Research Paper

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

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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 anthhelmintica* 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

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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 dahuric*[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.

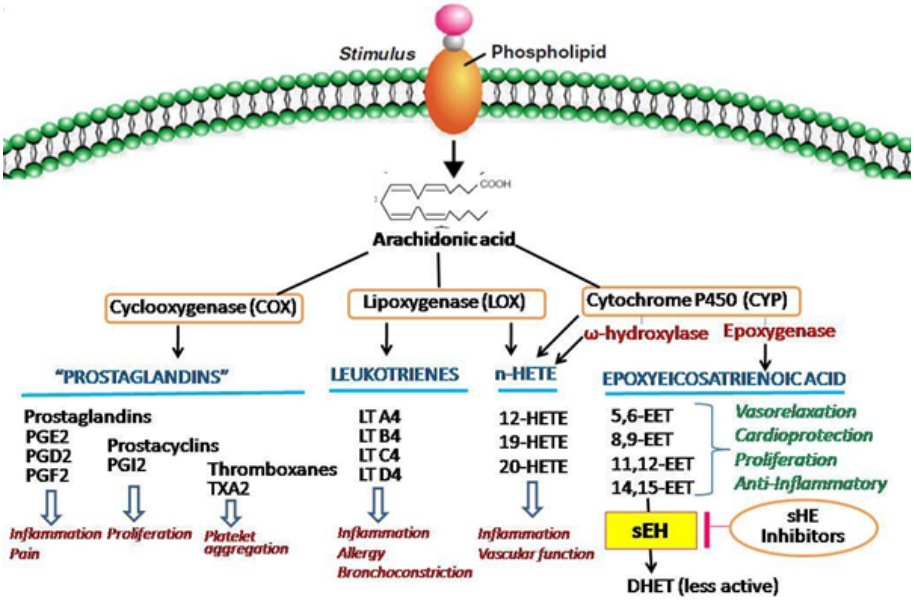

Fig. 1: Schematic representation of bioactive eicosanoids synthesis from the arachidonic acid cascade through the COX, LOX and CYP pathways

TABLE 1: LIST OF PLANTS USED IN THE STUDIES WITH THEIR DETAILS

Botanical name	Family	Common names	Chemical constitutes	Traditional uses	Reference
Acalypha indica	Euphorbiaceae	Indian copper leaf	Mauritianin, clitorin, nicrotiflorin, biorobin	Laxative, anthelmintic, emetic, expectorant, to treat scabies, earache, syphilitic ulcers and snake bites etc.,	[24]
Adhatoda vasica	Acanthaceae	Malabar nut tree	Alkaloids like vasicine, vasicinone, deoxyvasicine, vasicol, adhatodinine, vasicinol	Expectorant, malarial fever, chronic fever, intrinsic haemorrhage, cough, asthma, leprosy, skin diseases, piles	[25]
Andrographis paniculata	Acanthaceae	Creat or green chiretta	Diterpenoids, diterpene glycosides, lactones, flavonoids, and flavonoid glycosides	Snakebite, bug bite, diabetes, dysentery, fever, malaria, common cold, diarrhoea, jaundice, as a health tonic etc.,	[26]
Azadirachta indica	Meliaceae	Neem tree	Gedunin, nimbin, nimbolide, azadirone, neemfruitin etc.,	Infection, metabolic diseases, cancer, diabetes mellitus	[27]
Bergamia koengii	Rutaceae	Curry Leaf	Vitamin A and calcium	Digestive, tonic, stimulant, diarrhoea, dysentery and vomiting	[27]
Cardiospermum halicababum	Sapindaceae	Kanphuti/Ballon plant	Flavones, aglycones, fatty acids, glycosides, terpenoids	Diuretic, demulcent, emetic, laxative, treatment of rheumatism, stiffness of limbs, snakebite	[28]
Cassia alata	Fabaceae	Ketepeng	Flavones, flavonols, flavonoids glycosides, alatinon, alanonal and ß-sitosterol-ß-D-glucoside	Anti-allergic, anti-inflammatory, antioxidant, anticancer, antidiabetic and antifungal	[29]
C. paniculatus	Celastraceae	Jyotishmati	Alkaloids, Glycoside, sterols, dipalmitoyl glycerol	Emollient, thermogenic, stimulant, digestive, laxative, emetic, expectorant, appetizer, aphrodisiac, cardiotonic, anti-inflammatory	[30]
Centella asiatica	Apiaceae	Indian pennywort, Asiatic pennywort	Saponins, asiaticoside and madecassoside and their aglycones, asiatic acid and madecassic acids	Leprosy, lupus, varicose ulcers, eczema, psoriasis, diarrhoea, fever, amenorrhea	[31]
Clerodendrum phlomidis	Lamiaceae	Arni	Sesquiterpene, diterpenoids, triterpenoids, flavonoid and flavonoid glycosides, phenylethanoid glycosides, steroids and steroid glycosides, cyclohexylethanoids, anthraquinones, cyanogenic glycosides	Anti-inflammatory and anti-nociceptive, antioxidant, antihypertensive, anticancer, antimicrobial, anti-diarrheal, hepatoprotective, neuroprotective hypoglycemic, hypolipidemic	[32]
Corollocarpus epigaeus	Cucurbitaceae	Jungali suran	Tannins, alkaloids, saponins, steroids and phenolic compound	Laxative, anti-diabetic	[33]

