ORIGINAL ARTICLE e-ISSN: 2349-0659 p-ISSN; 2350-0964

Effects of Various Phytochemical Fractions of *Mundulea sericea* on Free Radical Scavenging and Inhibition of Inflammatory Agents

S. Gangadevi[1], D. Sheeba Gnanadeebam[2], K. Kalimuthu[1], V. Chinnadurai[3], P. Viswanathan[1]*

Abstract

Antioxident and antiinflammatory activities of *Mundulea sericea* (Willd.) A. Chev. is a medicinally important species belong to the family *Fabaceae*. This species contains various phytocompounds of entire parts which act as antimicrobial activity, anti-inflammatory activity, anticancer activity, antidiabetic activity, etc. Hence, the present study was aimed to analysis of phytochemical constitutes through Fourier transform infrared (FTIR) and gas chromatography mass spectrometry (GCMS) analysis by six various solvent extracts of hexane (MSBH), petroleum ether (MSBPE), chloroform (MSBC), ethyl acetate (MSBEA), ethanol (MSBE), and methanol (MSBM). The result of phytochemical analysis reported that the MSBE and MSBM extracts are contain more phytochemical constitutes than other extracts. In FTIR and GCMS study, MSBE and MSBM extracts are reported various phytochemical constitutes and out that many bioactive compounds are present of these extracts. Further, MSBE extract was subjected antioxidant and anti-inflammatory activities. In antioxidant activity, four different type assays such as 2,2-diphenyl-1-picryl-hydrazyl-hydrate radical scavenging, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate), hydrogen peroxide, and nitric oxide (NO) were used. Among this antioxidant study, NO was reported 74.63 inhibition percentage of free radical than other assays. In anti-inflammatory activity, albumin denaturation and human red blood cell membrane stabilization assay were performed by MSBE extract which result reported that the MSBE extract has good anti-inflammatory activity with more than 60% inhibition capacity on both assays. From this study, *M. sericea* bark extracts contain highly therapeutic phytocompounds which compounds possess good antioxidant and inflammatory activities.

Keywords: Antioxidant and anti-inflammatory, Ethanol extract, *Mundulea sericea*, Phytochemical

Asian Pac. J. Health Sci., (2021); DOI: 10.21276/apjhs.2021.8.3.21

Introduction

Medicinal plants play an important role in human life to control disease and as a valuable source of new drugs. The World Health Organization (WHO) has estimated that up to 80% of people still rely on herbal remedies for their health care.[1,2] As stated by the International Union for Conservation of Nature and the World Wide Fund, around 50,000–80,000 flowering plant species are used worldwide for therapeutic properties. Due to the low cost and easy availability of traditional drugs, the WHO also encouraging usage of herbal drugs for various human diseases.[3] The plant genus *Mundulea* belong to *Fabaceae* family is known for wide uses in traditional medicinal practices.[4-7] This family has highly contain secondary metabolites of flavonoids and isoflavonoids which phytocompounds reported as anticancer,[8,9] antimicrobial,[10] antioxidant, and antiplasmodial[11,12] activities.

Mundulea sericea (Willd.) A. Chev. (*M. sericea*) is a medicinally important species belong to *Fabaceae* family. This species is shrub or small tree habit located in dry forests and rocky hills of West and South India. It also widely distributed in central and southern tropical Africa.[13] The entire part of the plant contains various phytochemical constitutes which used as various human diseases. The entire parts of this plant such as bark, leaves, seeds, and roots are used as problem such as fish poison,[14] insecticide,[15] and an aphrodisiac.[16] The previous studies are reported that the isolated phytocompounds of rotenoids and isoflavanones,[17] flavanones, chalcones,[18] and an imidazole derivative[19] which are work as many biological activities. Furthermore, the whole plant has antimicrobial, analgesic, antioxidant, cytotoxic, and cancer chemopreventive activities. The present work was aimed to analysis of phytochemical constitutes in various solvent extracts such as hexane (MSBH), petroleum ether (MSBPE), chloroform (MSBC), ethyl acetate (MSBEA), ethanol (MSBE), and methanol (MSBM). Further, MSBE extract subjected to scavenging assays of antioxidant and anti-inflammatory activities.

[1]Department of Botany, Government Arts College (Autonomous), Coimbatore, Tamil Nadu, India

[2]Department of Botany, Gobi Arts and Science College (Autonomous), Gobichettipalayam, Tamil Nadu, India

[3]Department of Botany, Sri Vidya Mandir Arts and Science College (Autonomous), Katteri, Tamil Nadu, India

Corresponding Author: P. Viswanathan, Department of Botany, Government Arts College (Autonomous), Coimbatore, Tamil Nadu, India. E-mail: rpviswanathan68@gmail.com

How to cite this article: Gangadevi S, Gnanadeebam DS, Kalimuthu K, Chinnadurai V, Viswanathan P. Effects of Various Phytochemical Fractions of *Mundulea sericea* on Free Radical Scavenging and Inhibition of Inflammatory Agents. Asian Pac. J. Health Sci., 2021;8(3):122-130.

