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Phytoconstituents Analysis of Hydroalcoholic Root Extract of *Premna integrifolia* L.: An Important Ingredient of Herbal Formulation Dashamula

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ABSTRACT: Hydroalcoholic extract of root of *Premna integrifolia* was analyzed for polyphenol content and antioxidant potential because it is an important ingredient of widely used herbal medicine Dashamula. This extract is enriched with polyphenol with good antioxidant activity. Chemo profiling of this extract using GC-MS confirmed the presence of several valuable terpenoids such as lanugone A, podocarp-12-en-14-ol, ferruginol, sandaracopimaradien-18-al, communic acid and (-) β caryophyllene epoxide. The anti oxidative property of the extract might be due to presence of these several terpenoids. Further it needs isolation of these valuable terpenoids for bioactivity studies.

Keywords: Antioxidant, Flavonoids, Free radical scavenger, *Premna integrifolia,* Terpenoids, Phytoconstituents.

Abbreviations: ANOVA, One-way analysis of Variance; DPPH, 1, 1-diphenyl-2-picrylhydrazyl; GC-MS, Gas chromatography mass spectroscopy.

I. INTRODUCTION

Premna integrifolia L. also known as *Premna serratifolia* belongs to Verbenaceae family. Its root is an essential constituent of Ayurvedic formulation "Dashamula". In folklore its root decoction is used to cure swellings, bronchitis, dyspepsia, liver disorders, piles, constipation, and fever [1]. It is also used as cardiotonic, anticoagulant, stomachic, carminative [2] and anti microbial [3]. According to literature survey, the root of the plant used for various pharmacological purposes such as anti inflammatory and immune modulatory [4], anti atherosclerosis [5], cytotoxic [6], anti diabetic [7], antioxidant [8], antiobesity [9] and antihyperlipidemic [10]. The root also has mitigating effect against H_2O_2 induced oxidative stress in human leucocytes and erythrocytes [11]. The plant also attenuates hepatotoxic effects induced by cyclophosphamide [12] and aflatoxin [13]. The plant's parts like leaves, stem, stem barks, root, root barks and wood also has significant medicinal properties for the treatment of several diseases [14]. Phytoconstituents like tetrahydrofuran lignans, taungtangyiols A & B [15], 11,12,16-trihydroxy-2-oxo-5-methyl-10-demethyl-abieta-1[10], 6, 8, 11, 13-pentene [6], verbacoside [16], β,3α,8β-trihydroxy-pimara-15-ene, 6α,11,12,16-tetrahydroxy-7-oxoabieta-8,11,13-triene, 2α,19-dihydroxy-pimara-7,15-diene [17], premnacorymboside A, scutellarioside II, premnacorymboside B [18] were isolated from different parts of the plant. The aim of present study was to analyze antioxidant potential and identification of major phytoconstiuents present in hydroalcoholic extract of root of *P. integrifolia* by Gas chromatography mass spectroscopy (GCMS). Till date, there is no report of antioxidant activity and major phytoconstituent profile of hydro alcoholic extract of roots of *P. integrifolia.*

II. MATERIAL AND METHODS

A. Chemicals

1,1-Diphenyl, 2-picryl hydrazyl (DPPH) (SRL), Ascorbic acid (Hi-Media Ltd.), Gallic acid (Hi-Media Ltd.), Rutin LR (SDFCL), Folin-Ciocalteu reagent (Hi-Media Ltd.), Sodium carbonate (SRL), Aluminium chloride anhydrous, Potassium acetate(SRL), ethanol (Merck), Methanol (Merck)and Sodium sulphate anhydrous (SRL). All reagents are of analytical grade.

B. Plant collection and extract preparation

The roots of *P. integrifolia* were collected from Ayurvedic garden, Department of Dravyaguna, Banaras Hindu University, Varanasi, India. A voucher specimen (BSI/CRC/2016-17) is deposited in the herbarium of Botanical Survey of India (BSI), Allahabad, India after authentication under the accession number 97879. The roots are thoroughly washed under running tap water, shade dried and ground to coarse powder. 50 gram root powder is soaked in petroleum ether (125 ml) at room temperature to remove fats and macerated with hydro alcohol (60:40 v/v) for 72 hrs at 25±2ºC and 120 rpm. Afterward, extract is filtered (Whatman No.1 filter paper). The filtrate is evaporated at 20ºC to dryness and stored at 4ºC in air tight jar [19]. To study antioxidant potential of hydroalcoholic extract, 5mg/ml stock was prepared in double distilled water.

C. Free radical scavenging activity

To determine free radical scavenging activity of the extract, method described by William *et al.* [20] was followed. 3ml solution of DPPH (0.004% in methanol) was added in different concentrations of extract. The reaction mixture was incubated at 37ºC for 15 minute and its absorbance was recorded at 517 nm by using

spectrophotometer (SHIMADZU UV 1800ENG240V, SOF).

D. Measurement of total phenol (TP)

Total phenol content was evaluated by modified Folin ciocalteu assay as described by Upadhyay *et al*. [21]. 100µl extract of different concentration was added in 1ml double distilled water. 200µl Folin ciocalteu solution (1:1 Folin ciocalteu reagent: distilled water) was added in reaction mixture, mixed and allowed to stand (5-8 minute) at room temperature. 2ml sodium carbonate (7%) solution was added. Further, 700µl double distilled water was added into reaction mixture to make the volume up to 3ml. Mixed and allowed to stand for 15 minute. Absorbance of reaction mixture was recorded at 750nm. Phenolic content was estimated by using a standard curve from various concentration of gallic acid and expressed as milligram per gram of gallic acid equivalent (GAE).

