In vitro Assessment on Anti-Inflammatory and Anti-Lipidemic Properties of Selected Plant Species

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Preliminary assessment for anti-inflammatory and anti-lipidemic properties was done with different solvent extracts derived from *Urtica urens* and *Polygonum chinense* leaves through *in vitro* experimentation. To evaluate anti-inflammatory properties, the stability of human red blood cells membranes and the denaturation activity of proteins were assessed. For anti-lipidemic effects, an assay was conducted to measure the inhibition of HMG-CoA reductase. The results of membrane stabilization showed IC_{50} values of 480.96 ± 0.02 and 319.41 ± 0.19 µg/mL for ethyl acetate extract of *U. urens* and *P. chinense*, respectively. The standard drug Diclofenac sodium exhibited IC_{50} value of 240.37 ± 0.04 µg/mL. For protein denaturation, IC_{50} values were determined as 221.75 ± 0.2 and 315.76 ± 0.19 µg/mL for *U. urens* and *P. chinense*, respectively. The IC_{50} value of the standard drug was calculated as 126.7 ± 0.34. The IC_{50} values towards HMG-CoA reductase inhibition were subsequently determined as 29.84 ± 0.35 µg/mL for *U. urens* and 24.34 ± 0.04 µg/mL for *P. chinense* against the standard drug Diclofenac sodium (7.52 ± 0.43 µg/mL). GC-MS chromatograms revealed the presence of bioactive compounds in ethyl acetate extract of *P. chinense* leaves. This work is substantiation for the traditional therapeutic utilization of these extracts.

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INTRODUCTION

Natural products serve as a reservoir for both synthetic and traditional herbal therapies. Medicinal plants represent a remarkable endowment bestowed upon us by the natural world, serving as a vast repository of diverse pharmaceutical agents found throughout the globe (Agada *et al.* 2022). Herbal medicines are commonly marketed and distributed as dietary supplements, thereby subjecting their therapeutic indications, efficacy, and safety to varying perspectives that are contingent upon the clinical or traditional knowledge associated with diverse folk medicines prevalent in individual nations (Calapai 2007; Monica *et al*. 2022). There has been a significant shift in perspectives on the therapeutic uses of ethnopharmacology during the past decade (Saraswathi *et al*. 2021). Inflammation is a physiological reaction of the body that occurs in response to injury, infection, or tissue death characterized, by the presence of heat,

redness, discomfort, swelling, and disruptions in normal physiological activities. The initiation of this process occurs as a result of the liberation of chemical mediators from damaged tissue and migratory cells (Chandra *et al*. 2012; Prasathkumar *et al*. 2021).

Inflammatory disorders, encompassing all forms of rheumatic conditions, are highly prevalent and impact around 1% of the global adult population (Gabriel 2001). The condition results in notable impairment and subsequent decline in the overall well-being, resulting in a considerable socio-economic burden (Buch and Emery 2002). Steroidal and non-steroidal anti-inflammatory drugs (NSAIDs) are the current therapeutic approaches for managing inflammatory illnesses (Langman 1998; Khuda *et al*. 2022). The prolonged use of these medications results in detrimental side effects and compromises the integrity of the human biological system, including the liver, gastrointestinal tract, and other relevant systems. Consequently, there exists a persistent necessity for the development of novel anti-inflammatory pharmaceuticals that are both safe and powerful, with reduced or negligible toxicity. Numerous botanical sources and therapeutic substances possess anti-inflammatory properties, which function by expediting the degradation or inhibiting the activity of inflammatory mediators, therefore modulating the inflammatory response. Nevertheless, prolonged inflammatory reactions can lead to severe ailments such as cancer, diabetes, and Alzheimer's disease (Lee 2011; Ham and Moon 2013; Prasathkumar *et al*. 2022). Multiple studies have demonstrated a strong association between hyperlipidemia and inflammation, which plays a substantial role in the development of specific cardiovascular disorders, including atherosclerosis (Tunon *et al*. 2018; Mena-Vazquez *et al*. 2022).

Hyperlipidemia is a prevalent metabolic syndrome that is characterized by elevated levels of total cholesterol (TC), total triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C), as well as reduced levels of high-density lipoprotein cholesterol (HDL-C). These lipid abnormalities collectively contribute to the development of disturbances in lipid metabolism. Elevated lipid levels pose a significant risk factor for the development of hypertension, coronary heart disease, and cerebrovascular damage. The existing body of research indicates that the harmful impact of postprandial hyperlipidemia is likely caused by the activation of oxidative stress (Bae *et al*. 2001). The prevalence of hyperlipidemia has been increasing in recent years, primarily attributed to reduced levels of physical activity and the growing intake of calorie-dense fast foods that are low in fibre (Saklayen 2018). Therefore, it is crucial to highlight the significance of dietary interventions that target the reduction of hyperlipidemia and the associated oxidative stress. Previous studies have demonstrated the inhibitory effects of plant extracts, including phytochemicals, such as phenolic compounds and flavonoids, on HMG-CoA reductase, both in *ex vivo* (Kwon *et al*. 2010; Reddy *et al*. 2014) and *in vivo* studies (Qinna *et al*. 2012).