Source of support: Nil

Conflicts of interest: None.

Received: 12/04/21 **Revised:** 20/05/21 **Accepted:** 05/06/21

Materials and Methods

Chemicals

Analytical research grade chemicals of 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), butylated hydroxyl anisole, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS$^+$), potassium persulfate, ascorbic acid, TPTZ, hydrochloric acid, ferric chloride,

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Trolox, dextrose, sodium citrate, and citric acid were purchased and used for this work.

Plant Collection and Authentication

M. sericea was collected from Western Ghats region, Coimbatore, Tamil Nadu, India. The species name authenticated by BSI, Coimbatore, India, and reference number is BSIS/RC/5/23/2017/Tech./455.

Phytochemical Analysis

Preliminary phytochemical screening

M. sericea bark six extracts were subjected to preliminary phytochemical screening to find out the secondary metabolites of alkaloids, flavonoids, terpenoids, tannins, glycosides, etc. For alkaloids test, four different screening tests such as Dragendorff's test, Mayer's test, Wagner's test, and Hager's test[20] were analyzed. Further analysis of secondary metabolites such as flavonoids in 10% HCl and 5% NaOH test and alkaline test,[21] tannins used 5% $FeCl_3$ test,[20] steroids is Liebermann–Burchard test,[20] triterpenes using the Liebermann–Burchard test and Salkowski's test,[22] saponins foam test,[23] glycosides Killer and Kilian test,[24] gum and mucilages test,[25] fixed oils spot test,[23] and finally anthraquinones used in NH_4OH test[26] were studied by six solvent extracts of MSBH, MSBPE, MSBC, MSBEA, MSBE, and MSBM by standard procedure.

Fourier transform infrared (FT-IR) spectroscopy

FTIR analysis of *M. sericea* extracts was carried out through the potassium bromide pellet (FTIR grade) method in 1:100 ratio and spectrum was recorded using Jasco FT/IR-6300 FTIR equipped with JASCO IRT-7000 Intron Infrared Microscope applying dissemination mode employing at a resolution of 4 cm−1 (JASCO, Tokyo, Japan).

Gas chromatogram–mass spectroscopy (GC–MS) analysis

GC–MS analysis of *M. sericea* bark extracts was analyzed. The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250 μm df) and using helium gas at continuous flow of 1 ml/min, and with injector temperature of 260°C, the components were isolated. The 1 μL of extract sample injected into the instrument the oven temperature was 60°C (2 min) subsequently 300°C held for 6 min. The conditions of mass detector were 240°C temperature and ion source temperature at 70 eV ionization impact, 0.2 s scan time with 0.1 s scan interval. The components were correlated with GC–MS NIST (2008) library.

Biological Studies

Antioxidant activity

The *in vitro* antioxidant activity of MSBE extract was carried out by standard methods. Four different antioxidant methods (DPPH radical scavenging activity, ABTS radical scavenging assay, ferric reducing ability of plasma (FRAP), and nitric oxide (NO) radical inhibition activity) were studied by different concentration. Each antioxidant assay absorbance was measured by various wavelengths and the percentage of inhibition was calculated using the following formula.

$$\text{Percentage of inhibition } (\%) = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

DPPH radical scavenging method

The DPPH radical scavenging activity of MSBE extracts was evaluated by the method of Szabo *et al*.[27] Twenty-one milligrams of each sample extract or standard were dissolved individually in 1 mL dimethyl sulfoxide (DMSO) to a solution of 21 mg/mL concentration. Ten microliters extracts or standard with 200 μl of 100 mM DPPH solution was mixed separately in 96-well microtiter plate and incubated at 37°C for 20 min. The each plate well absorbance was measured at 490 nm by used ELISA reader and the percentage of inhibition was calculated.

