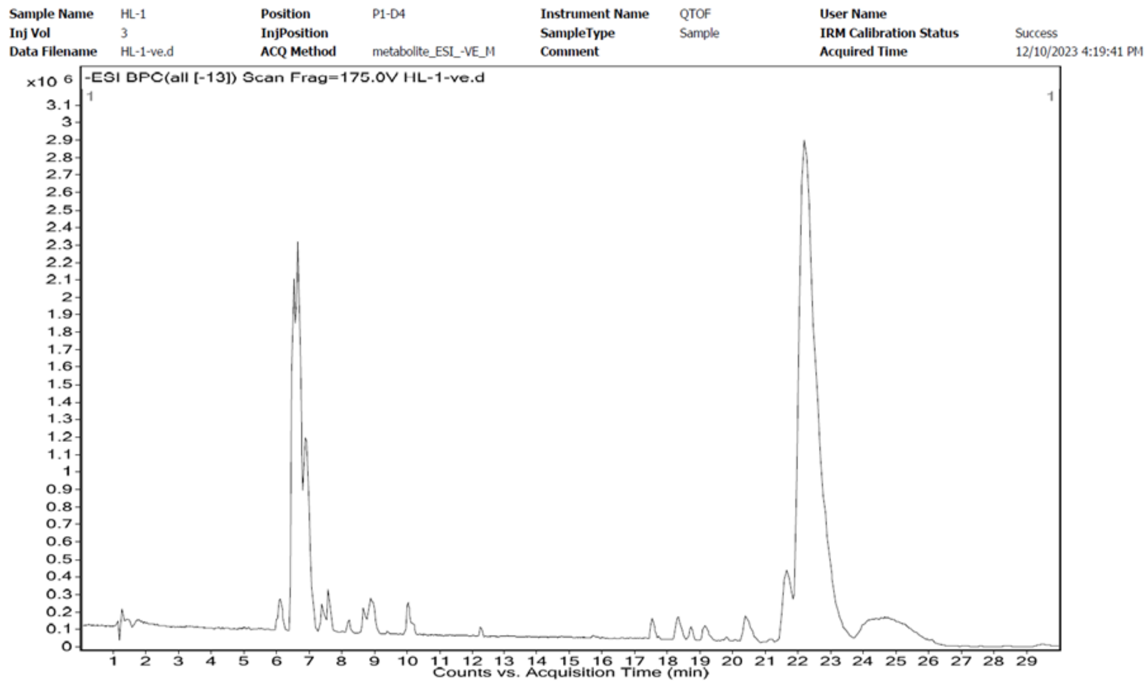


Fig 3: HR-LCMS profiling of LEE showing negative ion mode chromatogram

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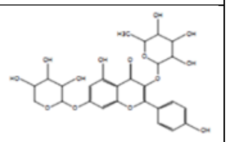
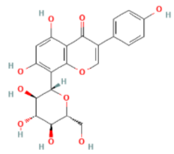
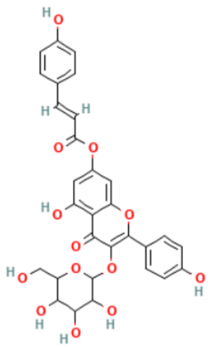
Table 3: Phytoconstituents of LEE obtained from Negative ion mode of HR-LCMS study.

HLEE (-)	Formula	Score	RT	Mass (DB)	DB Hits	Structure
Kaempferol 3-rhamnoside 7-xyloside	$C_{26}H_{28}O_{14}$	96.54	6.54	564.14	10	
Genistein 8-C-glucoside	$C_{21}H_{20}O_{10}$	97.87	7.45	432.10	10	
7-O-(4-Hydroxycinnamoyl) astragalin	$C_{30}H_{26}O_{13}$	95.11	10.03	594.13	10	

The HRLCMS analysis of the *Hibiscus lobatus* leaf ethanol extract (LEE) identified a variety of bioactive compounds with significant therapeutic potential. Notable compounds include Isoamyl nitrite, Oxacyclotetradecan-2-one, 6-Deoxyfagomine (Min, Im et al., 2013)[23], Carisoprodol (Mandana Hasanzad et al., 2024)[24], Pirbuterol (Richards, D. M., et al 1985)[25] (R.S. Vardanyan et al., 2006)[26], Quinaldic acid (Langner, E et al., 2015)[27] and Isocarbostyril (Laurent Ingrassia, et al., 2008)[28], all of which exhibit important pharmacological properties. For instance, Isoamyl nitrite act as vasodilators and antihypertensive agents, contributing to cardiovascular health. 6-Deoxyfagomine has potent antihyperglycemic activity, while Carisoprodol functions as a skeletal muscle relaxant. Pirbuterol, a bronchodilator, is effective in promoting respiratory health, and Quinaldic acid showed antitumor activity, particularly in colon cancer cells.

