Pain healing potential of famous traditional fermented Rhododendron (*Lali-Guras*) beverages from Indo-Nepal Himalaya: *In vitro* and *in silico* evaluation

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Abstract

This *in vitro* and *in silico* study was designed to validate acclaimed anti-inflammatory or pain-relieving properties of fermented ethnic beverages prepared from Rhododendron or *Guras* flowers in the Singalila ridge- the famous Rhododendron growing region of the Himalayas. Traditional beverages *Guras* wine and its distilled version *Guras Raksi* were considered in this study which were collected from Gairibas, a village situated in Indo-Nepal Singalila Ridge of the Himalayas. *In vitro* protein (albumin) denaturation inhibition assay was conducted to evaluate anti-inflammatory activity of the samples and later GC-MS analysis was carried out to identify anti-inflammatory compounds present in those beverages. From GC-MS results, eleven major metabolites such as 5-hydroxymethylfurfural; quinic acid; clionasterol; l-(+)-ascorbic acid, 2,6-dihexadecanoate; d-sorbitol; cis-cinnamic acid; tyrosol; lupeol; methyl commate A; 2-hydroxy-gamma-butyrolactone; and 1,3-propanediol, 2-(hydroxymethyl)-2-nitro- were chosen for molecular docking with human cyclooxygenase-1 (hCOX-1), an important targets in the drug-design for nonsteroidal anti-inflammatory drugs. Among all query compounds, phytosterol- clionasterol and triterpenoid- lupeol and methyl commate A exhibited considerably high binding energy scores (<-8 kcal/mol) even compared to anti-inflammatory drugs- acetaminophen and ibuprofen. Outcome of this research affirmed the potential of *Guras*-based traditional drinks in the healing of different forms of high-altitudinal stress induced pain.

Keywords: *Rhododendron wine, ethnic beverages, molecular docking, anti-inflammatory, GC-MS analysis*

Introduction

Pain or inflammation in high-altitude is a response of various altitudinal stresses (Majumder et al., 2023a). Native mountain people, trekkers and tourists sometimes encounter severe muscle and joint pain due to high altitude-induced physical stresses where remedies like ethno-medicinal herbal preparations, foods and beverages function as therapeutic (Majumder et al., 2021). Earlier, traditional fermented beverages *Tongba*, *Chhang*, and coumarin rich ethnomedicinal *Khokim Raksi* of this region were reported for anti-inflammatory and other medicinal properties (Majumder et al., 2021, 2022a, 2024). Traditionally, rhododendron or *Lali Guras* (*Rhododendron arboreum* Sm.) flowers are also used as substrates to prepare fermented beverages like wine and *raksi*. In the rhododendron growing regions of the Himalayas (Uttarakhand, Himachal Pradesh, West Bengal- Singalila ridge and Sikkim), *Guras* wine prepared by fermenting rhododendron's petal decoction using ethnic starter *marcha* is a popular drink for local inhabitants and tourists in the Himalayan region (Majumder et al., 2023b). This traditional product also hold cultural importance, often celebrated and sold during festivals (example: *Guras* festival in Singalila ridge of Indo-Nepal and Sikkim). Rhododendron decoction and its wine are often served and consumed to treat fever, body ache, muscular pain etc. (Singh et al., 2002; Majumder et al., 2023b). Majumder et al. (2023a) reported *in silico* validation of various high-altitude sickness (HAS) healing potential of various Himalayan ethnic beverages that included a range of diseases and disorders including acute mountain sickness

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(AMS), peripheral edema, high-altitude cerebral edema (HACE), high-altitude pulmonary edema (HAPE), hypoxia, infections commonly encountered at high altitude, pain and inflammation; cardiovascular diseases, gastrointestinal or digestive problems; neurological and psychiatric disorders and diseases etc. Liang et al. (2018) and Yeerjiang et al. (2023) also explored efficacy of natural products in combating high-altitude sicknesses by molecular docking.