ABTS radical scavenging method

13.5 mg of MSBE extracts and ascorbic acid were dissolved in 2 mL of DMSO and this solution was serially diluted with DMSO to get lower concentrations. 0.2 mL of each extract or standards mixed with 1 mL of DMSO and 0.16 mL of ABTS then it take final volume of 1.36 mL. After 20 min, the absorbance was measured in enzyme-linked immunosorbent assay (ELISA) reader at 734 nm.[28]

NO radical inhibition activity

Forty-two milligrams of MSBE extract were separately dissolved in 2 mL of DMSO to get 21 mg/mL concentration. The reaction mixture 6 mL containing 4 mL of 10 mM SNP, 1 mL of DMSO, and 1 mL of sample were incubated at 25°C for 90 min. After incubation, 1 mL of sulfanilic acid reagent was added and allowed to stand for 5 min for completion of diazotization, then 1 mL of NEDD was added and another 1 time allowed to stand for 30 min at room temperature. The absorbance was measured at 540 nm using ELISA reader.[29,30]

FRAP assay

The total antioxidant potential of sample was determined using FRAP assay as a measure of antioxidant power. The FRAP reagent consists of TPTZ solution (5 ml), ferric chloride solution (2.5 ml), and acetate buffer (25 ml). Then, 900 μl FRAP reagent was mixed with 90 μl water and 30 μl MSBE extract. The reaction mixture was then incubated at 37°C for 30 min and the absorbance was recorded at 593 nm.[31]

Anti-inflammatory Activity

Inhibition of albumin denaturation

The anti-inflammatory activity was studied using inhibition of albumin denaturation technique[32,33] followed with minor modifications. The reaction mixture (0.5 ml; pH 6.3) consisted of 0.45 ml of bovine serum albumin (5% aqueous solution) and 0.05 ml of distilled water and pH 6.3. Different concentrations of MSBE extract were added to the reaction mixture and were incubated at 37°C for 20 min and then heated at 57°C for 5 min after cooling the samples, 2.5 ml of phosphate buffer saline was added. Turbidity was measured spectrophotometrically at 600 nm. The protein suppression percentage was determined as follows:

$$\text{Percentage of inhibition } (\%) = \frac{(\text{AbsControl} - \text{AbsSample})}{(\text{AbsControl})} \times 100$$

Human red blood cell (HRBC) membrane stabilization method

MSBE extract various concentrations of 100, 200 300, 400, and 500 μg/ml were incubated separately with HRBC solution. Blood was collected (2 mL) from healthy volunteers and was mixed with equal volume of sterilized Alsever's solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid, and 0.42% NaCl in distilled water) and centrifuged at 3000 rpm. The packed cells were washed with isosaline solution and a 10% v/v suspension was prepared with normal saline and kept at 4°C undisturbed before use. Different concentrations of extract (50, 100, 200, 300, 400, and 500 μg/0.5 ml) in normal saline and aspirin as standard (100, 200, 300, 400, and 500 μg/0.5 ml) were separately mixed with 1 ml of phosphate buffer, 2 ml of hyposaline, and 0.5 ml of 10% HRBC suspension was added to prepared. The mixture was kept at 37°C for 30 min. After centrifugation at 3000 rpm for 20 min, the supernatant solution was evaluated spectrophotometrically at 560 nm.[34] Using the following formula, the HRBC membrane stabilization was calculated.

$$\text{Percentage of inhibition } (\%) = \frac{(\text{AbsControl} - \text{AbsSample})}{(\text{AbsControl})} \times 100$$

Results and Discussion

Phytochemical Screening

Preliminary phytochemical qualitative screening was carried out by various solvent extracts of *M. sericea* and the result is given in Table 1. The result reported that each extracts contain different types of secondary metabolites. All extracts were showed steroids and triterpenoids secondary metabolites. Unlike, as anthraquinones are not present of these extracts. Among the six extracts, MSBE and MSBM extracts were found more number of phytochemicals than other extracts. The both extracts were obtained alkaloids, flavonoids, tannins, steroids, and terpenoids [Table 1]. Natural compounds work as vital role of various pharmacological activities. Alkaloids are used as antimicrobial properties due to their intercalation of the DNA of the microorganism.[35] Like as flavonoids and tannins are major group of phenolic compounds which act as an antioxidants and anti-inflammatory properties. They have reported to possess anti-carcinogenic and anti-mutagenic activities.[35] Further, triterpenoids are reported various biological activities such as pain relieving, antipyresis, hepatoprotective, cardiotonic, soothing, tonic impacts, and also have hypocholesterolemic and antidiabetic properties.[36] Saponins are antimicrobial action.[37] In general, this effectiveness of medicinal plants may not be due to the one main active principle, but may be due the combined effect of more than 1 compound present in the plant.[38] The presence of more bioactive compounds of *M. sericea* extracts it may potential drug by further phytochemistry studies.