Curcuma longa	Zingiberaceae	Termeric	Curcumin, curcumenol, camphor, germacrone, ß-pinene, isocurcumenol	Anti-inflammatory, antiviral, anticancerous, carminative, antiproliferative, hypocholesterolemic, diuretic, antidiabetic, antihepatotoxic, antidiarrheal, antirheumatic, hypotensive, antioxidant, antimicrobial, insecticidal, larvicidal, anti-venomous, antithrombotic	[34]
Cynodon dactylon	Poaceae	Bermudagrass	Cynodin, hydrocyanic acid and triticin	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	[35]
Cyperus rotundus	Cyperaceae	Purple nutsedge	Cyperolone, ß-cyperone, ρ-cymol, calcium, camphene, copaene, cyperene, cyperenone, cyperol, cyperolone, caryophyllene, cyperotundone, d-copadiene, d-epoxyguaiene, isocyperol, isokobusone, kobusone, limonene, linoleic-acid, linolenic-acid, mustakone, myristic acid, oleanolic acid, oleic acid, ß-pinene, patchoulenone, rotundene, rotundenol, rotundone, α-rotunol, ß-rotunol, ß-selinene, selinatriene, sitosterol, stearic acid, sugeonol and sugetriol	Analgesic, anti-allergic, anti-arthritic, 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, larvicidal, hepatoprotective, neuroprotective, wound healing	[36]
Elettaria cardomomum	Zingiberaceae	Cardamom	a- pinene,R- pinene, sabinene, myrcene, limonene, y-terpinene, methyl eugenol	Aromatic stimulant, carminative, stomachic and diuretic	[37]
Embelia ribes	Myrsinacae	False black pepper	Embelin, volatile oil, fixed oil, resin, tannin, christembine, caffeic acid, vanillic acid, chrorogenic 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	[38,39]
Glyczirizha glabra	Fabaceae	Liquorice	Liquirtin, isoliquertin liquiritigenin and rhamnoliquirilin	Anti-inflammatory, asthma, expectorant, controls coughing, detoxifies the liver, bronchitis, peptic ulcer, arthritis	[40]

