Evaluation of Soluble Epoxide Hydrolase Inhibition Activity of 50 Traditional Medicinal Plants: Exploring Old Drugs for the New Pharmacological Target

KANNOTH MUKUNDAN GEETHA AND KONDAREDDYPALLY NANJUNDAPPA ANITHA*

Department of Pharmacology, College of Pharmaceutical Sciences, Dayananda Sagar University, Bangalore 560078, 1Department of Pharmacology, Government College of Pharmacy, Rajiv Gandhi University of Health Sciences, Bangalore, Karnataka 560027, India

Geetha et al.: Soluble Epoxide Hydrolase Inhibition Activity of Traditional Medicinal Plants

Soluble epoxide hydrolase inhibitors have been reported as antihypertensive, anti-inflammatory, antiulcer and anticancer actions and protect the brain, heart and kidney from damage. Exploring soluble epoxide hydrolase inhibition activity of traditional medicinal plants helps to uncover the new target to treat various complications associated with inflammatory mediators. The present study was planned to explore soluble epoxide hydrolase inhibition activity of fifty medicinal plants commonly used in the traditional medicinal system. The dried plant material of each species was grounded into a coarse powder and separately macerated with absolute methanol and ethyl acetate for 7 d. Then the solvents were evaporated and obtained extracts were solubilized in dimethyl sulfoxide (10 mg/ml) and soluble epoxide hydrolase enzyme inhibitory potencies were evaluated using a fluorescent reporting system. Moreover, preliminary phytochemical screening and high-performance thin layer chromatography analysis were carried out for two extracts that showed potent soluble epoxide hydrolase inhibition activities. The results revealed that 10 methanolic extracts and 20 ethyl acetate extracts were potentially effective in suppressing soluble epoxide hydrolase activity with inhibitory concentration value of less than 10 µg/ml. Among the 30 potentially effective plants, four seed extracts (Celastrus paniculatus, Nigella sativa, Wrightia tinctorial, Vernonia anthelmintica and Embelia ribes), one leaves extracts (Bergera koengii), one rhizome extracts (Curcuma longa) and one root extracts (Vetiveria zizanioides) were common in both methanolic and ethyl acetate extract. In conclusion, the study report suggests that various natural products used in the traditional medicinal system have many promising soluble epoxide hydrolase inhibitors. Methanolic extracts of seeds of Celastrus paniculatus and Nigella sativa have potent soluble epoxide hydrolase inhibition activities. Further research is warranted to identify a greater number of medicinal plants and active principles responsible for soluble epoxide hydrolase inhibition activities.

Key words: Medicinal plants, Ayurveda, traditional medicine, inflammation, soluble epoxide hydrolase

Eicosanoids are implicated in a variety of inflammation-plagued disorders such as atherosclerosis, stroke, hypertension, renal disease, asthma, arthritis, inflammatory bowel disease, ulcers and neurodegenerative disorders[1,2]. Eicosanoids are derived from Arachidonic Acid (ARA) through three pathways; Cyclooxygenase (COX) pathway, Lipoxygenase (LOX) pathway and Cytochrome P450 (CYP) pathway[2,3]. These three pathways of metabolism of ARA to derive various eicosanoids are shown in fig. 1.

The COX pathway yields prostaglandins and thromboxane and LOX pathway yield pro-inflammatory leukotrienes. The present strategies for treating inflammation and associated complications mainly target these two pathways. Plenty of drugs including non-steroidal anti-inflammatory drugs are available in the market and shows antipyretic, analgesic and anti-inflammatory actions by inhibiting COX pathway. LOX inhibitors have been used therapeutically in arthritis, seasonal allergies and asthma etc.[4-6].

The CYP enzyme-mediated eicosanoids synthesis is

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms

Accepted 11 November 2022
Revised 12 April 2022
Received 14 January 2022

Indian J Pharm Sci 2022;84(6):1429-1443
accompanied by two distinct pathways; the Omega (ω)-hydroxylase and epoxygenase pathways and converts ARA to Hydroxyeicosatetraenoic Acids (HETEs) and Epoxyeicosatrienoic Acids (EETs), respectively[3,6].

EETs are physiologically active compounds that exert various beneficial effects in the body including vasodilation, vascular smooth muscle cell anti-migratory actions, anti-inflammatory actions and suppress hyperthermia, pathological fibrosis, generation of reactive oxygen species, apoptosis, pain and platelet aggregation[1,7-11]. Literature review reveals that EETs also have a role in the modulation of angiogenesis, regulation of cerebral blood flow and mediation of neuroendocrine signaling[12]. Hence, EETs exhibit various beneficial actions like anti-inflammatory, analgesic, antihypertensive, cardio-protective and organ-protective properties.

In mammals, EETs have a very short half-life and the enzyme, soluble Epoxide Hydrolase (sEH) plays a central role in their metabolism[13]. The substrate-specific sEH hydrolyzes EETs to the corresponding Dihydroxyeicosatrienoic Acids (DHETs) whereby the biological effects of EETs are diminished, eliminated or altered[13]. Therefore, inhibiting sEH enzyme increases the half-life EETs and enhances its beneficial properties. Hence, sEH enzyme inhibition is an emerging and promising therapeutic strategy for addressing a variety of diseases[3,13].

Scientific literature survey indicates that various synthetic and natural origin sEH inhibitors have antihypertensive, anti-inflammatory, antiulcer and anticancer actions and protect the brain, heart and kidney from damage[14].