Rhododendron arboreum Sm., locally called *Lali-Guras* in eastern Nepal and Darjeeling and *Buransh* in Uttarakhand, is an important flower species whose flowers are used in brewing of wine and *Raksi* in the Himalayas, particularly in high-altitude settlements of Darjeeling (West Bengal), Sikkim, Uttarakhand, Himachal Pradesh, etc. (Majumder et al., 2023b; Lingwan et al., 2023). "The land of Rhododendrons"- the Singalila Ridge of the Himalayas (altitude: 2400-3600 meters) nurtures a rich and diverse flora of *Rhododendron* sp. where the red flowers of *Rhododendron arboreum* Sm. or *Lali Guras* is commonly used as fermentation substrate. Being rich in bioactive carbohydrates, chlorogenic acids, quinic acid, polyphenols, flavones, ursolic acid, etc., the juice and wine made from it possess ethnomedicinal properties, including acclaimed antioxidant and anticancer, anti-inflammatory, cardioprotective, antidiabetic, antiallergic, hepatoprotective etc. activities (Majumder et al., 2023b; Lingwan et al., 2023). *In vivo* anti-inflammatory and antinociceptive effects of the methanolic, ethanolic and ethyl acetate extracts of *R. arboreum* leaf and its fractions were reported (Rawat et al., 2017; Verma et al., 2010). Researchers revealed presence of flavonoids (hyperin), tannins, saponins and other phytochemicals to be responsible for anti-inflammatory activity (Nisar et al., 2014; Verma et al., 2010; Sharma et al., 2009). During the last covid-19 pandemic, scientists also researched and found anti-coronavirus potentials of *Guras* (Lingwan et al., 2023) due to presence of quinic acid and other chlorogenic acids. Earlier, antioxidant and antibacterial potential of these beverages were analyzed through biochemical tests and *in vitro* assays (Majumder et al., 2023b). Such recent concern on biological activities of this particular flower prompted this research to evaluate the underexplored ethnobiologically acclaimed pain-reliving potential (Rawat, 2022; Rawat et al., 2017) of its products which has not yet been validated scientifically. The process of brewing fermented beverages from *Lali Guras* includes harvesting fully bloomed *Lali Guras* or red *Rhododendron arboreum* Sm. flower, preparation of decoction by boiling the petals vigorously in water, addition of starter culture- *Marcha*, incubation for two – three weeks (depending on season) to facilitate the fermentation of wine, and finally distillation process to prepare its *Raksi*. In Singalila region, the drink is even praised by local people and tourists (Majumder et al., 2023b). Hence, this current study aimed to evaluate the anti-inflammatory property of different *Guras*-based beverages through *in vitro* (protein denaturation inhibition) and *in silico* (molecular docking) assays.

Materials and Methods

Collection of ethnic beverage samples

Rhododendron-based beverages were sampled from a small old brewing cottage located in Gairibas- one of the oldest human settlements in Singalila National Park situated at Indo-Nepal region of the Himalayas (27°02'52"N, 88°01'47"E; Elevation: 2581 m). Depending on brewing stages, three different samples such as, unfermented Rhododendron flower or *Guras* decoction (UGD), fermented *Guras* wine (GW), and the distilled version *Guras Raksi* obtained from a same fermentation batch were collected in sterilized screw-cap tubes (Plate I, Fig. 1) with replicas. The juice or unfermented decoction is not generally consumed in this region, but that was collected aiming for a comparative analysis.

In vitro anti-inflammatory test (protein denaturation inhibition assay)

Following the protocol of Dharmadeva et al. (2018) *in vitro* anti-inflammatory activity was evaluated through analysing albumin denaturation inhibition power. Reaction mixtures were prepared using 2.8 ml of phosphate-buffered saline (pH 6.4) and 0.2 ml of egg albumin (from fresh hen's egg). Then 2 ml of sample (each beverage

sample) were mixed gently with reaction mixtures. A similar procedure was followed only replacing sample with distilled water (for control) and with different concentration of ascorbic acid (to prepare standard curve). Similarly, a control set was prepared using distilled water as sample l. Reaction mixture was incubated in a hot-bath at 37±2°C for 20 min followed by heating at 70°C for 5 min. Then, the reaction mixture was allowed to cool down at room temperature for 15 min. Absorbance of reaction mixture after denaturation was measured at 680 nm using a spectrophotometer. Each test was carried out in triplicates and the mean absorbance was recorded. Ascorbic acid was used as reference, the results were determined from standard curve: y=-0.0077x + 0.7743; R^2 = 0.9949 and expressed as µg AAE/ ml. The protein denaturation inhibition percentage was determined using the following formulae:

$$Inhibition\ of\ albumin\ denaturation\ (\%) = \frac{Control_{Abs} - Sample_{Abs}}{Control_{Abs}} \times 100$$