Urtica urens L., also known as stinging nettle, is a plant belonging to the Urticaceae family. It is characterized as a dioecious herbaceous plant that can either be an annual or perennial species. The decoction derived from nettle leaves has been shown to have laxative, diuretic, and menorrhagia properties. Nettle has historically been employed in folk medicine for the treatment of iron deficiency anemia, because of its substantial iron concentration. According to Rezaei *et al*. (2012), nettle is utilized for its anti-inflammatory, blood sugar-lowering, analgesic, diuretic, and local anesthetic properties. It has also been shown to alleviate inflammation of the prostate.

Polygonum chinense L. is a perennial plant species belonging to the Polygonaceae family. It is found in habitats such as damp valleys, mixed woods, thickets, as well as

grassland or mountain slopes. This plant possesses potential therapeutic properties for the treatment of gastritis, anti-inflammatory effects, promotion of blood circulation, management of dysentery and diuretic properties, and potential efficacy in managing hemorrhage. While there are claims that several medicinal plants have been utilized as traditional remedies for a wide range of ailments, their efficacy lacks scientific substantiation. Therefore, the current preliminary investigation was conducted to objectively validate the traditional claim by assessing not only the anti-inflammatory properties of three distinct solvent extracts derived from *U. urens* and *P. chinense* leaves via membrane stabilization and protein denaturation assays but also the anti-lipidemic effects by inhibiting HMG-CoA reductase.

EXPERIMENTAL

Materials and Methods

Collection and extraction of plant material

The study utilized an ethnomedicinal and traditional approach to obtain freshly collected leaves of *U. urens* and *P. chinense* that were free from pathogens. The plant sample collection was conducted in January from the Wokha district situated in the Nagaland district, India. The leaves were first washed with clean water, followed by desiccation in a shade setting, and then pulverized into granules with a coarse texture. The dried and finely ground leaves powder was subjected to solvent extraction using hexane, ethyl acetate, and methanol at a ratio of 1:3 relative to the mass of the sample, based on their respective polarity. Indeed, a precise amount of 750 g (*U. urens*) and 1000 g (*P. chinense*) of the material was employed for the extraction operation. Whatman filter sheets were utilized to purify the extracts through the process of solvent evaporation. The extracted yield was preserved by chilling in order to maintain its integrity for future research experiments.

Solvents and chemicals

The organic solvents and the chemicals of analytical grade procured from Hi-Media chemicals and Merck Ltd. Mumbai, India, were used throughout the experiments.

Anti-inflammatory Activity

Membrane stabilization assay: Preparation of red blood cells (RBCs) suspension

A sample of 5 mL of freshly obtained solely human blood was taken using a heparinized tube and afterwards transferred to centrifuge tubes. The tubes underwent centrifugation at a speed of 3000 rpm for a duration of 10 min, followed by three subsequent washes using an equivalent volume of normal saline. The blood volume was quantified and subsequently diluted to form a suspension with a concentration of 40% v/v using an isotonic solution, namely 10 mM sodium phosphate buffer (Vasanthkumar *et al.* 2017).

Heat-induced hemolytic assay

Various concentrations (50, 100, 200, 400, 800, and 1600 µg/mL) of test samples and a reference standard (Diclofenac sodium) were combined with 0.1 mL of a 40% solution of RBCs. The control group was comprised of 0.1 mL of RBCs that were mixed only with an isotonic solution. The mixture was incubated in a water bath at a temperature

of 56 °C for a duration of 30 min. Following the incubation period, the tubes were then allowed to come down to the ambient temperature of the laboratory. The reaction mixture underwent centrifugation at a speed of 2500 rpm for a duration of 5 min, following which the absorbance of the resulting supernatant was determined at a wavelength of 560 nm. The percentage of membrane stabilization activity was determined by the utilization of the subsequent formula:

$$\% \text{ Inhibition of Haemolysis} = [(\text{OD of test} - \text{OD of control}) / \text{OD of test}] \times 100 \quad (1)$$

Protein Denaturation Assay

The experiment was conducted as per the modified methodology of Priya *et al*. (2011). Various concentrations (50, 100, 200, 400, 800, and 1600 µg/mL) of the test sample, reference standard (Diclofenac sodium), and control (without standard/test sample) were prepared by adding them to 4 mL of a phosphate buffer solution (0.2 M, pH 7.4). A volume of 1 mL of 1 mM albumin solution in phosphate buffer was combined with all the tubes and subjected to incubation at a temperature of 37 °C within an incubator for a duration of 15 min. The process of denaturation was initiated by subjecting the reaction mixture to a temperature of 60 °C in a water bath for a duration of 15 min. Following the process of cooling, the level of turbidity was assessed at a wavelength of 660 nm. The calculation of the percentage inhibition of denaturation was performed using the following formula:

$$\% \text{ Inhibition} = [(\text{OD of test} - \text{OD of control})/\text{OD of test}] \times 100 \quad (2)$$

Anti-lipidemic Assay

Inhibition of HMG-CoA Reductase

The experiment involved the preparation of a test and standard drug solution, which were then divided into five concentration series, ranging from 10 to 160 µg/mL. Each reaction mixture consisted of 200 µL, comprising 12 µL of HMG-CoA substrate, 164 µL of buffer, 5 µL of reductase enzyme NADPH (Nicotinamide Adenine Dinucleotide Phosphate Hydrogen), and 19 µL of test solution. Furthermore, the solution comprised a combination of the enzyme reaction, control, and test components. The reaction was performed in triplicate, subjected to incubation at a temperature of 37 °C for a duration of 10 min, and the absorbance was quantified at a wavelength of 340 nm using a microplate reader (Setyowati *et al.* 2021). Atorvastatin was used as a standard drug. Inhibition was recorded using the below mentioned formula:

$$\% \text{ Inhibition} = [(\text{OD of control} - \text{OD of test})/ (\text{OD of control})] \times 100 \quad (3)$$

