Contents lists available at UGC-CARE

International Journal of Pharmaceutical Sciences and Drug Research

[ISSN: 0975-248X; CODEN (USA): IJPSPP]

Available online at www.ijpsdronline.com

Research Article

In-vitro Anti-oxidant Activity and Gas Chromatography–Mass Spectrometry Analysis of Methanolic Extracts of *Saussurea lappa Clarke* and *Premna mucronata* Roxb

Humera Sadia, Govindu Sumalatha*

Chaitanya deemed to be University, Hanamkonda, Warangal, Telangana, India

ARTICLE INFO

Article history:

Received: 02 October, 2022

Revised: 22 October, 2022

Accepted: 28 October, 2022

Published: 30 November, 2022

Keywords:

GC-MS, *In-vitro* antioxidant activity, Phytocomponents, Premna *mucronata*, *Saussurea lappa clarke*, Vitamin E acetate and fucosterol.

DOI:

10.25004/IJPSDR.2022.140617

ABSTRACT

The main aim of the present investigation is identification of bioactive compounds from methanolic extraction of *Saussurea lappa clarke* and *Premna mucronata*, by GC–MS analysis and also evaluated the phytochemical screening. Methanolic extraction of *S. lappa clarke* and *P. mucronata* revealed the presence of flavonoids, phenolic compounds, triterpenoids, tannins, saponins, amino acids, proteins, steroids, gums and carbohydrates. Gas chromatography-mass spectrometry (GC-MS) analysis of the methanol extracts of *S. lappa clarke* and *P. mucronata* was performed on a GC-MS equipment. *In-vitro* antioxidant activity (hydroxyl radaical scavenging activity, determination of reducing power, metal chelating activity, carbon tetrachloride (ccl_4) induced lipid peroxidation, inhibitory test on protein oxidative modification, lipid peroxidation assay, catalase assay and reduced glutathione assay) was evaluated by adopting different methods. The GC-MS analysis has shown the presence of different bio active compounds in the methanolic extract of *S. lappa clarke* A total of 10 compounds were identified in that the main active compounds are 2-nonynoic acid, lupeol and dodecanal and *P. mucronata* GC-MS analysis showed the presence 15 bio active compounds in that the main compounds are L-cysteine, undecanoic acid, tetradecanoic acid, gamma.-sitosterol, vitamin E acetate and fucosterol. From the results, it is evident that *S. lappa clarke* and *P. mucronata* contains various phytocomponents and is recommended as a plant of phytopharmaceutical importance. Methanolic extraction of *S. lappa clarke* and *P. mucronata* possesses antioxidant activity.

Introduction

Medicinal plants represent a rich source of novel lead compounds that contribute to various therapeutic and pharmacological activities.[1] Around 25% of the pharmaceutical products used in the modern era were developed from plants.[2] According to WHO, nearly 80% of the world population consume the products of medicinal plants to cure different diseases.[3] In many studies, it is reported that antioxidant, anti-inflammatory, anticancer, antiviral, antibacterial, antifungal, insecticidal, antimalarial, anti-aging, and various other therapeutic activities depend on a significant variety of secondary metabolites (glucosinolates, lycopenes, anthocyanidins, flavonoids, isoflavonoids, polyphenols, limonoids, carotenoids, phytoestrogens, and omega-3 fatty acids, etc.) that are isolated from potential medicinal plants with the help of advanced, sensitive, and sophisticated equipment. Under these characteristics, about 20,000 plant species have been explored for their medicinal purposes.[4]

Reactive oxygen species (ROS) are formed by cellular metabolism or some exogenous factors, such as drugs, chemicals, smoke, and environmental stress conditions. The ROS structure contains at least one unpaired electron.[5] The risk is related to the accumulation of these agents in the body, resulting in a radical reactions chain, which degrades many biological vital molecules, namely DNA, proteins, lipids, and carbohydrates.[6] It has been revealed that ROS are associated with some diseases, such

***Corresponding Author:** Dr. Govindu Sumalatha

Address: Chaitanya deemed to be University, Hanamkonda, Warangal, Telangana, India.

Email ✉**:** sumalatha2k@gmail.com

Tel.: +91-7842450582

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

as diabetes mellitus, insulin resistance, cardiovascular diseases, Alzheimer's disease, Parkinson's disease, and some types of cancer.[7] Indeed, antioxidants of natural origin have received significant interest regarding exploration to identify secondary metabolites for the health and food industry. Antioxidants can maintain health by scavenging radicals and reactive oxygen species.[8] It is reported that two-thirds of all plant species have medicinal value and antioxidant potential.[9]

The extraction and characterization of these bioactive compounds have resulted in the delivery of specific medications with a high-activity profile.[10] Fourier-transformed infrared (FTIR) and gas chromatography-mass spectrometry (GC–MS) have been widely used for observation of functional groups and identification of various bioactive compounds present in plants.[11,12] GC–MS is a reliable technique for the identification of various compounds such as alkaloids, flavonoids, organic acids, amino acids etc. from plant extracts.[13] Also, computer-based tools have evolved as sophisticated drug discovery approaches that may be used to screen medicines from bioactive compounds present in medicinal plants.[14] Computational prediction models are utilized in the *in-silico* prediction of pharmacological, pharmacokinetic and toxicological production and play a crucial role in the selection of procedure leading to pharmaceutical and technological advancement.[15] Molecular docking is an efficient and low-cost approach for creating and testing pharmaceuticals. This technique gives the knowledge on drug-receptor interactions that may be used to anticipate how the drug model will bind to the target proteins[16] leading to reliable binding at the binding sites of ligands.[17]

S. lappa clarke (Compositae) is a traditionally known and potent plant which is well considered for its medicinal uses in different indigenous Indian systems of medicine.[18] It is popularly known as kuth root or costus and used in various traditional system of medicine for its anti-ulcer, anti-convulsant, anti-cancer, hepatoprotective, anti-arthritic, anti viral activities.[19] Several of its activities are well proved and established through *in-vitro, in-vivo* methods which gave a rationale scientific approach to the traditional claims. Phytochemical compounds isolated from this plant such as costunolide, Isodihydrocostunolide, cynaropicrin etc. were proven to be bio-active and potential source for developing new molecules for Alzheimer's disease.[20] Due to the significant proven activities *S. lappa* is having considerable chance for new drug discovery.[21, 22]