Some of the herbal components like roots of Cimicifuga dahurica[15], Lepidium meyenii and Carica papaya[16] and Sophora flavescens[17] are explored to possess sEH inhibitors with accountable medicinal values. Many herbal products were reported for their anti-inflammatory properties but very less focus was given to the sEH inhibition mediated anti-inflammatory actions[15-17]. Hence, exploring sEH inhibition activity of more traditional medicinal plants helps to uncover a new pharmacological target for old drugs to develop a safe and effective therapeutic strategy from natural sources[18-23].

With the literature knowledge, the present study was planned to explore the sEH inhibition activity of fifty medicinal plants commonly used in Ayurveda. As per our knowledge, the plants selected for this study are not reported for their sEH inhibitory potential. The ethno botanical description of plants used in this study with their Ayurvedic uses and chemical compositions are given in Table 1. Moreover, preliminary phytochemical screening and High-Performance Thin Layer Chromatography (HPTLC) analysis were carried out for two extracts that showed potent sEH inhibition activities.

![Fig. 1: Schematic representation of bioactive eicosanoids synthesis from the arachidonic acid cascade through the COX, LOX and CYP pathways](image-url)
<table>
<thead>
<tr>
<th>Botanical name</th>
<th>Family</th>
<th>Common names</th>
<th>Chemical constitutes</th>
<th>Traditional uses</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acalypha indica</td>
<td>Euphorbiaceae</td>
<td>Indian copper leaf</td>
<td>Mauritianin, clitorin, nicrotiflorin, biorobin</td>
<td>Laxative, anthelmintic, emetic, expectorant, to treat scabies, earache, syphilitic ulcers and snake bites etc.,</td>
<td>[24]</td>
</tr>
<tr>
<td>Adhatoda vasica</td>
<td>Acanthaceae</td>
<td>Malabar nut tree</td>
<td>Alkaloids like vasicine, vasicinone, deoxyvasicine, vasicol, adhatodinine, vasicinol</td>
<td>Expectorant, malarial fever, chronic fever, intrinsic haemorrhage, cough, asthma, leprosy, skin diseases, piles</td>
<td>[25]</td>
</tr>
<tr>
<td>Andrographis paniculata</td>
<td>Acanthaceae</td>
<td>Creat or green chiretta</td>
<td>Diterpenoids, diterpene glycosides, lactones, flavonoids, and flavonoid glycosides</td>
<td>Snakebite, bug bite, diabetes, dysentery, fever, malaria, common cold, diarrhoea, jaundice, as a health tonic etc.,</td>
<td>[26]</td>
</tr>
<tr>
<td>Azadirachta indica</td>
<td>Meliaceae</td>
<td>Neem tree</td>
<td>Gedunin, nimbin, nimbolide, azadirone, neemfruitin etc.,</td>
<td>Infection, metabolic diseases, cancer, diabetes mellitus</td>
<td>[27]</td>
</tr>
<tr>
<td>Bergamia koengii</td>
<td>Rutaceae</td>
<td>Curry Leaf</td>
<td>Vitamin A and calcium</td>
<td>Digestive, tonic, stimulant, diarrhoea, dysentery and vomiting</td>
<td>[27]</td>
</tr>
<tr>
<td>Cardiospermum halicababum</td>
<td>Sapindaceae</td>
<td>Kanphuti/Ballon plant</td>
<td>Flavones, glycones, fatty acids, glycosides, terpenoids</td>
<td>Anti-allergic, anti-inflammatory, antioxidant, anticancer, antidiabetic and antifungal</td>
<td>[28]</td>
</tr>
<tr>
<td>Cassia alata</td>
<td>Fabaceae</td>
<td>Ketepeng</td>
<td>Flavones, flavonols, flavonoids glycosides, alatinon, alanol and B-sitosterol-B-D-glucoside</td>
<td>Anti-inflammatory, antioxidant, antihypertensive, anticancer, anti-diarrheal, hepatoprotective, neuroprotective hypoglycemic, hypolipidemic</td>
<td>[29]</td>
</tr>
<tr>
<td>C. paniculatus</td>
<td>Celastraceae</td>
<td>Jyotishmati</td>
<td>Alkaloids, Glycoside, sterols, dipalmitoyl glycerol</td>
<td>Emollient, thermogenic, stimulant, digestive, laxative, emetic, expectorant, appetizer, aphrodisiac, cardiotoxic, anti-inflammatory</td>
<td>[30]</td>
</tr>
<tr>
<td>Centella asiatica</td>
<td>Apiaceae</td>
<td>Indian pennywort, Asiatic pennywort</td>
<td>Saponins, asiaticoside and madecassoside and their glycones, asiatic acid and madecassic acids</td>
<td>Anti-inflamatory and anti-nociceptive, antioxidant, anti-hypertensive, anticancer, antimicrobial, anti-diarrheal, hepatoprotective, neuroprotective hypoglycemic, hypolipidemic</td>
<td>[31]</td>
</tr>
<tr>
<td>Clerodendrum phlomoidis</td>
<td>Lamiaceae</td>
<td>Arni</td>
<td>Sesquiterpene, diterpenoids, triterpenoids, flavonoid and flavonoid glycosides, phenylethanoid glycosides, steroids and steroid glycosides, cyclohexyllethanoids, anthraquinones, cyanogenic glycosides</td>
<td></td>
<td>[32]</td>
</tr>
<tr>
<td>Corollocarpus epigaeus</td>
<td>Cucurbitaceae</td>
<td>Jungali suran</td>
<td>Tannins, alkaloids, saponins, steroids and phenolic compound</td>
<td>Laxative, anti-diabetic</td>
<td>[33]</td>
</tr>
</tbody>
</table>
**Curcuma longa** Zingiberaceae
Termeric

