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GC-MS ANALYSIS, ADME TOXICITY and *in silico* STUDIES OF SOME ISOLATED COMPOUNDS FROM *Melastoma malabathricum* LEAVES AGAINST SPLA$_2$ INHIBITION

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ABSTRACT

The bioactive components of *Melastoma malabathricum* leaves and the chemical compositions of ethanol-based *M. malabathricum* leaf extract were studied using gas chromatography-mass spectrometry (GC-MS) analysis. Twelve compounds obtained were: phthalic acid hept-4-yl isobutyl ester, oleic acid, n-hexadecanoic acid, melezitose, hexadecanoic acid methyl ester, hexadecanoic acid ethyl ester, D-mannose, 9,10 secocholesta-5,7,10(19) triene-3,24,25, triol, (3β,5Z,7E), 2-myristynoyl pantetheine, propanamide, 3-[3,5di(tetra-butyl)-4hydroxyphenyl]-N-(2hydroxyethyl), cholesta 4,6-dien-3-ol and desulphosinigrin. These compounds were characterized and analyzed for sPLA$_2$ inhibition by molecular docking using the standard inhibitors. Among these, 2-myristynoyl pantetheine showed better interactions with sPLA$_2$IIA enzyme with 'e' value of -8.62 (PubChem id: 535560), followed by desulphosinigrin (-7.19) and D-mannose (-4.96). ADME toxicity studies proved that 2-myristynoyl pantetheine is better for *in vivo* administration. The 2-myristynoyl pantetheine was found to exhibit anti-inflammatory activity.

Keywords: ADME toxicity, GC-MS, *Melastoma malabathricum*, molecular docking, phytocomponents, sPLA$_2$ inhibition

INTRODUCTION

Inflammation is the healing process of injured tissues but sustained inflammation results in the undesirable consequences such as systemic shock, sepsis, circulatory collapse, arthritis and local tissue injury in many organs (Nevalainen *et al.,* 1993, 2000). Inflammation is mainly mediated by secretory phospholipase A_2 enzyme which catalyzes the production of arachidonic acid and lysophospholipid. Elevated levels of sPLA$_2$ enzymes have been identified in many inflammatory disorders (Nanda *et al.,* 2007). Further, arachidonic acid is catalyzed by cyclooxygenase 1 and 2 (COX 1 & 2) and lipoxygenase (LOX) into pro-inflammatory mediators such as prostaglandins, leukotrienes and thromboxanes, respectively (Krug *et al.,* 1994). The lysophospholipid is converted into platelet activation factors (PAF) by acetyltransferase that continues to cause inflammation (Stafforini, 2014). At present various non-steroidal anti-inflammatory drugs (NSAID'S) have been used to reduce the inflammation in many inflammatory diseases such as rheumatoid arthritis, coronary heart disease, diabetes, asthma, cancer, etc., by blocking the metabolism of arachidonic acid

by an isoform of cyclooxygenase enzyme (COX-1 and/or COX-2), thereby reducing the production of pro-inflammatory mediators (Osafo *et al.,* 2017). Unfortunately, there are many side effects such as intestinal ulceration, bleeding and cardiovascular complications associated with the administration of nonsteroidal anti-inflammatory drugs (De Gaetano *et al.*, 2003). It appears rationale that the effective inhibitors of $sPLA_2$ could deplete the sources of arachidonic acid. Therefore, its downstream metabolites and PAF would not affect the homeostasis of COX-1/2 and LOX enzymes. There is a great demand for natural products for PLA_2 inhibition rather than NSAIDs.

The perusal of literature suggests that many medicinal plants possess a variety of pharmacological properties, and these have frequently been used in traditional medicine to cure various inflammatory ailments (Mintah *et al.,* 2019). Screening of active compounds from medicinal plants has led to the discovery of new medicinal drugs which provide effective protection against various diseases (Sheeja *et al.,* 2007). There are several endogenous and exogenous agents that reportedly inhibit $sPLA_2$ enzymes (Dharmappa *et al.*, 2009). Several laboratories are involved in synthesizing the molecules to inhibit $sPLA_2$ (Sadashiva *et al.*, 2005)) this, in turn, implies the importance of $sPLA_2$ inhibitors. In view of above *Melastoma malabathricum,* was chosen for the present study. It is medicinally important plants belonging to the genus *Melastoma* which is commonly known as "Doddanekkarika" in Kannada. The leaves of *M. malabathricum* are traditionally used to treat diarrhea, hemorrhoids, cuts, wounds, toothache, stomachache, etc. (Lin *et al.,* 2005). It has been reported that it possesses several pharmacological properties like antibacterial, antiviral, anti-parasitic, anti-coagulant, platelet-activating factor inhibitory, wound healing, anti-ulcer, anti-diarrheal, anti-venom, anti-inflammatory, anti-nociceptive and antipyretic properties when used at different concentrations (Zakaria *et al.*, 2012). In our previous study, *M. malabathricum* extracts in different solvents such as hexane, chloroform, ethanol, methanol and water were subjected to *in vitro* assays like antioxidant activity, anti-inflammatory activity, $sPLA_2$ inhibition and anti-cancer activity; and among all the extracts ethanol extract showed greater activities (Sophiya *et al.,* 2019). Further studies were planned to analyze and identify the phytoconstituents from ethanol-based *M. malabathricum* extract responsible for above pharmacological activities. Ethanolic extract of *M. malabathricum* was subjected to gas chromatography-mass spectrometry (GC-MS) to determine the potential phytoconstituents, molecular docking to know enzyme-inhibitor compatibility and ADME (absorption, distribution, metabolism, and excretion)-toxicity. A prediction was done to examine the profile of absorption, distribution, metabolism, excretion and toxicity of potential compounds.