E. Measurement of total flavonoid (TF)

Total flavonoid content was determined by using method described by Chang *et al*. [22]. 100µl extract was mixed with 100µl AlCl (2%) solution. Further, 1M Potassium acetate (100µl) was added to ethanol (2.7ml) and mixed in reaction mixture. Reaction mixture was incubated at 37ºC for 30 minute. Absorbance of the reaction mixture was recorded at 415nm. Total flavonoid content was calculated using rutin as standard and expressed as milligram per gram of rutin equivalents (RE).

F. Non targeted GCMS analysis

Wide spectrum of phytoconstituents present in root extract was identified by using non targeted GCMS-QP2010 Ultra. For separation of phtyo constituents present in the extract through GCMS requires polar column. Therefore, 100mg extract was dissolved in 1ml of methanol and sonicated for 1 hour at 20±2ºC. Sodium sulphate anhydrous (150mg) was added to methanol stock to remove traces of water. GCMS experimental condition was as follow: Temperature of GC column oven was programmed at 60ºC for 3 min, raised to 250ºC at 10ºC/min rate and held for 2 min again raised to 280ºC at 15ºC/min rate and held for 19 min. 2.0 µl sample was injected by split mode at 260ºC. Mobile phase (carrier gas) total flow rate was 16.3ml/min at 73.3kPa. The MS ion source and interface source temperature was 230ºC and 270ºC respectively. The total ran time for GCMS was 41.98 min. The result obtained was matched with NIST 14 (National Institute of Standards and Technology) and WILEY8 library.

III. RESULTS

Maceration of dry root powder in hydro alcoholic solvent for 72 hours yielded its 4.026% (w/w) of the extract.

A. DPPH radical scavenging assay

Table 1 depicts the effective concentration of extract that scavenge DPPH free radical. The EC_{50} (half maximal effective concentration) value for extract was 568.51µg/ml, higher than ascorbic acid. Though, the antioxidant potentiality of the root extract was lower than ascorbic acid.

B. Total phenol and flavonoid content

Total phenol and flavonoid content in the extract was given in Table 2. Total phenol content was calculated through standard curve (y =0.003x R^2 = 0.983). The total phenol content was expressed as milligram of gallic acid equivalent per gram.

Table 1: Free radical scavenging potential of hydroalcoholic root extract of *Premna integrifolia.*

Concentration (µg/mL)	Percent inhibition (%)
50	3.779±0.007
100	12.515±0.017
200	23.296±0.014
400	42.131±0.012
600	57.931±0.006
800	68.340±0.014
1000	79.678±0.008
EC_{50}	568.51

EC_{50} of Ascorbic acid 395.17

Each value represented in table is represented as mean±S.D (n=3).

The total phenol content present in sample depends on solvent selection. The Phenolic content in the extract was 110.24±0.012 mg/g GAE [20]. The total flavonoid content was estimated using standard curve (y = 0.004x, R^2 = 0.971). The total flavonoid content was expressed as milligram rutin equivalent per gram. The total flavonoids content in the extract was 80.66±0.015 mg/g rutin.

Table 2: Total phenol and flavonoid content in hydroalcoholic root extract of *Premna integrifolia.*

Concentration (µg/mL)	Total Phenol content (mg/g gallic acid equivalent)	Total Flavonoid content (mg/g rutin equivalent)
50	21.24±0.010	34.91±0.015
100	29.91±0.005	40.91±0.085
200	38.41±0.001	48.79±0.002
400	54.74±0.008	53.41±0.045
600	82.07±0.009	64.91±0.125
800	94.24±0.010	72.79±0.012
1000	110.24±0.012	80.66±0.015

Each value represented in table is represented as mean±S.D (n=3).

C. Phytoconstituents characterisation using GC-MS

GC-MS chromatogram (Fig. 1) of extract revealed the presence of several phytoconstituents responsible for antioxidant activity. Total fifty four compounds (Table 3) were identified in the extract which accounts 100% area. Out of fifty four, eight phytoconstituents, like tris nitro (35.83%), lanugone A (10.46%), 1-(4-isopropylphenyl)-2-methylpropyl acetate (8.88%), aristolene epoxide (4.43%), ethylene brassylate (2.79%), 4-oxo-β-isodamascol (2.30%), 8,8-dimethyl-9-methylene-1,5-cycloundecadiene (2.10%) and ethyl 1-thio-α-d-arabinofuranoside (1.49%) were reported as a major compounds present in the extract (Fig. 2). Phtyo constituent present in the extract were reported for various biological activities such as bactericide, slimicide and crossing linking agent [23]. Ethylene brassylate present in extract is a macro cyclic musk used as fragrance and flavoring agent. Number of minor phyto steroids such as γ- sitosterol (0.81%), stigmasta-3, 5-dien-7-one (0.37%), stigmasterol (0.35%) and stigmasteryl acetate (0.24%) were present in the

extract. Compounds like methyl androgenic steroid (stanolone, 0.79%), jasmonate (3-oxo-2-pent-2-enyl-cyclopentyl)-acetic acid methyl ester, (0.51%), and pathalate ester (diethyl phthalate, 0.44%) were also present in extract.