- Curcumin, curcumenol, camphor, germacrone, β-pinene, isocurcumenol

- Anti-inflammatory, antiviral, anticancerous, carminative, antiproliferative, hypocholesterolemic, diuretic, antidiabetic, antitumour, antidiarrheal, anti-inflammatory, hypotensive, antioxidant, antimicrobial, insecticidal, larvicide, anti-venomous, antithrombotic

- Anasarca, calculus, cancer, carbuncles, convulsions, cough, cramps, cystitis, diarrhoea, dropsy, dysentery, epilepsy, headache, haemorrhage, hypertension, hysteria, insanity, kidneys, laxative, measles, rubella, snakebite, sores, stones, tumours, urogenital disorders, warts and wounds

**Cynodon dactylon** Poaceae
Bermudagrass

- Cynodin, hydrocyanic acid and triticin

- Analgesic, anti-allergic, anti-arthritis, anti-candida, anti-cariogenic, anti-convulsant, anti-diarrheal, anti-emetic, anti-helminthic, anti-hyperglycemic, anti-hypertensive, anti-inflammatory, anti-malarial, anti-obesity, antioxidant, anti-platelet, anti-pyretic, anti-ulcer, anti-viral, ovicidal, gastroprotective, larvicide, hepatoprotective, neuroprotective, wound healing

**Cyperus rotundus** Cyperaceae
Purple nutsedge

- Cyperolone, B-cyperone, p-cymol, calcium, camphene, copaene, cyperene, cypereone, cyperol, cyperolone, caryophyllene, cyperotundone, d-copadiene, d-epoxyguaiene, isocyperol, isokobusone, kobusone, limonene, linoleic-acid, linolenic-acid, mustakone, myristic acid, oleanolic acid, oleic acid, β-pinene, patchoulenone, rotundene, rotundenol, rotundone, α-rotunol, β-rotunol, β-selinene, selinatriene, sitosterol, stearic acid, sugeonol and sugetriol

- Analgesic, anti-allergic, anti-arthritis, anti-candida, anti-cariogenic, anti-convulsant, anti-diarrheal, anti-emetic, anti-helminthic, anti-hyperglycemic, anti-hypertensive, anti-inflammatory, anti-malarial, anti-obesity, antioxidant, anti-platelet, anti-pyretic, anti-ulcer, anti-viral, ovicidal, gastroprotective, larvicide, hepatoprotective, neuroprotective, wound healing

**Elettaria cardamomum** Zingiberaceae
Cardamom

- a- pinene, R- pinene, sabinene, myrcene, limonene, y-terpinene, methyl eugenol

- Aromatic stimulant, carminative, stomachic and diuretic

**Embelia ribes** Myrsinaceae
False black pepper

- Embelin, volatile oil, fixed oil, resin, tannin, christembine, cafffeic acid, vanillic acid, chlorogenic acid, cinnamic acid, o-cumaric acid

- Antibacterial, antifertility activities, antiprotozoal, abdominal disorders, lung diseases, constipation, indigestion, fungus infections, mouth ulcer, sore throat, pneumonia, heart disease, analgesic, anti-inflammatory, antioxidant