P. mucronata Roxb commonly known as, "Ganiar", "Bari arani", "Agnimatha", Agethu" and "Agyon", is a low bushy tree (with trunk up to 1.2 m height) of great ethnobotanical value belonging to the family Verbenaceae.[23] Furthermore, *P. mucronata* is a medicinal and aromatic plant and one of the important ingredients of "Dashamula" herbal preparation.[24] The bark finds application in curing boils while the leaves have been applied externally to treat dropsy as diuretic. Savasani *et al.* reported that *P. mucronata* showed cardioprotective activity in myocardial infarction due to its antioxidants composition.[25] It also possesses larvicidal properties. Antimicrobial, hypocholestremics and wound healing ability as well as anti-inflammatory activities have been demonstrated by this plant.[26] Recent studies showed that its essential oil has remarkable antifeedant properties.[27] The present study was aimed for GC- MS analysis of bio- active compounds and phytochemical screening for methanolic extraction of *S. lappa clarke* and *P. mucronata.*

Materials And Methods

Plant Material

Saussurea lappa clarke and *Premna Mucronata* Roxb were authenticated by Dr. K. Madhava chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati and voucher specimen was (Pt 0823&Pt 0754) preserved in the herbarium.

Parentage Yield

The percentage of yield was calculated using the following formula: -

$$\text{Yield (g/100 g)} = (W_1 \times 100)/W_2$$

Where,

W_1 = weight of the crude extract residue obtained after solvent removal

W_2 = weight of plant powder packed in the Soxhlet

Table 1: Percentage yield.

Extract	*n-hexane*	*Ethyl acetate*	*Methanol*
Saussurea lappa	2.13	3.18	5.11
Premna Mucronata Roxb	1.84	2.96	5.07

Table 2: Results of Phytochemical screening of n-Hexane, Ethyl acetate and Methanolic extraction of *Saussurea lappa*

S. No	*Name of the Phytochemical*	*n-Hexane,*	*Ethyl acetate*	*Methanol*
1	Carbohydrates	+	+	+
2	Amino acids	+	+	+
3	Proteins	-	-	+
4	Alkaloids	+	+	+
5	Cardiac glycosides	-	-	+
6	Triterpenoids	+	+	+
7	Saponins	+	+	+
8	Flavonoids	-	-	+
9	Phenolic compounds	+	+	+
10	Tannins	+	-	+
11	Steroids	+	+	+
12	Gums	-	-	-

Where, + means positive and - means negative.

Table 3: Results of Phytochemical screening of n-Hexane, Ethyl acetate and Methanolic extraction of *Premna Mucronata* Roxb

S. No	*Name of the Phytochemical*	*n-Hexane,*	*Ethyl acetate*	*Methanol*
	Carbohydrates	+	+	+
	Amino acids	+	+	+
	Proteins	-	-	+
	Alkaloids	+	+	+
	Cardiac glycosides	-	-	+
	Triterpenoids	-	+	+
	Saponins	+	-	+
	Flavonoids	-	-	+
	Phenolic compounds	+	+	+
	Tannins	+	-	+
	Steroids	+	+	+
	Gums	-	-	-

Where, + means positive and - means negative.

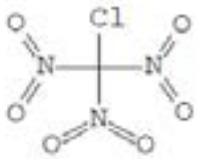
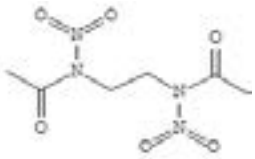
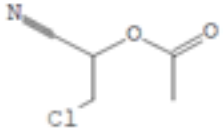
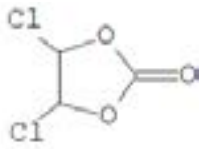
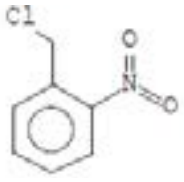
Extraction of Plant Material

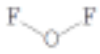
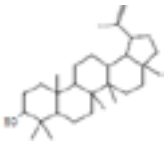
The 5 kg of the fresh leaves was shade dried at temperatures 25–35°C for 7 days. The dried plant material was powdered in a grinder. The dried plant powder was subjected to soxhlet extraction using n-hexane, ethyl acetate and methanol to get the respective extracts. Then each of the extracts was filtered using cotton plugs followed by Whatman no. 1 filter paper. The filtrates were then concentrated, dried under reduced pressure in the rotary evaporator and lyophilized to get in powder form.

Preliminary Phytochemical Analysis

All the extract/fractions of *Saussurea lappa clarke* and *Premna mucronata* Roxb were analyzed for their primary and secondary metabolites to confirm the presence of various primary metabolites, such as carbohydrates, amino acids, proteins, and lipids, and secondary metabolites, such as alkaloids, tannins, phenols, flavonoids, saponins,

Table 4: Bioactive compounds found in methanolic extract of *Saussurea Lappa Clarke* (MESC)

S. No	*R. Time*	*Area%*	*Compound name*	*Molecular Formula*	*M.W g/ mol*	*Structure of Compound*
1	0.035	10.37	2-nonynoic acid	$C_9H_{14}O_2$	154	
2	0.390	7.57	Methane, chlorotrinitro-	$CClN_3O_6$	185	
3	2.121	0.44	N,N'-Ethylenebis(N-nitroacetamide)	$C_6H_{10}N_4O_6$	234	
4	0.985	0.39	Lactonitrile, 3-chloro-, acetate	$C_5H_6ClNO_2$	147	
5	1.165	7.89	4,5-Dichloro-1,3-dioxolan-2-one	$C_3H_2Cl_2O_3$	156	
6	2.455	6.85	Benzene, 1-(chloromethyl)-2-nitro-	$C_7H_6ClNO_2$	171	