FTIR Analysis

FTIR spectroscopy can give information about the molecular structure of organic and inorganic components, and one of the most multifaceted analytical techniques for the non-destructive, chemical characterization of samples.[39-41] Hence, the present work six solvent extracts of *M. sericea* were examined to FTIR analysis. The results were reported various function groups which are given in Figure 1 and Table 2. Each extracts contain different types of functional group such as alcohol, phenols, ketones, aromatic compounds, alkyl halides, carboxylic acid, aliphatic amides, amines, and alkenes [Table 2]. As per the nature of the shape, intensity, and position of the peaks, the vital phytochemical constituents of the extracts may be identified.[42] The presence of alkanes, aliphatic and amides, and amides compounds were responsible for the potential pharmacological activities.[43] Similar kind functional group compounds are present in this extract [Table 2]. Hence, those extracts may act as potentially of various pharmacological activities.

GC–MS Analysis

The medicinal plants are exhibiting foundation of various phytochemical constitutes which are determined by GC–MS spectra analysis.[44] Current work was examined the presence of phytochemical constitutes in various extracts of *M. sericea* through GC–MS analysis. Different kinds of phytochemicals were identified along with molecular weight formal and peak of this extracts. Table 3 given some of the high peak compounds including its molecular formula and molecular weight of each extract. Based on the GC–MS report, MSBPE, MSBE, and MSBM extracts were reported more number higher peak compounds [Figure 2]. Moreover, all solvent extract phytocompounds were reported many biological activities such as antioxidant, anticancer, and antimicrobial.

Table 1: Preliminary phytochemical screening of various extracts of *M. sericea*

Compounds	*Tests*	*MSBH*	*MSBPE*	*MSBC*	*MSBEA*	*MSBE*	*MSBM*
Alkaloids	Dragendorff's test	–	–	–	–	+	+
	Mayer's test	–	–	–	–	+	+
	Wagner's test	+	+	–	+	+	–
	Hager's test	–	–	–	–	+	+
Flavonoids	10% HCl and 5% NaOH test	–	–	+	–	+	+
	Alkaline test	–	–	–	+	+	+
Tannins	5% $FeCl_3$ test	–	–	–	–	+	+
Steroids	Liebermann–Burchard's test	+	+	+	+	+	+
Triterpenoids	Liebermann –Burchard's test	+	+	+	+	+	+
	Salkowski's test	+	+	–	+	+	+
Saponins	Foam test	–	–	–	–	+	–
Glycosides	Killer and Kilian test	+	–	+	+	–	–
Gum and mucilages	Whistler and BeMiller test	+	+	–	+	–	–
Fixed oils	Spot test	+	–	+	–	–	–
Anthraquinones	NH_4OH test	–	–	–	–	–	–

(+): Indicates presence of phytocompounds, (–): Indicates absent of phytocompounds, MSBH: Hexane, MSBPE: Petroleum ether, MSBC: Chloroform, MSBEA: Ethyl acetate, MSBE: Ethanol, MSBM: Methanol