The flavonoids such as kaempferol 3-rhamnoside 7-xyloside and genistin (Goh Yu Xian et al., 2022)[29] were identified in the negative ion spectra (Table 3), both known for their strong antioxidant, anti-inflammatory, and anticancer activities. Kaempferol and its derivatives are also recognized for their antimicrobial properties and potential in inhibiting cancer cell proliferation. These compounds also exhibit cardioprotective, neuroprotective, and anti-diabetic effects, broadening the therapeutic applications of the leaf extract.

The analysis also detected Genistein 8-C-glucoside and 7-O-(4-Hydroxycinnamoyl), astragalin (Rajesh et al., 2019)[30], compounds with anti-inflammatory, antimutagenic, anticancer, and antiatherosclerotic properties. 7-O-(4-Hydroxycinnamoyl) astragalin reduces NF-κB activation and inflammatory responses, supports the treatment of atopic dermatitis, and offers protection against liver oxidative stress. Furthermore, it

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plays a role in cancer prevention and treatment, inhibits adipogenesis, promotes lipolysis, and mitigates neuropathy by reducing ER stress and apoptosis. The combination of these bioactive compounds underscores the potential of *Hibiscus lobatus* leaf ethanol extract as a valuable resource in the development of treatments for various chronic diseases and dermatological applications.

Table 4: *In vitro* antioxidant potential of *Hibiscus lobatus* leaf extracts.

Activity	LEE (µg/ml)	**% Inhibition**	LCE (µg/ml)	**% Inhibition**	LEE (µg/ml)	**% Inhibition**
DPPH radical Scavenging activity	200	15.02	200	16.25	200	16.89
	400	22.45	400	21.45	400	27.41
	600	38.6	600	43.58	600	45
	800	47.2	800	51.02	800	52.75
	1000	54.21	1000	58.25	1000	64.31
IC_{50} (µg/ml)		**872.81**		**805.3049**		**744.7**
ABTS Radical Scavenging activity	20	18.02	20	19.42	20	21.04
	40	26.22	40	28.42	40	30.18
	60	34.45	60	39.22	60	41.5
	80	43.44	80	44.58	80	49.34
	100	54.63	100	58.45	100	69.23
IC_{50}(µg/ml)		**90.20493**		**83.86**		**45.97**
Metal Chelating activity	20	8.56	20	9.59	20	13.48
	40	14.25	40	16.18	40	22.96
	60	27.54	60	32.65	60	36.33
	80	34.25	80	38.76	80	49.93
	100	51.45	100	54.75	100	65.37
IC_{50} (µg/ml)		**105.02**		**95.73573**		**79.04**
Hydroxyl radical scavenging assay	100	10.25	100	**12.54**	100	**14.52**
	200	21.12	200	**23.56**	200	27.42
	300	32.66	300	36.54	300	40.12
	400	43.25	400	**44.52**	400	56.12
	500	52.66	500	54.28	500	68.12
IC_{50} (µg/ml)		**477.5215**		**447.0322**		**364.3243**

The antioxidant potential of *Hibiscus lobatus* leaf extracts—LPE, LCE, and LEE was evaluated using DPPH, ABTS, metal chelating, and hydroxyl radical scavenging assays shown in Table 4. All extracts demonstrated dose-dependent antioxidant activity across the tested assays. In the DPPH radical scavenging assay, LEE showed the highest inhibition of 64.31% at 1000 µg/mL with an IC_{50} value of 744.70 µg/mL, followed by LCE (IC_{50}: 805.30 µg/mL) and LPE (IC_{50}: 872.81 µg/mL), indicating superior free radical scavenging efficiency in the ethanol extract. A similar trend was observed in the ABTS assay, where LEE again recorded the lowest IC_{50} value (45.97 µg/mL), significantly outperforming LCE (83.86 µg/mL) and LPE (90.20 µg/mL). In the metal chelating assay, LEE exhibited the strongest activity with an IC_{50} of 79.04 µg/mL, whereas