GC-MS analysis and selection of metabolites for molecular docking

To prepare sample extracts, 1 mL of each sample was evaporated in room temperature and dissolved in 1 mL methanol, the widely accepted organic solvent for extraction. The polarity of methanol shows proximity with water/ethanol, the foundation of any edible beverage (Majumder et al., 2021, 2022a). GC-MS analysis was carried out following Majumder et al. (2020) and Acharyya et al. (2021). GCMS-QP2010 Plus (Shimadzu Co., Japan) was used in this research which was packed with a DB-5 fused-silica capillary column (30 m × 0.25 mm × 0.25 µm). 1 µL of each sample was split injected with a ratio of 20:1. Column oven temperature: 80 °C, carrier gas: helium (99.9995% purity), injection temperature: 260 °C, total flow rate: 16 mL/min, and column flow rate: 1.2 mL/min were considered. Flow control mode was at a linear velocity of 40.5 cm/sec. The interface temperature and ion source temperature were set to 270 °C and 230 °C respectively. Total ion chromatogram (TIC) was based on the intensity of fragments produced by the ionization. A single-quadrupole mass spectrometer was used in this research. Mass spectra were recorded at 5 scan/sec with a scanning rate of 40-600 m/z. For compound identification, the probability-based matching method was used. Here, the obtained spectra were matched with Wiley and the NIST databases for identification. Data acquisition and control of the chromatograph were carried out using the GCMSsolution software. Detected compounds with relatively large peak area were considered as major compounds (Majumder et al., 2024) and chosen for follow-up molecular docking analysis.

Retrieval of protein and ligands and docking analysis

Inflammatory receptor protein- human cyclooxygenase-1 (hCOX-1) was chosen by considering inflammation induced from diseases and disorders encountered in high-altitude places. The protein (PDB: 6Y3C) was retrieved from Protein data bank (Berman et al., 2000). The structures of selected eleven major compounds detected in *Guras* beverages and two standard anti-inflammatory drugs- acetaminophen (paracetamol) and ibuprofen were extracted from ZINC (Irwin et al., 2020). Auto Dock Tools (AutoDock4 and AutoDock Vina n.d.) (Majumder et al., 2023a; Ghosh et al., 2022) and SwissDock (Grosdidier et al., 2011) were used for molecular docking analysis. The condition parameters were adjusted following Majumder et al. (2023a) such as, default grid size was configured as x=40, y=40 and z=40 and distance between the grid points were 0.375 Å. The results of docking by Auto Dock tools and Vina were visualized with PyMOL molecular visualization tool. 2-D representations of protein-ligand complexes were obtained from LigPlot+ software (Laskowski and Swindells, 2011; Ghosh et al., 2022).

Results

In vitro anti-inflammatory test (protein denaturation inhibition assay)

Results of this analysis revealed highest anti-inflammatory activity from fermented sample GW (65.63±0.67% or ~70 µg AAE/ ml) followed by unfermented decoction-UGD (39.42±0.51% or ~46 µg AAE/ ml). A fermentation process induced increase of anti-inflammatory activity has been seen in the result, graphically represented in (Plate I, Fig. 2). However, distilled sample GR exhibited the least inhibition power with showing only 19.12±0.18% (~28 µg AAE/ ml). Furthermore, GC-MS analysis and molecular docking were carried out that elucidated more.

GC-MS analysis and metabolites for docking

GC-MS analysis revealed thirty-seven metabolites in UGD which was rich in phytochemicals like quinic acid and other phenolics (total peak area: 41.19%); fatty acid derivatives (total peak area: 19.14%); terpenoids and steroids (total peak area: 14.43%);

and amino acid derivatives (total peak area: 1.81%) were present. Results revealed that a large number of molecules in UGD originated as derivatives of compounds exclusively found in Rhododendron flowers, devoid of metabolites synthesized due to involvements of external organisms. Phytochemicals (plant origin biomolecules) were also detected in the fermented distilled sample GR, but the peak areas were comparatively very low. In the unfermented sample UGD, presence of unfermented and derivatized sugars or other carbohydrates were also reflected due to caramelization of sugar and other carbohydrate metabolites (total peak area: 23.16%) while fermented samples were rich in microbial biosynthesized metabolites or fermented products of those substrate carbohydrates. A total of 66.22% peak area in GW were composed of various fermented carbohydrate derivatives; for GR a higher value (85.68%) has been recorded led by glycerol derivative-1,3-propanediol, 2-(hydroxymethyl)-2-nitro- (52.62%). The percentage shares (based on total peak area) of different types of metabolites area have been graphically represented in (Plate II, Fig. 3, Fig. 4).