MATERIALS AND METHODS

Based on previous study ethanol extract of *Melastoma malabathricum* leaves which exhibited greater antioxidant activity, anti-inflammatory activity, $sPLA_2$ inhibition and anti-cancer activity than other solvent extracts (Sophiya *et al.,* 2019) was used in the present study.

GC MS analysis

M. malabathricum ethanolic extract (5 mg powder) was dissolved in 10 mL ethanol and GC-MS analysis performed using GCMS equipment (Agilent Technologies 7890B Triple quad 7000C). The equipment has an HP-5 MS UI 30 m, 0.25 mm, 0.25 μm composed of (5%-phenyl)-methyl-polysiloxane. Helium was used as a carrier gas at the flow rate of 1.0 mL min^{-1}. The injector was operated at 250°C and the oven temperature programmed at 60°C for 15 min and then gradually increased to 280°C for 3 min. The identification of compounds was based on MS Library Agilent Mass Hunter Qualitative Analyses (NIST) B.07.00.

Ligand preparation

The compounds reported in GCMS analysis of the ethanolic extract of *M. malabathricum* leaves were used. The structures of twelve ligand compounds were retrieved from PubChem database.

Protein preparation and active site prediction

The target protein non-pancreatic secretory phospholipase A_2 (IIA) was retrieved from Protein Data Bank (*www.rcsb.org*) and the water molecules were removed from the protein. To identify the ligand binding capacity active site prediction with the determined model, CASTp server was used (Tian *et al.*, 2018).

Optimization of lead molecules by Lipinski rule

The identified plant compounds structures were downloaded and re-evaluated for their drug likeliness by following the "Lipinski Rule of Five" by Backman (2011).

Docking studies

Docking was performed between the crystal structure of protein non-pancreatic secretory phospholipase A_2 (IIA) and the twelve phytochemical compounds (ligands) retrieved from the PubChem (*https://www.rcsb.org/structure/1KQU*) and protein data bank (*https://pubchem.ncbi.nlm.nih.gov*). Docking studies were carried out by using AutoDock Tools 1.5.6 (Sanner, 1999). The protein and ligand files were converted to "pdbqt" format, and grid parameters were set as per the result provided by CASTp. AutoDock tool produces 10 runs of ligand interacting with protein, which has different binding affinities. The results of AutoDock were visualized by Accelrys Discovery Studio (Ferdous, 2013; Li *et al.*, 2016).

ADME toxicity prediction

The ADME toxicity prediction for selected three high mean energy valued potential compounds 2-myristynoyl pantetheine, Desulphosinigrin, Geneisten known inhibitor was conducted using ADMETSAR server which included the prediction of absorption, metabolism, distribution, excretion and toxicity.

RESULTS AND DISCUSSION

The compounds present in ethanolic extract of *M. malabathricum* leaves were identified by GC-MS analysis (Fig. 1) and peaks observed (Table 1). Fig. 2-4 show the expanded pictures of peaks of Fig. 1, representing the individual mass spectrum of identified compounds. Twelve compounds identified in the ethanolic extract of *M. malabathricum* leaves were phthalic acid hept 4-yl isobutyl ester, oleic acid, n-hexadecanoic acid, melezitose, hexadecanoic acid methyl ester, hexadecanoic acid ethyl ester, D-mannose, 9,10 secocholesta-5,7,10(19)triene-3,24,25 triol, (3β,5Z,7E), 2-myristynoyl pantetheine, propanamide, 3-[3,5di(tetr-butyl)-4hydroxyphenyl]-N-(2hydroxyethyl), cholesta 4, 6-dien-3-ol, and desulphosinigrin. The compounds retention time (RT), molecular formula, molecular weight, percent-