Hibiscus rosasinensis	Malvaceae	China rose	Stigmasterol, ß-sitosterol, taraxeryl acetate	Cough cold, hair loss, reduction of cholesterol, mild laxative, expectorant and diuretic	[41]
Indigofera aspalathoides	Fabaceae	Wiry Indigo	Tannin, alkaloids, triterpenes, flavones, saponin and steroids	Anti-cancerous and antioxidant activity. anti-microbial, hypoglycemic, hepatoprotective, anti-inflammatory and antiviral	[42]
Indigofera tinctoria	Fabaceae	True indigo	Alkaloids, flavonoids, tannins and phenols, saponins, glycosides and terpenoids, indigotine, indiruben, rotenoids	Anti-diarrheal, antiviral, antipyretic, antidiabetic, antipsoriatic, antioxidant, antifungal, antineoplastic, antiparasitic, antiseborrheic, anticataract, antithyroid, anticarcinogenic, antileprosy, hair growth stimulant.	[43]
Lawsonia alba	Lythraceae	Henna	Lawsone, esculetin, fraxetin, isoplumbagin, scopoletin, betulin, betulinic acid, hennadiol, lupeol, lacoumarin, laxanthone, flavone glycosides, two pentacytic triterpenes	Headache, hemicranias, lumbago, bronchitis, boils, ophthalmia, syphilis, sores, amenorrhea, scabies, dysuria, bleeding disorder, diuretic, antifungal, antibacterial, anti-amoebiasis, astringent, anti-hemorrhagic	[44]
Leucas aspera	Lamiaceae	Thumbai	Triterpenoids, oleanolic acid, ursolic acid and b-sitosterol, nicotine, sterols, glucoside, diterpenes, phenolic compounds (4-(24-hydroxy-1-oxo-5-n-propyltetracosanyl)-phenol)	Antifungal, antioxidant, antimicrobial, antinociceptive and cytotoxic	[45]
Mentha piperita	Lamiaceae	Peppermint	Menthol, menthyl acetate, menthone, pulegone, menthofuran, limone	Astringent, antiseptic, antipruritic, antiemetic, carminative, vermifuge, diaphoretic, analgestic	[46]
Mollugo cerviana	Molluginaceae	Thread stem carpet weed	Carbohydrates, saponins, tannins, terpenoids, flavonoids, steroids, phenols, proteins and alkaloids	Antimicrobial, anti-inflammatory, antioxidant activity and spermicidal activity	[47]
N. sativa	Ranunculaceae	Fennel flower	Thymoquinone, thymohydroquinone, dithymoquinone, p-cymene,carvacrol,4-terpineol, t-anethol	Antidiabetic, anticancer, immunomodulator, analgesic, antimicrobial, anti-inflammatory, spasmolytic, bronchodilator, hepatoprotective, renal protective, gastro-protective, antioxidant	[48]
Ocimum basilicum	Lamiaceae	Basil	Linalool, methyl chavicol or citral and 1,8-cineole, camphor, thymol, methyl cinnamate, eugenol, methyl eugenol, methyl isoeugenol and elemicine	Headaches, coughs, diarrhoea, constipation, warts, worms and kidney malfunctions	[49]

