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Antioxidant Capacity and Lipophilic Constitution of *Alternanthera bettzickiana* Flower Extract

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ABSTRACT

Alternanthera bettzickiana (Regel) Nicolson is an erect herb and an ornamental pot plant, which is recorded to be useful in purifying and nourishing blood. It is also claimed to be a soft laxative, a galactagogue and an antipyretic in addition to its wound healing property. The leaves are reported to be used like spinach and in soups. The lipophilic acetone extract of the fresh flowers exhibited *in vitro* antioxidant/radical scavenging (ABTS$^{\bullet+}$ and FRAP assays) and metal (Ferrous ion) chelating capacities. The extract has been analysed to contain eighteen saturated and four unsaturated hydrocarbons, ten saturated, three monounsaturated and one polyunsaturated esters of fatty acid, in addition to a saturated and an unsaturated higher alcohol and a fatty aldehyde, together with a monoterpene and nine acyclic diterpenes and a steroid. γ-Tocopherol and α-tocopherol-β-D-mannoside are the principal vitamin E identified in combination with pairs of cyclohexenones and benzofuranones as well as five phthalates.

Key words: *Alternanthera bettzickiana*,*Amaranthaceae*,
Antioxidant capacity, Lipophilic constitution, phytometabolites.

INTRODUCTION

Alternanthera Forsk[1,2], belonging to the family *Amaranthaceae* and comprising of *ca.* 80 species[3], is a genus of evergreen, perennial herbs that are native to tropical and sub-tropical regions. The taxa occur abundantly in Australia and Tropical America and about nine of them are reported from South India[2]. The leaves of *A. sessilis* (L.) and *A. bettzickiana* (Regel) Nicolson are reported to be used like spinach and in soups[4]. *A. brasiliana*, *A. philoxeroides*, *A. pungens*, *A. sessilis* and *A. tenella* have been pharmacologically investigated[5-13] to exhibit antiviral (HSV-1 and HIV), antihistaminic, anticarcinogenic, antileukaemic, antiulcer, antihepatotoxic and diuretic activities. *A. philoxeroides* Griseb is being prescribed clinically in the People's Republic of China for the treatment of viral hepatitis, epidemic parotitis, hemorrhagic fever and influenza. *A. sessilis* (L.) R. Br. ex DC is

said to be recommended against fever and also used as a galactogogue. The stem and the leaves are claimed to help in snake-bite and the young shoots are reported to be nutritious[14].

A. bettzickiana (Regel) Nicolson (syn.*Telanthera bettzickiana* Regel[2], is an erect herb and an ornamental pot plant in houses and public gardens. The whole plant is reported to be useful in purifying and nourishing blood and is claimed to be a soft laxative, a galactagogue and an antipyretic, in addition to its wound healing property. The acetone extract has been found to possess lipoxygenase, tyrosinase and xanthine oxidase inhibitory activities[15]. Earlier studies have reported the characterisation of simple and acylated betacyanins from the leaves[16]. The recent understanding of the multiple roles of the diverse array of secondary metabolites (mediated by reduction, reactive species-scavenging and pro-oxidant metal chelation) by which they protect against the pathogenesis of a number of degenerative disorders[17,18], has resulted in viewing these food plants as functional foods. Hence, characterisation of the dietary antioxidants present in them and their capacities provide better insight into their functionality, as these dietary constituents are necessary to cope up with the initiation or propagation of the reactive oxidants[18]. In continuation of our investigations on the South Indian species of *Alternanthera*[19], the reactive species scavenging and pro-oxidant metal chelating capacities as well as the lipophilic constitution of the fresh flowers of *A. bettzickiana*, collected from the wild habitat, are reported in the present paper.

MATERIALS AND METHODS

Materials

Fresh flowers of *A. bettzickiana*, collected from the wild population were extracted using acetone (3x4 L, 48 h, ambient), after establishing the identity of the taxon, and concentrated under reduced pressure to get the lipophilic extract. 2,2'-Azino*bis*(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) diammonium salt, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), 3-(2-pyridyl)-5,6-di(4-phenylsulphonic acid)-1,2,4-triazine (ferrozine) sodium salt, ethanol, ferrous chloride and rutin were obtained from Sigma-Aldrich Inc. All other chemicals/reagents were of analytical/laboratory grades from Himedia/Merck/Loba Chemie. GC-MS was recorded using SHIMADZU QP2010.