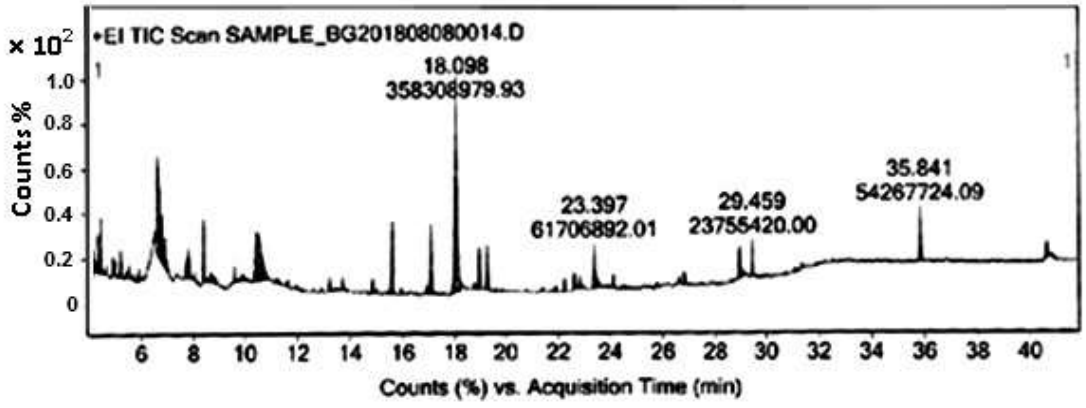

Fig. 1: GC-MS chromatogram of the ethanolic extract of *M. malabathricum* leaves

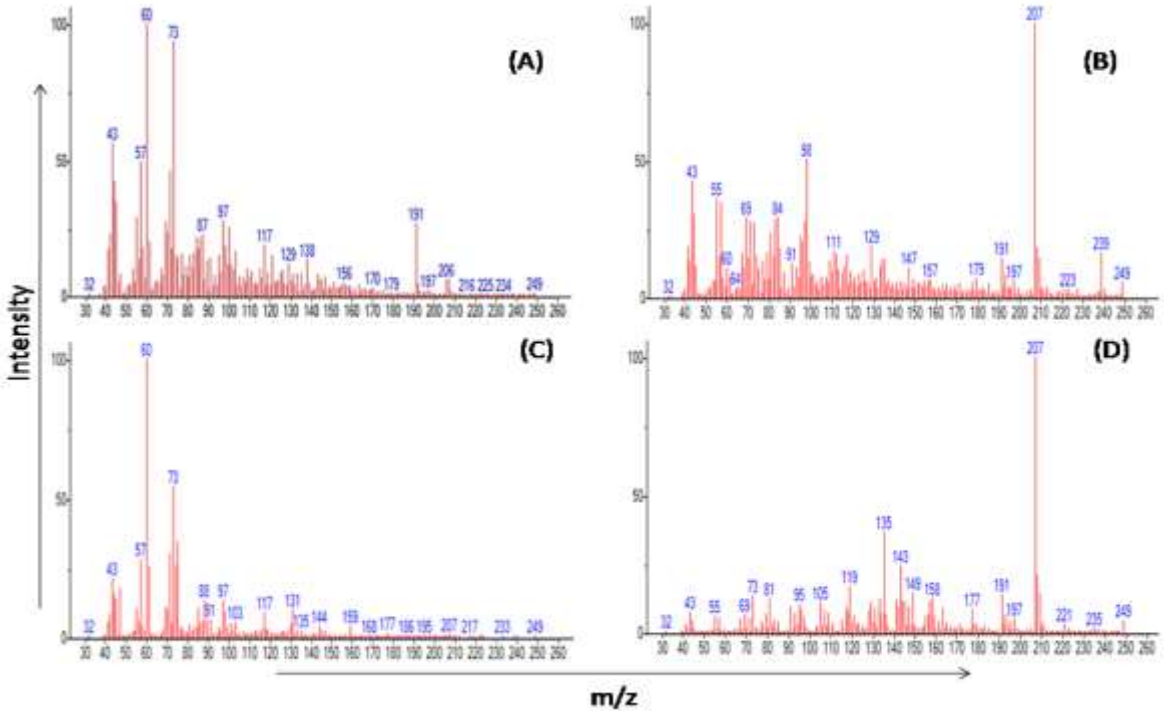

Fig. 2: Mass spectrum analysis of the ethanolic extract of *M. malabathricum;* (A) 2-myristynoyl pantetheine at RT: 8.469 min (B) 9,10 secocholesta-5,7,10(19)triene-3,24,25, triol, (3beta,5Z,7E) at RT: 29.023 min (C) Desulphosinigrin at rt: 10.616 min (D) Cholesta 4,6-dien-3-ol at RT: 35.857 min

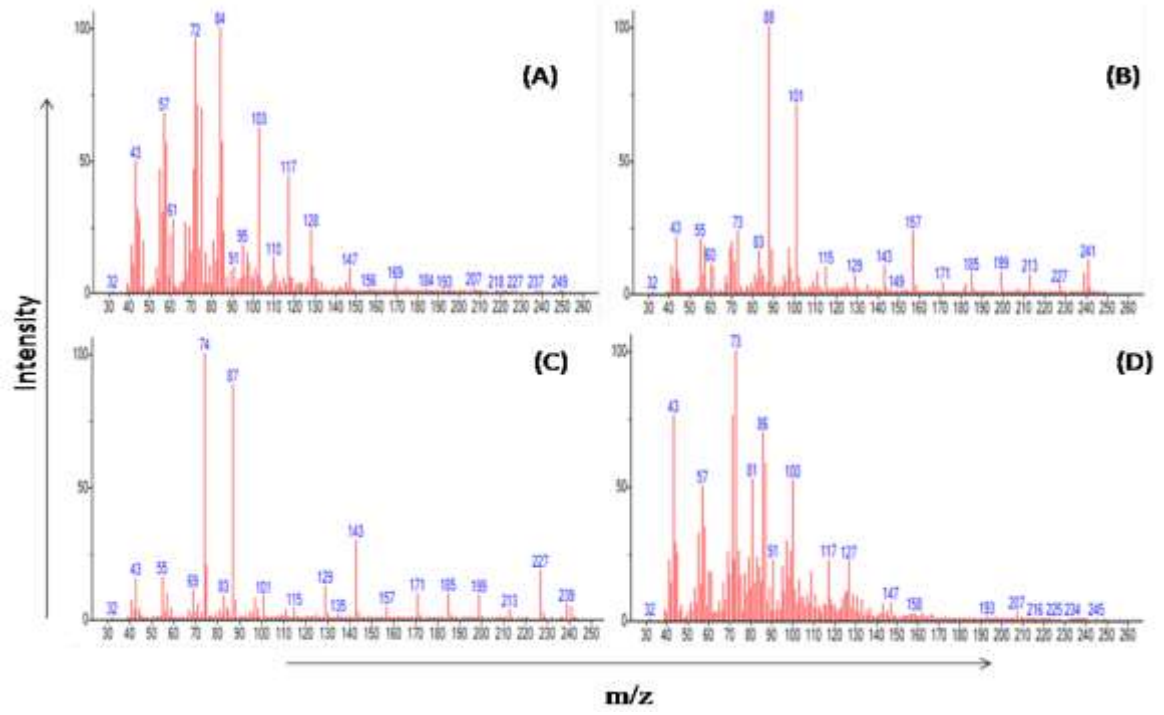

Fig. 3: Mass spectrum analysis of the ethanolic extract of *M. malabathricum;* (A) D-mannose at RT: 6.725 min (B) Hexadecanoic acid, ethyl ester at RT: 18.945 min (C) Hexadecanoic acid, methyl ester at RT: 17.102 min, and (D) Melezitose at RT: 7.822 min.