Ocimum sanctum	Lamiaceae	Tulsi	Eugenol, euginal, urosolic acid, carvacrol, linalool, limatrol, caryophyllene, methyl carvicol	Antimicrobial, anti-diarrheal, anti-oxidant, anti-cataract, anti-inflammatory, hepato-protective, 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	[50]
Phyllanthus emblica	Euphorbiaceae	Amla	tannins, gallic acid, ellagic acid, emblicol, phyllembin, lupeol, essential oil, fixed oil	Antidiabetic, hypolipidemic, antibacterial, antioxidant, antiulcer, hepatoprotective, gastroprotective	[51]
Phyllanthus niruri	Euphobiaceae	Stonebreaker	Alkaloid, flavonoid, terpenoids, cardiac glycoside, saponins, tannins, cyanogenic glycosides	Hepatitis B, lithiasis, hyperlipidemia, diabetes, hyperuricemia, nephrotoxicity, platelet aggregation, algesia, unwanted pregnancy, vasoconstriction, hepatotoxicity	[52]
Piper betel	Piperaceae	Betel leaf	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	Obstructed urination, weakness of nerves, sore throat, respiratory disorders, constipation, problem of breast milk secretion, inflammation, antimicrobial, antifungal, antihistaminic	[53]
Piper longum	Piperaceae	Long pepper	Alkaloids, amides, lignans, esters, volatile oils	Cancer, diabetes, obesity, hyperlipidemia, asthma, fungal infection, arthritis, pain, amoebiasis, ulcer, depression, inflammation	[53]
Piper nigrum	Piperaceae	Peppercorn	Piperic acid, piperlonguminine, pellitorine, piperolein B, piperamide, piperettine and (-)-kusunokinin	Anti-inflammatory, analgesic, anticonvulsant and neuroprotective, antidiabetic	[54]
Plectranthus vettiveroides	Lamiaceae	Hribera	Androstan-17-one 3-ethyl-3-hydroxy-(5α) (-) spathulenol	Antipyretic, diuretic, antibacterial, antioxidant, anticancer, antidiabetic, hepatoprotective	[55]
Psoralea corylifolia	Fabaceae	Babchi	Bakuchiol, psoralen, isopsoralen, corylifolin, corylin, psoralidin	Antitumor, antihyperglycemic, antidepressant, antioxidant, antibacterial, diuretic, anthelmintic, laxative, wound healing, stomachic, stimulant, aphrodisiac, diaphoretic, asthma, cough, nephritis	[56]
Pungamia glabra	leguminosae	Karanj	Karangin, pongamol, pongagalabrone, and pongapin, pinnatin and kanjone	Antiseptic, anti-inflammatory, anti-plasmodial, anti-nociceptive, anti-hyperglycemic, anti-lipidoxidative, antidiarrhoeal, anti-ulcer, anti-hyperammonic, CNS depressant and antioxidant	[57]

Santalum album	Santalaceae	Sandal wood	Alpha-santalol, tannins, terpenes, resins	Anti-inflammatory, antimicrobial, antiproliferative, acne, psoriasis	[57]
Sesamum indicum	Pedaliaceae	Benne	sesamol, sesamolin and sesamin	Antioxidants, anti-inflammatory, anti-microbial, anti-pyretic.	[58]
Smilax chinensis	Smilacaceae	China root	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	Antimicrobial, anthelmintic, antioxidant, anticancer, hepatoprotective	[59]
Solanum trilobatum	Solanaceae	Climbing brinjal	Sobatum,β-solamarine, solasodine,solaine, glycoalkaloid and diosogenin	Hepatoprotective, antimicrobial, antioxidant, cytotoxic, haemolytic, immunomodulatory and anti-inflammatory	[60]
Terminalia chebula	Combretaceae	Chebulic myrobalan	Gallic acid, chebulagic acid, punicalagin, chebulanin, corilagin, neochebulinic acid, ellagic acid, chebulinic acid, 1,2,3,4,6-penta-O-galloyl-β-D-glucose, 1,6-di-o-galloyl-D-glucose, casuarinin, 3,4,6-tri-o-glloyl-D-glucose, terchebulin	Antioxidant, antibacterial, antifungal, antiviral, antiprotozoal, antiulcer, anticarcinogenic, purgative, radioprotective, antiallergic. hepatoprotective, anti-inflammatory, antispasmodic	[61]
Tinospora cordifolia	Menispermaceae	Guduchi	Berberine, palmatine, isocolumbine, tinocordiside, palmatoside, beta sitosterol, tinosporides	Anti-diabetic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic, antioxidant, anti-allergic, anti-stress, anti-leprotic, anti-malarial, hepatoprotective, immunomodulatory	[62]
Trichosanthes cucumerina	Cucurbitaceae	snake gourd	Proteins, fat, fibre, carbohydrates, minerals, and vitamins A and E in high levels	Antidiabetic, antibacterial, anti-inflammatory, anthelmintic, antifebrile, gastroprotective, and antioxidant activity	[63]
Trigonella foenum	Fabaceae	Fenugreek	Diosgenin, trigonelline, fenugreekine, galactomannan and 4-hydroxy isoleucine	In the treatment of diabetes, microbial and cancer disease	[64]
Vernonia anthhelmintica	Asteraceae	Purple fleabane	Brassicasterol, stigmasterol, resin, myristic acid palmitic acid, stearic acid, oleic acid linoleic acid vernolic acid and methyl vernolate	Diabetes mellitus, leukoderma, skin disease, fever, worm infection and kidney trouble	[65]