**Glycyrrhiza glabra** Fabaceae
Liquorice

- Liquiritin, isoliquiritigenin and rhamnoliquiritin

- Anti-inflammatory, asthma, expectorant, controls coughing, detoxifies the liver, bronchitis, peptic ulcer, arthritis
<table>
<thead>
<tr>
<th><strong>Plant</strong></th>
<th><strong>Family</strong></th>
<th><strong>Common Name</strong></th>
<th><strong>Chemical Constituents</strong></th>
<th><strong>Medical Uses</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Hibiscus rosasinensis</em></td>
<td>Malvaceae</td>
<td>China rose</td>
<td>Stigmasterol, β-sitosterol, taraxeryl acetate</td>
<td>Cough cold, hair loss, reduction of cholesterol, mild laxative, expectorant and diuretic</td>
</tr>
<tr>
<td><em>Indigofera aspalathoides</em></td>
<td>Fabaceae</td>
<td>Wiry Indigo</td>
<td>Tannin, alkaloids, triterpenes, flavones, saponin and steroids</td>
<td>Anti-cancerous and antioxidant activity, antimicrobial, hypoglycemic, hepatoprotective, anti-inflammatory and antiviral</td>
</tr>
<tr>
<td><em>Indigofera tinctoria</em></td>
<td>Fabaceae</td>
<td>True indigo</td>
<td>Alkaloids, flavonoids, tannins, saponins, glycosides and terpenoids, indigotin, indirubin, rotenoids</td>
<td>Anti-diarrheal, antiviral, antipyrctic, antidiabetic, antifungal, antineoplastic, antiparasitic, antiseborrhoeic, antihypertensive, antineurogenic, antileprosy, hair growth stimulant</td>
</tr>
<tr>
<td><em>Lawsonia alba</em></td>
<td>Lythraceae</td>
<td>Henna</td>
<td>Lawsone, esculetin, fraxetin, isopelargonin, scopoletin, betulin, betulinic acid, hennadiol, lupeol, luteolin, flavone glycosides, two pentacyclic triterpenes</td>
<td>Headache, hemicranias, lumbago, bronchitis, boils, ophthalmia, phylis, sores, amenorrhea, scabies, dysuria, bleeding disorder, diuretic, antifungal, antibacterial, anti-amoeobiasis, astringent, antimeningitic</td>
</tr>
<tr>
<td><em>Leucas aspera</em></td>
<td>Lamiaceae</td>
<td>Thumbai</td>
<td>Triterpenoids, oleanolic acid, ursolic acid and b-sitosterol, nicotine, sterols, glucosides, diterpenes, phenolic compounds (4-(24-hydroxy-1-oxo-5-n-propyltetracosanyl)-phenol)</td>
<td>Antifungal, antioxidant, antimicrobial, antinoceptive and cytotoxic</td>
</tr>
<tr>
<td><em>Mentha piperita</em></td>
<td>Lamiaceae</td>
<td>Peppermint</td>
<td>Menthol, methyl acetate, menthone, pulegone, methylophuran, limone</td>
<td>Astringent, antisepctic, antipruritic, antiemetic, carminative, vermifuge, diaphoretic, analgestic</td>
</tr>
<tr>
<td><em>Mollugo cerviana</em></td>
<td>Molluginaceae</td>
<td>Thread stem carpet weed</td>
<td>Carbohydrates, saponins, tannins, terpenoids, flavonoids, steroids, phenols, proteins and alkaloids</td>
<td>Antimicrobial, anti-inflammatory, antioxidant activity and spermicidal activity</td>
</tr>
<tr>
<td><em>N. sativa</em></td>
<td>Ranunculaceae</td>
<td>Fennel flower</td>
<td>Thymoquinone, thymohydroquinone, dithymoquinone, p-cymene, carvacrol, 4-terpineol, t-anethol</td>
<td>Antidiabetic, anticancer, immunomodulator, analgesic, antimicrobial, anti-inflammatory, spasmyloytic, bronchodilator, hepatoprotective, renal protective, gastro-protective, antioxidant</td>
</tr>
<tr>
<td><em>Ocimum basilicum</em></td>
<td>Lamiaceae</td>
<td>Basil</td>
<td>Linalool, methyl chavicol or citral and 1,8-cineole, camphor, thymol, methyl cinnamate, eugenol, methyl eugenol, methyl isoeugenol and elemicin</td>
<td>Headaches, coughs, diarrhoea, constipation, warts, worms and kidney malfunctions</td>
</tr>
<tr>
<td><strong>Ocimum sanctum</strong></td>
<td>Lamiaceae</td>
<td>Tulsi</td>
<td>Eugenol, euginal, urosolic acid, carvacrol, linalool, limatrol, carvophyllene, methyl carvicol</td>
<td>Antimicrobial, anti-diarrheal, anti-oxidant, anti-cataract, anti-inflammatory, hepatoprotective, analgesic, anti-pyretic, anti-allergic, CNS depressant, anti-asthmatic, anti-tussive, diaphoretic, anti-thyroid, anti-fertility, anti-ulcer, anti-emetic, anti-spasmodic, anti-arthritic, adaptogenic, anti-stress</td>
</tr>
<tr>
<td><strong>Phyllanthus emblica</strong></td>
<td>Euphorbiaceae</td>
<td>Amla</td>
<td>Tannins, gallic acid, ellagic acid, embicol, phylllembin, lupeol, essential oil, fixed oil</td>
<td>Antidiabetic, hypolipidemic, antibacterial, antioxidant, antiulcer, hepatoprotective, gastroprotective</td>
</tr>
<tr>
<td><strong>Phyllanthus niruri</strong></td>
<td>Euphorbiaceae</td>
<td>Stonebreaker</td>
<td>Alkaloid, flavonoid, terpenoids, cardiac glycoside, saponins, tannins, cyanogenic glycosides</td>
<td>Terpinine, P-cymene, carvacrol, chavicol and its derivatives, allyl catechol, eugenol, estragol, oxalic acid, malic acid and amino acids. Leaves contain good amounts of vitamins particularly nicotinic acid, ascorbic acid and carotin</td>
</tr>
<tr>
<td><strong>Piper betel</strong></td>
<td>Piperaceae</td>
<td>Betel leaf</td>
<td>Alkaloids, amides, lignans, esters, volatile oils</td>
<td>Cancer, diabetes, obesity, hyperlipidemia, asthma, fungal infection, arthritis, pain, amoebiasis, ulcer, depression, inflammation</td>
</tr>
<tr>
<td><strong>Piper longum</strong></td>
<td>Piperaceae</td>
<td>Long pepper</td>
<td>Piperic acid, pipertlonguminine, pellitorine, piperolen B, piperamide, pipertetine and (-)-kusunokinin</td>
<td>Anti-inflammatory, analgesic, anticonvulsant and neuroprotective, anti-diabetic</td>
</tr>
<tr>
<td><strong>Plectranthus vettiveroides</strong></td>
<td>Lamiaceae</td>
<td>Hribera</td>
<td>Androstan-17-one 3-ethyl-3-hydroxy-(5α) (-) spathulenol</td>
<td>Antipyretic, diuretic, antibacterial, antioxidant, anticancer, anti-diabetic, hepatoprotective</td>
</tr>
<tr>
<td><strong>Psoralea corylifolia</strong></td>
<td>Fabaceae</td>
<td>Babchi</td>
<td>Bakuchiol, psoralen, isopsoralen, corylifolin, corylin, psoraldin</td>
<td>Antitumor, antihyperglycemic, antidepressant, antioxidant, antibacterial, diuretic, anthelmintic, laxative, wound healing, stomachic, stimulant, aperidiatic, diaphoretic, asthma, cough, nephritis</td>
</tr>
<tr>
<td><strong>Pungamia glabra</strong></td>
<td>leguminosae</td>
<td>Karanj</td>
<td>Karangin, pongamol, pongagalabrone, and pongapin, pinnatin and kanjone</td>
<td>Antiseptic, anti-inflammatory, anti-plasmodial, anti-nociceptive, anti-hyperglycemic, anti-lipidoxidative, antidiarhoeal, anti-ulcer, anti-hyperammonic, CNS depressant and antioxidant</td>
</tr>
<tr>
<td><strong>Santalum album</strong></td>
<td>Santalaceae</td>
<td>Sandal wood</td>
<td>Alpha-santalol, tannins, terpenes, resins</td>
<td>Anti-inflammatory, antimicrobial, antiproliferative, acne, psoriasis</td>
</tr>
<tr>
<td><strong>Sesamum indicum</strong></td>
<td>Pedaliaceae</td>
<td>Benne</td>
<td>sesamol, sesaminol and sesamin</td>
<td>Antioxidants, anti-inflammatory, anti-microbial, anti-pyretic.</td>
</tr>
<tr>
<td><strong>Smilax chinensis</strong></td>
<td>Smilacaceae</td>
<td>China root</td>
<td>Beta-sitosterol, caffeic acid, catechin, daucosterin, daucosterol, engeletin, epicatechin, friedelin, heloniosides, hydroxyflavan, isoengeletin, naringenin, piceid, quercetin, resin, resveratrol, rutin, saponin, scirpusin, seiboldogenin, smilacin, smilasides, tannin, taxifolin, trihydroxystibene, vanillic acid, flavonoids and stilbenes</td>
<td>Antimicrobial, anthelmintic, antioxidant, anticancer, hepatoprotective</td>
</tr>
<tr>
<td><strong>Solanum trilobatum</strong></td>
<td>Solanaceae</td>
<td>Climbing brinjal</td>
<td>Sobatum, β-solamarine, solasodine, solaine, glycoalkaloid and diosogenin</td>
<td>Hepatoprotective, antimicrobial, antioxidant, cytotoxic, haemolytic, immunomodulatory and anti-inflammatory</td>
</tr>
<tr>
<td><strong>Terminalia chebula</strong></td>
<td>Combretaceae</td>
<td>Chebulic myrobalan</td>
<td>Gallic acid, chebulagic acid, punicalagin, chebulanin, corilagin, neochepbulinic acid, ellagic acid, chebulinic acid, 1,2,3,4-penta-O-galloyl-D-glucose, 1,6-di-O-galloyl-D-glucose, casuarinin, 3,4,6-tri-O-galloyl-D-glucose, terchebulin</td>
<td>Antioxidant, antibacterial, antifungal, antiviral, antiprotozoal, antiulcer, anticarcinogenic, purgative, radioprotective, antiallergic, hepatoprotective, anti-inflammatory, antispasmodic</td>
</tr>
<tr>
<td><strong>Tinospora cordifolia</strong></td>
<td>Menispermaceae</td>
<td>Guduchi</td>
<td>Berberine, palmatine, isocolumbine, tinocordiside, pantoside, beta sitosterol, tinosporides</td>
<td>Anti-diabetic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arithmetic, antioxidant, anti-allergic, anti-stress, leprotic, anti-malarial, hepatoprotective, immunomodulatory</td>
</tr>
<tr>
<td><strong>Trichosanthes cucumerina</strong></td>
<td>Cucurbitaceae</td>
<td>snake gourd</td>
<td>Proteins, fat, fibre, carbohydrates, minerals, and vitamins A and E in high levels</td>
<td>Antidiabetic, antibacterial, anti-inflammatory, anthelmintic, antifebrile, gastroprotective, and antioxidant activity</td>
</tr>
<tr>
<td><strong>Trigonella foenum</strong></td>
<td>Fabaceae</td>
<td>Fenugreek</td>
<td>Diosgenin, trigonelline, fenugreekine, galactomannan and 4-hydroxy isoleucine, brassicasterol, stigmasterol, resin, myristic acid palmitic acid, stearic acid, oleic acid linoleic acid, vernolic acid and methyl vernolate</td>
<td>In the treatment of diabetes, microbial and cancer disease</td>
</tr>
<tr>
<td><strong>Vernonia anthelmintica</strong></td>
<td>Asteraceae</td>
<td>Purple fleabane</td>
<td>Diabetes mellitus, leukoderma, skin disease, fever, worm infection and kidney trouble</td>
<td></td>
</tr>
</tbody>
</table>
Vetiveria zizanioides, Poaceae, Vetiver