7	2.500	0.10	Difluorine monoxide	OF_2	54	
8	3.120	0.71	Ethane, 1-chloro-1-fluoro-	C_2H_4ClF	82	
9	32.745	1.97	Lupeol	$C_3H_{50}O$	426	
10	34.131	0.11	Dodecanal	$C_{12}H_{24}O$	184	

steroids, glycosides, and resins, according to standard methods.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC-MS analysis was carried out in a combined 7890A gas chromatograph system (GCMSQP2010, SHIMADZU) and mass spectrophotometer, fitted with a HP-5 MS fused silica column (5% phenyl methyl siloxane 30.0 m ×250 µm, film thickness 0.25 µm), interfaced with 5675C inert MSD with triple-axis detector. Helium gas was used as carrier gas and was adjusted to column velocity flow of 1.0 mL/min. Other GC-MS conditions are ion-source temperature, 250°C; interface temperature, 300°C; pressure, 16.2 psi; out time, 1.8 mm; and 1-µL injector in split mode with split ratio 1:50 with injection temperature of 300°C. The column temperature started at 36°C for 5 minutes and changed to 150 V at the rate of 4°C/min. The temperature was raised to 250°C at the rate of 20°C/min and held for 5 minutes. The total elution was 37 minutes. The relative percent amount of each component was calculated by comparing its average peak area to total areas. MS solution software provided by supplier was used to control the system and to acquire the data.

Identification of Compounds

Identification of components was achieved based on their retention indices and interpretation of mass spectrum was conducted using the database of National Institute of Standards and Technology (NSIT). The database consists of more than 62,000 patterns of known compounds. The spectra of the unknown components of *S. lappa clarke* and *Premna mucronata* Roxb fraction obtained were compared with the standard mass spectra of known components stored in NIST library (NISTII).

In-vitro Antioxidant Studies

Hydroxyl Radical Scavenging Activity

The hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the extract for hydroxyl radicals generated from the Fe^{3+}/ascorbate/EDTA/H_2O_2 system. The hydroxyl radicals attack deoxyribose, which eventually results in TBARS formation. The reaction mixture contained deoxyribose (2.8 mM), $Fecl_3$ (0.1 mM), H_2O_2 (1 mm), ascorbate (0.1 mM), KH_2PO_4-KOH buffer (20 mM, pH 7.4) and various concentrations (MESC, MEPM 100, 200, and 300 µg/mL and standard mannitol 100 µg/mL) of the drug in a final volume of 1-mL. The reaction mixture was incubated for 1-hour at 37°C. Deoxyribose degradation was measured at 532 nm.[28]

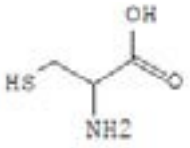
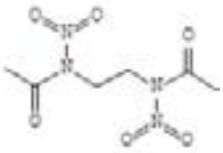
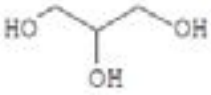
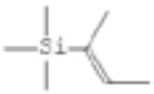
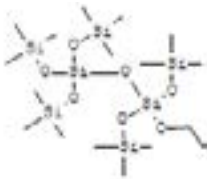
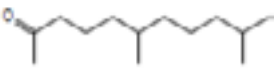
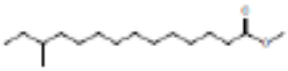
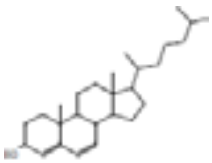
Determination of Reducing Power

The reducing power of MESC and MEPM was determined according to the following method. Various concentrations (125, 250, 175 and 500 µg/mL) of extract of MESC, MEPM in 1-mL of distilled water was mixed with phosphate buffer (2.5 mL, 0.2M, pH 6.6) and potassium ferricyanide ($K_3Fe(CN)_6$) (2.5 mL, 1%). The mixture was incubated at 50°C for 20 minutes. A portion (2.5 mL) of trichloroacetic acid (15%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and ferric chloride (0.5 mL, 0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicates increased reducing power.[29]

Metal Chelating Activity

Metal chelation property for ferric ion (Fe^{3+}) was estimated by using thiocynate method. Here different ratio of the extract (1:0.25 to 1:10 ratio) was mixed with a fixed concentration of ferric chloride (10 µg). The mixture was incubated for 30 minutes. At the end of the incubation, 1-mL of potassium thiocyanate (25%) was added and absorbance of ferric-thiocyanate complex (reddish brown complex) was measured at 460 nm. The results were compared with EDTA (1:10). Metal chelation property for ferrous ion (Fe^{2+}) was estimated by using 2, 2-bipyridyl method. Here different concentrations of the extract were mixed with a fixed concentration of ferrous sulphate (10 µg).

Table 5: Bioactive compounds found in Methanolic extract of Premna Mucronata (MEPM)

S. No	*R. Time*	*Area%*	*Compound name*	*Molecular Formula*	*M.W g/mol*	*Structure of Compound*
1	1.065	0.31	N,N-Dinitro-1,3,5,7-tetrazabicyclo[3,3,1]nonane	$C_5H_{10}N_6O_4$	218	
2	1.315	0.50	L-Cysteine	$C_3H_7NO_2S$	121	
3	2.113	0.08	N,N'-Ethylenebis(N-nitroacetamide)	$C_6H_{10}N_4O_6$	234	
4	6.091	0.29	Glycerin	$C_3H_8O_3$	92	
5	10.389	0.12	Silane, trimethyl(1-methyl-1-propenyl)-, (E)-	$C_7H_{16}Si$	128	
6	12.666	0.08	3-Ethoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy) tetrasiloxane	$C_{17}H_{50}O_7Si_7$	562	
7	13.736	0.16	Undecanoic acid	$C_{11}H_{22}O_2$	186	
8	19.438	0.09	2-Undecanone, 6,10-dimethyl-	$C_{13}H_{26}O$	198	
9	27.985	0.26	Tetradecanoic acid	$C_{16}H_{32}O_2$	256	
10	26.762	0.23	Eicosanoic acid	$C_{21}H_{42}O_2$	326	
11	30.399	0.16	Cholesta-4,6-dien-3-ol, (3.beta.)-	$C_{27}H_{44}O$	384	