METHODS

Determination of Vitamin C equivalent antioxidant capacity

ABTS radical cation ($ABTS^{\bullet+}$) scavenging capacity and ferric-reducing/antioxidant power (FRAP) of the lipophilic extract were determined by the procedures described previously[20] and expressed as Vitamin C equivalent antioxidant capacity (VCEAC). Vitamin C standard curves were constructed by plotting the absorbances of 1.25, 2.5, 5, 10, 15, 20, 25 mg/L of L-ascorbic acid against the corresponding concentrations. The VCEAC of the extract of increasing concentrations (50, 100, 200, 400 mg/L) and standard rutin (10 mg/L) were determined from the standard graph and expressed as percentage, as detailed before. All data were recorded as mean ± SD, computed from three replications.

Determination of Transition metal ion chelating capacity

Pro-oxidant metal chelating capacities of the lipophilic extract/standard were evaluated using Fe^{II} and the percentage inhibition of the ferrozine–Fe^{II} complex formation was calculated adopting the protocol described earlier[18,20], in triplicate. The ubiquitous flavonoid rutin was used as positive standards as before.

Separation and Identification of the lipophilic metabolites

GC-MS was recorded using a SHIMADZU QP2010 gas chromatographic system, equipped with a split injection port, a flame ionization detector and a GC-MS solution version 2.53 software. Column: 30.0 m × 0.25 mm, 0.25 ìm capillary column (100% Dimethylpolysiloxane) and carrier gas: He (99.9995% purity) at 1.50 mL/min with a split ratio of 10:1. Injector and detector temperatures maintained at 260°C. Oven temperature: initially at 70°C for 2 min. and increased to 300°C at a rate of 5°C/min. Mass spectra were recorded at 70eV with scan range of 40 – 1000 m/z. Interpretation of mass spectra were made using the databases of National Institute Standard and Technique (NIST08s), WILEY8 and FAME.

RESULTS AND DISCUSSION

Determination of *in vitro* antioxidant capacity

Oxidative stress is created when there is an imbalance between the generation of reactive species and their quenching. Oxidative stress due to high flux of oxidants has been implicated in the pathogenesis of several modern human ailments[20]. Antioxidant protection against damages that could be caused by free radicals is vital for the integrity of cellular structures and macromolecules. All plants synthesise a vast array of chemical compounds that are not necessarily involved in the plant's metabolism but instead serve a variety of functions that enhance the plant's survivability, including their ability to combat oxidative stress. A number of these bioactive exogenous dietary antioxidants have been demonstrated to be effective in preventing reactive species-mediated damages and the consequent chronic disease states[18,20]. Food industries are also concerned with oxidative processes since lipids, the natural constituents of cellular membranes, are oxidised during peroxidation, producing partial or total changes in food sensorial properties and in nutritional values. Plant-based antioxidants and colourants are the order of the day to preserve food quality because of safety concerns. In the recent past, there have been growing interests in functional foods, which not only offer the basic nutrition and energy, but also added physiological benefits to the consumers. The functionality of a food usually has a close relationship with some of its ingredients and those ingredients that could be derived from food/natural sources are preferred over synthetic ones, whose applications are restricted due to suspected harmful health effects. Characterisation of the dietary antioxidants and their capacities are also essential to validate the safety and traditional uses and to standardise preparations of these plants. As a result, widespread screening of medicinal and food plants with antioxidant potentials has become a common practice.