Docking results

Docking score mimics the potential energy change when the protein and ligand are about to bind. A more negative energy scores or estimated ΔG (kcal/mol) indicates a stronger binding, while a less negative or potentially positive score indicates a weak or absent binding. Docking energy scores of *Guras*-based ethnic beverages' metabolites while binding with hCOX-1 protein are included in Table 1. Some representative images of docking analysis results (visualized with PyMOL molecular visualization tool and LigPlot+) are given in (Plate III, Fig. 5).

Table 1. Docking energy scores or estimated ΔG (kcal/mol) of metabolites of different *Guras*-based beverages while binding with hCOX-1 (PDB: 6Y3C)

Index	Name of the molecule	Sample source (GC peak area%)	ΔG (kcal/mol)
G1	5-Hydroxymethylfurfural	UGD (4.35%)	-6.4
G2	Quinic acid	UGD (34.97%)	-6.6
G3	Clionasterol	UGD (5.47%)	-8.5*
G4	l-(+)-Ascorbic acid 2,6-dihexadecanoate	GW (20.74%)	-6.4
G5	D-Sorbitol	GW (28.01%)	-5.6
G6	cis-Cinnamic acid	GW (1.09%)	-6.2
G7	Tyrosol	GW (1.65%)	-5.4
G8	Lupeol	GW (3.69%)	-8.2*
G9	Methyl commate A	UGD (3.14%), GW (3.15%), GR (2%)	-8.8*
G10	2-Hydroxy-gamma-butyrolactone	GR (4.46%)	-4.7
G11	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-	GR (52.62%)	-4.9
CD1	Acetaminophen/ Paracetamol	Control drug	-6.9
CD2	Ibuprofen	Control drug	-8.1

* Scores higher than control drugs

Discussion

Albumin denaturation assay is one of the popular, quick and cost-effective *in vitro* anti-inflammatory assessments which helps to determine whether a sample with anticipated anti-inflammatory property can inhibit thermal denaturation of protein (Dharmadeva et al., 2018). Denaturation refers to the structural and functional changes in a protein that results in loss of bioactivity of that protein. Fresh egg white (albumin) was employed as

a model protein in this experiment and denaturation of the albumin (*in vitro* inflammatory response) was brought about by inducing heat. This experiment was based on the concept where it is considered that substances with anti-inflammatory properties can stabilize protein structures and inhibit denaturation, a process often associated with inflammatory response and tissue damage. Therefore, tested sample significantly inhibiting albumin denaturation is considered as anti-inflammatory (Dharmadeva et al., 2018). Scientists have also reported that chemicals or agents, specifically NSAIDs, which prevent protein denaturation can potentially inhibit the COX enzyme (human inflammation receptors) simultaneously (Madhuranga and Samarakoon, 2023).

The results of *in vitro* anti-inflammatory activity corresponded the same trend determined through antioxidant (DPPH and iodometric) assays by Majumder et al. (2023b) where highest bioactivity was noticed in *Guras* wine was followed by unfermented decoction and distilled *Raksi*. In comparison with anti-inflammatory reference drug ascorbic acid or Vitamin-C, the *Guras* beverage claimed their potential as albumin denaturation inhibitor as shown in Fig. 2. Thus, this *in vitro* anti-inflammatory analysis somewhat validated the ethnobiological claims of pain relieving activity of *Guras*. Furthermore, molecular docking provided biomolecular insight into this property as described below.

As this study exclusively focused on evaluating anti-inflammatory properties, therefore, a brief literature study has been done to identify the anti-inflammatory biomolecules. This revealed fifteen compounds with reported anti-inflammatory properties: UGD's major phenolic compound quinic acid (Lingwan et al., 2023; Majumder et al., 2022b); 2-hydroxy-gamma-butyrolactone (Salat et al., 2012); 2,3-dihydrobenzofuran (Closse et al., 1981; Chen et al., 2019; Nousheen et al., 2022); GW's tyrosol (Hu et al., 2020; Majumder et al., 2022b;); UGD's terpenoid metabolites- cis-citral (Ganjewala et al., 2012), patchoulane (Chavan et al., 2012), beta-copaene (Al-Rekaby, 2020), alpha-curcumene (Lenfeld et al., 1986), squalene, vitamin E, methyl commate A (detected in all samples) (Majumder et al., 2020), fucosterol (Abdul et al., 2016); exclusive GW metabolites- bornyl acetate and lupeol (major terpenoid of GW) (Majumder et al., 2020); and exclusive GR metabolite- cis-α-irone (Lim, 2016). Among these fifteen biomolecules eleven signature compounds (Fig. 4) with considerably high relative peak areas were used in the docking analysis.