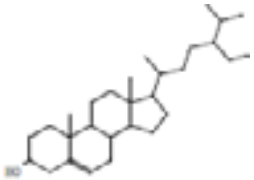
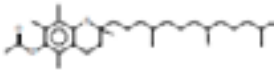
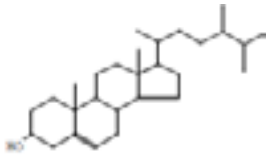
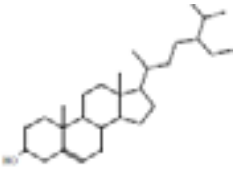
12	32.093	1.35	gamma.-Sitosterol	$C_{29}H_{50}O$	414	
13	30.762	0.08	Vitamin E acetate	$C_{31}H_{52}O_3$	472	
14	31.408	0.064	5-Cholestene-3-ol, 24-methyl-	$C_{28}H_{48}O$	400	
15	32.200	0.19	Fucosterol	$C_{29}H_zO$	412	

Table 6: Hydroxyl radical scavenging activity of MEPM, MESC and Mannitol

S. No	*Concentration (µg/mL)*	*% inhibition of hydroxyl radical*
1	Control	-
2	MEPM (200)	75.3 ± 4.12
3	MEPM (400)	81.45 ± 6.52
4	MESC (200)	74.91 ± 5.82
5	MESC (400)	80.62 ± 7.62
6	Standard (Mannitol 100 µg)	81.56 ± 5.85

Table 7: Determination of reducing power of MEPM, MESC and BHT

S. No	*Concentration (µg/mL)*	*Absorbance*
1	Control	0.088 ± 0.00025
2	MEPM (400)	1.013 ± 0.00036
3	MEPM (375)	0.978 ± 0.00052
4	MEPM (200)	0.534 ± 0.00025
5	MEPM (125)	0.352 ± 0.00041
6	MESC (400)	1.063 ± 0.00025
7	MESC (375)	0.968 ± 0.00036
8	MESC (200)	0.542 ± 0.00048
9	MESC (125)	0.348 ± 0..00026
10	BHT (400)	0.634 ± 0.00025
11	BHT (375)	0.512 ± 0.00042
12	BHT (200)	0.395 ± 0.00015
13	BHT (125)	0.496 ± 0.00036

The mixture was incubated for 30 minutes. At the end of the incubation, 2 mL of 2, 2-bipyridyl (1 mM) was added and absorbance of ferrous – bipyridyl complex (pink-colored complex) was measured at 525 nm. The results were compared with EDTA.[30]

Carbon Tetrachloride (CCl_4) Induced Lipid Peroxidation

Rat liver (30%w/v) homogenate in ice–cold 0.15 M potassium chloride was prepared in homogenizer. Aliquots of 0.5 mL of homogenate were taken in different small conical flasks. These flasks were incubated at 37°C in a constant shaker bath (150 cycles/min) for 45 minutes with 1.5 mL of potassium phosphate buffer (pH 7.4), 2 mL of 0.15 M potassium chloride , MESC and MEPM at (25, 50, 100, 200 and 300 µg/mL) and vitamin – E 100 µg/mL in different flasks and finally 10 µL of carbon tetrachloride (CCl_4) was added. In case of control, both CCl_4 and drugs were not added and in some flasks only drug was excluded. The reaction was stopped by the addition of 4 mL of 10%(w/v) tri chloro acetic acid and after incubation, the contents were centrifuged at 4000 rpm for 10 minutes and about 2 mL of clear supernatant was transferred to a graduated tube and 2 mL of 0.67% w/v of thiobarbituric acid was added and heated in a boiling water bath for 15 minutes. The tubes were cooled, bringing the mixture to P^H 12-12.5 with potassium hydroxide, stabilized the colour developed, and the absorbency was measured at 543 nm.[31]

Inhibitory Test on Protein Oxidative Modification

Albumin oxidative modification by copper was performed by the following method. The test sample (MESC and MEPM 100–1000 µg/mL) and vitamin- E (100–1000 µg/mi), was added to the reaction mixture containing albumin (10 µg/mL) and 100 µM $CuCl_2$ in 50 mM tris- HCL buffer (pH 7.4) in a total volume 0.3 mL. The mixture was incubated at 37°C for 2 hours. Next 1.6 mL of 0.125 M phosphate buffer (PH 8.0) containing 12.5 mM EDTA and 10.0 M urea, and 0.1 mL of 50 mm phosphate buffer (pH 7.0) containing 10 mm DTNB were added to the reaction mixture. This

Table 8: Effect of MEPM, MESC and EDTA on Fe^{2+}/ Fe^{3+} metal chelation

Iron : Drug	*OD at 525 nm*	*% Chelation of Fe^{2+}*	*OD at 460 nm*	*% Chelation of Fe^{3+}*
1:00(control)	0.310	0	1.054	0
1:0.25 MEPM	0.234	24.26 ± 2.15*	0.865	13.85 ± 1.52*
1:0.5 MEPM	0.217	33.68 ± 3.62*	0.765	22.52 ± 2.65*
1:1 MEPM	0.215	35.42 ± 2.45*	0.787	29.56 ± 2.69*
1:2.5 MEPM	0.197	42.56 ± 2.74*	0.645	31.85 ± 2.54*
1:5 MEPM	0.176	53.62 ± 2.46*	0.675	36.84 ± 3.25*
1:10 MEPM	0.135	65.24 ± 3.56*	0.438	55.17 ± 3.95*
1:0.25 MESC	0.254	25.25 ± 2.64*	0.952	15.25 ± 2.84
1:0.5 MESC	0.214	3634 ± 2.95*	0.852	24.32 ± 2.45
1:1 MESC	0.210	38.78 ± 2.48*	0.814	30.14 ± 2.74
1:2.5 MESC	0.195	44.95 ± 2.41*	0.754	34.61 ± 2.14
1:5 MESC	0.172	52.78 ± 2.65*	0.716	37.48 ± 2.65
1:10 MESC	0.142	64.95 ± 2.14*	0.541	57.61 ± 3.54
(1:10) Standard (EDTA)	0.067	78.62 ± 4.26*	0.1455	85.41 ± 3.48*

Table 9: Inhibition of lipid peroxidation –induction by CCl_4 system of MEPM, MESC and Vitamin-E