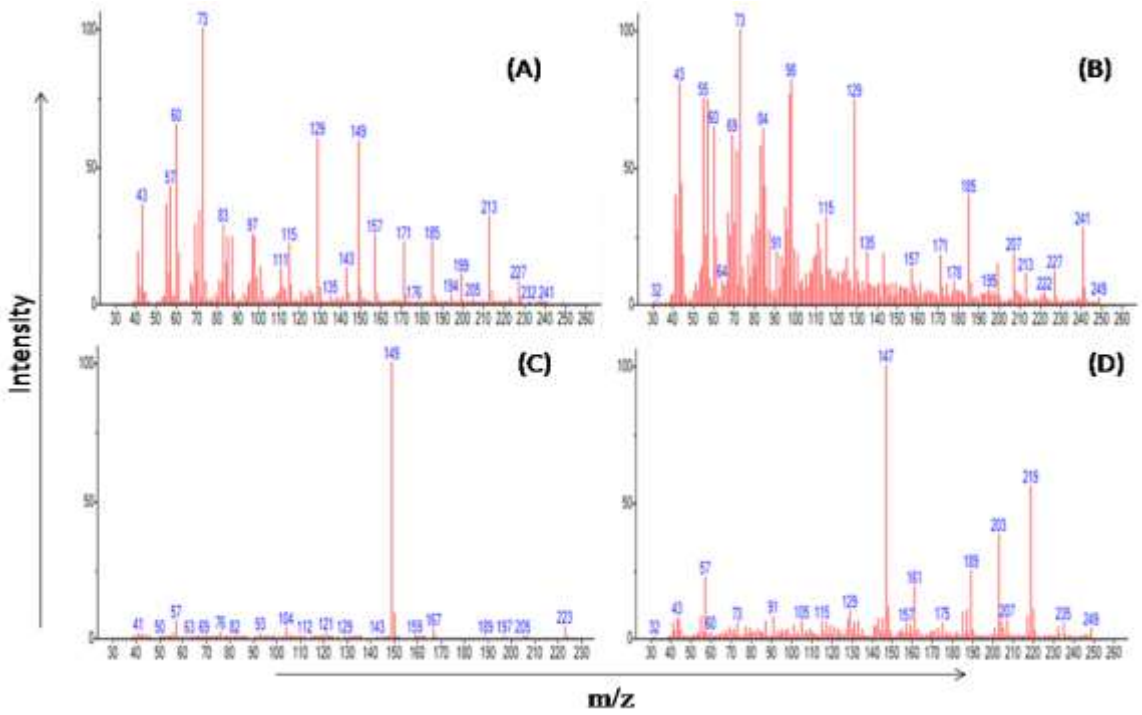

Fig. 4: Mass spectrum analysis of the ethanolic extract of *M. malabathricum;* (A) n-Hexadecanoic acid at RT: 18.098 min (B) Oleic acid at RT :23.438 min (C) Pthalic acid, hept 4-yl isobutyl ester at RT: 15.628 min, and (D) Propanamide, 3-[3,5di(tetr-butyl)-4hydroxyphenyl]-N-(2hydroxyethyl) at RT: 19.269 min.

Table 1: GC/MS peaks observed in the ethanolic extracts of *M. malabathricum*

Integration peak list						
Peak	Start	Retention time (min)	End	Height	Area	Area (%)
1	4.458	4.475	4.504	15074406.83	19480234.35	5.44
2	6.597	6.659	6.792	26708173.22	190167386.6	53.07
3	6.792	6.821	6.891	13144234.77	40795287.1	11.39
4	8.363	8.415	8.527	16861420.51	44135945.9	12.32
5	10.375	10.475	10.931	13173811.98	197783568.4	55.20
6	15.536	15.636	15.729	19399576.67	76156959.0	21.25
7	17.010	17.110	17.225	19093313.90	77835003.2	21.72
8	17.895	18.098	18.252	57815835.02	358308979.9	100.0
9	19.161	19.278	19.362	11804685.22	51092369.3	14.26
10	37.758	38.841	35.961	14291270.47	54267724.1	15.15

age composition and different medicinal activities in ethanol extract of *M. malabathricum* leaves are presented in Table 2.

Protein preparation and active site prediction

The crystal structure of human phospholipase A_2 protein was retrieved from RCSB Protein Data Bank (PDB Id: pdb1KQU). CASTp online server (*http://sts.bioe.uic.edu/castp/index.html?1ycs*) was used to predict the active sites and surface area 174.217 Å and volume (SA) 45.962 Å on human phospholipase A_2 protein. Among all the active sites, one of the pockets had residues interact with ligand molecules. The identified active sites were LEU2, PHE5, HIS6, ILE9, ALA17, ALA18, TYR21, GLY22, HIS27, CYS28, GLY29, VAL30, GLY31, CYS44, HIS47, ASP48, TYR51, LYS52, GLU55, THR61, LYS62, ALA94 and PHE98; hence the protein pocket (TYR21, HIS47 and ASP46) was selected as the active site for further experimentation.