The extract has its characteristic fragrance. Phytoconstituent profile study of the extract revealed that it possesses commercially popular flavor and fragrant agents like ethylene brassylate (musk fragrance) (2.79%) [24], galaxolide (polycyclic musk) (1.15%) [25], acetyl cedrene (1.07%) [26], furaneol (0.63%) [27], aromadendrene (0.55%) and β caryophyllene epoxide (0.44%) [28] (Fig. 3).

GCMS profile of extract exhibited the presence of important terpenoids such as lanugone A (10.46%), podocarp-12-en-14-ol (1.53%), ferruginol (1.13%) [29], sandaracopimaradien-18-al (0.98%), communic acid (0.52%) and (-) β caryophyllene epoxide (0.44%) [30] (Fig. 4).

Table 3: Major compounds present in the hydro alcoholic root extract of *P. integrifolia* analysed using GC-MS.

Peak	Retention time (min)	Area (%)	Identified compound	Molecular formula	Molecular weight	Nature	Reported activity	References
1	5.343	2.11	pentyl acetate	$C_7H_{12}D_2O_2$	132	ester	-	-
2	6.384	0.40	2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	144	-	-	-
3	7.963	0.63	Furaneol	$C_6H_8O_3$	128	furan	flavor, fragrance	[27]
4	8.501	1.66	4-amino-5-nitroso uracil	$C_4H_4N_4O_3$	156	pyrimidine	-	-
5	9.364	1.68	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	$C_6H_8O_4$	144	pyranone	-	-
6	10.970	0.58	3-ethyl-4,4-dimethyl-2-Pentene	C_9H_{18}	126	alkene	-	-
7	11.151	0.99	3,4,4a,5,8,8a methyl-hexahydro-1(2H)-naphthalenone	$C_{11}H_{16}O$	164	-	-	-
8	12.814	0.08	1-tridecene	$C_{13}H_{26}$	182	olefin	-	-
9	13.01.	0.15	3-[N'-(3H-Indol-3-ylmethylene)-hydrazino]-5-methyl-[1,2,4]	$C_{12}H_{13}N_7$		-	-	-
10	13.750	0.27	Isoeugenol	$C_{10}H_{12}O_2$	164	phenol	antibacterial	[37]
11	14.460	35.83	tris nitro	$C_4H_9NO_5$	151	-	bactericide	[23]
12	15.127	0.42	Sucrose	$C_{12}H_{22}O_{11}$	342	carbohydrate		
13	15.377	0.44	diethyl phthalate	$C_{12}H_{14}O_4$	222	ester	antimicrobial	[38]
14	15.808	0.28	2-(bromomethyl)-2-adamantanol	$C_{11}H_{17}BrO$	244	alcohol	-	-
15	15.966	0.57	caryophylla-3,8(13)-dien-5α-ol	$C_{15}H_{24}O$	220	alcohol	-	-
16	16.100	2.10	8,8-dimethyl-9-methylene-1,5-cycloundecadiene	$C_{14}H_{22}$	190	alkene	-	-
17	16.292	8.88	1-(4-isopropylphenyl)-2-methylpropyl acetate	$C_{15}H_{22}O_2$	234	-	-	-
18	16.496	1.49	ethyl 1-thio-α-D-arabinofuranoside	$C_7H_{14}O_4S$	194	carbohydrate	-	-
19	17.097	0.63	7-oxabicyclo[4.1.0]heptane, 1-(1,3-dimethyl-1,3-butadienyl)-2,2,6-trimethyl-, (E)-	$C_{15}H_{24}O$	220	alkane	-	-
20	17.291	1.22	4-9(1E)-3-hydroxy-1-propenyl)-2-methoxyphenol	$C_{10}H_{12}O_3$	180	phenol	-	-
21	17.508	1.07	acetyl cedrene	$C_{17}H_{26}O$	246	alkyl cyclic ketones	Flavor, fragrance	[26]
22	17.668	0.44	β caryophyllene epoxide	$C_{15}H_{24}O$	220	terpenes	Flavor, fragrance, anti termite	[28], [39]
23	18.269	1.15	Galaxolide	$C_{18}H_{26}O$	258	benzopyran	fragrance	[25]
24	19.274	0.32	3,9B-epoxy-9BH-benz[E]indene, dodecahydro-3,3A,6,6,9A-pentamethyl-	$C_{18}H_{30}O$	262	-	-	-
25	19.416	1.61	n-hexadecanoic acid	$C_{16}H_{32}O_2$	256	fatty acid	anti-inflammatory	[40]
26	20.014	2.79	ethylene brassylate	$C_{15}H_{26}O_4$	270	ketone	fragrance	[24]
27	20.431	0.42	13-hexyloxacyclotridec-10-en-2-one	$C_{18}H_{32}O_2$	280	ketone	-	-
28	21.097	0.51	9,12-octadecadien-1-ol	$C_{18}H_{34}O$	266	alcohol	-	-
29	21.195	4.43	aristolene epoxide	$C_{15}H_{24}O$	220	cyclic alkane	-	-
30	21.522	0.30	heptadecanoic acid, ethyl ester	$C_{19}H_{38}O_2$	298	ester	-	-
31	21.914	0.19	3α,7β-dihydroxy-5α,6β-epoxycholestane	$C_{27}H_{46}O_3$	418	-	-	-
32	22.333	0.98	sandaracopimaradien-18-al	$C_{20}H_{30}O$	286	diterpenoids	-	-
33	22.445	0.79	Stanolone	$C_{19}H_{30}O_2$	290	steroid	-	-
34	22.537	0.55	Aromadendrene	$C_{15}H_{24}$	204	scsquiterpenes	-	-
35	22.831	0.17	podocarp-12-en-14-ol	$C_{17}H_{28}O$	248	diterpene	-	-
36	22.971	1.13	Ferruginol	$C_{20}H_{30}O$	286	abietane diterpenoid	neuroprotective	[35]
37	23.497	1.53	13,13-dimethyl-podocarp-7-en-3β-ol	$C_{19}H_{32}O$	276	diterpene	-	-
38	23.730	0.88	aristolene epoxide	$C_{15}H_{24}O$	220	cyclic alkane	-	-
39	24.622	2.30	4-oxo-β-isodamascol	$C_{13}H_{20}O_2$	208	ketone	-	-
40	24.790	0.52	communic acid	$C_{20}H_{30}O_2$	302	diterpenes	-	-
41	25.011	0.37	1,2-benzene dicarboxylic acid,	$C_{24}H_{38}O_4$	390	phthalate ester	-	-