S. No	*Concentration (µg/mL)*	*% Inhibition*
1	Control	-
2	MEPM (25)	21.53 ± 2.48*
3	MEPM (50)	38.14 ± 2.62*
4	MEPM (100)	42.96 ± 2.84*
5	MEPM (200)	51.75 ± 2.54*
6	MEPM (300)	60.97 ± 3.54*
7	MESC (25)	20.36 ± 3.56*
8	MESC (50)	37.45 ± 3.45*
9	MESC (100)	40.25 ± 2.84*
10	MESC (200)	47.15 ± 2.14*
11	MESC (300)	58.41 ± 2.48*
12	Standard (Vit E)	66.24 ± 2.56*

Statistical significant test for comparison was done by ANOVA, followed by Dunnet 's 't' test (n=6), *p < 0.001, when test and standard are compared against control, values are Mean ± SEM.

solution was allowed to stand at room temperature for 5 minutes. The absorbency was read at 412 nm as cysteine-SH residue.[32]

Results

Percentage yields of n-hexane, ethyl acetate and methanolic extractions of *S. lappa and P. Mucronata* Roxb results were showed in Table 1.

In the present study, the investigation of n-hexane, ethyl acetate and methanolic extraction of S*milax perfoliata* and *Breynia retusa* revealed the presence of of various presences of various phytoconstituents like flavonoids, phenolic compounds, triterpenoids, tannins, saponins, amino acids, proteins, steroids, gums and carbohydrates results were showed in Table 2 and 3. Based on the phytochemical analysis and percentage yield of extractions selected methanolic extraction was selected both plants for further GC-MS analysis.

GC-MS investigation of methanolic extraction of *S. lappa clarke* and *P. mucronata* showing different phytochemicals. The chromatograms displayed in Figure 1 and 2 whereas the chemical constituents with their retention time (RT), atomic equation, molecular weight (MW) and area (%) within the MESC and MEPM are displayed in Table 4 and 5. GC-MS analysis carried on methanolic fraction of *S. lappa clarke* showed bio active compounds like 2-Nonynoic acid, lupeol and dodecanal and *breynia retusa* showed bio active compounds like L-cysteine, undecanoic acid, tetradecanoic acid, gamma.-sitosterol, vitamin e acetate and fucosterol.

In-vitro Anti-oxidant Study of MEPM and MESC

Hydroxyl Radical Scavenging Activity

When compared to the control, the MEPM and MESC considerably (p 0.001) scavenged the hydroxyl radicals produced by the EDTA/H2O2 system (at all tested doses of 100, 200, and 300 g). The amount of OH radicals that MEPM was able to scavenge increased in a dose-dependent way. comparable results were obtained using mannitol 100 ug (p 0.001). The results are displayed in Table 6.

Determination of Reducing Power

With higher MEPM and MESC concentrations reducing power increased. Significant (p 0.001) activity was seen at all tested MEPM and MESC concentrations compared to the control. The outcomes were similar to the benchmark (BHT) (p 0.001). The results are displayed in Table 7.

Effect of MESC and MEPM on fe^{2+} and fe^{3+} Metal Chelation

At a 1:10 ratio of iron was found in MEPM chelated Fe^{2+} (65.24 ± 3.56%) and Fe^{3+} (55.17 ± 3.95) and MESC chelated Fe^{2+} (64.95 ± 2.14%) and Fe^{3+} (57.61 ± 3.54). MEPM and MESC increased dose-dependently, as did the chelating power for mental transition ions (Fe^{2+}, Fe^{3+}). When compared to the control, MEPM and MESC showed considerable (*p < 0.001) chelation at all tested doses. When compared to the control, EDTA showed considerable (*p < 0.001) chelation of Fe^{2+} and Fe^{3+} of 78.64% and 85.42%, respectively, under the same circumstances. The results are displayed in Table 8.

Lipid Peroxidation Induced By CCl_4

When compared to the control, MEPM and MESC significantly (*p < 0.001) reduced the generation of lipid peroxide from CCl_4 at all tested dose levels (25, 50, 100, 200, and 300 g). In a dose-dependent way, the percentage inhibitions of peroxide generation rose. The outcomes were comparable to those of the norm. The results are displayed in Table 9.

Table 10: Inhibitory test on protein oxidative modification of MEPM, MESC and Vitamin-E

S. No	*Concentration (µg/mL)*	*% inhibition*	*IC50 Value (µg/mL)*
1	MEPM (100)	23.48 ± 2.56	
2	MEPM (200)	44.52 ± 2.51	
3	MEPM (400)	57.26 ± 2.74	427.65 ± 7.56
4	MEPM (600)	66.23 ± 3.56	
5	MEPM (800)	72.96 ± 3.57	
6	MEPM (1000)	80.56 ± 3.98	
7	MESC (100)	24.26 ± 2.56	
8	MESC (200)	45.25 ± 2.48	
10	MESC (400)	57.45 ± 2.47	431.26 ± 5.62
11	MESC (600)	62.56 ± 3.64	
12	MESC (800)	76.25 ± 3.79	
13	MESC (1000)	81.54 ± 6.45	
14	Standard (Vitamin-E100)	32.54 ± 2.85	
15	Vitamin-E(200)	51.24 ± 2.96	
16	Vitamin-E(400)	63.54 ± 2.96	264.53 ± 5.94
17	Vitamin-E(600)	72.18 ± 3.54	
18	Vitamin-E(800)	80.65 ± 3.69	
19	Vitamin-E(1000)	81.55 ± 3.84	

Values are Mean ± SEM.

Inhibitory Test on Protein Oxidative Modification

At a concentration of 1000 g/mL, the inhibitory ratio of MEPM and MESC on albumin oxidative modification reached a maximum of 78.94 and increased in a concentration-dependent way. It was discovered that MEPM IC_{50} was 427.65 ± 7.56 g/mL and MESC IC_{50} was 431.26 ± 5.62g/mL. At a concentration of 1000 g/mL, the results were equivalent to the industry standard (mannitol), with a percentage inhibitory ratio of 81.99%. Mannitol's IC_{50} was determined to be 263.35 7.41 g/mL. The results are displayed in Table 10.