Table 2: The compounds detected in the ethanolic extract of *Melastoma malabathricum* leaves

S. No.	Pubchem ID	Name of the compound	Molecular formula (Mol. Wt., g mol^{-1})	R T (min)	Structure probability (%)	Reported biological activity	Reference
1.	535560	2-Myristynoyl pantetheine	$C_{25}H_{44}N_2O_5S$ (484)	8.469	15.4	Not reported	Srivastava *et al.* (2015)
2	6439679	9,10 secocholesta-5,7,10(19) triene-3,24,25, triol, (3beta,5Z,7E)	$C_{27}H_{44}O_3$ (416)	29.023	44.8	Anticancer	Zmijewski *et al.* (2008)
3	18950	D-Mannose	$C_6H_{12}O_6$ (180)	6.725	33.2	Antitumor agent	Kamel *et al*., (2010)
4	12366	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$ (284)	18.945	46.1	Antioxidant	Ponnamma *et al.* (2012); Guerrero *et al.* (2017)
5	26249298	Hexadecanoicacid, methyl ester	$C_{17}H_{34}O_2$ (270)	17.102	44.9	Antibacterial & anti-fungal	Chandrasekaran *et al.* (2011); Abubakar *et al.* (2016)
6	92817	Melezitose	$C_{18}H_{32}O_{16}$ (504)	7.822	41.8	Antibacterial	Landry *et al.* (2016)
7	985	n-hexadecanoic acid	$C_{16}H_{32}O_2$ (256)	18.098	57.5	Anti-inflammatory antioxidant, nematicide & pesticide	Aparna *et al.* (2012); Abubakar *et al.* (2016)
8	10494	Oleic acid	$C_{18}H_{34}O_2$ (282)	23.438	12.4	Antibacterial	Zheng *et al*. (2005)
9	6423815	Pthalicacid, hept 4-yl isobutyl ester	$C_{19}H_{28}O_4$ (320)	15.628	7.8	Antimicrobial & antifungal	Liu *et al*. (2010); Dulara *et al.* (2019)
10	6578	Propanamide, 3-[3,5di(tetr-butyl)-4hydroxyphenyl]-N-(2 hydroxyethyl)	$C_{19}H_{31}NO_3$ (321)	19.269	11.8	Antibacterial	Olgen *et al*. (2014)
11	85700	Cholesta 4,6-dien-3-ol	$C_{27}H_{44}O$ (384)	35.857	12.6	Antimicrobial	Zhu *et al.* (2018)
12	9601716	Desulphosinigrin	$C_{10}H_{17}NO_6S$ (279)	10.616	16.7	Antiasthmatic	Khadim *et al.* (2017)

Table 3: Evaluation of Lipinski's rule of plant extracted compounds

Pubchem ID	Compound name	Atoms	HBA	HBD	logP	MR	MW	TPSA
9601716	Desulphosinigrin	35	8	5	-1.1167	65.3337	279.31	148.04
6578	Propanamide	12	2	1	0.582	19.4424	73.09	43.09
535560	2-Myristynoyl pantetheine	77	8	4	3.9542	136.309	484.69	141.03
26249298	Hexadecanoic acid, methyl ester	56	5	3	2.5547	88.6034	318.45	86.99
18950	D-Mannose	24	6	5	-3.2214	35.736	180.15	110.38

Note: HBA: Hydrogen bond acceptor; HBD: Hydrogen bond donor; LogP: Lipophilicity; MR: Molar refractivity; MW: Molecular weight in dalton; and TPSA: Topological polar surface area in Å

Lipinski rule and docking analysis of selected compounds

Lipinski's rule of five, also known as Pfizer's rule, was to evaluate the drug likeness with a certain pharmacological activity that would make it expected orally active drug in humans. All the ligands

satisfy Lipinski rule as shown in Table 3. Based on their docking results two compounds were found to be more interactive with targeted protein and showed more inhibitory activity. They are 2-myristynoyl pantetheine (-8.62) and desulphosinigrin (-7.19) when compared with genistein, a known inhibitor (-7.44). The interaction between phytochemical compound with targeted protein has shown docking values as depicted in Table 4 and Fig. 5.