			diethyl ester					
42	25.109	0.51	(5,5-dimethyl-6-[(1E)-3-methyl-1,3-butad ienyl]-7-oxabicyclo[4.1.0] hept-1-yl)methyl acetate	$C_{16}H_{24}O_3$	264	-	-	-
43	25.408	0.76	5-isopropyl-4-[(7-isopropyl-1-methyl-4-azulenyl)methyl]-3,8-dimethyl-1,4-dihydro-2-azulenecarbaldehyde	$C_{31}H_{36}O$	424	-	-	-
44	26.091	0.65	(3,3-dimethyl-2,3-dihydro-1H benzo[f]chromen-8-yloxy) trimethylsilane	$C_{18}H_{24}O_2Si$	300	-	-	-
45	26.760	0.51	(3-oxo-2-pent-2-enyl-cyclopentyl)-acetic acid methyl ester	$C_{13}H_{20}O_3$	224	methyl jasmonate	-	-
46	27.307	0.95	Δ^9 tetrahydrocannabinol	$C_{21}H_{30}O_2$	314	cannabinoids	antibacterial	[41]
47	27.564	0.52	squalene	$C_{30}H_{50}$	410	triteroene	anti gastric cancer	[42]
48	28.180	10.46	lanugone A	$C_{20}H_{24}O_3$	312	cycloaibetiane, abeoabietaine diterpenoids	-	-
49	29.056	0.69	kaur-16-ene	$C_{20}H_{32}$	272	diterpene	-	-
50	31.025	0.24	stigmasteryl acetate	$C_{31}H_{50}O_2$	454	phytosterol	-	-
51	34.526	0.35	stigmasterol	$C_{29}H_{48}O$	412	phytosterol	antimicrobial, anti cancer	[43], [44]
52	35.997	0.81	γ sitosterol	$C_{29}H_{50}O$	414	plant steroid	-	-
53	37.421	0.32	methyl commate A	$C_{32}H_{52}O_4$	500		-	-
54	38.452	0.37	stigmasta-3,5-dien-7-one	$C_{29}H_{46}O$	410	ketone	-	-

"-" : Activity of the compound is not reported.

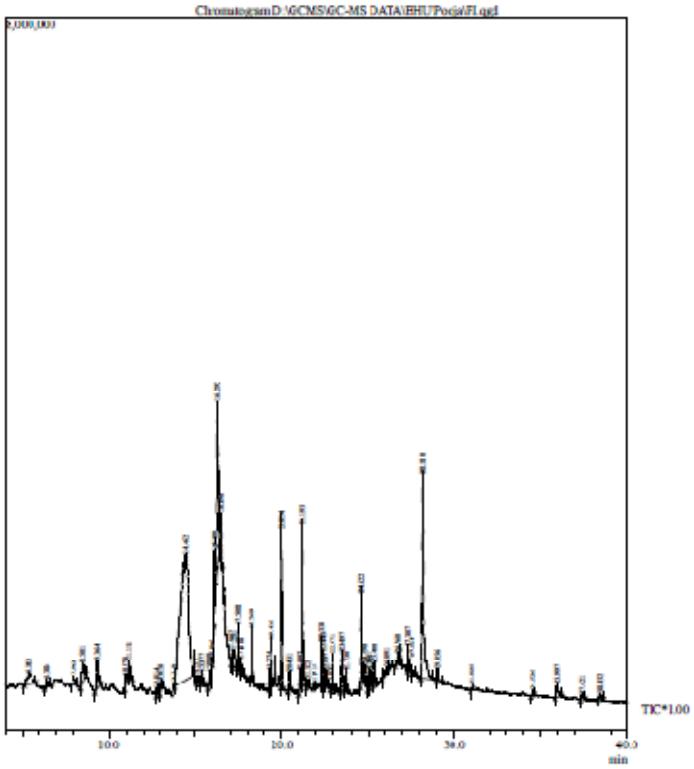

Fig. 1. GC-MS chromatogram of *Premna integrifolia* hydroalcoholic root extract.