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LCE and LPE showed slightly weaker chelating capacities with IC_{50} values of 95.73 µg/mL and 105.02 µg/mL, respectively. Furthermore, the hydroxyl radical scavenging assay revealed consistent results, where LEE displayed the highest inhibition percentage (68.12% at 500 µg/mL) and the lowest IC_{50} value (364.32 µg/mL), suggesting its effectiveness in neutralizing reactive hydroxyl species. Overall, LEE consistently demonstrated the highest antioxidant activity across all assays, likely due to its higher content of polar phenolic and flavonoid compounds, which are known contributors to antioxidant mechanisms. These findings support the potential use of ethanol leaf extract of *Hibiscus lobatus* in therapeutic applications requiring antioxidant protection.

Total antioxidant capacity and Total Reductive capacity of LPE, LCE, LEE

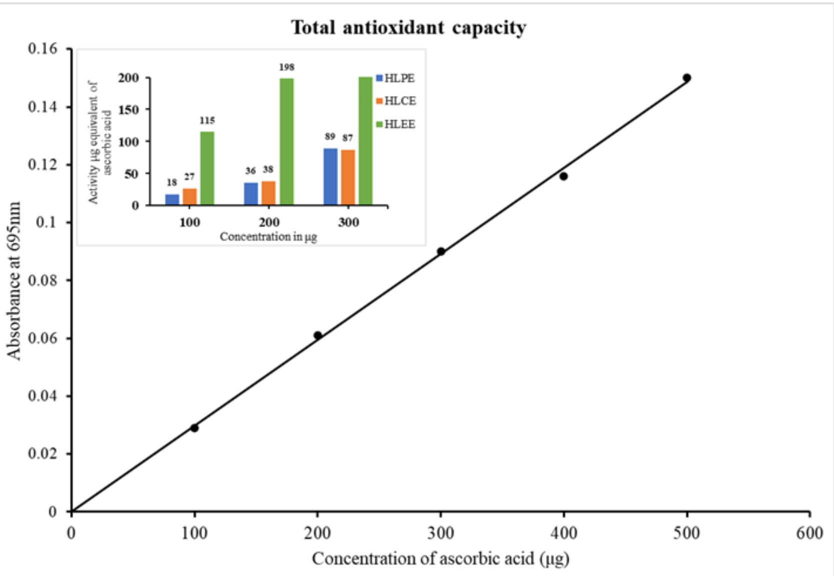

Fig 3: Total antioxidant capacity

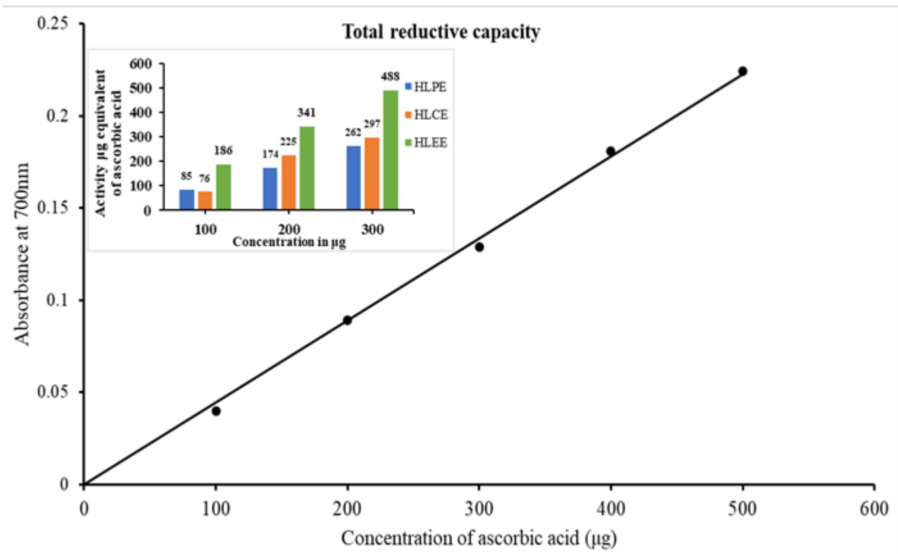

Fig 4: Total reductive capacity

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In addition to radical scavenging assays, the total antioxidant capacity and total reductive capacity of the *Hibiscus lobatus* leaf extracts were assessed to understand their overall reducing and antioxidant potential. The results demonstrated that LEE exhibited significant activity in both assays compared to LCE and LPE (Fig 3). In the total antioxidant capacity assay, LEE showed the highest activity, with 198 µg/mg ascorbic acid equivalents at 200 µg extract concentration and 198 µg at 300 µg concentration, indicating a dose-dependent increase. LCE followed closely, while LPE recorded the lowest activity. Similarly, in the total reductive capacity assay, LEE exhibited a sharp increase in reducing power, with 488 µg/mg ascorbic acid equivalents at 300 µg extract concentration (Fig 4). HLCE and HLPE showed moderate and lower activities, respectively. The linear standard curves for ascorbic acid in both assays confirmed the accuracy of these estimations. These results reinforce the earlier findings from DPPH, ABTS, metal chelation, and hydroxyl radical scavenging assays, confirming that the LEE of *Hibiscus lobatus* leaves possesses the strongest antioxidant potential among the tested extracts, likely due to its rich content of polar bioactive compounds such as phenols and flavonoids.