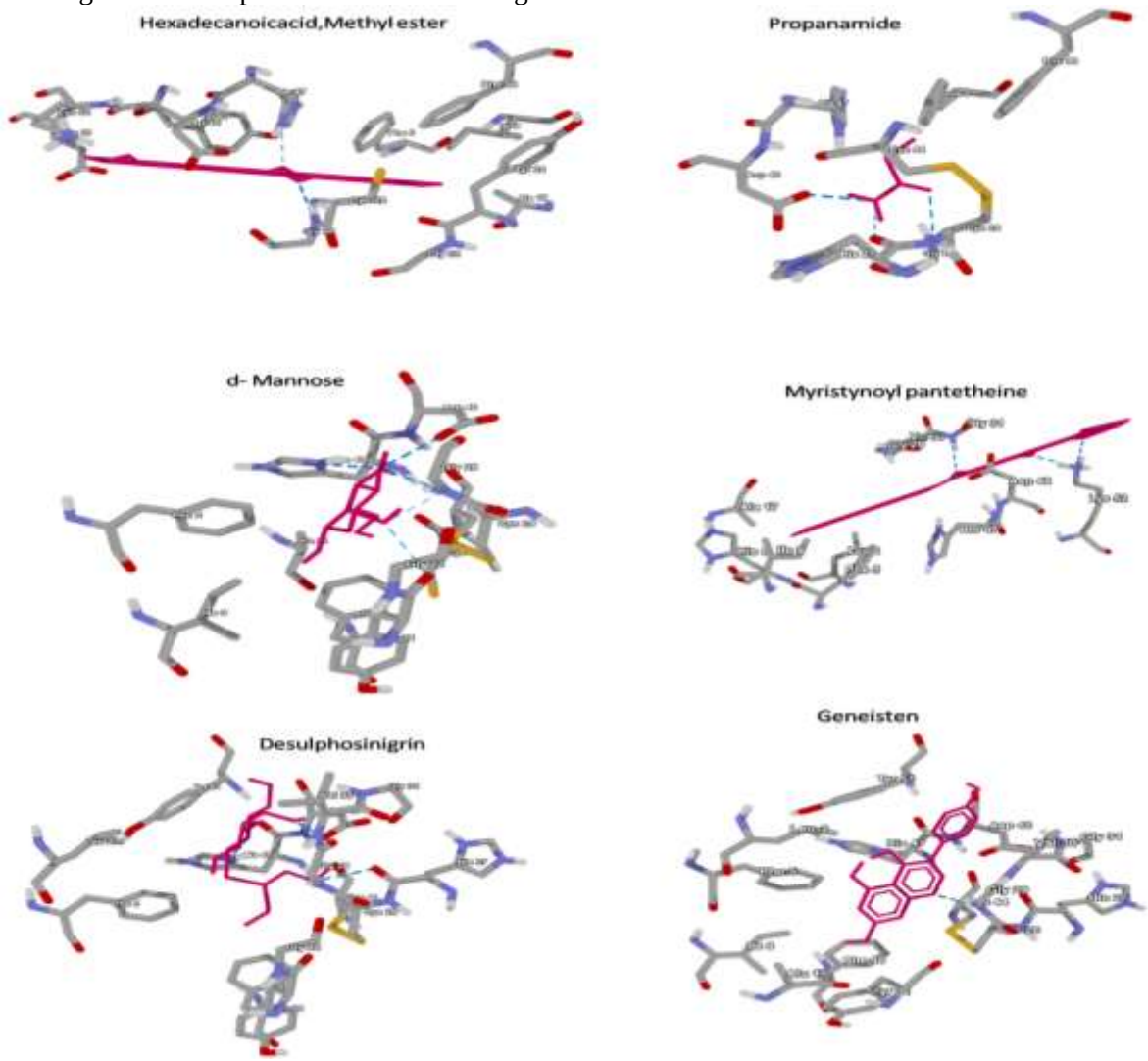

Fig. 5: Molecular docking of *Melastoma malabathricum* leaves training sets with secretory phospholipase A_2 receptor using AutoDock4.2

Table 4: Molecular docking of *M. malabathricum* (L) with human phospholipase A_2 protein using AutoDock4.2. The interactions are predicted on the basis of binding energy (ΔG = kcal mol^{-1})

Compound name	Pub Chem id	Docking energy	No of H atoms	Ligand interaction
Desulphosinigrin	9601716	-7.19	4	HIS27:O, GLY29:HN, HIS47, ASN114
Propanamide	6578	-2.82	3	ASP48:OD1, GLY29:HN, GLY29:O
2-Myristynoyl pantetheine	535560	-8.62	3	GLY31:HN, LYS52: HZ, LYS52: HZ
Hexadecanoic acid, methyl ester	26249298	34.26	1	GLY29:HN
D-Mannose	18950	-4.96	2	GLY29:HN, HIS47
Geneisten	5280961	-7.44	1	GLY29:HN

ADME toxicology study

Absorption, distribution, metabolism, excretion and toxicity (ADMET) properties of drug candidates play a key role in drug discovery. ADMET structure-activity relationship server, entitled ADMETSAR (*http://lmmd.ecust.edu.cn/admetsar1/predict/*) is a comprehensive knowledge and tool for predicting ADMET properties of drug candidates. The top highly interacted compounds 2-Myristynoyl pantetheine and desulphosinigrin were screened for ADMET and the detailed results are shown in Table 5.

Table 5: Predicted ADME toxicology of selected top two phytochemical compounds compared with genistein

Model	Result	Probability (Desulphosinigrin)	Probability (2-Myristynoyl pantetheine)	Probability (Genistein)
Absorption				
Blood-Brain barrier	BBB+	0.5354	0.6472	0.6785
Human intestinal absorption	HIA-	0.9359	0.6650	0.9877
Caco-2 permeability	Caco2-	0.6290	0.6716	0.7002
P-glycoprotein substrate	Non-substrate	0.7458	0.6724	0.5000
P-glycoprotein inhibitor	Non-inhibitor	0.7426	0.6537	0.9288
	Non-inhibitor	0.9879	0.6704	0.7828
Renal organic cation transporter	Non-inhibitor	0.7656	0.9625	0.9075
Distribution				
Subcellular localization	Mitochondria	0.5385	0.7276	0.7606
Metabolism				
CYP450 2C9 substrate	Non-substrate	0.8192	0.7775	0.7672
CYP450 2D6 substrate	Non-substrate	0.8105	0.7734	0.9105
CYP450 3A4 substrate	Non-substrate	0.5423	0.5418	0.6821
CYP450 1A2 inhibitor	Non-inhibitor	0.7341	0.8032	0.9254
CYP450 2C9 inhibitor	Non-inhibitor	0.7780	0.8172	0.8949
CYP450 2D6 inhibitor	Non-inhibitor	0.8836	0.8523	0.9232
CYP450 2C19 inhibitor	Non-inhibitor	0.7526	0.7250	0.8881
CYP450 3A4 inhibitor	Non-inhibitor	0.7727	0.5387	0.7960
CYP inhibitory promiscuity	Low CYP inhibitory promiscuity	0.8871	0.8289	0.8009
Excretion				
Total clearance	log mL min^{-1} kg^{-1}	0.6540	0.6000	0.1510
Toxicity				
Human ether-a-go-go-related gene inhibition	Weak inhibitor	0.9600	0.9986	0.9569
	Non-inhibitor	0.9043	0.8312	0.8917
AMES toxicity	Non-AMES toxic	0.5463	0.8568	0.9638
Carcinogens	Non-carcinogens	0.8616	0.8053	0.9276
Fish toxicity	High FHMT	0.7629	0.9621	0.9017
Tetrahymena pyriformis toxicity	High TPT	0.5405	0.9861	0.9810
Honey bee toxicity	High HBT	0.6375	0.6249	0.6794
Biodegradation	Not readily biodegradable	0.7810	0.9031	0.8572
Acute oral toxicity	II	0.5624	0.5951	0.5830
Carcinogenicity (three-class)	Non-required	0.5809	0.6482	0.7003