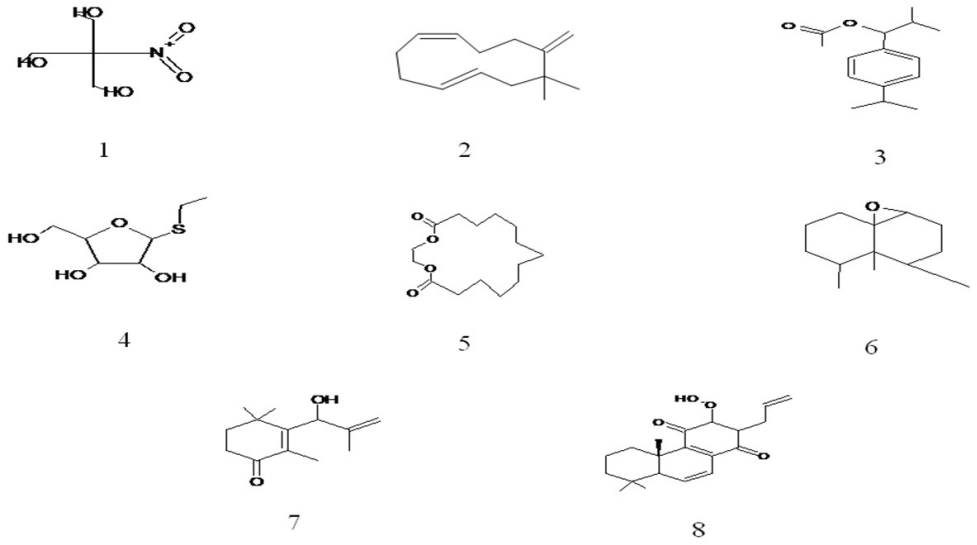

Fig. 2. Structures of major phytoconstiuents present in the extract (1) tris nitro, (2) 8, 8-dimethyl-9-methylene-1,5-cycloundecadiene, (3) 1-(4-isopropylphenyl)-2-methylpropyl acetate, (4) ethyl 1-thio-α-D-arabinofuranoside, (5) ethylene brassylate, (6) aristolene epoxide, (7) 4-oxo-β-isodamascol, 8) lanugone A.

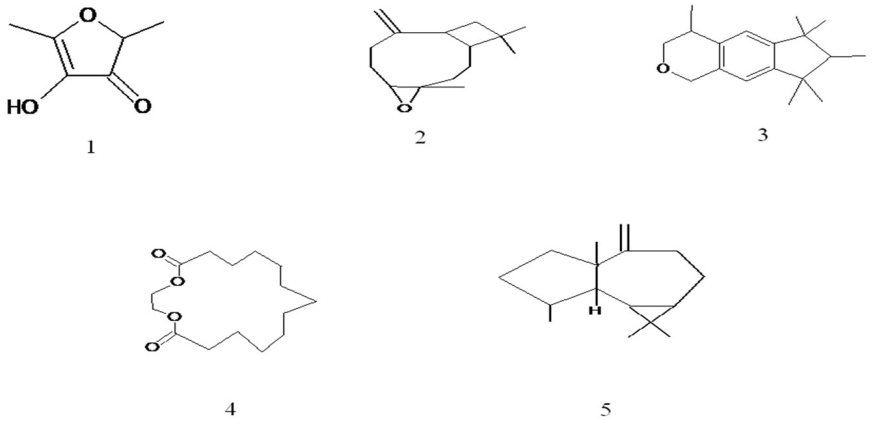

Fig. 3. Structures of imoprtant phytoconstituents present in the extract known for flavor and fragrance (1) Furaneol, (2) β caryophyllene epoxide, (3) galaxolide, (4) ethylene brassylate, (5) aromadendrene.

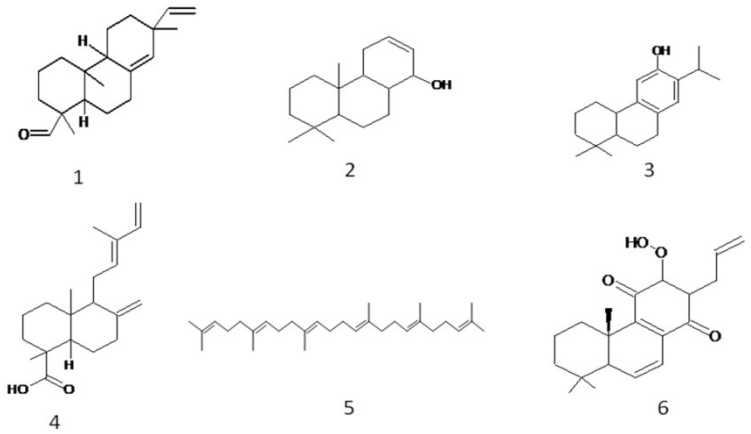

Fig. 4. Structures of important terpenoids present in the extract (1) sandaracopimaradien-18-al, (2) podocarp-12-en-14-ol, (3) ferruginol, (4) communic acid, (5) squalene, (6) lanugone A.