Table 5: Anti-inflammatory potential of *Hibiscus lobatus* leaf extracts

Activity	Conc	IC_{50} of Extracts						Standard IC_{50}
		LPE	LCE	LEE	LPE	LCE	LEE	Diclofenac
Protein Inhibitory Assay	100	21.25	22.45	27.12	**395.98**	**397.16**	**331.4585**	221.87
	200	35.15	34.24	37.15				
	300	42.84	43.21	48.66				
	400	49.54	52.36	56.75				
	500	56.87	54.32	68.45				
Inhibition of Albumin Denaturation	100	22.02	11.06	29.18	**305.2668**	**419.8168**	**293.417**	188.44
	200	38.45	24.44	37.65				
	300	54.48	34.08	55.44				
	400	65.42	48.25	61.12				
	500	71.75	59.86	78.88				
HRBC Membrane Stabilization	100	17.56	18.04	22.2	**415.776**	**353.3684**	**315.7286**	204.8
	200	25.45	27.64	35.44				
	300	39.34	41.44	44.56				
	400	48.12	56.88	59.56				
	500	57.64	70.46	79.74				

The anti-inflammatory potential of *Hibiscus lobatus* leaf extracts were evaluated using three in vitro assays: protein denaturation inhibition, inhibition of albumin denaturation, and HRBC membrane stabilization shown in Table 5. Among the three extracts tested LPE, LCE, and LEE. The leaf ethanol extract consistently exhibited superior anti-inflammatory activity across all assays, as reflected by its lower IC_{50} values. In the protein denaturation assay, LEE showed the highest inhibitory activity with an IC_{50} of 331.46 µg/ml, followed by LPE and LCE with IC_{50} values of 395.98 µg/ml and 397.16 µg, respectively. This indicates a better potential of LEE to stabilize proteins against heat-induced denaturation. Similarly, in the albumin denaturation assay, LEE again demonstrated the lowest IC_{50} value (293.42 µg/ml), indicating stronger activity compared to LPE (305.27 µg/ml) and LCE (419.82 µg/ml). The standard drug used for comparison had an IC_{50} of 188.44 µg/ml, showing that while the extracts were less potent than the standard, LEE showed a relatively closer profile. In the HRBC

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membrane stabilization assay, which reflects the extract's capacity to prevent lysis of erythrocyte membranes a model for anti-inflammatory action LEE recorded the highest activity with an IC_{50} of 315.73 µg/ml. LCE followed with 353.37 µg/ml, while LPE had the least effect with 415.78 µg/ml. Although none of the extracts outperformed the standard (IC_{50}: 204.8 µg/ml), the ethanol extract showed the most promising results in all three models.