Vetiveria zizanioides	Poaceae	Vetiver	Cycloisolongifolene, isoledene, isolongifolene, longifolene, sativene	Antimicrobial	[66]
Withania somnifera	Solanaceae	Ashwagandha/ winter cherry	Isopelletierine, anaferine, cuseohygrine, anahygrine, withanolides, withaferins	Anti-epileptic, anti-inflammatory, anti-arthritic, anti-depressant, anti-coagulant, antioxidant, anti-diabetic, anti-pyretic	[67]
W. tinctoria	Apocynaceae	Sweet Indrajao	Lupeol, α- and ß- amyrin, indigotin, indirubin, tryptanthrin, isatin, rutin, ß-sitosterol, triacontanol, myristic acid, palmitoleic acid, palmetic acid, stearic acid, behenic acid, arachidic acid	Antidiarrhoeal, aphrodisiac, anthelmintic, febrifuge, stomachic, toothache, tonic and dog bite	[68]
Zingiber officinalae	Zingiberaceae	Ginger	Gingerol, diarylheptanoids, glutamate, aspartic acid, serine, glycine, threonine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, arginine, proline	Antioxidant, anti-inflammatory, antimicrobial, anticancer, antiobesity, antidiabetic, antinausea, antiemetic, antiallergic, neuroprotective, hepatoprotective, cardiovascular protective and respiratory protective	[69]

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*, *Glyczirizha 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 anthhelmintica*, *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 Cyano (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), 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.

$$\text{Total flavanoids}=(A\times M_0)/(A_0\times M)$$

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:

Prisom 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 anthhelmintica* 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.

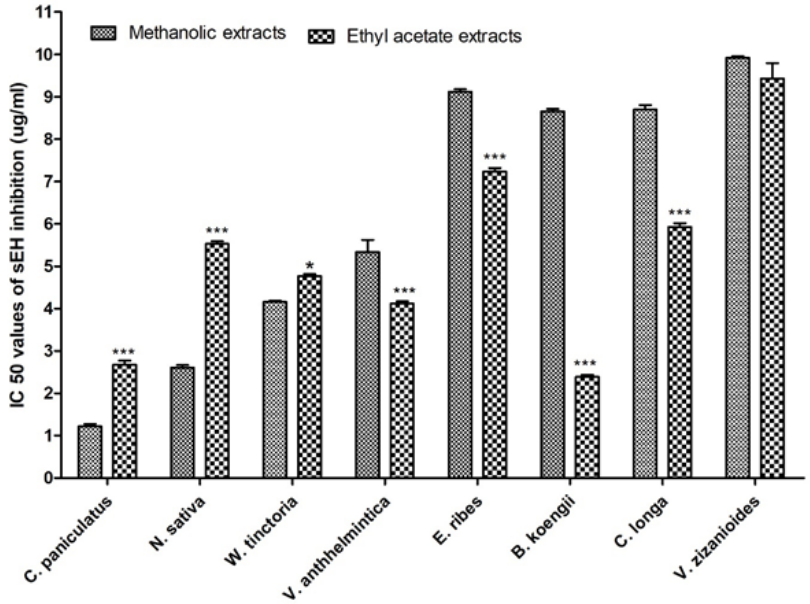

Fig. 2: Comparison of sEH inhibition activity of methanolic and ethyl acetate extracts
Note: The test was conducted in triplicate (n=3) and the data were represented as mean±SEM. *p<0.05 and ***p<0.001 when compared with methanolic extract. Two-way ANOVA followed by Bonferroni post hoc tests, (▨): Methanolic extract and (▩): Ethyl acetate extracts