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Since, *P. chinense* leaves extracts exhibited better anti-inflammatory and anti-lipidemic activities as compared to *U. urens* leaves extracts, the ethyl acetate extract of *P. chinense* leaves was selected for GC-MS analysis. The extract was analysed using GC-MS (SHIMADZU QP2020). The carrier gas utilised in this investigation was helium gas of high purity, with a purity level of 99.99%. The flow rate was consistently maintained at 1.20 mL/min. To facilitate the identification of GC-MS spectra, a methodology incorporating electron ionisation energy was implemented, employing substantial ionisation energy of 70 eV (electron Volts). The scan time was established to be 0.30 seconds, while the identified pieces spanned a range of 50 to 500 *m*/*z*. The injection utilized

a volume of 1 µL, with a split ratio of 10:0. The injector temperature was continually maintained at 250 °C. The temperature of the column oven was initially set at 50 °C and maintained at this level for a period of 5 min. Following that, the temperature was elevated at a pace of 6 °C per min until it attained a value of 280 °C. The process of identifying phytochemicals in the test samples involved comparing their retention time (measured in minutes), peak area, peak height, and mass spectral patterns with the spectrum database of authentic compounds maintained by the National Institute of Standards and Technology (NIST) library 60.

Statistical Analysis

All the experiments were carried out in triplicate and results were expressed as mean ± SD.

RESULTS AND DISCUSSION

Anti-inflammatory Activity

The release of lysosomal components from active neutrophils is effectively inhibited, thereby limiting the extent of inflammatory reactions and subsequently reducing the tissue damage. The precise method by which the extract stabilizes the membrane remains unclear. However, it is evident that the extract effectively prevents the osmotic leakage of internal electrolytes and fluid components during induced haemolysis. Inflammation may be categorized into two main types: acute inflammation and chronic inflammation (Ferrero-Miliani *et al*. 2007). The body's initial response to damaging stimuli is known as acute inflammation, which is characterized by an augmented migration of plasma and leukocytes from the bloodstream into the wounded tissues. Chronic inflammation refers to an extended duration of the inflammatory response, resulting in a gradual alteration in the cellular composition at the site of inflammation. This condition is distinguished by the simultaneous occurrence of tissue death and repair as a consequence of the inflammatory process (Sabine *et al*. 2007). There is epidemiological and clinical evidence that supports the notion that chronic inflammation plays a significant role as a risk factor in a range of human illnesses (Khatami 2009). Hence, the inhibition of pro-inflammatory molecule synthesis and signaling factors is a crucial pathway to be targeted for the prevention and treatment of many illnesses.

To evaluate the anti-inflammatory properties, two assays were conducted to measure the ability of *U. urens* and *P. chinense* to inhibit inflammation. These assays included the assessment of membrane stabilization and protein denaturation activity. In a concentration-dependent way, all solvent extracts exhibited the ability to inhibit the mentioned activities. The aforementioned extracts resulted in a notable reduction in the hemolysis of RBCs seen throughout the course of the experiment. Ethyl acetate extract exhibited the highest level of inhibition among the three extracts, with the methanol extract following closely after. Hexane extract exhibited the lowest inhibitory activity across all tested concentrations. Table 1 and Figs. 1, 2, 3, and 4 provide a summary of the impacts of several extracts derived from both the selected plants on heat-induced haemolysis and protein denaturation. The IC_{50} values for each assay were determined using linear regression. The study evaluated the inhibitory effects of membrane stabilization in the extracts obtained from the leaves of *U. urens* and *P. chinense*. The IC_{50} values of the dry extract were measured as 798.64 ± 0.34, 480.96 ± 0.02, and 698.48 ± 0.14 from *U. urens*

and 590.78 ± 0.60, 319.41 ± 0.19, and 427.92 ± 0.17 from *P. chinense* for the hexane, ethyl acetate, and methanol extract, respectively. The IC_{50} value for the standard Diclofenac sodium was estimated as 240.37 ± 0.04 µg/mL, as shown in Table 1 and Fig. 1.