ANOVA was used as the statistically significant test for comparison, followed by the Dunnet's "t" test, $*p < 0.001$, when the test and the standard were compared against the control, and the values were mean and SEM.

ANOVA was used as the statistically significant comparison test, with the Dunnet's t test ($*p < 0.001$) used to compare the results to the control. Fe^{3+} to Fe^{2+} transition deduced *via* spectrophotometry.

ANOVA was used to perform a statistically significant comparison test, followed by a Dunnet's t test. The concentrations of Fe^{2+} and Fe^{3+} were measured using the Fe^{2+}-dipyridyl complex (525 nm) and the Fe^{3+}-thiocynate complex (460 nm), respectively. When test and standard are compared to control, EDTA (ethylene diamine tetraacetic acid) is used as the control.

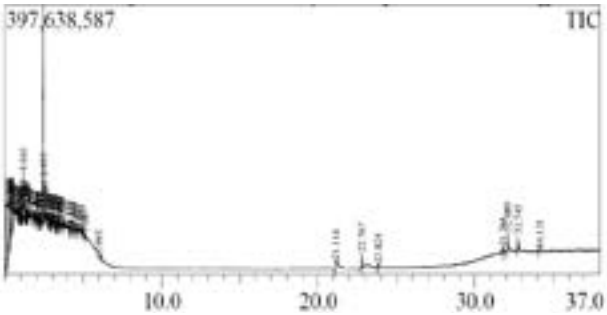


Fig. 1: GC-MS chromatogram of Methanolic extract of saussurea lappa clarke (MESC).

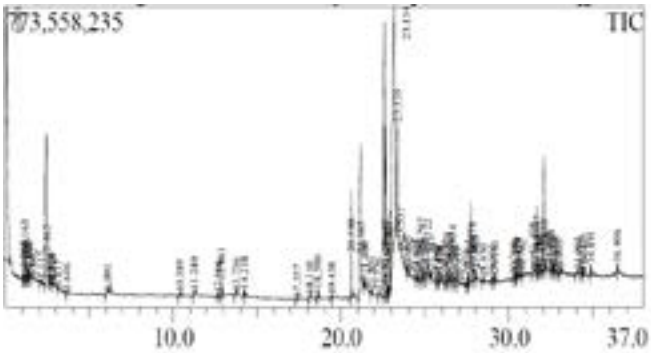


Fig. 2: GC-MS chromatogram of Methanolic extract of Premna Mucronata (MEPM).

Discussion

Medicinal plants are an affluent source of phytochemical compounds that can play a vital role to treat several chronic diseases.[33] An extensive number of potent biomolecules come from a diverse number of medicinal plants in recent times.[34] Scientists believed that these potent chemical constituents obtained from nature are used for treating many disorders with fewer side effects.[35] These potent compounds are highly capable of inhibiting the harmLess act of a multiple number of chronic diseases. Few chronic diseases are so critical and there are no specific drugs for those diseases.[36,37] In such cases, medicinal plants should be applied and they give an effective result in pharmacologically and phytochemically.[38]

In the present study, the investigation of methanol extracts of *S. lappa clarke* and *P. mucronata* revealed the presence of various phytoconstituents, including flavonoids, carbohydrates, cardiac glycosides, tannins, phenols, amino acids, alkaloids, steroids, proteins and terpenoids. These bioactive phytoconstituents could be responsible for the therapeutic ability of various extracts of *S. lappa clarke* and *P. mucronata*. The analysis was carried out by gas chromatography-mass spectrometry (GC–MS), one of the most widely used techniques for separation of phytoconstituents. The GC–MS investigation of *S. lappa clarke* extract revealed the presence of 10 phytochemical compounds, which could contribute to the medicinal properties of this plant species. In that lupeol has a complex

pharmacology, displaying antiprotozoal, antimicrobial, anti inflammatory, antitumor and chemopreventive properties. It is an effective inhibitor in laboratory models of prostate and skin cancers. As an anti-inflammatory agent, lupeol functions primarily on the interleukin system. Lupeol to decreases IL-4 (interleukin 4) production by T-helper type 2 cells. Another bio active compound dodecanal, also known as lauraldehyde or dodecyl aldehyde, is an organic compound with the chemical formula $CH_3(CH_2)_{10}CHO$. This colourless liquid is a component of many fragrances. It occurs naturally in citrus oils, but commercial samples are usually produced from dodecanol by dehydrogenation.[39] The GC–MS investigation *P. mucronata* extract revealed the presence of 15 phytochemical compounds, which could contribute to the medicinal properties of this plant species. In that cysteine is a semi essential proteinogenic amino acid with the formula HOOC-CH-(NH_2)-CH_2-SH. The thiol side chain in cysteine often participates in enzymatic reactions as a nucleophile. The cysteine sulfhydryl group is nucleophilic and easily oxidized. The reactivity is enhanced when the thiol is ionized, and cysteine residues in proteins have pK_a values close to neutrality, so are often in their reactive thiolate form in the cell.[40] Because of its high reactivity, the sulfhydryl group of cysteine has numerous biological functions. Another bio active compound undecylic acid (systematically named undecanoic acid) is a carboxylic acid with chemical formula $CH_3(CH_2)_9COOH$. It is often used as an antifungal agent, to treat ringworm and athlete's foot, for example. Like decanoic acid, it has a distinctive, unpleasant odor. Also present gamma-sitosterol, it is a naturally occurring plant steroid isolatable from plants of the genus *Lagerstroemia*. Gamma-sitosterol is a potent inhibitor of the complement component C1 complex, and it has demonstrated potential as a diabetic treatment in rats. Gamma-sitosterol is a stereoisomer of beta-sitosterol, which sees wide use as an over the counter natural supplement. However, plant extracts containing gamma-sitosterol have demonstrated toxicity on *in-vitro* human cell assays; which may discourage use as a natural supplement. High doses of vitamin E (400 units or more per day) may increase the chance of rare but very serious side effects. There is no proof that high doses of vitamin E help to prevent or treat heart disease. There is very little evidence that it helps prevent or treat Alzheimer's disease. In some people, taking these high doses may even be harmful. Talk to your doctor or pharmacist and discuss the risks and benefits before taking vitamin E supplements. Fucosterol (24-ethylidene cholesterol) is a bioactive compound belonging to the sterol group that can be isolated from marine algae. Fucosterol of marine algae exhibits various biological activities including anti-osteoarthritic, anticancer, anti-inflammatory, anti-photoaging, immunomodulatory, hepatoprotective, anti-neurological, antioxidant, algicidal, anti-obesity and antimicrobial.