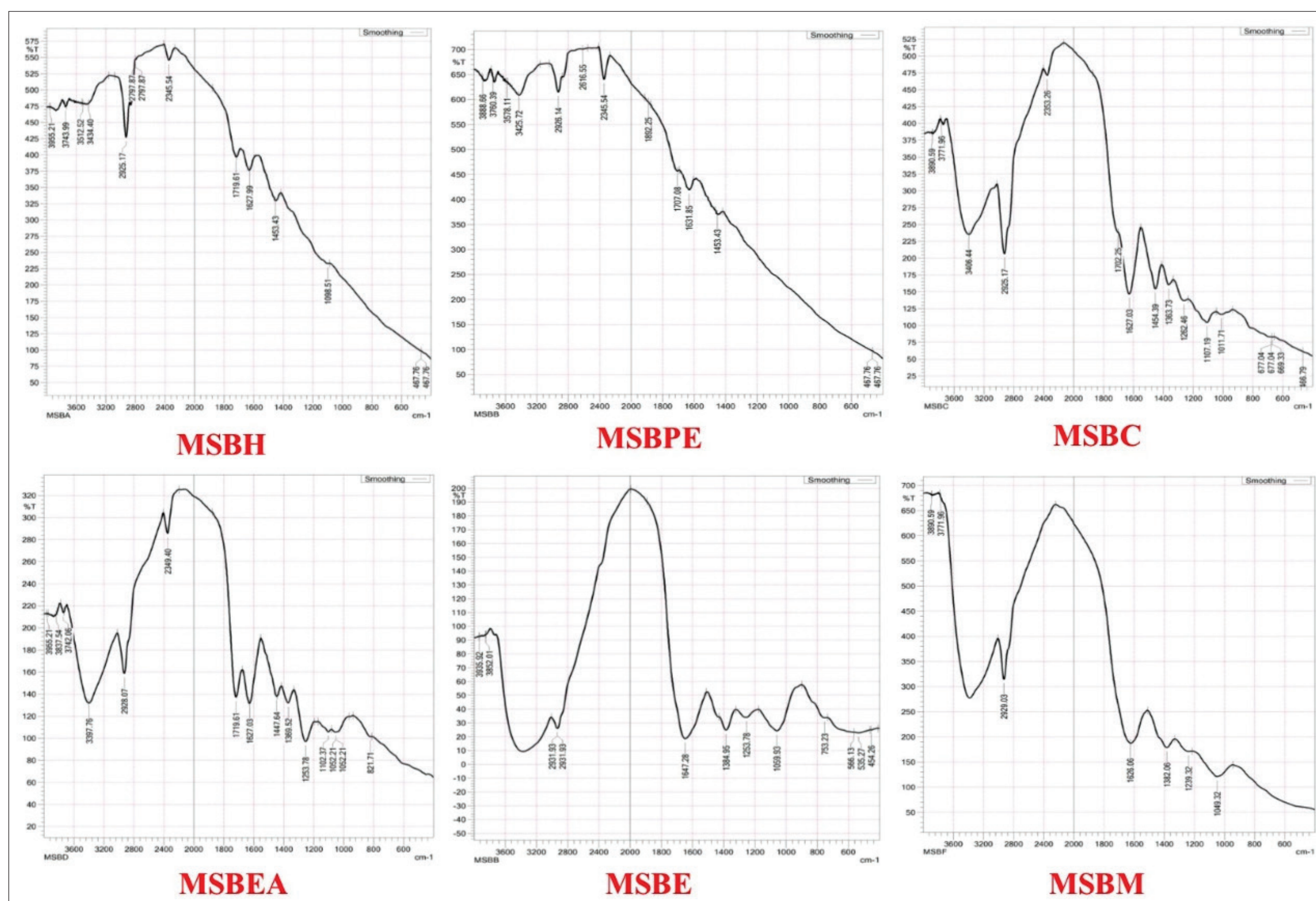

Figure 1: Fourier transform infrared functional group of various solvent extracts of *Mundulea sericea*

Table 2: FTIR functional group of various solvent extracts of *M. sericea*

Functional groups name	*Bond*	*Test samples and its functional group presence*					
		MSBH	*MSBPE*	*MSBC*	*MSBEA*	*MSBE*	*MSBM*
Alcohols, phenols	O-H stretch	+	+	+	+	−	+
Alkanes	C-N stretch	−	+	+	+	+	−
Ketones	C=O stretch	+	+	+	−	−	−
Aromatics	C-H stretch	+	−	+	−	−	−
Alkyl halides	C-H wag	−	−	+	+	+	−
Alcohol ester	C-O stretch	−	−	+	+	−	−
Carboxylic acid	C-O stretch	−	−	+	+	−	−
Aliphatic amines	C-O stretch	+	−	−	+	+	−
1, 2 amines, amides	N-H stretch	+	−	−	−	−	+
Alkynes	H-C=H	+	−	−	−	−	−
Amines	N-H bend	+	+	−	+	+	−
Alkenes	C-H bend	+	−	−	+	−	−
Aromatic amine	C-N bend	+	−	−	−	−	+

MSBH: Hexane, MSBPE: Petroleum ether, MSBC: Chloroform, MSBEA: Ethyl acetate, MSBE: Ethanol, MSBM: Methanol, FTIR: Fourier transform infrared

Bioactive Studies

Antioxidant activity

Phytochemical constitutes are work against free radical scavenging and have vital antioxidant property. Hence, the present study was aimed to MSBE extract subjected to antioxidant assay for analysis of free radical scavenging percentage. Table 4 shows MSBE extract inhibition percentage of free radical inhibition at different concentrations. MSBE extract was reported that the maximum percentage of free radical scavenging at high concentration of 250 µg/ml in all assays. Among these assays, hydrogen peroxide (H_2O_2) was reported maximum antioxidant activity (74.63 ± 0.71) compared to other assay. Followed ABTS assay showed second leading antioxidant activity (72.66 ± 0.16). Those result nearby equal to standard of ascorbic acid value (87.87 ± 0.14) [Figure 3]. Several article reports have mentioned that the free radical scavenging activity is greatly influenced by the secondary metabolites such as phenolic compounds, flavonoid, and hydroxycinnamic acids.[45-47] Similarly, this plant ethanol extract is showed many secondary metabolites [Table 1] which phytocompounds may act as free radicals scavenging.