The process of protein denaturation has been characterized as a pathogenic phenomenon that entails the disruption of protein structure, leading to the subsequent loss of its functional properties (Sridevi *et al*. 2015). A number of anti-inflammatory medicines exhibit a dose-dependent capacity to reduce thermally-induced protein denaturation (Padmanabhan and Jangle 2012). The current investigation observed notable protein denaturation inhibitory activity in the various solvent extracts of *U. urens* leaves. This activity exhibited a dose-dependent increase within the range of 50 to 1600 µg/mL. The doses necessary to achieve 50% inhibition of hexane, ethyl acetate, and methanol extracts were determined as 800.26 ± 0.19, 221.75 ± 0.2, and 501.63 ± 0.32 µg/mL, respectively. This result suggests that ethyl acetate is more effective than the other extracts, as shown in Table 1 and Fig. 3. The study also investigated the protein denaturation inhibitory activity of extracts of *P. chinense*. The concentrations required for 50% inhibition were determined as 760.98 ± 0.53, 315.76 ± 0.19, and 591.28 ± 0.47 µg/mL for the respective extracts mentioned. In comparison, the standard Diclofenac sodium exhibited an IC_{50} value of 126.7 ± 0.34 µg/mL, indicating its superior efficacy compared to the other extracts being studied (Table 1 and Fig. 4). The findings from the carrageenan-induced paw edema experiment, a commonly used and reliable animal model for evaluating anti-inflammatory properties, support the notion that nettle leaves extract possesses anti-inflammatory effects, as reported by Hajhashemi and Klooshani (2013). However, there is a scarcity of information regarding the anti-inflammatory activity of *P. chinense*. Previous research conducted by Li *et al*. (2004) identified fatty acid esters, namely phthalic acid, dibutyl ester, bis (2-ethyl hexyl) maleate, and 1,2 benzenedicarboxylic acid, as compounds associated with anti-inflammatory properties. The reported anti-inflammatory effects are commonly attributed to the presence of tannins, flavonoids, and other chemical components inside the plant. Therefore, it was unsurprising that the crude extracts derived from these plants exhibited significant inhibition of protein denaturation, suggesting their potential as anti-inflammatory agents.

Table 1. *In vitro* Anti-inflammatory Activity of Hexane, Ethyl Acetate, and Methanol Extracts of *U. urens* and *P. chinense* Leaves in Comparison with Standard Drug Diclofenac Sodium

Plant	Anti-inflammatory Assays	Hexane	Ethyl Acetate	Methanol	Standard
U. urens	Membrane stabilization	798.64 ± 0.34	480.96 ± 0.02	698.48 ± 0.14	240.37 ± 0.04
U. urens	Protein denaturation	800.26 ± 0.2	221.75 ± 0.2	501.63 ± 0.32	126.7 ± 0.34
P. chinense	Membrane stabilization	590.78 ± 0.6	319.41 ± 0.17	427.92 ± 2.04	240.37 ± 0.04
P. chinense	Protein denaturation	760.98 ± 0.53	315.76 ± 0.19	591.28 ± 0.47	126.7 ± 0.34

Each IC_{50} value was obtained by calculating the average and data are expressed as mean ± SD

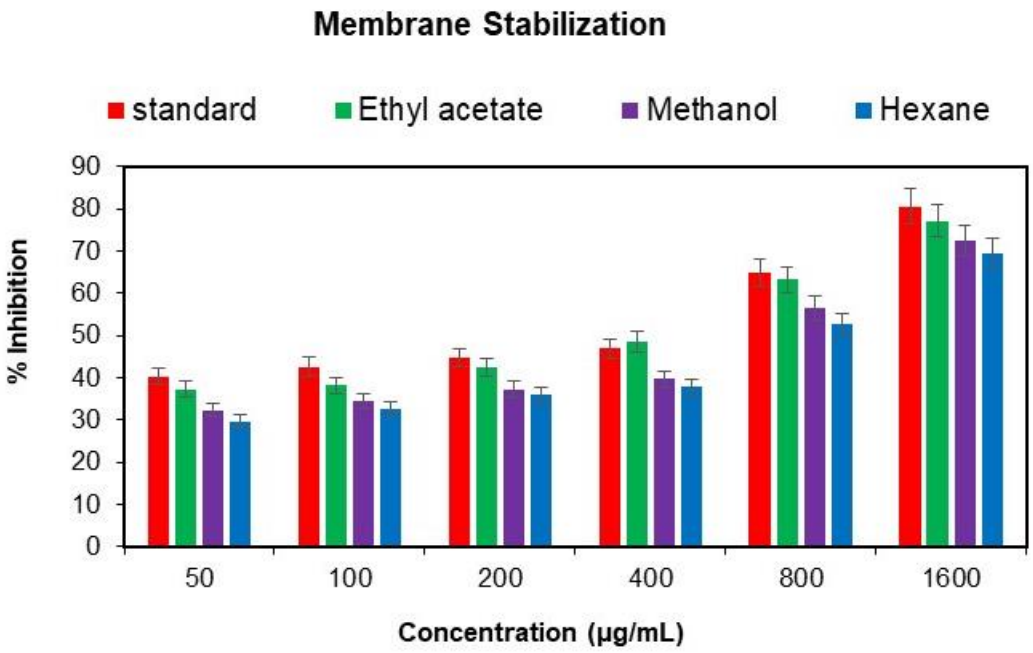

Fig. 1. Membrane stabilization inhibitory activity of different concentration (50 to 1600 µg/mL) of *U. urens* (hexane, ethyl acetate, methanol extracts, and standard Diclofenac sodium). Each value represents the mean ± SD of triplicate experiments.