Cyclooxygenase enzymes convert arachidonic acid to prostaglandins that promote inflammation, pain, and fever; thus, considered useful as targets for designing non-steroidal anti-inflammatory drugs (Smith et al., 1998; Tegeder, 2013). Human cyclooxygenase-1 or hCOX-1 maintains many physiological functions like blood flow regulation, platelet aggregation and mucus production in the stomach by producing prostaglandins and thromboxanes. Wallace et al. (1998) and Tegeder (2013) demonstrated the expression of COX-1 through immediate release of PGE2 in the spinal cord and periphery a response of injury which helps to activate and sensitize nociceptive neurons. Normally, nociception and feeling of pain are mediated by toxic molecules, inflammatory mediators and noxious thermal stimuli at temperature extreme conditions which can harm tissues (Dubin and Patapoutian, 2010; Schaible, 2015; Middleton et al., 2021). These high threshold physical stimuli are detected by nociceptors- specialized peripheral sensory neurons (Dubin and Patapoutian, 2010; Middleton et al., 2021) that simultaneously alert human from such potentially damaging stimuli (Dubin and Patapoutian, 2010). Therefore, finding out potential of the molecules present in high-altitude's *Guras*-beverages in alleviating inflammation or pain and deteriorating effect of extreme cold temperature on pain was necessary in extreme cold Himalaya's perspective. Tegeder et al. (2001) in a study reported that COX-2 inhibitor (celecoxib) had no antinociceptive effect, whereas a COX-1 inhibitor (ibuprofen) was effective. So, hCOX-1 has been targeted to find out probable nociceptor blockers (substances that can block pain signalling) in this molecular docking

analysis. Moreover, Ibuprofen being a potential inhibitor of COX-1 and much less effective as COX-2 inhibitor was chosen as standard drug in this study.

COX-1 also contributes to neuropathic pain or neuroinflammation (Choi et al., 2009; Tegeder, 2013). This pain is different from nociception because its development does not involve physical damage or response to any specific circumstance or outside stimulus. Neuropathic pain or nerve pain is referred to injury or medical condition that damages the nervous system or prevents it from working properly which is usually chronic. Symptoms such as numbness and tingling, loss of balance and coordination, vertigo, muscle weakness, loss of alertness, confusion, irrational behaviour, hallucinations, tightness in the chest, nausea and vomiting, loss of appetite, insomnia, constipation, anxiety etc. are symptoms of neuropathic pain in high-altitude condition (Ericsson et al., 2001; Chen et al., 2018; Majumder et al., 2023a). Earlier, Majumder et al. (2021, 2023a) classified neuropathic pain or nerve pain or neuroinflammation as a typical high-altitude sickness and also evaluated the role of various ethnic beverages other than *Guras* wine or *Raksi* in combating this sickness. Majumder et al. (2023a) also targeted hCOX-1 in an *in silico* study to evaluate the anti-inflammatory potential of signature molecules from different Himalayan beverages.

Moreover, Yi et al. (2020) reported efficiency of COX inhibitors in alleviating the symptoms of acute mountain sickness. Distinct role of COX-1 on neuroinflammatory response (hippocampal inflammation) and associated high-altitude hypoxia induced cognitive deficits has also been reported by Chauhan et al. (2019). Therefore, in this *in silico* study, selection of hCOX-1 over other proteins was reasonable as it not only associates inflammation but also a range of complications occurred due to high-altitudinal inflammation.