Figure 2: Gas chromatogram–mass spectroscopy analysis of various solvent extracts of *Mundulea sericea*

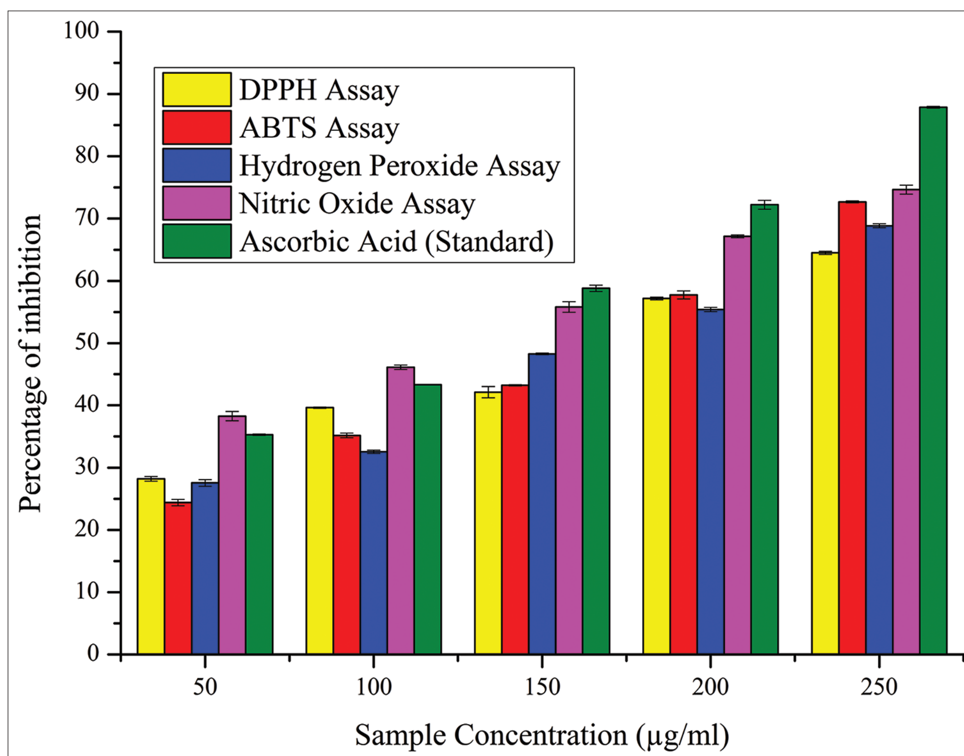

Figure 3: Various antioxidant activities of ethanol extract

Table 3: Phytochemical compounds of various solvent extracts of *M. sericea*

Extract	*Compound*	*Formula*	*Weight*
MSBH	Cyclopentane, 1,1′-Thiobis-	$C_{10}H_{18}S$	170
	1,13-Tetradecadiene	$C_{14}H_{26}$	194
	9,15-Octadecadienoic acid, methyl ester, (Z,Z)-	$C_{19}H_{34}O_2$	294
	Bicyclo[4.1.0]heptane, 7-pentyl-	$C_{12}H_{22}$	166
	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312
	Cyclopentadecanone, 2-hydroxy	$C_{15}H_{28}O_2$	240
	Eicosanoic acid, ethyl ester	$C_{22}H_{44}O_2$	340
	Docosanoic acid, ethyl ester	$C_{24}H_{48}O_2$	368
MSBPE	Oxacyclotridecan-2-one	$C_{12}H_{22}O_2$	198
	1,22-Docosanediol	$C_{22}H_{46}O_2$	342
	Oxacycloheptadec-8-en-2-one	$C_{16}H_{28}O_2$	252
	9,15-Octadecadienoic acid, methyl ester, (Z,Z)-	$C_{19}H_{34}O_2$	294
	Bicyclo[4.1.0]heptane, 7-Pentyl-	$C_{12}H_{22}$	166
	(1S,15S)-Bicyclo[13.1.0]hexadecan-2-one	$C_{16}H_{28}O$	236
	Methyl 17-methyl-Octadecanoate	$C_{20}H_{40}O_2$	312
	Cyclopentadecanone, 2-hydroxy-	$C_{15}H_{28}O_2$	240
	Eicosanoic acid, Ethyl ester	$C_{22}H_{44}O_2$	340
	Cyclohexadecane	$C_{16}H_{32}$	224
	Benzenesulfonic acid, 2-Butoxy-5-(1,1,3,3-Tetramethylbutyl)-	$C_{18}H_{30}O_4S$	342
MSBC	Bicyclo[7.2.0]undec-4-ene, 4,11,11-Trimethyl-8-Methylene-,[1R-(1R*,4Z,9S	$C_{15}H_{24}$	204
	2-Butenoic acid, 3,7-Dimethyl-6-Octenyl ester	$C_{14}H_{24}O_2$	224
	11-Tricosene	$C_{23}H_{46}$	322
	Oxalic acid, Butyl cyclohexylmethyl ester	$C_{13}H_{22}O_4$	242
	2-Propenamide, N-(4-Chlorophenyl)-3-Phenyl	$C_{15}H_{12}ONCl$	257
	3,3,6,6-Tetramethyl-1,2,3,4,5,6,7,8-Octahydro-1,8-Acridinedione	$C_{17}H_{21}O_2N$	271
MSBEA	1,3,4-Tri-O-acetyl-D-glycero-tetrulose	$C_{10}H_{14}O_7$	246
	Cis-1-acetoxy-1-cyano-2-methyl-2-Phenylcyclopropane	$C_{13}H_{13}O_2N$	215
	Bis[4-N-butylbenzyl]sulfone	$C_{22}H_{30}O_2S$	358
	Pentanenitrile, 4,4-dimethyl-5-oxo-	$C_7H_{11}ON$	125
	Trans-1,2,5,5-Tetramethyl-3,7,9-Trioxabicyclo(4,2,1)nonane	$C_{10}H_{18}O_3$	186