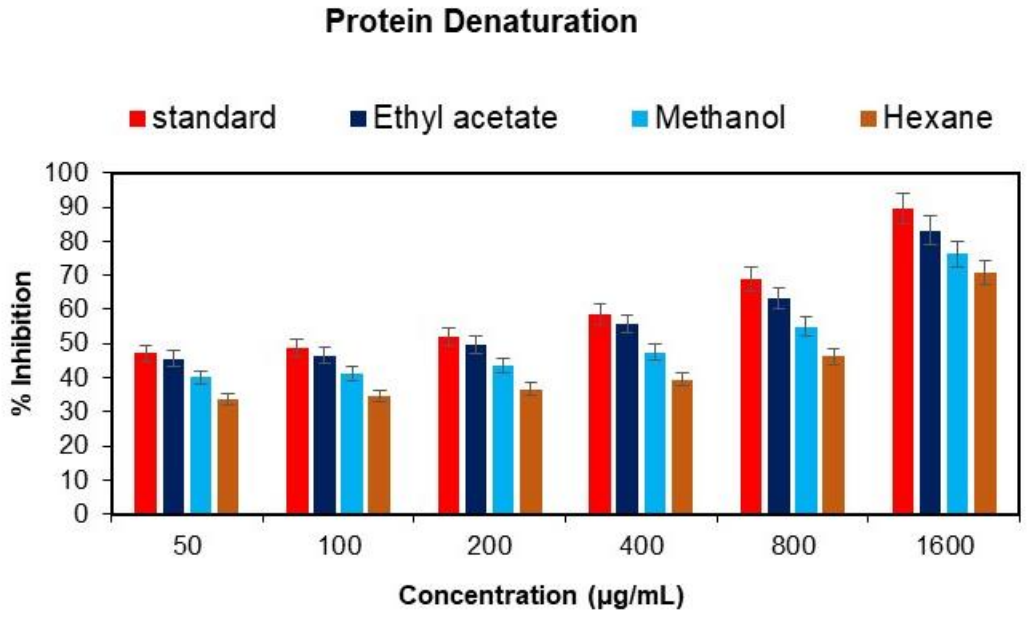

Fig. 2. Protein denaturation inhibitory activity of different concentration (50 to 1600 µg/mL) of *U. urens* (hexane, ethyl acetate, methanol extracts, and standard Diclofenac sodium). Each value represents the mean ± SD of triplicate experiments.

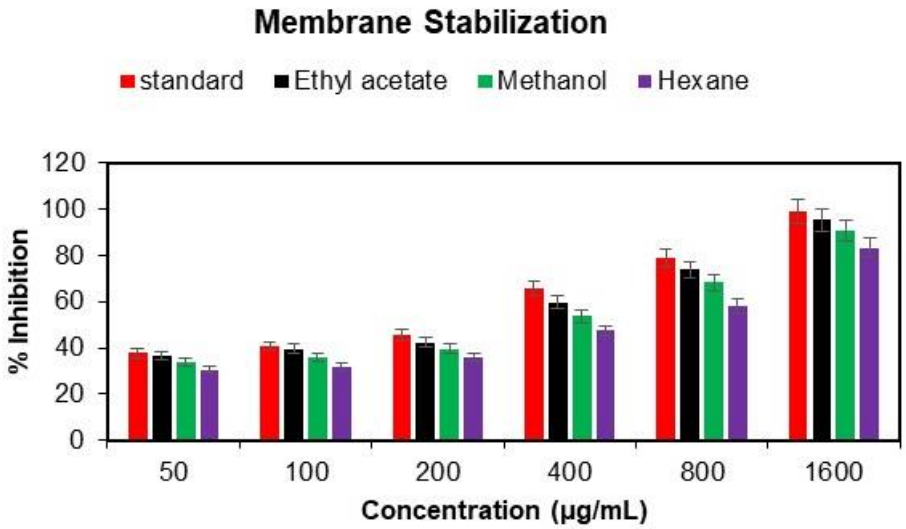

Fig. 3. Membrane stabilization inhibitory activity of different concentration (50 to 1600 µg/mL) of *P. chinense* (hexane, ethyl acetate, methanol extracts, and standard Diclofenac sodium). Each value represents the mean ± SD of triplicate experiments.

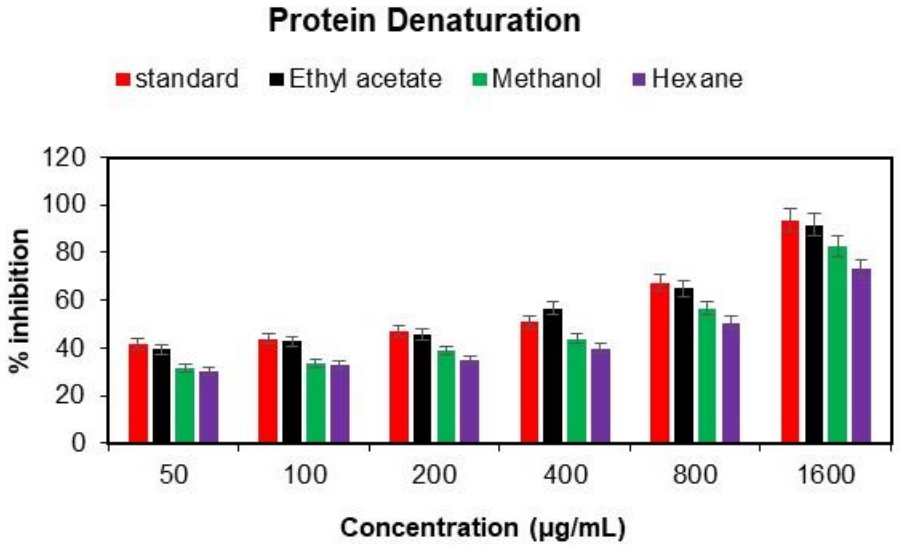

Fig. 4. Protein denaturation inhibitory activity of different concentration (50 to 1600 µg/mL) of *P. chinense* (hexane, ethyl acetate, methanol extracts, and standard Diclofenac sodium); Each value represents the mean ± SD of triplicate experiments.