Methyl commate A was detected as the major compound of terpenoid class in all three samples (UGD, GW and GR). Protein-ligand binding energy score with selected hCOX-1 protein generated by this pentacyclic triterpene metabolite was the strongest among all (energy score: -8.8 kcal/mol) (Fig. 5-A and A'). This result followed by another two metabolites derived from terpenoid pathway such as, UGD's abundant phytosterol clionasterol (energy score: -8.5 kcal/mol) and GW's major compound pentacyclic triterpene alcohol- lupeol (energy score: -8.2 kcal/mol) (Fig. 5-B and B'). Terpenoid biosynthesis pathway was earlier established as a source of various anti-inflammatory compounds (terpenoids, tocopherol, steroids/phytosterols, etc.) which have been used for therapeutic purposes for centuries (Majumder et al., 2020). These three terpenoid metabolites even scored stronger binding energy compared to scores of two tested anti-inflammatory standard drugs acetaminophen or paracetamol and ibuprofen which also affirmed their potential as anti-inflammatory candidate unlike other query molecules. Moreover, other terpenoids detected in UGD, GW, and GR (apart from the three major compounds selected for docking) were also reported to possess anti-inflammatory activity as mentioned above which might be associated with natural pain-killing property of *Guras*, one of the acclaimed medicinal properties for which the drink is valued ethnomedicinally (Rawat, 2022). Following terpenoids, phenolic acids such as UGD's major compound quinic acid (Fig. 5-C and C') and GW's cis-cinnamic acid, UGD's Maillard reaction product 5-hydroxymethylfurfural, and GW's major carbohydrate catabolite ascorbic acid derivative l-(+)-ascorbic acid 2,6-dihexadecanoate (Fig. 5-D and D') retuned significant outcome (binding energy score: <-6 kcal/mol) followed by GW metabolites D-sorbitol and tyrosol (binding energy score: -5.6 and -5.4 kcal/mol). Two of the selected exclusive GR metabolites- 1,3-propanediol, 2-(hydroxymethyl)-2-nitro- (major compound with highest peak area) and 2-hydroxy-gamma-butyrolactone scored the least binding energy score (Table 1) which corresponded the least *in vitro* anti-inflammatory activity as exhibited by the crude sample of *Guras Raksi* (Fig. 2).

Conclusion

This article highlighted a very specific ethnomedicinal value i.e., anti-inflammatory or pain relieving property linked to traditional Rhododendron or *Guras* based beverages. In this research, *in vitro* analysis demonstrated anti-inflammatory activity, which was validated through detection of anti-inflammatory terpenoids, phenolics and some sugar metabolites by analytical GC-MS analysis. Later *in silico* (docking) experiment revealed all selected molecules of *Guras* as potential candidates to bind with COX-1 protein and to be its probable inhibitors. Even, the three selected terpenoids- methyl commate A (detected in all samples), clionasterol (of UGD), and lupeol (of GW) performed better by showing stronger binding affinity compared to control drugs paracetamol and ibuprofen. These alcoholic beverages not only offer a refreshing experience and medicinal properties but also boost tourism and micro-economy of this region. However, it is noteworthy that the region lacks a large-scale production unit or industry dedicated to local alcoholic beverages. It is only confined to each ethnic group or community of the respective state, especially women who are associated with preparing these beverages. By embracing advanced food biotechnology or fermentation technology, the region can unlock the potential for large-scale production of these culturally significant beverages. This research validated anti-inflammatory potential of *Guras* beverages through *in vitro* and computational techniques which may advise future pharmacological experiments and *in vivo* analysis to investigate the medicinal properties in depth.

Conflict of interest:

None

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Authors' contributions:

SM designed the study, carried out sample collection, preparation and metabolite profiling. SM and SS carried out *in vitro* analysis. SM, SC and AG carried out molecular docking. SM wrote the manuscript. SM and MB revised the manuscript. MB supervised this research.

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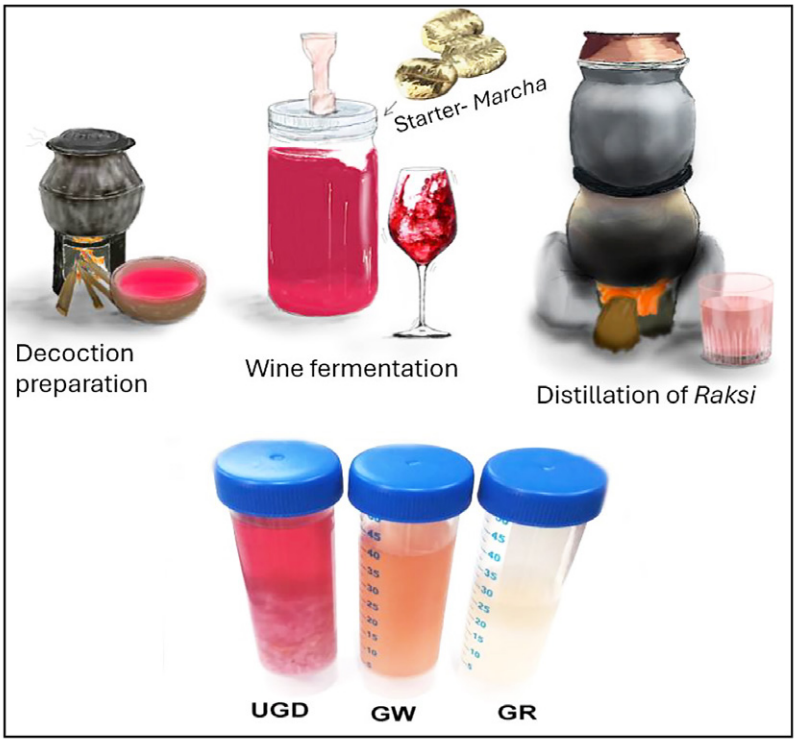