- Cycloisolongifolene, isoleledene, isolongifolene, longifolene, sativene

- Isopelletierine, anafarine, cuseohygrine, anahygrine, withanolides, withaferins

Withania somnifera, Solanaceae, Ashwagandha/winter cherry

- Lupeol, α- and β- amyrin, indigotin, indirubin, trypanthrin, isatin, rutin, B-sitosterol, triacantanol, myristic acid, palmitoleic acid, palmetric acid, stearic acid, behenic acid, arachidic acid

- Gingerol, diaryleheptanoids, glutamate, aspartic acid, serine, glycine, threonine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, arginine, proline

W. tinctoria, Apocynaceae, Sweet Indrajao

- Antidiarrhoeal, aphrodisiac, anthelmintic, febrifuge, stomachic, toothache, tonic and dog bite

- Antioxidant, anti-inflammatory, antimicrobial, anticancer, antiobesity, anti-diabetic, antioxidant, anti-arthritis, anti-depressant, anti-coagulant, anti-diabetic, anti-pyretic

Zingiber officinalae, Zingiberaceae, Ginger

- Antioxidant, anti-inflammatory, antimicrobial, anticancer, antiobesity, anti-diabetic, antioxidant, anti-arthritis, anti-depressant, anti-coagulant, anti-diabetic, anti-pyretic

- Cardiovascular protective and respiratory protective

MATERIALS AND METHODS

Materials:

The human 5-HETE enzyme-linked immunosorbent assay kit was procured from Fine Test (Fine Biotech, Wuhan, China). Dimethyl Sulfoxide (DMSO), methanol and ethyl acetate were purchased from Hi-Media Laboratories Pvt. Ltd. (Mumbai, India). All other chemicals and reagents used in the study are of analytical grade.