Anti-lipidemic Activity

The maintenance of lipid metabolism homeostasis is achieved through the equilibrium between lipid synthesis and breakdown. Any disturbance in this equilibrium has the potential to result in hyperlipidemia (Goldstein *et al*. 1973). Hyperlipidemia is a medical condition characterized by abnormalities in lipid metabolism, which has been associated with an increased susceptibility to respiratory infections (Thompson *et al*. 2016). Therefore, the mitigation of hyperlipidemia represents an important therapeutic approach with the objective of diminishing the occurrence of atherosclerosis and mitigating the risk of various vascular events such as coronary heart disease and cerebrovascular illness (Collins *et al*. 2004). The synthesis of cholesterol commences with the condensation of two

acetyl-coenzyme A (acetyl-CoA) molecules, resulting in the formation of the intermediate acetoacetyl-CoA. This process involves the joining of these acetyl-CoA molecules. The catalytic facilitation of this process is mediated by an enzyme called thiolase, which is specifically referred to as acetyl-CoA acetyltransferase (ACAT). The reaction serves as the rate-determining step in the comprehensive process of cholesterol production. Extensive research has been conducted on this reaction due to its pivotal role in defining the trajectory of the overall reaction. It is commonly referred to as the committed step and is regulated by the enzyme HMG-CoAR. The anti-lipidemic action of extracts from *U. urens* and *P. chinense* was observed by the suppression of lipid production, digestion, and absorption *in vitro*. These effects were demonstrated by the use of different solvent extracts. The lipid synthesis inhibition caused by the leaves solvent extracts of *U. urens* and *P. chinense* has been shown to be a result of the suppression of the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase.

The current investigation evaluated the inhibition of HMG-CoA reductase by extracts obtained from the leaves of *U. urens*. The IC_{50} values of the extracts were determined as 49.2 ± 0.45, 29.84 ± 0.35, and 49.05 ± 0.62 µg/mL for hexane, ethyl acetate, and methanol extract, respectively. For *P. chinense*, IC_{50} values of 48.78 ± 0.19, 24.34 ± 0.04, and 48.55 ± 0.1 µg/mL were determined for hexane, ethyl acetate, and methanol extract, respectively. The IC_{50} value for standard drug Diclofenac sodium was reported as 7.52 ± 0.43 µg/mL, as shown in Table 2 and Fig. 5. As previously indicated, a critical stage in the process involves the transformation of HMG-CoA into mevalonate, which is facilitated by the enzyme HMG-CoAR. The significance of this enzyme within the mevalonate pathway has been assessed through a range of experimental investigations. For instance, Chappell *et al*. (1995) conducted a study wherein an HMG-CoAR gene from a hamster, which was expressed constitutively, was introduced into tobacco plants. As a result, the enzyme's activity became down-regulated, leading to a 3 to 10 folds increase in the accumulation of sterols. Hence, the use of enzyme inhibitors holds significant potential in the effective control and treatment of hyperlipidemia (Corsini *et al*. 1995; Carbonell and Freire 2005). Therefore, it is imperative to conduct further research and advance the development of therapeutic interventions for hyperlipidemia. Given the high prevalence of dyslipidemia among individuals with diabetes, it is crucial to address lipid abnormalities to mitigate the risk of complications. Previous studies have demonstrated the inhibitory effects of plant extracts, including phytochemicals, such as phenolic compounds and flavonoids, on HMG-CoA reductase, both in *ex vivo* experiments (Kwon *et al*. 2010; Reddy *et al*. 2014) and *in vivo* studies (Qinna *et al*. 2012). The results of the current study indicated that treatment with Atorvastatin inhibited HMG-CoA reductase activity, which is consistent with earlier research, indicating the substantial inhibitory effects of statins on HMG-CoA reductase (Reddy *et al*. 2014). Consequently, this preliminary study offers pharmacological substantiation for the traditional utilization of these extracts in the treatment of diabetes, inflammation, cancer, and other health ailments. Furthermore, the innovative findings will make a valuable contribution to the advancement of future functional foods and dietary supplements. The results of the current findings extend to the manufacturing of neutraceuticals and cosmeceuticals, as well as their potential application in adjuvant therapy for a range of chronic illnesses.

Table 2. *In vitro* Anti-lipidemic Activity of Hexane, Ethyl Acetate, and Methanol Extracts of *U. urens* and *P. chinense* Leaves in Comparison with Standard Drug Atorvastatin for HMG-CoA Reductase

Anti-lipidemic Assays (HMG-CoA Reductase)		
U. urens	Hexane	49.2 ± 0.45
	Ethyl acetate	29.84 ± 0.35
	Methanol	49.05 ± 0.62
P. chinense	Hexane	48.78 ± 0.19
	Ethyl acetate	24.38 ± 0.04
	Methanol	48.55 ± 0.1
Standard	Atorvastatin	7.52 ± 0.43

Each IC_{50} value was obtained by calculating the average and data are expressed as mean ± SD.