Fig. 1. Different brewing stages of Rhododendron or *Guras* fermentation i.e., decoction, fermentation and distillation; collected samples *Guras* decoction (UGD), fermented *Guras* wine (GW), and the distilled version *Guras Raksi*

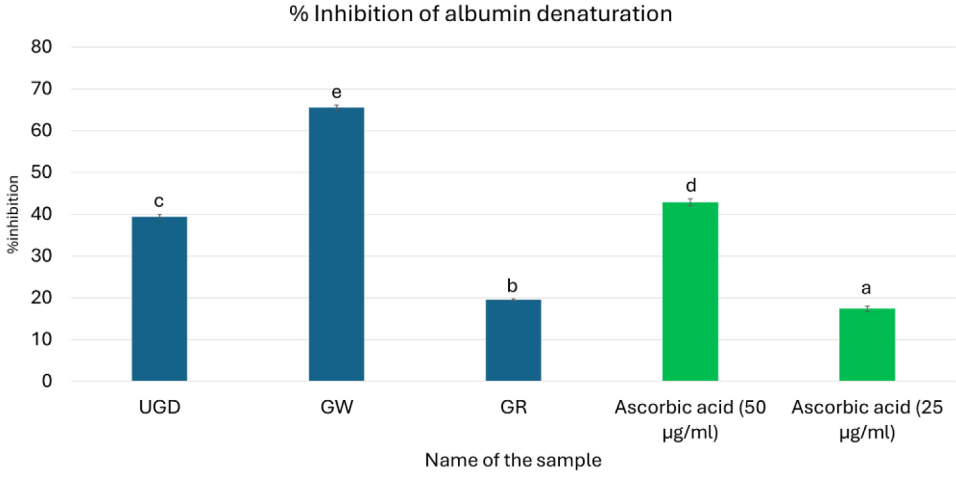

Fig. 2. *In vitro* anti-inflammatory activity (%protein denaturation inhibition power) shown by samples- UGD (unfermented *Guras* decoction), GW (*Guras wine*) and GR (*Guras Raksi*). Different letters above the error bars signify significant difference among samples ($P < .05$).

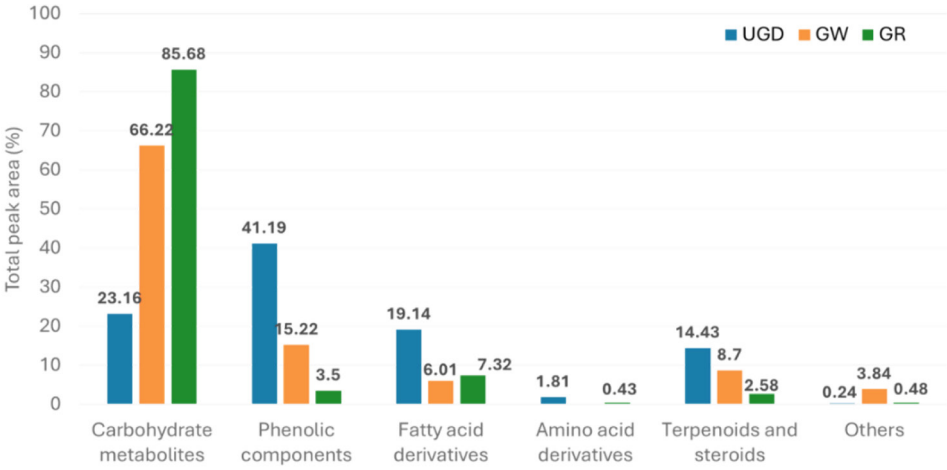

Fig. 3. Percentage shares (peak area) of different types of metabolites in UGD (unfermented *Guras* decoction), GW (*Guras* wine) and GR (*Guras Raksi*)