Plant material and extraction procedure:

Plant materials of 50 plant species (Acalypha indica, Adhatoda vasica, Andrographis paniculate, Azadirachta indica, Bergamia koengii, Cardiospermum halicababum, Cassia alata, Celastrus paniculatus (C. paniculatus), Centella asiatica, Clerodendrum phlomidis, Corollocarpus epigaeus, Curcuma longa, Cynodon dactylon, Cyperus rotundus, Elettaria cardomomum, Embelia ribes, Glycyrrhiza glabra, Hibiscus rosasinensis, Indigofera aspalathoides, Indigofera tinctorial, Lawsonia alba, Lawsonia alba, Leucas aspera, Mentha piperita, Mollugo cerviana, Nigella sativa (N. sativa), Ocimum basilicum, Ocimum sanctum, Phyllanthus emblica (P. emblica), Phyllanthus niruri, Piper betel, Piper longum, Piper nigrum, Plectranthus vettiveroides, Psoralea corylifolia, Pungamia glabra, Santalum album, Sesamum indicum, Smilax chinensis, Solanum trilobatum, Terminalia chebula, Tinospora cordifolia, Trichosanthes cucumerina, Trigonella foenum, Vernonia anthelmintica, Vetiveria zizanioides, Withania somnifera, Wrightia tinctorial (W. tinctoria) and Zingiber officinalae) included in this study were collected from the local market of Tirupati and authenticated from Department of Botany, Sri Venkateshwara University, Tirupati, India. The voucher specimens were kept in the department herbarium for future reference. Based on traditional and Ayurvedic use mentioned in the literature part of the plant selected for this study. The detailed information about a plant family, chemical constituents, traditional medicinal uses and part of the plant used in the present study is given in Table 1[24-69].

The dried plant materials of each plant were grounded into a coarse powder. 100 g of coarse powder was equally divided into two parts and separately soaked in 200 ml of absolute methanol and 200 ml of ethyl acetate for 7 d. Then the extracts were filtered through double layers of muslin, centrifuged at 7000 rpm for 10 min and finally filtered again through Whatman filter paper no. 41 to attain a clear filtrate. The clear
filtrates were evaporated and dried at 40° under reduced pressure using a rotatory vacuum evaporator (LabTech). The extract yields were weighted and stored in glass bottles at 5°.

sEH inhibition activity of plant extracts:

Dried plant extracts were solubilized at 10 mg/ml in DMSO and inhibitory potencies were measured against the human sEH using a fluorescent reporting system (BMG Labtech) as per the method prescribed by Jones et al. Shortly, half maximal Inhibitory Concentration (IC_{50}) values were determined using Cyan (2 Methoxynaphthalen-6-yl) Methyl Trans-(3-phenyl-oxyran-2-yl) Methyl Carbonate (CMNPC) as a fluorescent substrate. Recombinant sEH (1 nM) was incubated with inhibitors for 5 min in 100 mM sodium phosphate buffer (pH 7.4) containing 0.1 mg/ml of BSA at 30° prior to substrate introduction ((S)=5 μM). The activity was measured by determining the appearance of the 6-methoxy-2-naphthaldehyde with an excitation wavelength of 330 nm and an emission wavelength of 465 nm for 10 min. The DMSO without plant extracts and treated in the same manner as the test was used as a negative control for reference.

Preliminary phytochemical screening of C. paniculatus Methanolic Extract (CPME) and N. sativa Methanolic Extracts (NSME):

Based on the in vitro sEH inhibition activity, two potent extracts: CPME and NSME were evaluated for the following qualitative and quantitative preliminary phytochemical tests.

Qualitative tests: The preliminary qualitative tests for selected extracts (CPME and NSME) were performed for the detection of the presence of alkaloids (Mayer’s test, Dragendorff’s test, Wagner’s test and Hager’s test), carbohydrates (Molisch’s test, Fehling’s test and Benedict’s test), glycosides (Legal’s test and Libermann-Burchard’s test), phytosterols (Liebermann-Burchard’s test), fixed oils and fats (Spot test), saponins (Foam test), phenolic compounds and tannins (ferric chloride test, lead acetate test), proteins and free amino acids (Millon’s test and ninhydrin test), gums and mucilage, flavonoids (Shinoda test, Alkaline reagent test)\[18,19\].

Quantitative tests: For estimation of total flavonoids, 1 ml of (0.1 % w/v) solution of plant extract in ethanol was mixed with 1 ml Aluminium chloride (AlCl\(_3\)) (2 % w/v in ethanol) and 1 drop of acetic acid was added to it and made up to 25 ml with distilled water. Similarly, the standard quercetin in 0.1 % w/v in ethanol was treated in the same manner as the sample. The sample and the standard were allowed to stand at room temperature for 40 min. The absorbance was measured at 415 nm and the readings were recorded\[18,19\]. Total flavonoid content was calculated using the formula shown below.

\[\text{Total flavanoids}=\frac{(A\times M_0)}{(A_0\times M)}\]

Where, \(A\) was absorbance of extract, \(A_0\) was absorbance of standard, \(M\) was weight of extract and \(M_0\) was weight of standard.