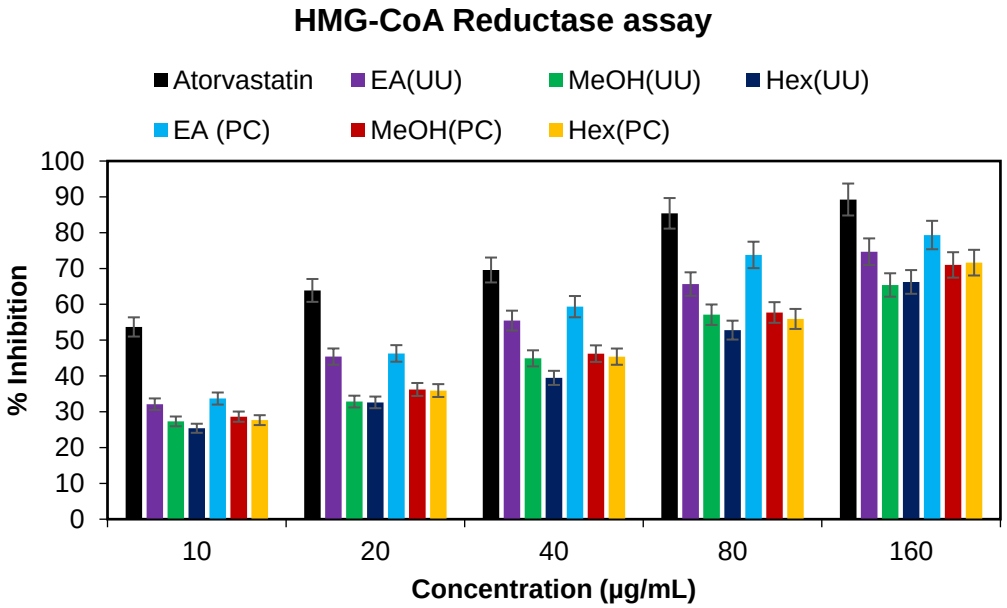

Fig. 5. HMG-CoA reductase inhibitory activity of different concentration (10 to 160 µg/mL) of *U. urens* and *P. chinense* (Hexane, ethyl acetate, methanol extracts, and standard Atorvastatin). Each value represents the mean ± SD.

GC-MS Analysis

The GC-MS chromatogram of ethyl acetate extract of *P. chinense* leaves revealed the presence of 55 phytochemical constituents, as shown in Fig. 6. The compound name, molecular formula, molecular weight, retention time (RT), and peak area of each compound is summarized in Table 3. Among these bioactive components, elaidic acid, etretinate, guggulsterone, bis(2-ethylhexyl) phthalate, eburicoic acid, 10,12-pentacosadiynoic acid, octadecamethylcyclononasiloxane, and 1-(trans-4-pentylcyclohexyl)-4-propylbenzene were predominantly present in the ethyl acetate extract, which might be the reason to exhibit anti-inflammatory and anti-lipidemic activities of this very extract.

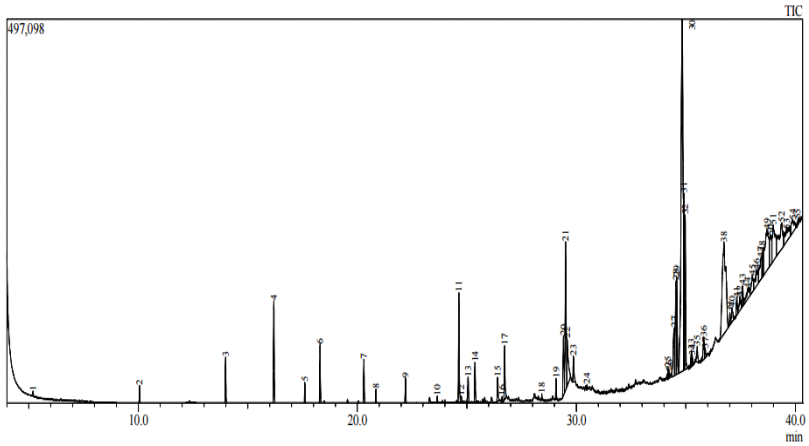

Fig. 6. GC-MS chromatogram of ethyl acetate extract of *P. chinense* leaves

Table 3. List of 55 Phytoconstituents Present in Ethyl Acetate Extract of *P. chinense* Leaves