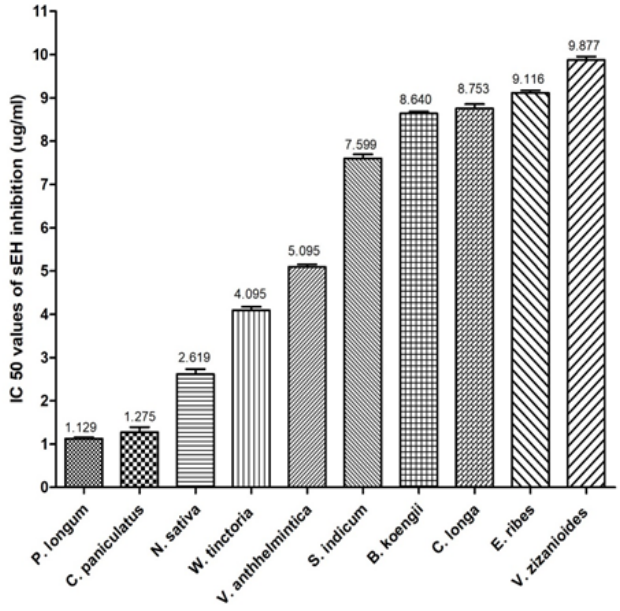

Fig. 3: The methanolic extract showing potent sEH inhibition activity

TABLE 2: RESULTS OF PHYTOCHEMICAL EVALUATION OF CPME AND NSME

Test components	Test methods	CPME	NSME
Alkaloids	Mayer's Test	+	+
	Dragendorff's test	+	+
	Wagner's test	+	+
	Hager's test	+	+
Carbohydrates	Molisch's test	+	+
	Fehling's test	+	+
	Benedict's test	+	+
Glycosides	Legal's test	+	+
	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	+	-
	Ninhydrin test	+	+
Gums and mucilage	Swelling property	-	-
Flavonoids	Shinoda test	+	+
	Alkaline reagent test	+	+

Note: (+): Presence and (-): Absence of that particular component

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 R_f value of standard linoleic acid was found to be 0.87 at 366 nm. Both CPME and NSME revealed spots having R_f 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[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[6]. However, the 3rd enzymatic pathway of endogenous production of lipid autacoids mediated through CYP system is neglected[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[2,3]. Whereas the endogenous enzyme sHE found in a variety of organs, including the liver, heart, spleen, lung and kidney[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[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[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.,[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[22]. These intriguing findings support the idea that boosting epoxy eicosanoids by sEH inhibitors or EET analogues could be a useful treatment for a variety of chronic conditions. Recent research suggests that aberrant sEH levels may play a role in the development of certain psychiatric illnesses and that sEH inhibitors have antidepressant and antipsychotic action[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_{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 sEH 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 sEH inhibition activities.

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

Components	Quantities present	
	CPME	NSME
Total flavonoids (equivalent to quercetin)	10.6913 QE/g	8.3185 QE/g
Total phenolic content (equivalent to gallic acid)	2.25 µg/ml	2.75 µg/ml
Total saponin content (equivalent to aescin)	1 µg/ml	0.7 µg/ml
Alkaloid content	0.1324	0.142

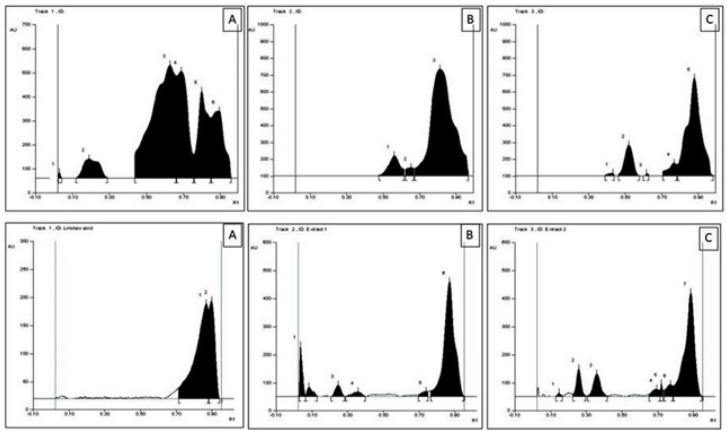

Fig. 4: HPTLC chromatogram of (A): Standard linoleic acid; (B): CPME and (C): NSME

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.

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