The acetone extract of *A.bettzickiana* has been recorded to possess lipoxygenase, tyrosinase and xanthine oxidase inhibitory activities[15]. Consequently, the fresh flowers of *A.bettzickiana* (Fig. 1) were extracted with acetone at ambient temperature for 48 h, concentrated at 40 ± 2°C to get the lipophilic extract. The antioxidant capacity has been evaluated based on the measurement of the capacities of increasing concentrations of the extract (i) to scavenge stable $ABTS^{\bullet+}$ radicals, (ii) to reduce Fe^{III} to Fe^{II} and (iii) to inhibit Fe^{II} -ferrozine complex formation. The most commonly employed assay of antioxidant capacity measurements is the one that involves the generation of the coloured radical cationic oxidant $ABTS^{\bullet+}$ and determining the ability of an extract/a metabolite to scavenge the same[21]. Since $ABTS^{\bullet+}$ is soluble in both aqueous and organic phases and is not affected by ionic strength, it is capable of reacting with both lipophilic and hydrophilic metabolites. The extract scavenged the radicals dose-dependently, even though it possessed only 57.43% of the VCEAC of the standard rutin at the highest concentration, namely 400 mg/L (Table 1). The FRAP assay also measured the antioxidant capacity to vary linearly with the concentrations and was found to have 47.8% of

Table 1. Reactive species scavenging and Ferrous ion chelating capacities of *A. bettzickiana* lipophilic flower extract

Analyte	Concentration (mg/L)	Relative Percentage[a]		
		VCEAC		Fe(II)
		ABTS	FRAP	Chelation
Lipophilic Extract	50	10.4 ± 0.3	8.6 ± 0.5	21.4 ± 0.7
	100	18.7 ± 0.8	14.4 ± 0.4	35.6 ± 1.2
	200	30.1 ± 1.6	25.2 ± 1.1	52.3 ± 2.2
	400	48.3 ± 1.5	38.5 ± 1.6	68.7 ± 0.8
Rutin	10	84.1 ± 2.4	80.6 ± 3.1	72.9 ± 1.6

[a]Mean ± standard deviation (n=3)

Table 2: Lipophilic constitution of *A. bettzickiana* flowers

Compound		#	Rt	Peak area
1	Tetradecane	5	10.580	4866768
2	Hexadecane	16	13.057	7492617
3	Octadecane	24	15.285	4946679
4	Eicosane	38	17.355	2821134
5	Tetracosane	48	21.155	900282
6	Pantacosane	64	26.467	14931855
7	Heptacosane	53	23.515	3198293
8	Octacosane	63	26.151	10826544
9	Nonacosane	59	24.931	9056357
10	Tetratriacontane	51	22.764	364711
11	5-Methyltetradecane	13	12.486	412731
12	3-Methyltetradecane	14	12.712	809771
13	3-Methylheptadecane	22	14.978	625702
14	2-Methylheptadecane	46	20.923	225445
15	5-Butylnonane	2	8.987	1187747
16	9-Ethyl-9-n-heptyloctadecane	55	23.966	316146
17	3,5,23-Trimethyltetracontane	9	11.828	2220585
18	3,5,24-Trimethyltetracontane	61	25.460	1115753
19	1-Tetradecene	4	10.481	1014929
20	1- Hexadecane	15	12.969	942787
21	1-Nonadecene	23	15.213	722763
22	1-Methyl-1-tetradecene	21	14.480	819147
23	Methyldodecanoate	10	12.154	1286351
24	Methylpalmitate	33	16.602	14065962
25	Methylstearate	42	18.662	1104505
26	Methylicosanoate	44	20.550	461677
27	Methylbehenate	49	22.233	1307049
28	Methyltricosanote	52	23.013	450734
29	Methyllignocerate	54	23.757	624791
30	Isopropylhexadecanoate	43	20.251	191806
31	Ethylpalmitate	37	17.296	1638189
32	Cyclohexylpalmitate	47	20.993	344521
33	Methylelaidate	40	18.414	1956366
34	Methyl-cis-12-octadecenoate	41	18.472	777620
35	Methylnervonate	56	24.012	369710
36	Methyllinoleate	39	18.358	5799387
37	2-Hexyl-1-octanol	3	10.189	1209413
38	(2E)-2-Tetradecen-1-ol	19	14.203	875559
39	1-Tetradecanal (Myristylaldehyde)	20	14.381	586873
40	1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane (1,8-Cineole)	7	11.384	562439
41	(2E,6E)-3,7,11-Trimethyl-2,6,10-dodecatrien-1-ol (Farnesol)	11	12.241	550559
42	3,7,11- Trimethylpentadecan-2-en-1-ol	30	16.163	1192539
43	3,6,10,14-Tetramethylpentadecan-2-en-1-ol	28	15.974	1196852
44	3,7,11,15-Tetramethylhexadecan-1-ol (Dihydrophytol)	17	13.927	388105
45	(2E)-3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Phytol)	31	16.261	1192198