For the estimation of Total Phenolic Content (TPC), 1 ml of (0.1 % w/v) solution of plant extract in ethanol was mixed with 1.5 ml of Folin Ciocalteu’s reagent and 8.5 ml of water. Allow it to stand for 5 min. Then 4 ml of 20 % sodium carbonate was added in it. The absorbance was measured at 765 nm and TPC was estimated using gallic acid as standard\[18,19\].

Total alkaloid content was estimated by the gravimetric method. Briefly, a quantity of extract was weighed and transferred to a separating funnel. 10 ml of chloroform was added and the contents were shaken well for 30 min to extract the alkaloids completely. The contents were dried in a china dish and the residue was weighed to calculate the mass of alkaloids\[18,19\].

HPTLC analysis of CPME and NSME:

HPTLC studies were carried out using a Camag HPTLC system with a Linomat V sample applicator, a Camag 3 TLC scanner and winCATS 4 software for the interpretation of the data. An aluminium plate (20×10 cm) precoated with silica gel 60F254 (E Merck) was used as the adsorbent. The plates were developed using n-hexane:ethyl acetate (5:4) as the mobile phase for plant extracts in a Camag twin trough chamber and scanned at 254, 366 and 425 nm. The Retention factor \((R_f)\) values of the extracts were determined using winCATS 4 software. The developed plates were photo-documented at 254 nm, 366 nm and 425 nm using a Camag 3 Reprostar. The \(R_f\) values of extracts were compared with the \(R_f\) value of standard Linoleic acid and confirmed by an overlay of spectra.

Statistical analysis:

Prism software was used for statistical analysis. All the values are expressed in mean±Standard Error of the Mean (SEM). (n=3), Two-way Analysis of
Variance (ANOVA) followed by Bonferroni post hoc tests. p<0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The extract of 100 g of dried plant materials yielded plant extract residues ranging from 1% to 25% with methanol and 0.2% to 14% with ethyl acetate. With both the solvents highest extract yield was obtained from fruits of *P. emblica* while *Cynodon dactylon* gives the lowest extract yield.

Fifty plant species were investigated to evaluate their sEH enzyme inhibition potential against the human sEH enzyme using a fluorescent reporting system. The results revealed that 10 methanolic extracts and 20 ethyl acetate extracts were potentially effective in suppressing sEH activity with IC$_{50}$ value of less than 10 µg/ml. The highest potency was observed with methanolic extract of *Piper longum* fruit (IC$_{50}=1.108$ µg/ml) but the ethyl acetate extracts of the same were very poor in inhibiting sEH activity (IC$_{50}>50$ µg/ml). In case of ethyl acetate, *Bergera koengii* leaves extract showed the highest potency with IC50 value of 2.384 µg/ml. Among the 30 potentially effective plants, four seed extracts (*C. paniculatus*, *N. sativa*, *W. tinctoria*, *Vernonia anthelmintica* and *Embelia ribes*), one leaves extracts (*Bergera koengii*), one rhizome extracts (*Curcuma longa*) and one root extracts (*Vetiveria zizanioides*) were common in both methanolic and ethyl acetate extracts. When comparing the sEH inhibition activities of these plants it was observed that methanolic extracts of three plants (*C. paniculatus*, *N. sativa* and *W. tinctoria*) were more potent than ethyl acetate extract, whereas for all other plants ethyl acetate extracts were more potent. A comparison of sEH inhibition activity of methanolic and ethyl acetate extracts of the plants is represented in fig. 2.

Out of 50 plant extracts, 31 methanolic extracts and 6 ethyl acetate extracts showed very poor sEH inhibition activity with IC$_{50}$ values >50 µg/ml. Five plants (*Azadirachta indica*, *P. emblica*, *Piper nigrum*, *Psoralea corylifolia* and *Pungamia glabra*) showed sEH inhibition activity with IC$_{50}$ value >50 µg/ml) in both methanolic and ethyl acetate extracts.

The methanolic extract which showed potent sEH inhibition activity is shown in fig. 3.

Preliminary phytochemical screening indicates that CPME contains components such as alkaloids, carbohydrates, glycosides, proteins, free amino acids and flavonoids, whereas phytosterols, fixed oils, fats, saponins, gums and mucilage were absent. NSME contains components such as alkaloids, carbohydrates, glycosides, proteins, free amino acids and flavonoids, whereas phytosterols, fixed oils, fats, saponins, gums and mucilage were absent. The qualitative phytochemical analysis results of CPME and NSME are shown in Table 2.

![Fig. 2: Comparison of sEH inhibition activity of methanolic and ethyl acetate extracts](image-url)

Note: The test was conducted in triplicate (n=3) and the data were represented as mean±SEM. *p<0.05 and ***p<0.001 when compared with methanolic extract. Two-way ANOVA followed by Bonferroni post hoc tests, ( ): Methanolic extract and ( ): Ethyl acetate extracts.
The quantitative test results (Table 3) indicate that CPME contains 10.6913 (Quercetin Equivalent (QE)/g of total flavonoids, 2.25 µg/ml (equivalent to gallic acid) TPC and 13.24 % of alkaloid content and total saponin content of 1 µg/ml (equivalent to aescin). NSME contains 8.3185 QE/g of total flavonoids, 2.75 µg/ml (equivalent to gallic acid) TPC, 14.20 % of alkaloid content and total saponin content of 0.7 µg/ml (equivalent to aescin).

The HPTLC fingerprint studies were carried out for establishing the presence of the biomarker compound linoleic acid. The Rf value of standard linoleic acid was found to be 0.87 at 366 nm. Both CPME and NSME revealed spots having Rf 0.87 at 366 nm. The presence of linoleic acid was confirmed by the overlay spectrum of standard linoleic acid at 366 nm. The linoleic acid, CPME and NSME exhibited blue fluorescence at 254 nm, bright blue fluorescence at 366 nm and no fluorescence at 425 nm. Both extracts...
were also evaluated for the presence of linoleic acid but could not be confirmed by the overlay spectrum of standard linoleic acid at 254 nm. The HPTLC chromatograms are shown in fig. 4.