Conclusions: The authors performed GC-MS phytochemical screening against human secretory phospholipase A_2 and also compared with genistein, a potent inhibitor. Based on pharmacophore,

pharmacokinetic and molecular docking study, two compounds namely 2-myristynoyl pantetheine (-8.62 $kcal^{-1}$ mol^{-1}) and desulphosinigrin (-7.19 kcal mol^{-1}) showed strong interaction with human secretory phospholipase A_2. Further, the phytochemicals isolated from *Melastoma malabathricum* leaf extract showed potential targets to human secretory phospholipase A_2 receptor as well as developed anti-inflammatory active drug molecules. Clinical and wet laboratory studies are required to find the efficiency and off-target interactions of ligand. The presence of various bioactive compounds justifies the use of *M. malabathricum* for various ailments by traditional practitioners. It is recommended as a plant of pharmaceutical importance. More studies are needed to fully ascertain its bioactivity.

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Conflict of interest: The authors declare thar they have no conflict of interest.

Ethical statement: This research article does not contain any studies with human participants or animals performed by any of the authors.

REFERENCES

Abubakar, M.N. and Majinda, R.R. 2016. GC-MS analysis and preliminary antimicrobial activity of *Albizia adianthifolia* (Schumach) and *Pterocarpus angolensis* (DC). *Medicines*, **3***:* 3 (DOI: 10.3390/medicines3010003).

Aparna Vasudevan, Kalarickal V.D., Mandal, P.K., Karthe, P., Sadasivan, C. and Haridas, M. 2012 Anti-inflammatory property of n-hexadecanoic acid: Structural evidence and kinetic assessment. *Chemical Biology and Drug Design,* **80:** 434-439.

Backman, Y., Cao, Y. and Girke, T. 2011. ChemMine tools: An online service for analyzing and clustering small molecules. *Nucleic Acids Research,* **39:** W486-W491.

Chandrasekaran, A., Senthilkumar and Venkatesalu, V. 2011. Antibacterial and antifungal efficacy of fatty acid methyl esters from the leaves of *Sesuvium portulacastrum* L. *European Review for Medical and Pharmacological Sciences,* **15:** 775-780.

De Gaetano, M.B., Donati, B. and Cerletti, C. 2003. Prevention of thrombosis and vascular inflammation: Benefits and limitations of selective or combined COX-1, COX-2 and 5-LOX inhibitors. *Trends in Pharmacological Sciences,* **24:** 245-252.

Dharmappa, K.K., Kumar, R.V., Nataraju, A., Mohamed, R., Shivaprasad, H.V. and Vishwanath, B.S. 2009. Anti-inflammatory activity of oleanolic acid by inhibition of secretory phospholipase A2. *Planta Medica,* **75:** 211-215.

Dulara, B.K., Godara, P. and Barwer, N. 2019. *In vivo* and *in vitro* phytochemical GC-MS analysis of volatile constituents of *Andrographis paniculata* (Burm. f.) Nees. *The Pharma Innovation Journal*, **8:** 255-261.

Ferdous, S., Mirza, M.U. and Saeed, U. 2013. Docking studies reveal phytochemicals as the long searched anticancer drugs for breast cancer. *International Journal of Computer Applications,* **67:** 1-5.

Guerrero, R.V., Vargas, R.A. and Petricevich, V.L. 2017. Chemical compounds and biological activity of an extract from bougainvillea x buttiana (var. rose) Holttum and Standl. *International Journal of Pharmacy and Pharmaceutical Science**s*, **9:** 42-46.

Kadhim, M.J., Al-Rubaye, A.F. and Hameed, I.H. 2017. Determination of bioactive compounds of methanolic extract of *Vitis vinifera* using GC-MS. *International Journal of Toxicological and Pharmacological Research*, **9:** 113-126.

Kamel, M.M., Ali, H.I., Anwar, M.M., Mohamed, N.A. and Soliman, A.M. 2010. Synthesis, antitumor activity and molecular docking study of novel sulfonamide-Schiff's bases, thiazolidinones, benzothiazinones, and their C-nucleoside derivatives. *European Journal of Medicinal Chemistry*, **45**: 572-580.

Krug, H.F., Mattern, D., Bidault, J. and Ninio, E. 1994. Effects of organometals on cellular signaling. II. Inhibition of reincorporation of free arachidonic acid and influence on paf-acether synthesis by triethyl lead. *Environmental Health Perspectives*, **102**: 331-334.

Landry, B.K., Moumita, S., Jayabalan, R. and François, Z.N. 2016. Honey, probiotics and prebiotics: Review. *Research Journal of Pharmaceutical Biological and Chemical Sciences*, **7:** 2428-2438. (*https://www.rjpbcs.com/2016_7.5.html*).

Li, D., Tian, X., Wang, A., Guan, L., Zheng, J., Li, F., Li, S., Zhou, H., Wu J. and Tian, Y. 2016. Nucleic acid-selective light-up fluorescent biosensors for ratiometric two-photon imaging of the viscosity of live cells and tissues. *Chemical Science*, **7:** 2257-2263.