46	3,7,11,15-Tetramethyl-1-hexadecen-3-ol (Isophytol)	34	16.846	10425228
47	3-Methylene-7,11,15-trimethyl-1-hexadecene (Neophytadiene)	26	15.714	3214724
48	(6E)-6,10-Dimethyl-5,9-undecadien-2-one (Geranyl acetone)	6	11.298	581180
49	6,10,14,-Trimethylpentadecan-2-one (Hexahydrofarnesyl acetone)	27	15.777	10829732
50	Ergost-7-en-3-ol	62	25.648	1731027
51	3-Methylphenol	1	6.108	24357443
52	2,3-Dimethylphenol	25	15.398	512614
53	³-Tocopherol	58	24.676	15142652
54	±-Tocopherol-²-D-mannoside	60	25.372	858467
55	(3E)-4-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-3-buten-2-one	8	11.771	2153452
56	2,4,4-Trimethyl-3-[(1E)-3-oxo-1-butenyl]-2-cyclohexen-1-one	18	13.975	442306
57	4,4,7a- Trimethyl-5,6,7,7a-tetrahydro-1-benzofuran -2-(4H)-one	12	12.384	2606569
58	3,6-Dimethyl-5,6,7,7a-tetrahydro-1-benzofuran-2-(4H)-one	45	20.837	1342797
59	1,2-Benzenedicarboxylic acid (Phthalic acid)	32	16.539	1645250
60	Dibutylphthalate	35	17.029	4126463
61	Diisobutylphthalate	29	16.054	1408793
62	Mono-2-ethylhexylphthalate	50	22.426	9851493
63	Butyl-2-ethylhexylphthalate	36	17.243	1373311

Peak number in the GC-Mass Spectrum (Fig. 1)

the rutin VCEAC at 400 mg/L (Table 1). The per cent Fe^{II} chelating capacities of the extract was found to be encouraging with 94.2% as efficient as the standard (Table 1). Considerable evidence has emerged from clinical studies to show that increases in cellular free iron concentrations have been associated with oxidative stress and that genetic and non-genetic iron misregulations in the brain contribute to neuronal death in certain neurodegenerative disorders[18]. Even mildly elevated iron levels have been linked to increased cardiovascular disease and cancer incidences in humans and hence should be maintained within the optimum level. Moreover, in chronic anaemia associated with iron overload such as thalassemia major, Fe-chelating therapy is the only method available for preventing early death, caused predominantly by myocardial and hepatic iron toxicity or to prevent endocrinal abnormalities like diabetes and hypothyroidism. Persuasive epidemiological evidences, today, have brought to light that regular intake of bioactive dietary phytometabolites promises a wide range of benefits, including the regulation of transition metals such as iron**.**

Separation and Identification of the lipophilic metabolites

The acetone extract of *A.bettzickiana* has been further subjected to GC-MS analysis (Fig. 2) to analyse its chemical composition. The phytometabolites, belonging to various chemical classes (Fig. 3 and 4), that have been identified from the lipophilic fraction are tabulated (Table 2). Eighteen saturated[1-18] and four unsaturated[19-22] hydrocarbons, ten saturated[23-32], three monounsaturated[33-35] and one polyunsaturated[36] esters of fatty acid, including a cyclohexyl derivative[32], in addition to a saturated[37] and an unsaturated[38] higher alcohol and a fatty aldehyde[39] have been identified. A monoterpene[40] and nine acyclic diterpenes[41-49], together with a phytosterol, ergost-7-en-3-ol (24-Methyl-5-β-cholest-7-en-3-β-ol) [50] were also resolved. Phytosterols are the

cholesterol homologues and it is reported that low-doses of phytosterol-supplementation has produced significantly lowered plasma total cholesterol[22]. Such lipid-lowering effect of phytosterols is claimed to be mediated by competitive inhibition of cholesterol absorption and by transcriptional induction of genes implicated in cholesterol metabolism in both enterocytes and hepatocytes. The reduced absorption stimulates LDL-receptor formation, which, in turn, increases the hepatic uptake of LDL and thus decreases LDL levels[23]. Though the biological and ecochemical functions of terpenes have not been fully investigated yet, plants generally produce volatile terpenes in order to attract specific insects for pollination, to protect the plants from herbivores that feed on these plants, and also play an important role as signal compounds and growth regulators. Less volatile but strongly bitter-tasting or toxic terpenes also act as antifeedants.