COX and LOX are two well-studied enzymatic pathways for synthesizing lipid autacoids endogenously\cite{1-3}. Modulation of these two enzymatic pathways is utilized as a target for treating various pathological conditions associated with inflammatory responses.

Many drugs are available in the market and used extensively for treating inflammation and associated conditions which shows their action through COX and LOX pathways\cite{6}. However, the 3rd enzymatic pathway of endogenous production of lipid autacoids mediated through CYP system is neglected\cite{3}. The EETs produced through the epoxygenase pathways play important role in controlling inflammation as EETs are actively involved in suppressing inflammation through their action on vascular smooth muscles, platelet aggregation, reactive oxygen species generation, nociception and other inflammatory responses\cite{2,3}. Whereas the endogenous enzyme sHE found in a variety of organs, including the liver, heart, spleen, lung and kidney\cite{20}, converts EETs to corresponding DHET, a less active compound. Hence, inhibiting sHE spares the highly active EETs and helps for imparting their beneficial anti-inflammatory effects\cite{8,10}. In preclinical models, sEH inhibitors have a substantial anti-inflammatory effect and prevent a variety of pathologic processes, including lung fibrosis, thrombosis and acute respiratory distress syndrome\cite{21}. In addition, sHE inhibition also shows its beneficial effects in many chronic pathological conditions like neurodegeneration and Central Nervous System (CNS) disorders, cardiovascular complications, renal disorders, ulcers, asthma, cancer etc.,\cite{8-12}. Extensive animal hypertension investigations have revealed that EETs vascular, epithelial transport and anti-inflammatory activities lower blood pressure and slow the course of renal and cardiovascular illness\cite{22}. These intriguing findings support the idea that boosting epoxy eicosanoids by sHE inhibitors or EET analogues could be a useful treatment for a variety of chronic conditions. Recent research suggests that aberrant sHE levels may play a role in the development of certain psychiatric illnesses and that sHE inhibitors have antidepressant and antipsychotic action\cite{23}.

Hence, in recent decades scientists are focusing on sHE enzyme inhibition as a treatment strategy in many areas. In a similar path in the present study 50 plants with ethno pharmacological importance and previously not reported for sHE enzyme inhibition were evaluated for their action against human sHE activity.

From the studied plants ten methanolic extracts and twenty ethyl acetate extracts have shown potent sHE inhibition potency with IC\textsubscript{50} value of less than 10 µg/ml. These potent plants have been proved beneficial for many chronic pathological conditions including cardiovascular system and CNS complications and cancer. The sHE inhibition potential activities of these plants may be one of the mechanisms through which showed their actions. Hence, the present study data uncover the newer pharmacological target of various plants used in this study. This research work is limited to identifying the plants having sHE inhibition properties in the selected medicinal plants. There is further research scope to screen more and more plants and identify the active constituents responsible for their sHE inhibition activities.

**TABLE 3: RESULTS OF QUANTITATIVE PHYTOCHEMICAL TESTS OF CPME AND NSME**

<table>
<thead>
<tr>
<th>Components</th>
<th>Quantities present</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total flavonoids (equivalent to quercetin)</td>
<td>10.6913 QE/g</td>
</tr>
<tr>
<td>Total phenolic content (equivalent to gallic acid)</td>
<td>2.25 µg/ml</td>
</tr>
<tr>
<td>Total saponin content (equivalent to aescin)</td>
<td>1 µg/ml</td>
</tr>
<tr>
<td>Alkaloid content</td>
<td>0.1324</td>
</tr>
</tbody>
</table>

1440 Indian Journal of Pharmaceutical Sciences November-December 2022
CPME and NSME showed the most potent sEH inhibition activities. The CPME contains components such as alkaloids, carbohydrates, glycosides, proteins, free amino acids and flavonoids, whereas phytosterols, fixed oils, fats, saponins, gums and mucilage were absent. NSME contains components such as alkaloids, carbohydrates, glycosides, proteins, free amino acids and flavonoids, whereas phytosterols, fixed oils, fats, saponins, gums and mucilage were absent.

The quantitative test results indicate that CPME contains 10.6913 QE/g of total flavonoids, 2.25 µg/ml TPC and 13.24 % of alkaloid content and total saponin content of 1 µg/ml. NSME contains 8.3185 QE/g of total flavonoids, 2.75 µg/ml TPC, 14.20 % of alkaloid content and total saponin content of 0.7 µg/ml. In both CPME and NSME the presence of linoleic acid was confirmed by the overlay spectrum of HPTLC with standard linoleic acid. Hence, the presence of these active compounds may be responsible for their sEH inhibition activities.

In conclusion, the study report suggests that various natural products used in the traditional medicinal system have many promising sEH inhibitors. In the present study, first time we reported sEH inhibition potentials of fifty traditional medicinal plants used in the various system of medicine. From the plants evaluated methanolic extracts of seeds of C. paniculatus and N. sativa has potent sEH inhibition activities. Further research is warranted to identify a greater number of medicinal plants and active principles responsible for sEH inhibition activities.

Conflict of interests:
The authors declared no conflict of interests.

REFERENCES
13. Das Mahapatra A, Choubey R, Datta B. Small molecule soluble epoxide hydrolase inhibitors in multitarget and combination therapies for inflammation and cancer. Molecules