Metal chelating capacity was significant as they reduced the concentration of the catalyzing transition metal in lipid peroxidation (Duh PD *et al.*, 1999). Through epidemiological studies, it was reported that phenolic compounds have been shown to act as natural antioxidants by helping to neutralize free radicals and as metal chelating agents.[41] Antioxidants inhibit interaction between metal and lipid through formation of insoluble metal complexes with ferrous ion. Hence the data obtained for *S. lappa Clarke* and *P. mucronata* reveals that some of the extracts demonstrate an effective capacity for iron binding, suggesting that its action as antioxidant may be related to its iron binding capacity that will prevent the free radical generation through fenton reaction.

Superoxide radical is known to be a very harmful species to cellular components as a precursor of more reactive oxygen species. The superoxide radical is known to be produced *in-vivo* and can result in the formation of H_2O_2 *via* dismutation reaction. Moreover, the conversion of superoxide and H_2O_2 into more reactive species, eg., the hydroxyl radical, has been thought to be one of the unfavourable effects caused by superoxide radicals.[42] Numerous biological reactions generate superoxide radical which is a highly toxic species. Although they cannot directly initiate lipid oxidation, superoxide radical anions are potent precursors of highly reactive species such as hydroxyl radical and thus the study of scavenging of this radical is important.[43] Since *S. Lappa Clarke* and *P. Mucronata* showed appreciable percentage of scavenging activity for hydrogen peroxide, it can be used against unfavourable effects caused in the body by hydrogen.

Hydrogen peroxide itself is not very reactive but sometimes it is toxic to cell because it may give rise to hydroxyl and peroxyl radicals in the cells through Fenton reaction. Therefore, removing of H_2O_2 is very important for antioxidant defence in cell or food systems. Dietary polyphenols have also been shown to protect mammalian and bacterial cells from cytotoxicity induced by hydrogen peroxide, especially compounds with the orthodihydroxy phenolic structure, quercetin, catechin, gallic acid ester and caffeic acid ester.[44] Therefore, the methanol extract of *S. lappa Clarke* and *P. Mucronata* can be used as a potent hydrogen peroxide scavenger in body systems nitric oxide or reactive nitrogen species, formed during their reaction with oxygen or with superoxides, such as NO_2, N_2O_4, N_3O_4, NO_3- and NO_2+ are very reactive. These compounds are responsible for altering the structural and functional behavior of many cellular components. The plant products may have the property to counteract the effect of NO formation and in turn may be of considerable interest in preventing the ill effects of excessive NO generation in the human body. Further, the scavenging activity may also help to arrest the chain of reactions initiated by excess generation of NO that are detrimental to the human health. Nitric acid is also implicated for inflammation, cancer, and

other pathological conditions.[45] Since the methanolic extracts of *S. lappa Clarke* and *P. Mucronata* showed good activity it is clear that it can be used for scavenging reactive nitrogen species in human body.

Conclusion

The present investigation was focused on identification of various bioactive compounds from the methanolic extraction of *S. lappa clarke* and *P. mucronata* for the first time by GC–MS analysis. These compounds are responsible for the different therapeutic and pharmacological properties. *S. lappa clarke* and *P. mucronata* showed good *in-vitro* anti-oxidant activity.