Conclusion

The present study establishes the significant anti-inflammatory potential of *Hibiscus lobatus* leaf ethanol extract, as evidenced by its superior performance across protein denaturation, albumin denaturation, and HRBC membrane stabilization assays. This enhanced activity is likely attributed to the presence of polar phytochemicals such as flavonoids, phenolics, and glycosides, known for their inflammation-modulating and membrane-stabilizing properties. Supporting these findings, HRLCMS analysis revealed a diverse array of bioactive compounds in LEE, including Isoamyl nitrite, 6-Deoxyfagomine, Carisoprodol, Pirbuterol, Quinaldic acid, and Isocarbostyril, along with potent flavonoids like kaempferol 3-rhamnoside 7-xyloside, genistin, genistein 8-C-glucoside, and 7-O-(4-Hydroxycinnamoyl) astragalin. These compounds exhibit a wide range of pharmacological activities, including anti-inflammatory, antioxidant, anticancer, and cardioprotective effects. Together, the biochemical and phytochemical evidence supports the therapeutic potential of *Hibiscus lobatus* leaf ethanol extract as a promising natural source for developing anti-inflammatory agents and managing chronic inflammatory conditions. Further in vivo studies and clinical evaluations are warranted to validate its efficacy and safety.

References:

1. Malhotra H, Garg V and Singh G: In-vitro antioxidant and anti-inflammatory evaluation of successive extracts of Ziziphus nummularia. Int J Pharm Sci & Res; 2023. 5354-66.
2. Priya, M.G.R., Girija, K. and Ravichandran, N., 2011. *Invitro* study of anti-inflammatory and antioxidant activity of 4-(3H)-quinazolinone derivatives. Rasayan J. chem, 4, p.418-424.
3. Zaynab, M., Fatima, M., Abbas, S., Sharif, Y., Umair, M., Zafar, M.H. and Bahadar, K. Role of secondary metabolites in plant defense against pathogens. Microbial pathogenesis; 2018. pp.198-202.
4. Chandra, S., Chatterjee, P., Dey, P. and Bhattacharya, S., 2012. Evaluation of in vitro anti-inflammatory activity of coffee against the denaturation of protein. Asian Pacific Journal of Tropical Biomedicine, 2(1), p. 178-S180.
5. Gandhi, Y., Kumar, R., Grewal, J., Rawat, H., Mishra, S.K., Kumar, V., Shakya, S.K., Jain, V., Babu, G., Sharma, P. and Singh, A. Advances in anti-inflammatory medicinal plants and phytochemicals in the management of arthritis: A comprehensive review. Food chemistry advances; 2022. p.100085.
6. Gunathilake, K.D.P.P., Ranaweera, K.K.D.S. and Rupasinghe, H.P.V. Influence of boiling, steaming and frying of selected leafy vegetables on the in vitro anti-inflammation associated biological activities; 2018. p.22.
7. Pandey, M.M., Rastogi, S. and Rawat, A.K.S. Indian traditional ayurvedic system of medicine and nutritional supplementation. Evidence-Based Complementary and Alternative Medicine; 2013. p.376327.
8. Gonfa, Y.H., Tessema, F.B., Bachheti, A., Rai, N., Tadesse, M.G., Singab, A.N., Chaubey, K.K. and Bachheti, R.K.Anti-inflammatory activity of phytochemicals from medicinal plants and their nanoparticles: A review. Current Research in Biotechnology.;2023. p.100152.
9. Lucci, P. and Martins, C.P. Liquid Chromatography–High-Resolution Mass Spectrometry in Environmental and Food Analysis. In Fast Liquid Chromatography–Mass Spectrometry Methods in Food and Environmental Analysis; 2015. pp. 325-345.
10. Brain, K.R., Jones, B.E. and Turner, T.D. Application of densitometry to the qualitative and quantitative evaluation of pharmaceutical colourants. Journal of Chromatography A; 1975. p.383-388.
11. Evans, W.C. and Trease, G.E. Trease and Evans' pharmacognosy. WB Saunders company; 1996.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(3), 824 - 837 | ISSN:2251-6727