IV. DISCUSSION

Wide ranges of phytochemicals are known for their antioxidant potential. These phytochemicals belong to different categories like polyphenol, terpenoids and tocopherol etc. These compounds not only provide protection to plants against oxidative stress but also has vital role in human health promotion. These phytoconstituents quenched the undesirable free radicals which are involved in hydrogen and electron transfer. Thus, it inhibits the oxidative induced damage of structural integrity of organelles, cells and tissues. During stress antioxidant defense system's expression enhanced to maintain the dynamics between free radicals production and antioxidant function. Antioxidant defense troops consist of enzymes like super oxide dismutase, catalase and glutathione reductase and free radical scavengers (ascorbic acid and tocopherol) [32]. To boost antioxidant defense, supplementary dietary intake rich with antioxidant could be increased. Fruits, vegetables and medicinal plant's products are rich source of wide spectrum of antioxidants [33] (quercetin, rutin, ascorbic acid and vitamin E etc.), it supports the enhancement of antioxidant defense process in human.

Ameliorative action of phytocompounds against oxidative injuries increased the attention of workers towards nutraceuticals and chemo profiling of plant extracts. *P. integrifolia* leaf extract contained good amount of phenol and flavonoid which provides its strong antioxidant potential. It was confirmed on the basis of DPPH free radical scavenging assay. Extract rich with polyphenol possess strong antioxidant activity was reported in several studies [34]. GC-MS analysis of extract of *P. integrifolia* revealed the presence of several terpenoids. Literature survey enlightened that terpenoids exhibited strong antioxidant action with significant biological functions. β carophyllene epoxide present in the extract is used as flavoring agent in food industry. The bicyclic sesquiterpene, β carophyllene epoxide is natural compound found in wide range of plants. It is reported to possess antioxidant, anti-inflammatory and anti cancerous property [28]. Ferruginol is natural diterpene was found in the extract. It provides potential neuro protection against Aβ oligomers induce neurodegenerative injuries like Alzheimer's disease [35]. It also exhibited free radical scavenging property [29]. Communic acids, natural diterpene mostly found in *Juniperus* genus. It possesses biological activities such as antioxidant, cytotoxic, anti-mycobacterial and testosterone 5α-reductase inhibitory activities [36].

V. CONCLUSIONS

Hydroalcoholic leaf extract of *Premmna integrifolia* enriched with polyphenol with strong free radical scavenging property. Phytoconstituent profiling of extract using GC-MS analysis revealed the presence of several important chemicals. Among these chemicals large number of terpenoids like ferruginol, communic acid and β carophyllene epoxide were reported which act as a good antioxidant. The free radical scavenging property of the hydroalcoholic extract might be due to presence of these terpenoids. Further studies required to isolate, purify and characterize these terpenoids for various biological applications.

VI. FUTURE SCOPE

Hydro alcoholic root extract of *Premna integrifolia* L. can be used in the preparation of several hebal formulations due to its antioxidant potential. It is an important ingredient of Dashamula formulation so, it can be utilized to ameliorate liver complications.

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Conflict of interest. No conflict of interest in the study.

REFERENCES

[1]. Gokani, R.H., Lahiri, S.K., Santani, D.D. and Shah, M.B., (2007). Evaluation of Immunomodulatory Activity of *Clerodendrum phlomidis* and *Premna integrifolia* Root. *International Journal of Pharmacology, 3*(4): 352-356.

[2]. Vadivu, R., Suresh, A.J., Girinath, K., Kannan, P.B., Vimala, R. and Kumar N.M.S., (2009). Evaluation of Hepatoprotective and *In-vitro* cytotoxic Activity of Leaves of *Premna serratifolia* Linn. *Journal of Scientific Research*, *1*(1): 145-152.

[3]. Rajendran, R. and Basha, N.S., (2010). Antimicrobial Activity of Crude Extracts and Fractions of *Premna serratifolia* Lin. Root. *Medicinal Plants, 1*(1): 1-6.

[4]. Azad, R., Babu, N.K., Gupta, A.D. and Reddanna, P., (2018). Evaluation of Anti-Inflammatory and Immunomodulatory Effects of *Premna integrifolia* Extracts and Assay-Guided Isolation of a COX-2/5-LOX Dual Inhibitor. *Fitoterapia, 131*: 189–199.

[5]. Subramani, C., Rajakkannu, A., Rathinam, A., Gaidhani, S., Raja, I. and Singh, D.V.K., (2017). Anti-Atherosclerotic Activity of Root Bark of *Premna integrifolia* Linn. In High Fat Diet Induced Atherosclerosis Model Rats. *Journal of Pharmaceutical Analysis, 7*: 123-128.

[6]. Habtemariam, S. and Varghese, G. K., (2015). A Novel Diterpene Skeleton: Identification of a Highly Aromatic, Cytotoxic and Antioxidant 5-Methyl-10-Demethyl-Abietane-Type Diterpene from *Premna serratifolia*. *Phytotherapy Research, 29*: 80–85.

[7]. Majumder, R., Akter, S., Naim, Z., Al-Amin, M.d. and Alam, M.d.B., (2014). Antioxidant and Anti-Diabetic Activities of the Methanolic Extract of *Premna integrifolia* Bark. *Advances in Biological Research, 8*(1): 29-36.