	Heptadecanoic acid, ethyl ester	$C_{19}H_{38}O_2$	298
MSBE	3-Octenoic acid, Butyl ester, (Z)-	$C_{12}H_{22}O_2$	198
	2,6-Octadienal, 3,7-Dimethyl	$C_{10}H_{16}O$	152
	Methyl Z-11-Tetradecenoate	$C_{15}H_{28}O_2$	240
	Heptacosanoic acid, 25-Methyl-, Methyl ester	$C_{29}H_{58}O_2$	438
	Cyclopropaneoctanal, 2-octyl-	$C_{19}H_{36}O$	280
	Bicyclo[4.1.0]Heptane, 7-pentyl-	$C_{12}H_{22}$	166
	2-Dodecylcyclobutanone	$C_{16}H_{30}O$	238
	Docosanoic acid, Methyl ester	$C_{23}H_{46}O_2$	354
	Eicosanoic acid, Ethyl ester	$C_{22}H_{44}O_2$	340
	Cyclohexane, 1-(1,5-Dimethylhexyl)-4-(4-Methylpentyl)-	$C_{20}H_{40}$	280
	Tetracosanoic acid, methyl ester	$C_{25}H_{50}O_2$	382
	Nonadecanoic acid, ethyl ester	$C_{21}H_{42}O_2$	326
MSBM	Pentafluoropropionic acid, Octadecyl ester	$C_{21}H_{37}O_2F_5$	416
	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312
	18-Nonadecen-1-ol	$C_{19}H_{38}O$	282
	(E)-9-Octadecenoic acid Ethyl ester	$C_{20}H_{38}O_2$	310
	Oleic acid	$C_{18}H_{34}O_2$	282
	1-Hexyl-2-Nitrocyclohexane	$C_{12}H_{23}O_2N$	213
	Docosanoic acid, Ethyl ester	$C_{24}H_{48}O_2$	368
	Pentadecanoic acid	$C_{15}H_{30}O_2$	242
	Docosanoic acid, ethyl ester	$C_{24}H_{48}O_2$	368
	T-Butyl Cyclopentaneperoxycarboxylate	$C_{10}H_{18}O_3$	186
	2,6-Lutidine 3,5-dichloro-4-dodecylthio	$C_{19}H_{31}NCl_2S$	375

MSBH: Hexane, MSBPE: Petroleum ether, MSBC: Chloroform, MSBEA: Ethyl acetate, MSBE: Ethanol, MSBM: Methanol

Table 4: Various free radical scavenging activity of MSBE extract

Assay	*Percentage of inhibition*				
	50 µg/ml	*100 µg/ml*	*150 µg/ml*	*200 µg/ml*	*250 µg/ml*
DPPH	28.22±0.38	39.62±0.11	42.12±0.91	57.17±0.21	64.51±0.24
ABTS	24.41±0.51	35.17±0.37	43.22±0.11	57.73±0.64	72.66±0.16
H_2O_2	27.57±0.54	32.55±0.27	48.27±0.14	55.41±0.34	68.83±0.31
NO	38.27±0.74	46.13±0.36	55.81±0.84	67.12±0.24	74.63±0.71
Ascorbic acid	35.27±0.12	43.31±0.05	58.81±0.52	72.20±0.73	87.87±0.14

MSBE: Ethanol, DPPH: 2,2-diphenyl-1-picryl-hydrazyl-hydrate, ABTS: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate), H_2O_2: Hydrogen peroxide, NO: Nitric oxide