Fig. 1: *A. bettzickiana* twig with flowers

The most abundant class of bioactive dietary metabolites are the biophenols[24], which are the extremely important components of the human diet with both nutritional and medicinal benefits reported for animals and humans, mediated largely by their redox property, free radical scavenging capacity and the ability to mitigate oxidative stress-induced tissue damage associated with chronic diseases. They also exhibit a remarkably diverse range of bio-physicochemical properties that makes them rather unique and intriguing natural products. Among the scores of reasons for their ever-increasing recognition, not only by the scientific community but also by the general public, is their capacity to scavenge oxidatively generated free radicals. Tocochromanols[24], which are the lipid-soluble dietary antioxidants, that belong to vitamin E group are the interesting biophenols identified in the present study. γ-Tocopherol [53], is the major

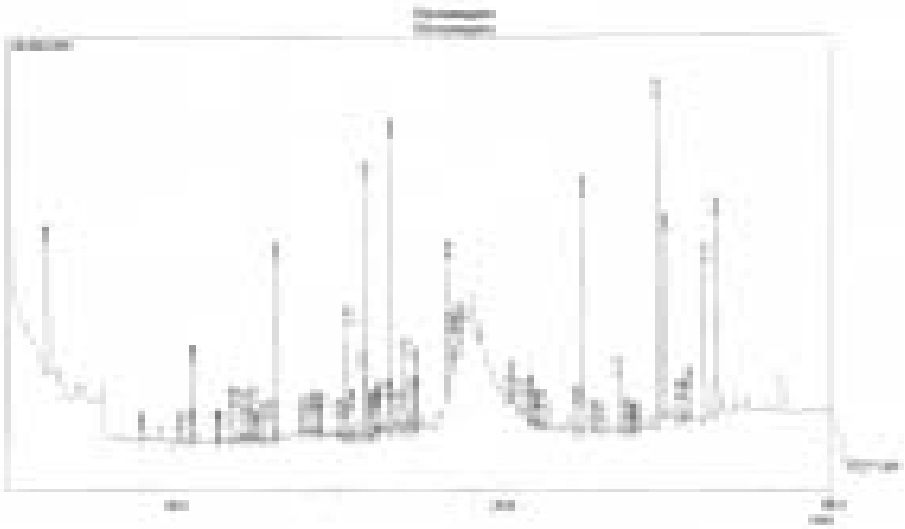

Fig. 2: Gas chromatogram of the lipophilic extract of *A. bettzickiana* flowers

tocopherol in circulation and has been found to be an unique antioxidant that protects cells from damages associated with nitrogen-based oxidants[23] γ-tocopherol is also reported to act as an antiinflammatory agent and may, therefore, reduce long-term damages to cells. This vitamin E component is found to co-exist with its analogue, α-tocopherol-β-D-mannoside [54]. Their antioxidant