[8]. Jain, S., Singh, M., Barik, R. and Malviya N., (2013). *In-Vitro* Antioxidant Activity of *Premna integrifolia* Linn. Roots. *Research Journal of Pharmacology and Pharmacodynamics, 5*(5): 293-296.

[9]. Mali, P.Y., Bigoniya, P., Panchal, S.S. and Muchhandi, I.S., (2013). Anti-Obesity Activity of Chloroform Methanol Extract of *Premna integrifolia* in Mice Fed with Cafeteria Diet. *Journal of Pharmacy and Bioallied Sciences, 5*(3): 229-243.

[10]. Patel, M.J. and Patel, J.K., (2012). Evaluation of The Anti-Hyperlipidaemic Activity of *Premna integrifolia* on Nicotine Induced Hyperlipidaemia in Rats. *International Journal of Pharma and Bio Sciences, 3*(2): 226-232.

[11]. Mali, P.Y., (2014). Beneficial Effect of Extracts of *Premna integrifolia* Root on Human Leucocytes and

Erythrocytes Against Hydrogen Peroxide Induced Oxidative Damage. *Chronicles of Young Scientists, 5*(1): 53-58.
[12]. Singh, C., Prakash, C., Tiwari, K.N., Mishra, S.K. and Kumar, V., (2018). *Premna integrifolia* Ameliorates Cyclophosphamide-Induced Hepatotoxicity by Modulation of Oxidative Stress and Apoptosis. *Biomedicine & Pharmacotherapy, 107*: 634–643.
[13]. Singh, C., Prakash, C., Mishra, P., Tiwari, K.N., Mishra, S.K., More, R.S., Kumar, V. and Singh, J., (2019). Hepatoprotective Efficacy of *Premna integrifolia* L. Leaves Against Aflatoxin B1- Induced Toxicity in Mice. *Toxicon, 166*: 88-100.
[14]. Rajendran, R. and kumar, E.K., (2010). Anti-Arthritic Activity of *Premna serratifolia Linn.,* Wood Against Adjuvant Induced Arthritis. *Avicenna Journal of Medical Biotechnology, 2*(2): 101-106.
[15]. Win, N.N., Woo, S.Y., Ngwe, H., Prema., Wong, C.P., Ito, T., Okamoto, Y., Tanaka, M., Imagawa, H., Asakawa, Y., Abe, I. and Morita, H., (2018). Tetrahydrofuran Lignans: Melanogenesis Inhibitors from *Premna integrifolia* Wood Collected in Myanmar. *Fitoterapia, 127*: 308–313.
[16]. Bose, L.V., Varghese, G.K. and Habtemariam, S., (2013). Identification of Acteoside as The Active Antioxidant Principle of *Premna serratifolia* Root Wood Tissues. *Phytopharmacology, 4*(2): 228-236.
[17]. Yadav, D., Tiwari, N. and Gupta, M.M., (2010). Diterpenoids from *Premna integrifolia*. *Phytochemistry Letters, 3*: 143–147.
[18]. Hang, N.T.B., Ky, P.H., Minh, C.V., Cuong, N.X., Thao, N.P. and Kiem, P.V., (2008). Study on The Chemical Constituents of *Premna integrifolia* L. *Natural Product Communications, 3*(9): 1449-1452.
[19]. Singh, S., Gupta, A., Kumari, A. and Verma, R., (2019). Antimicrobial and Antioxidant Potential of *Hibiscus rosa-sinensis* L. in Western Himalaya. *Biological Forum- An International Journal. 11*(1): 35-40.
[20]. Willliams, W.B., Cuvelier, M.E. and Berset, C., (1995). Use of Free Radical Method to Evaluate Antioxidant Activity. *Lebensmittel-Wissenschaft and Technology, 28*: 25-30.
[21]. Upadhyay, R., Chaurasia, J. K., Tiwari, K. N. and Singh, K., (2014). Antioxidant Property of Aerial Parts and Root of *Phyllanthus fraternus* Webster, An Important Medicinal Plant. *The Scientific World Journal, 2014*: 1-7.
[22]. Chang, C.C., Yang, M.H., Wen, H.M. and Chern, J.C., (2002). Estimation of Total Flavonoid Content in Propolis by Two Complementary Colorimetric Methods. *Journal of Food and Drug Analysis, 10*(3): 178-182.
[23]. Commercial Solvents Corporation' 245 Park AVE., New York, N. Y. – 10017. TRIS NITRO® Brand of Tris (Hydroxymethyl) Nitronlethane.NP Series TDS No. 5 (1968).1-6.
[24]. McGinty, D., Letizia, C.S. and Api, A.M., (2011). Fragrance Material Review on Ethylene Brassylate. *Food and Chemical Toxicology, 49*: S174–S182.
[25]. Frater, G., Müller, U. and Kraft, P., (1999). Preparation and Olfactory Characterization of the Enantiomerically Pure Isomers of the Perfumery Synthetic Galaxolide. *Helvetica Chimica Acta, 82*: 1656-1665.
[26]. Scognamiglio, J., Letizia, C.S., Politano, V. T. and Api, A.M., (2013). Fragrance Material Review on Acetyl Cedrene. *Food and Chemical Toxicology, 62*: S152–S166.