Lin, K.W. 2005. Ethnobotanical study of medicinal plants used by the Jah Hut peoples in Malaysia. *Indian Journal of Medical Sciences*, ***59*:** 156-161.

Liu, J., Yi, W., Hu, J., Wu, F., Zhao, L., Song, H. and Wang, Z. 2010. Design, synthesis and biological evaluation of 3-(4-halophenyl)-3-oxopropanal and their derivatives as novel antibacterial agents. *Chemical and Pharmaceutical Bulletin*, **58***:* 1127-1131.

Mintah, S.O., Asafo-Agyei, T., Archer, M.A., Junior, P.A.A., Boamah, D., Kumadoh, D., Appiah, A., Ocloo, A., Boakye, Y.D. and Agyare, C. 2019. Medicinal plants for treatment of prevalent diseases. *Pharmacognosy-Medicinal Plants*. IntechOpen. (DOI: 10.3772/intechopen.82049).

Nanda, B.L., Nataraju, A., Rajesh, R., Rangappa, K.S., Shekar, M.A. and Vishwanath, B.S. 2007. PLA2 mediated arachidonate free radicals: PLA2 inhibition and neutralization of free radicals by anti-oxidants - A new role as anti-inflammatory molecule. *Current Topics in Medicinal Chemistry*, **7:** 765-777.

Nevalainen, T.J., Grönroos, J.M. and Kortesuo, P. 1993. Pancreatic and synovial type phospholipases A2 in serum samples from patients with severe acute pancreatitis. *Gut*, **34:** 1133-1136.

Nevalainen,T.J., Haapamäki, M.M. and Grönroos, J.M. 2000. Roles of secretory phospholipases A2 in inflammatory diseases and trauma. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, **1488:** 83-90.

Osafo, N., Agyare, C., Obiri, D.D. and Antwi, A.O. 2017. Mechanism of action of nonsteroidal anti-inflammatory drugs. *Nonsteroidal Anti-Inflammatory Drugs*, Intechopen: 1-15, (*DOI:10.5772/68090*).

Olgen, S., Altanlar, N., Karatayli, E. and Bozdayi, M. 2014. Antimicrobial and antiviral screening of novel indole carboxamide and propanamide derivatives. *Zeitschrift für Naturforschung.* **63**: 3-4.

Ponnamma, S.U. and Manjunath, K. 2012. GC-MS analysis of phytocomponents in the methanolic extract of *Justicia wynaadensis* (Nees) T. Anders. *International Journal of Pharma and Bio-Sciences*, **3***:* 570-576.

Sadashiva, M.P., Nataraju, A., Mallesha, H., Rajesh, R., Vishwanath, B. and Rangappa, K. 2005. Synthesis and evaluation of trimethoxyphenyl isoxazolidines as inhibitors of secretory phospholipase A_2 with anti-inflammatory activity. *International Journal of Molecular Medicine*, **16:** 895-904.

Sanner, M.F. 1999. Python: A programming language for software integration and development. *Journal of Molecular Graphics and Modelling*, **17:** 57-61.

Sheeja, K. and Kuttan, G. 2007. Activation of cytotoxic T lymphocyte responses and attenuation of tumor growth *in vivo* by *Andrographis paniculata* extract and andrographolide. *Immunopharmacology and Immunotoxicology*, **29:** 81-93.

Srivastava, R., Mukerjee, A. and Verma, A., 2015. GC-MS analysis of phytocomponents in pet ether fraction of *Wrightia tinctoria* seed. *Pharmacognosy Journal*, **7***:* 4. (*DOI:10.5530/pj.2015.4.7*).

Sophiya, P., Lohith, N.S., Giresha, A.S., Narayanappa, M., Rajkumar S. Meti, Sathisha, A.D. and Dharmappa, K.K. 2019. Inhibitory effect of ethanol extract of *Melastoma malabathricum* leaves on inflammatory $sPLA_2$ enzyme and its ability to reduce the cell viability of PC3 cell line. *Interntional Journal of Pharmaceutical Sciences and Research,* **10**: 5041-5050.

Stafforini, D.M. and Zimmerman. G.A. 2014. Unraveling the PAF-AH/lp-PLA2 controversy. *Journal of Lipid Research,* **55 :** 1811-1814.

Tian, C., Chen, X., Lei, J. and Zhao, J.L. 2018. CASTp 3.0: Computed atlas of surface topography of proteins. *Nucleic Acids Research,* **46:** W363-W367.

Zakaria, Z.A., Hisam, E.A.,Norhafizah, M., Rofiee, M., Othman, F., Hasiah, A. and Vasudevan, M. 2012. Methanol extract of *Bauhinia purpurea* leaf possesses anti-ulcer activity. *Medical Principles and Practice,* **21:** 476-482.

Zheng, C.J., Yoo, J.S., Lee, T.G., Cho, H.Y., Kim, Y.H. and Kim, W.G. 2005. Fatty acid synthesis is a target for antibacterial activity of unsaturated fatty acids. *FEBS Letters,* **579**: 5157-5162.

Zhu, Y.Z., Liu, J.W., Wang, X., Jeong, I.H., Ahn, Y.J. and Zhang, C.J. 2018. Anti-BACE1 and antimicrobial activities of steroidal compounds isolated from marine *Urechisuni cinctus. Marine Drugs*, **16**: 94.

Zmijewski, M.A., Li, W., Zjawiony, J.K., Sweatman, T.W., Chen, J., Miller, D.D. and Slominski, A.T. 2008. Synthesis and photoconversion of androsta-and pregna-5, 7-dienes to vitamin D_3-like derivatives. *Photochemical and Photobiological Sciences*, **7***:* 1570-1576.