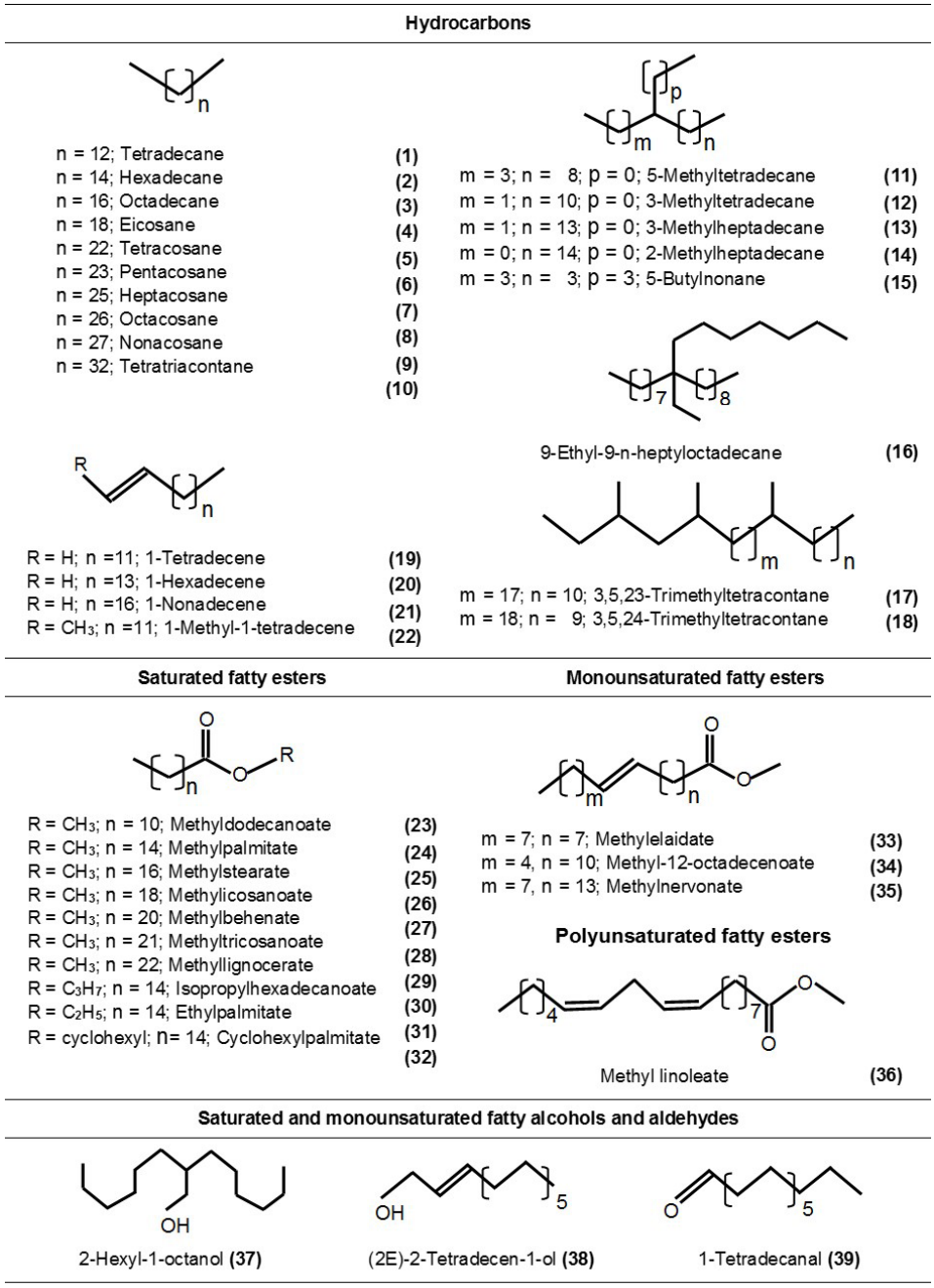

Fig. 3: Certain lipophilic classes of metabolites identified

activity has been attributed to the capacity of their heterocyclic chromanol ring system to donate the phenolic hydrogen to lipid free radicals[24]. The other classes of phytometabolites identified include the pairs of cyclohexenones [55-56], and benzofuranones [57-58] as well as the five phthalates [59-63].