[27]. Zhu, Li-Xia., Zhang, Meng-Meng., Liu, Zheng., Shi, Yin. and Duan, Chang-Qing., (2019). Levels of Furaneol in Msalais Wines: A Comprehensive Overview of Multiple Stages and Pathways of Its Formation During Msalais Winemaking. *Molecules. 24*: 1-12.
[28]. Fidyt, K., Fiedorowicz, A., Strzadała, L. and Szumny, A., (2016). β-Caryophyllene and β-Caryophyllene Oxide-Natural Compounds of Anticancer and Analgesic Properties. *Cancer Medicine, 5*(10): 3007-3017.
[29]. Stobiecka, A., (2019). A DFT Study on The Radical-Scavenging Properties of Ferruginol-Type Diterpenes. *Food Biophysics, 14:* 1–12.
[30]. Dahham, S.S., Tabana, Y.M., Iqbal, M.A., Ahamed, M.B.K., Ezzat, M.O., Majid, A.S.A. and A.M.S.A. Majid., (2015). The Anticancer, Antioxidant and Antimicrobial Properties of The Sesquiterpene β-Caryophyllene from The Essential Oil of *Aquilaria crassna. Molecules, 20*: 11808-11829.
[31]. Puri, R.S. and Pundir, A., (2019). Review on Ethanomedicinal plant: *Trillium govanianum* Wall. Ex D. Don. *International Journal of Theoretical and Applied Sciences. 11*(2): 04-09.
[32]. Graßmann, J., (2005). Terpenoids as Plant Antioxidants. *Vitamins and Hormones, 72*: 506-535.
[33]. Saini, R., Mishra, A. K., Mishra, S. K. and Tiwari, K.N., (2019). Evaluation of Polyphenolic Content and Anti Oxidant Potential of Poly Herbal Formulation. *International Journal on Emerging Technologies, 10*(2): 243-248.
[34]. Mohanraj, R., and Karuppusam, S., (2017) Preliminary Phytochemical Screening of an Endemic Plant *Nothopegia heyneana* Gamble (Anacardiaceae) in Southern India. *Bio Bulletin, 3*(2): 38-43.
[35]. Zolezzi, J.M., Lindsay, C.B., Serrano, F.G. and Ureta, R.C., (2018). Neuroprotective Effects of Ferruginol, Jatrophone and Junicedric Acid Against Amyloid-β Injury in Hippocampal Neurons. *Journal of Alzheimer 's Disease, 2020*: 1-20.
[36]. Barrero, A.F., Herrador, M.M., Arteaga, P., Arteaga, J.F. and Arteaga, A.F., (2012). Communic Acids: Occurrence, Properties and Use as Chirons for The Synthesis of Bioactive Compounds. *Molecules, 17*: 1448-1467.
[37]. Nielsen, C.K., Subbiahdoss, G., Zeng, G., Salmi, Z., Kjems, J., Mygind, T., Snabeand, T. and Meyer, R.L., (2017). Antibacterial Isoeugenol Coating on Stainless Steel and Polyethylene Surfaces Prevents Biofilm Growth. *Journal of Applied Microbiology, 124*: 179-187.
[38]. Premjanu, N. and Jaynthy, C., (2014). Antimicrobial Activity of Diethyl Phthalate: An Insilico Approach. *Asian Journal of Pharmaceutical and Clinical Research, 7*(4): 141-142.
[39]. Ashitania, T., Kusumotoa, N., Karlsonb, A.K.B., Fujitac, K. and Takahashia, k., (2013). Antitermite Activity of *β*-Caryophyllene Epoxide and Episulfi De. *Verlag Der Zeitschrift Für Naturforschung, Tübingen, 68*: 302–306.
[40]. Aparnal, V., Dileep, K. V., 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 Drug Design, 80*: 434–439.
[41]. Klingenren, B. V. and Han, M. T., (1976). Antibacterial Activity of Δ-Tetrhydrocannabinol. *Antonie Van Leeuwenhoek, 42*: 9-12.
[42]. Palaniyandi, T., Sivaji, A., Thiruganasambandam, R., Natarajan1, S. and Hari, R., (2018). *In Vitro* Antigastric Cancer Activity of Squalene, a Triterpenoid Compound Isolated from *Rhizophora mucronata* Mangrove Plant Leaves Against Ags Cell Line. *Magazine, 14*(57): S369- S376.
[43]. Yusuf, A.J., Abdullahi, M.I., Aleku, G.A., Ibrahim, I.A.A., Alebiosu, C.O., Yahaya, M., Adamu, H.W., *Pharmacognosy* Sanusi, A., Mailafiya, M.M. and Abubakar, H., (2018). Antimicrobial Activity of Stigmasterol from The Stem Bark of *Neocarya macrophylla. Journal of Medicinal Plants for Economic Development, 2*(1): 1-5.
[44]. Ali, H., Dixit, S., Ali, D., Alqahtani, S.M., Alkahtani, S. and Alarifi, S., (2015). Isolation and Evaluation of Anticancer Efficacy of Stigmasterol in a Mouse Model of DMBA-Induced Skin Carcinoma. *Drug Design, Development and Therapy, 9*: 2793–2800.

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