Reference

1. Aumeeruddy MZ, Mahomoodally MF. Combating breast cancer using combination therapy with 3 phytochemicals: Piperine, sulforaphane, and thymoquinone. Cancer. 2019; 125:1600–1611.
2. Aumeeruddy-Elalfi Z, Lall N, Fibrich B, Van Staden AB, Hosenally M, Mahomoodally MF. Selected essential oils inhibit key physiological enzymes and possess intracellular and extracellular antimelanogenic properties in vitro. J Food Drug Anal. 2018; 26:232–243.
3. Veiga M, Costa EM, Silva S, Pintado M. Impact of plant extracts upon human health: A review. Crit. Rev. Food Sci Nutr. 2020; 60:873–886.
4. Bursal E, Aras A, Kılıç Ö. Evaluation of antioxidant capacity of endemic plant Marrubium astracanicum subsp. macrodon: Identification of its phenolic contents by using HPLC-MS/MS. Nat Prod Res. 2019; 33:1975–1979.
5. Salehi B, Armstrong L, Rescigno A, Yeskaliyeva B, Seitimova G, Beyatli A, Sharmeen J, Mahomoodally MF, Sharopov F, Durazzo A. Lamium plants—A comprehensive review on health benefits and biological activities. Molecules. 2019; 24:1913.
6. Bursal E, Taslimi P, Gören AC, Gülçin İ. Assessments of anticholinergic, antidiabetic, antioxidant activities and phenolic content of Stachys annua. Biocatal. Agric. Biotechnol. 2020; 28:101711.
7. Hassan W, Noreen H, Rehman S, Gul S, Amjad Kamal M, Paul Kamdem J, Zaman B, BT da Rocha J. Oxidative stress and antioxidant potential of one hundred medicinal plants. Curr. Top. Med Chem. 2017; 17:1336–1370.
8. Chen Q, Wang Q, Zhu J, Xiao Q, Zhang L. Reactive oxygen species: Key regulators in vascular health and diseases. Br. J. Pharmacol. 2018; 175:1279–1292. Al Rashdi RSY, Hossain MA, Al Touby SSJ. Antioxidant and antibacterial activities of leaves crude extracts of Adenium obesum grown in Oman National Botanical Garden. Adv. Biomark. Sci. Technol. 2021; 3:8–14.
9. Yadav R, Khare RK, Singhal A. Qualitative phytochemical screening of some selected medicinal plants of Shivpuri District (MP). Int. J. Life Sci. Res. 2017;3:844–847.
10. Satapute P, Murali KP, Kurjogi M, Jogaiah S. Physiological adaptation and spectral annotation of Arsenic and Cadmium heavy metal-resistant and susceptible strain Pseudomonas taiwanensis. Envirom Pollut. 2019;251:555–563.
11. Fan S, Chang J, Zong Y, Hu G, Jia J. GC-MS analysis of the composition of the essential oil from Dendranthema indicum Var. Aromaticum using three extraction methods and two columns. Molecules. 2018;23:567.
12. Razack S, Kumar KH, Nallamuthu I, Naika M, Khanum F. Antioxidant, biomolecule oxidation protective activities of Nardostachys jatamansi DC and its phytochemical anlaysis by RP-HPLC and GC-MS. Antioxidants. 2018;4:185–203.
13. Sliwoski G, Kothiwale S, Meiler J, Lowe EW. Computational methods in drug discovery. Pharmacol. Rev. 2014;66:334–395.
14. Loza-Mejia MA, Salazar JR, Sanchez-Tejeda JR. In silico studies on compounds derived from Calceolaria:phenylethanoid glycosides as potential multi-target inhibitors for the development of pesticides. Biomolecules. 2018;8:121.
15. Ee K, Kim D. In silico molecular binding prediction for human drug targets using deep neural multi-task learning. Genes. 2019;10:906.
16. Harathi A. In silico molecular docking and in vitro antidiabetic studies of dihydropyrimido (4,5-a] acridin-2-amines). Bio Med Res Int. 2014;1:1–10.
17. Cho JY, Baik KU, Jung JH, Park MH. In vitro anti-inflammatory effects of cynaropicrin, a sesquiterpene lactone, from Saussurealappa. European Journal of Pharmacology. 2000; 398(3):399-407
18. Zahara K, Tabassum S, Sabir S, Arshad M, Qureshi R, Amjad MS, Chaudhari SK. A review of therapeutic potential of Saussurealappa-An endangered plant from Himalaya. Asian Pacific journal of tropical medicine. 2014;7:S60-9
19. Sutar N, Garai R, Sharma US, Singh N, Roy SD. Antiulcerogenic activity of Saussurealappa root. Int J Pharm Life Sci. 2011 Jan;2(1):516-20
20. Madhuri K, Elango K, Ponnusankar S. Saussurealappa (Kuth root): review of its traditional uses, phytochemistry and pharmacology. Oriental pharmacy and Experimental medicine. 2012;12(1):1-9.
21. Madhuri K, Elango K, Ponnusankar S. Saussurealappa (Kuth root): review of its traditional uses, phytochemistry and pharmacology. Oriental pharmacy and Experimental medicine. 2012;12(1):1-9
22. Palariya D, Singh A, Dhami A, Pant AK, Kumar R, Prakash O. Phytochemical analysis and screening of antioxidant, antibacterial and anti-inflammatory activity of essential oil of Premna mucronata Roxb. Leaves. Trends in Phytochemical Research. 2019; 3(4):275-86
23. Patel NG, Patel KG, Patel KV, Gandhi TR. Myocardial salvaging effect of Premna mucronata Roxb.(Verbenaceae) on isoproterenol induced myocardial necrosis in rats. Der Pharmacia Lettre. 2015;7(7):137-47
24. Mali PY. Premnaintegrifolia L.: A review of its biodiversity, traditional uses and phytochemistry. Ancient science of life. 2015;35(1):4
25. Leeratiwong CH, Chantaranothai PR, PATON AJ. A synopsis of the genus Premna L.(Lamiaceae) in Thailand. Tropical Natural History. 2009;9(2):113-42
26. Subedi L, Timalsena S, Duwadi P, Thapa R, Paudel A, Parajuli K. Antioxidant activity and phenol and flavonoid contents of eight medicinal plants from Western Nepal. Journal of Traditional Chinese Medicine. 2014 Oct 15; 34(5):584-90.
27. Mary NK, Shylesh BS, Babu BH, Padikkala J. Antioxidant, and hypolipidaemic activity of a herbal formulation-liposem. Ind J Exp Biol. 2002;40:901-904.
28. Gulcin I, Oktay M, Kufrevioglu OI, Aslan A. Determination of antioxidant activity of lichens Cetraria islandica (L) Ach. J Ethnopharmacol. 2002;79: 325-329.
29. Tripati YB, Singh AV, Dubey GP. Antioxidant property of the bulb of Scilla indica. Science. 2001;80:1267-1269.
30. Duh PD, Tu YY, Yen GC. Antioxidant activity of water extract of Harng Jyur Chrysanthemum morifolium Ramat. Lebens-mittel-Wissenchaft Und-technologie. 1999;32: 269-277.
31. Toda S, Shirataki Y. Inhibitory effects of Astragali Radix, a crude drug in Oriental medicines on lipid peroxidation and protein oxidative modification by copper. J Ethnopharmacol. 1999;68:331-333.
32. Adnan M, Chy M, Uddin N, Kama ATM, Azad M, Kalam O, Chowdhury KAA, Kabir MSH, Gupta SD, Chowdhury M. Comparative Study of Piper sylvaticum Roxb. Leaves and Stems for Anxiolytic and Antioxidant Properties through in vivo, in vitro, and in silico Approaches. Biomedicines. 2020;8:68.
33. Al Mahmud Z, Emran TB, Qais N, Bachar SC, Sarker M, Uddin MMN. Evaluation of analgesic, anti-inflammatory, thrombolytic and hepatoprotective activities of roots of Premna esculenta (Roxb) J. Basic Clin. Physiol. Pharmacol. 2016; 27:63–70
34. Rahman MS, Sultan RA, Emran T. Bin Evaluation of the anti-diarrheal activity of methanol extract and its fractions of Urena sinuata L.(Borss) leaves. J. Appl. Pharm. Sci. 2016;6:56–60
35. Emran TB, Rahman MA, Uddin MMN, Rahman MM, Uddin MZ, Dash R, Layzu C. Effects of organic extracts and their different fractions of