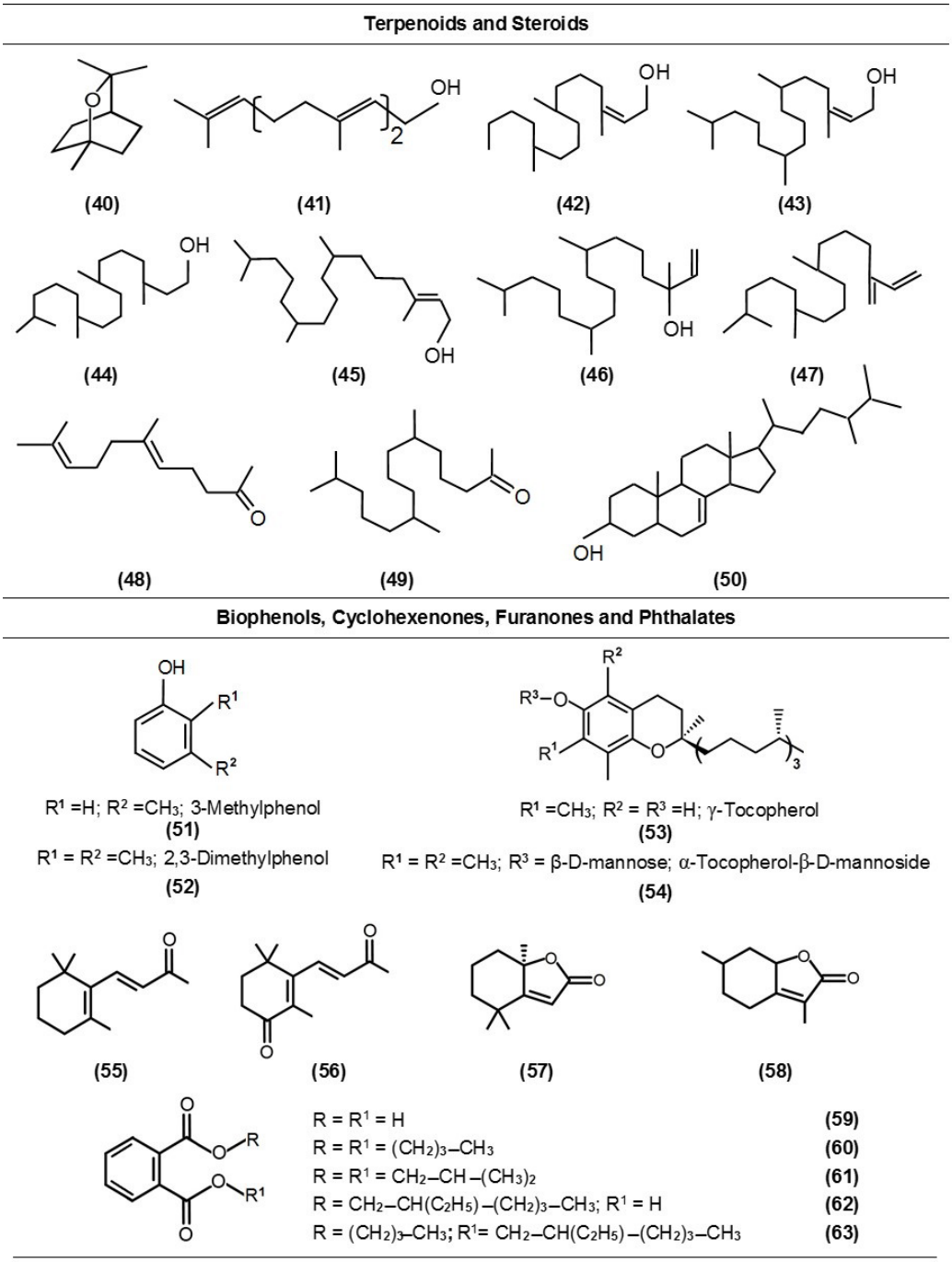

Fig. 4: Biologically significant classes of metabolites identified

CONCLUSION

Studies of the recent past decades have substantiated that increased consumption of wild sources of fruits and vegetables reduce the risk of chronic diseases including cardio- and cerebro-vascular diseases, certain forms of cancer, hypertension, type 2 diabetes and stroke, world-wide. The protection is due, largely, to the plethora of bioactive metabolites, both nutritive and non-nutritive, biosynthesised by these food plants. Consequently, the focus of nutrition research, today, is heading towards the concept of 'Preventive Medicine', and experts have predicted that nutrition will become the primary and the only accessible and the most affordable treatment modality in the 21st century. Hence, as a part of the continuing exercise, we have analysed and reported the antioxidant capacity and the composition of the lipophilic extract of the fresh flowers of the functional food plant, *A. bettzickiana*.

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