12. Zhishen, J., Mengcheng, T. and Jianming, W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food chemistry; 1999. p.555-559.
13. Chang, C.C., Yang, M.H., Wen, H.M. and Chern, J.C. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of food and drug analysis; 2002.
14. Braca, A., Fico, G., Morelli, I., De Simone, F., Tomè, F. and De Tommasi, N. Antioxidant and free radical scavenging activity of flavonol glycosides from different Aconitum species. Journal of Ethnopharmacology; 2003. p.63-67.
15. Qabaha, K., Abbadi, J., Yaghmour, R., Hijawi, T., Naser, S.A. and Al-Rimawi, F. Unveiling the antibacterial and antioxidant potential of Hedera helix leaf extracts: recent findings. Canadian Journal of Physiology and Pharmacology; 2023.p.26-32.
16. Klein, S.M., Cohen, G., Lieber, C.S. and Cederbaum, A.I., Increased microsomal oxidation of hydroxyl radical scavenging agents and ethanol after chronic consumption of ethanol. Archives of Biochemistry and Biophysics; 1983. p.425-432.
17. Ebrahimzadeh, M.A., Eslami, S., Nabavi, S.M., Nabavi, S.F. and Eslami, B. Antioxidant and antihemolytic activities of Leontodon hispidos. *Biotechnology & Biotechnological Equipment*, 2010, pp.2127-2131.
18. Umamaheswari, M. and Chatterjee, T.K. In vitro antioxidant activities of the fractions of Coccinia grandis L. leaf extract. African Journal of Traditional, Complementary and Alternative Medicines; 2008. pp.61-73.
19. Singhal, M., Paul, A., Singh, H.P., Dubey, S.K. and Gaur, K. Evaluation of reducing power assay of chalcone semicarbazones. J. Chem. Pharm. Res; 2011. p.639-645.
20. Mizushima, Y. and Kobayashi, M. Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. *Journal of Pharmacy and Pharmacology; 1968.* pp.169-173.
21. Sakat, S.S., Mani, K., Demidchenko, Y.O., Gorbunov, E.A., Tarasov, S.A., Mathur, A. and Epstein, O.I. Retracted article: Release-Active Dilutions of Diclofenac Enhance Anti-inflammatory Effect of Diclofenac in Carrageenan-Induced Rat Paw Edema Model. *Inflammation; 2014.* pp.1-9.
22. Parvin, M.S., Das, N., Jahan, N., Akhter, M.A., Nahar, L. and Islam, M.E. Evaluation of in vitro anti-inflammatory and antibacterial potential of Crescentia cujete leaves and stem bark. BMC Research; 2015. pp.1-7.
23. Kim, S.I., Hong, S., Kim, I.S. and Jung, Y.H. Total synthesis of D-fagomine and 6-deoxyfagomine. Tetrahedron; 2013. P.3901-390.
24. Mandana Hasanzad, Negar Sarhangi, Hamid Reza Aghaei Meybodi, Shekoufeh Nikfar, Pharmacogenetics and toxicology, Editor(s): Philip Wexler, *Encyclopedia of Toxicology (Fourth Edition)*, *Academic Press*, 2024, 467-491,
25. Richards, D.M. and Brogden, R.N. Pirbuterol: a preliminary review of its pharmacological properties and therapeutic efficacy in reversible bronchospastic disease. Drugs; 1985. P 6-21.
26. Vardanyan, R. and Hruby, V. Synthesis of essential drugs. Elsevier; 2006.
27. Langner, E., Walczak, K., Jeleniewicz, W., Turski, W.A. and Rajtar, G. Quinaldic acid inhibits proliferation of colon cancer ht-29 cells in vitro: effects on signaling pathways. European Journal of Pharmacology; 2015. p.21-27.
28. Ingrassia L, Lefranc F, Mathieu V, Darro F, Kiss R. Amaryllidaceae isocarbostyril alkaloids and their derivatives as promising antitumor agents. *Transl Oncol*. 2008 P 1-13.
29. Goh, Y.X., Jalil, J., Lam, K.W., Husain, K. and Premakumar, C.M. Genistein: A review on its anti-inflammatory properties. Frontiers in pharmacology; 2022. p.820969.
30. Shukla, R., Pandey, V., Vadnere, G. P., & Lodhi, S. Role of Flavonoids in Management of Inflammatory Disorders, *Bioactive food as dietary interventions for arthritis and related inflammatory diseases*. *Academic Press*, 2019. 293-322.