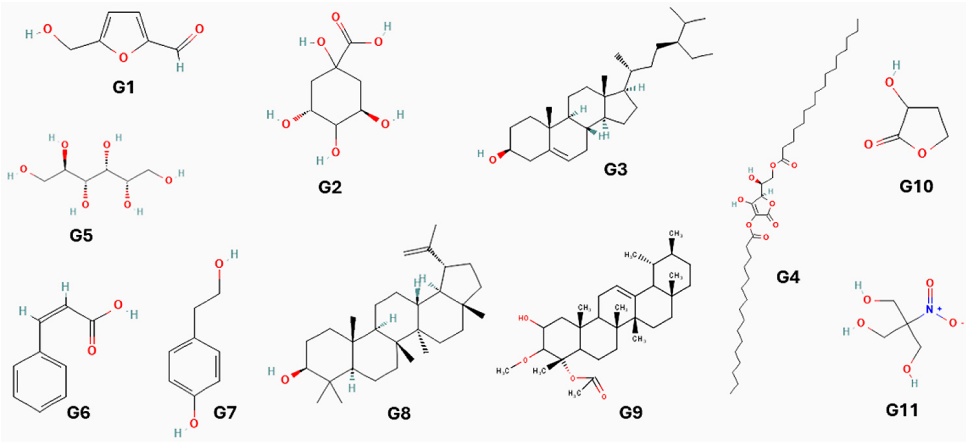

Fig. 4. Chemical structures of selected ligands i.e., G1: 5-Hydroxymethylfurfural; G2: Quinic acid; G3: Clionasterol; G4: l-(+)-Ascorbic acid 2,6-dihexadecanoate; G5: D-Sorbitol; G6: cis-Cinnamic acid; G7: Tyrosol; G8: Lupeol; G9: Methyl commate A; G10: 2-Hydroxy-gamma-butyrolactone; and G11: 1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-

Plate III

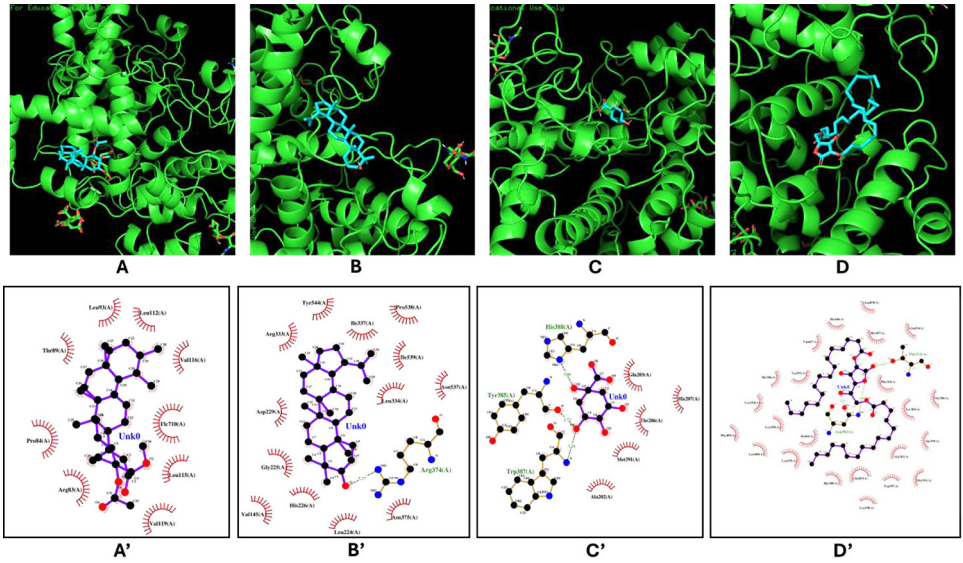

Fig. 5. Top: PyMOL representations of protein-ligand bindings achieved after docking of Methyl commate A (A); Lupeol (B); Quinic acid (C) and l-(+)-Ascorbic acid 2,6-dihexadecanoate (D) with COX-1 (PDB: 6Y3C). Bottom: 2-D representations of protein- ligand interactions from Ligplot+ program showing Methyl commate A (A'); Lupeol (B'); Quinic acid (C') and l-(+)-Ascorbic acid 2,6-dihexadecanoate (D') at respective binding sites of COX-1.