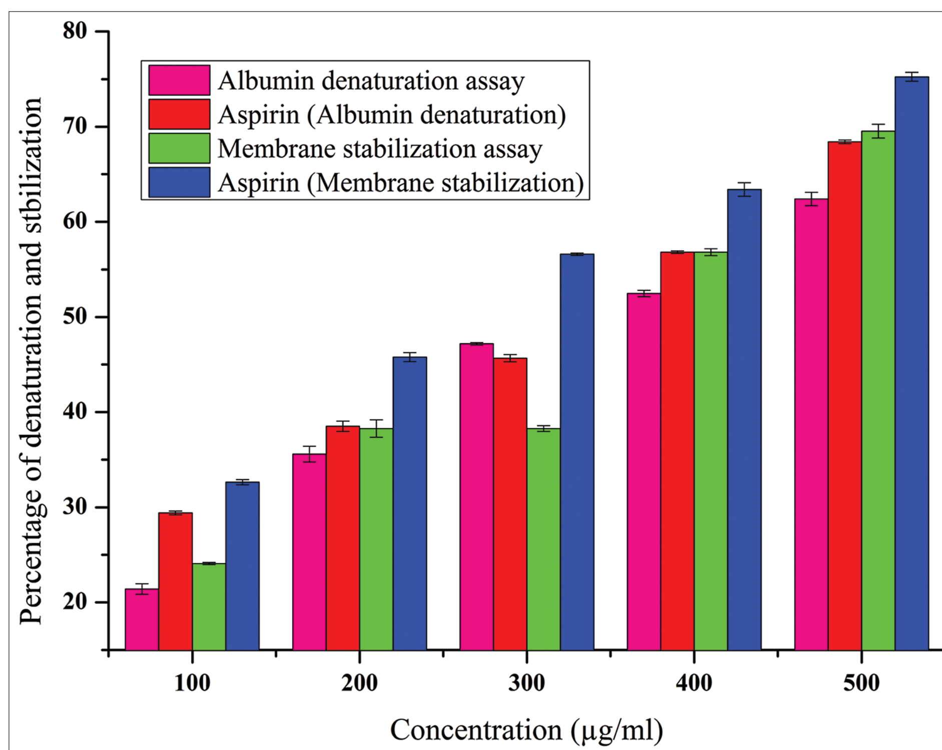

Figure 4: Anti-inflammatory activity of ethanol extract

Table 5: Anti-inflammatory activity of MSBE extract

Concentration (µg/ml)	Albumin denaturation		Membrane stabilization	
	MSBE	Aspirin	MSBE	Aspirin
100	21.42±0.55	29.42±0.20	24.10±0.11	32.65±0.28
200	35.58±0.82	38.51±0.54	38.27±0.91	45.79±0.48
300	47.19±0.12	45.67±0.38	44.28±0.31	56.61±0.12
400	52.48±0.33	56.82±0.13	56.82±0.36	63.40±0.72
500	62.40±0.71	68.42±0.19	69.53±0.72	75.25±0.46

MSBE: Ethanol

Anti-inflammatory Activity

Inflammation is a very common symptom which is a normal protective response to tissue injury and caused many diseases such as physical, noxious chemical, or microbial agents and also initiates the healing process for the tissue.[48] The result was compared with standard of aspirin. Table 4 mentions the inhibition percentage of albumin denaturation and membrane stabilization of MSBE extract. From the obtained result, the maximum inhibition percentage of albumin denaturation and membrane stabilization (62.4 and 69.53) is noticed at higher concentration of 500 µg/ml which results are more or less equal to standard of aspirin (68.42 and 75.25) [Table 5 and Figure 4]. Many articles report that plant flavonoids possess potent anti-inflammatory and antioxidant properties.[49-51] Their anti-inflammatory activities are probably due to their inhibitory effect on enzymes involved in the production of the chemical mediators of inflammation and metabolism of arachidonic acid.[52,53] Similarly, MSBE extract contains flavonoid compounds which phytocompounds may be work against inflammation agent.

Conclusion

In this study, we analyzed phytochemical constitutes of six solvent extracts through preliminary phytochemical screening, FTIR, and GC–MS analysis. Further, MSBE extract is subjected to biological studies such as antioxidant and anti-inflammatory activities which extract selected based on active and more phytochemical constitutes. Based on this study, MSBE extract was showed good antioxidant and anti-inflammatory activities. Further, investigation needs to isolation and separation of phytocompounds of MSBE extracts for analysis of antioxidant or anti-inflammatory drug.

Acknowledgment

The author thanks to Government Arts College (Autonomous), Coimbatore, Tamil Nadu, India, for necessary facilities.

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