Name of Compounds	Molecular Formula	Molecular Weight	RT (min)	Peak Area (%)
Dimethyl sulfone	$C_2H_6O_2S$	94.14	5.192	0.09
Decamethylcyclopentasiloxane	$C_{10}H_{30}O_5Si_5$	370.77	10.054	0.29
Dodecamethylcyclohexasiloxae	$C_{12}H_{36}O_6Si_6$	444.92	13.985	0.88
Tetradecane	$C_{14}H_{30}$	198.39	16.189	1.93
Tetradecamethylcycloheptasiloxane	$C_{14}H_{42}O_7Si_7$	519.07	17.604	0.38
Octadecane	$C_{18}H_{38}$	254.5	18.299	1.12
Octadecane	$C_{18}H_{38}$	254.5	20.3	0.84
Benzoic acid, 2,4-bis(trimethylsiloxy),trimethylsilyl ester	$C_{16}H_{30}O_4Si_3$	370.66	20.848	0.23
Octadecane	$C_{18}H_{38}$	254.5	22.199	0.51
2,5-Bis(trimethylsilyloxy)-bis(trimethylsilyl)mandelate	$C_{20}H_{40}O_5Si_4$	472.9	23.65	0.13
Neophytadiene	$C_{20}H_{38}$	278.5	24.636	2.55
Octane	C_8H_{18}	114.23	24.741	0.15
Neophytadiene	$C_{20}H_{38}$	278.5	25.058	0.59
Phytol	$C_{20}H_{40}O$	296.5	25.372	0.88
6-methoxy-3h-1,3-benzothiazole-2-thione	$C_8H_7NOS_2$	197.27	26.405	0.62
Trans-1-(phenylthio)-6-oxo-4-oxahept-1-ene	$C_{12}H_{14}O_2S$	222.31	26.609	0.11
Palmitic acid	$C_{16}H_{32}O_2$	256.42	26.724	1.35
Benzoic acid, 2,6-bis(trimethylsiloxy),trimethylsilyleste	$C_{16}H_{30}O_4Si_3$	370.66	28.422	0.09
Phytol	$C_{20}H_{40}O$	296.5	29.072	0.43
Linoleic acid	$C_{18}H_{32}O_2$	280.4	29.411	1.71
Elaidic acid	$C_{18}H_{34}O_2$	282.5	29.508	4.88
1-(methoxymethoxy)dodecane	$C_{14}H_{28}D_2O_2$	232	29.57	2.17
Oleic acid	$C_{18}H_{34}O_2$	282.5	29.867	0.73
Octadecamethylcyclononasiloxane	$C_{18}H_{54}O_9Si_9$	667.4	30.483	0.04
Hexadecamethylcyclooctasiloxane	$C_{16}H_{48}O_8Si_8$	593.2	34.186	0.22
(2-Chloroethyl) cyclohexane	$C_8H_{15}Cl$	146.66	34.24	0.24
Rhodopin	$C_{40}H_{58}O$	554.9	34.469	2.1

3(cyclopropylcarbonyl)8methyl2,3,3a,4,5,6hexahydro1hpyrazino[3,2,1jk]carbazole	$C_{19}H_{22}N_2O$	294.4	34.549	2.97
4-Hexylbenzoyl chloride	$C_{13}H_{17}ClO$	224.72	34.577	2.71
Etretinate	$C_{23}H_{30}O_3$	354.5	34.831	21.78
Guggulsterone	$C_{21}H_{28}O_2$	312.4	34.915	5.01
Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.6	34.97	3.74
1,3-Diethyl-2-thiobarbituric acid	$C_8H_{12}N_2O_2S$	200.26	35.251	0.46
Absinthin	$C_{30}H_{40}O_6$	496.6	35.29	0.28
Rhodopin	$C_{40}H_{58}O$	554.9	35.518	0.5
3,7-Dioxo-5beta-cholanoic acid	$C_{24}H_{36}O_4$	388.5	35.819	0.82
1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane	$C_{13}H_{40}O_5Si_6$	444.96	35.895	0.28
Eburicoic acid	$C_{31}H_{50}O_3$	470.7	36.739	10.36
Bis(2-ethylhexyl) glutarate	$C_{21}H_{40}O_4$	356.5	37.011	0.38
Dodecyl methacrylate	$C_{16}H_{30}O_2$	254.41	37.106	0.58
Glyceryl monostearate	$C_{21}H_{42}O_4$	358.6	37.329	0.54
Octadecamethylcyclononasiloxane	$C_{18}H_{54}O_9Si_9$	667.4	37.465	0.75
Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.6	37.597	0.59
2,5-dichloro-n-methyl-n-(1-methyl-4-piperidinyl)benzenesulfonamide	$C_{13}H_{18}Cl_2N_2O_2S$	337.3	37.815	1.07
Non-7-ynamide	$C_9H_{15}NO$	153.22	38.037	1
Allyltetramethoxybenzene	$C_{13}H_{18}O_4$	238.28	38.225	1.43
5,7-Dodecadiene-4,9-dione, 6,7-dihydroxy-2,11-dimethyl-	$C_{14}H_{22}O_4$	254.32	38.425	1.63
Methyl 5-amino-2-hydroxybenzoate	$C_8H_9NO_3$	167.16	38.485	0.87
10,12-Pentacosadiynoic acid	$C_{25}H_{42}O_2$	374.6	38.703	5.2
Carbofuran	$C_{12}H_{15}NO_3$	221.25	38.875	1.79
Octadecamethylcyclononasiloxane	$C_{18}H_{54}O_9Si_9$	667.4	38.992	3.25
1-(trans-4-Pentylcyclohexyl)-4-propylbenzene	$C_{20}H_{32}$	272.5	39.382	3.42
2,4-Dicyclopentylphenol	$C_{16}H_{22}O$	230.34	39.605	1.81
Methylephedrine	$C_{11}H_{17}NO$	179.26	39.876	1.28
12-Chlorobicyclo[8.2.0]dodecan-11-one	$C_{12}H_{19}ClO$	214.74	40.125	0.23

CONCLUSIONS

1. Based on these preliminary results, it can be inferred that the various solvent extracts of *U. urens* and *P. chinense* leaves exhibited remarkable anti-inflammatory and anti-lipidemic properties.
2. GC-MS chromatograms revealed the presence of several bioactive compounds in ethyl acetate extract of *P. chinense* leaves.
3. Subsequent investigations incorporating a more comprehensive analysis of the properties of the extracts, including their biological effects, hold the potential to facilitate the advancement of dietary interventions or the identification of innovative pharmaceutical agents or prototypes that exhibit pharmacological efficacy.
4. The results of this study demonstrate potential bioactive roles of plants extract. Therefore, further research is in process to isolate and characterize bioactive compounds, with the aim of providing valuable insights for pharmaceutical firms in addressing illness conditions within society.

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