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Phytochemical and GC-HRMS analysis of active compounds of methanol extract of whole plant of *Basella alba* and their *in vitro* antioxidant activities

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Abstract
Herbal medicines are getting more attention as conventional therapies showing resistance and other side effects. The plant *Basella Alba* is a leaf vegetable and traditionally used for different medicinal purpose. We investigated methanol extract of whole plant of *Basella alba* (MBA) for the presence of phytochemicals and antioxidant potential by *in vitro* methods. On phytochemical analysis, flavonoids, cardiac glycosides, phenols, saponins and diterpenes were detected. GC-HRMS analysis indicated the presence of the compounds belonging to class of pyrans, phytol, flavonoids, phenols, glycerides, esters, fatty acids, palmitic acid, diterpens and sterols. MBA showed significant ($p < 0.05$) concentration dependant DPPH, superoxide and nitric oxide free radical scavenging activity as well as total antioxidant activity. The half maximum inhibitory concentrations (IC_{50}) obtained for DPPH, superoxide and nitric oxide free radical scavenging assays were 22.51 ± 7.81, 105.54 ± 8.53 and 43.123 ± 7.36 µg/mL respectively. Also significant ($p < 0.05$) increase in absorbance with increase in concentrations was observed during total antioxidant assay. Therefore, the present study designated a dose dependent antioxidant potential of methanol extract of whole plant of *B. alba.*

Keywords: *Basella alba*, antioxidant, GC-HRMS, DPPH, superoxide, nitric oxide

1. Introduction
Traditional medicinal plants have been a great source of potential antioxidants with free radicals scavenging activity. Phytochemicals like phenols and flavonoids produced as secondary metabolites by these plants play an important role by reducing oxidative stress in biological systems (Acker *et al*., 1996; Nabavi *et al.*, 2008) [26, 12]. The plant *Basella alba* belongs to the family Basellaceae and commonly it is known Malabar Spinach. It is found abundantly in tropical Asia and Africa where it is widely used as a leaf vegetable. It is a fast-growing, soft-stemmed vine, reaching up to 10 m (33ft) in length. This plant has been reported for its antimicrobial, anti-inflammatory, cytotoxic, anticonvulsant, antioxidant and various other medicinal properties by local tribes as well as researchers in recent years (Anandarajagopal *et al*., 2011; Kumar *et al*., 2011; Premakumari *et al*., 2010; Sushila *et al*., 2010) [1, 8, 15, 22]. Hence, present study was aimed to identify the active phytochemicals in *B. alba* plant and investigation of antioxidant potential by *in vitro* methods.

2. Materials and Methods
2.1 Chemicals
The chemicals used for the study *viz*., 1,1-Diphenyl-2- picryl- hydrazyl (DPPH), phosphate buffer, nitroblue tetrazolium (NBT), nicotinamide adenine dinucleotide (NADH), phenazine meth sulphate (PMS), sodium nitroprusside, Griess Reagent (sulphanilamide, orthophosphoric acid, naphthyl ethylene diamine hydrochloride), vitamin C, sulphuric acid, sodium phosphate and ammonium molybdate were of analytical grade.

2.2 Collection of plant material and authentication
The whole plant of *Basella Alba* (Vallicheera) was collected from Vashi market area, Mumbai, India. The plant material was taxonomically identified and authenticated by Botanical Survey of India, Southern Regional Centre, T.N.A.U. Campus, Coimbatore, Tamil Nadu, India as Collection No. BIS/SRC/5/23/2019/Tech./2932.Voucher specimens of the plant has been deposited at the Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India (HERB/VPT/CVASMTY/1/2018).

Fig 1: Basella Alba (Vallicheera)

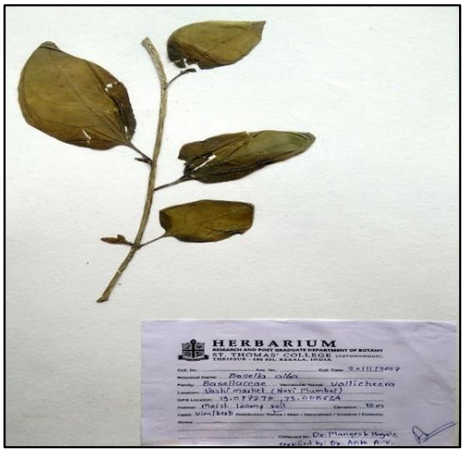

Fig 2: *Herbarium* of Basella Alba

2.3 Methanol extraction

The whole plant of *B. Alba* was air dried at room temperature and coarsely powdered using an electric pulverizer. The powder obtained was extracted by a Soxhlet apparatus using methanol at 55º C. A rotary vacuum evaporator was used to concentrate the methanol extract, under reduced pressure and temperature (55º C). After complete evaporation of the solvent it was kept under refrigeration in airtight container.

2.4 Qualitative phytochemical analysis

The methanol extract of whole plant of *B. Alba* (MBA) was tested for the presence of various active phytoconstituents namely steroids, alkaloids, tannins, phenolic compounds, flavonoids, diterpenes, triterpenes and saponins (Harborne, 1998) [6].

2.5 Gas chromatography high resolution mass spectroscopy (GC-HRMS) analysis of MBA.

The active phytochemical principles of MBA were analysed using GC-HRMS system of Sophisticated Analytical Instrument Facility (SAIF), Indian Institute of Technology (IIT), Bombay, Maharashtra, India. Gas Chromatography (Agilent, USA, 7890) with FID detector, EI/CI source and time of flight analyser with a mass range of 10- 2000 amu and a mass resolution of 6000 was used. Helium was used as the carrier gas at flow rate of 1 mL/ min. The oven temperature was maintained at 70º C for 1 min and then increased to 200º C in 5 min. The injector temperature was 250 ºC and total analysis time was 50 minutes. Extracts aliquots of 1 µL were injected into the chromatographic column (capillary column, length - 30m, inner diameter: 0.25mm, film thickness: 0.25µm) after a clear baseline had been obtained. Major constituents were identified by using mass spectrum library (NIST MS search 2.0 library) (Muthukrishnan *et al*., 2016) [11].

2.6 DPPH free radical scavenging activity

1, 1-Diphenyl-2- picryl- hydrazyl (DPPH) radical scavenging activity was measured using the method of Cotelle *et al*. (1996) [4] with some modifications. To 3 mL of the reaction mixture containing 2.4 mL of DPPH (100µM in methanol), 1.6 mL of test solution at 3.9, 7.81, 15.625, 31.25, 62.5, 125, 250 and 500 µg/mL concentrations of MBA was incubated at 37º C for 30 min and absorbance of the resulting solution was measured at 517 nm using UV/VIS/NIR Spectrophotometer (Lambda 750, Perkin Elmer, Singapore). Ascorbic acid was used as the reference standard. The percentage inhibition of DPPH radical was calculated by comparing the absorbance of the test with those of control (not treated with extract) using the following equation:

Per cent inhibition = (1- AT/AC) x 100

AC= Absorbance of control, AT= Absorbance of extracts/ standard

2.7 Superoxide anion free radical scavenging activity

Superoxide anion scavenging activity was measured according to the method of Robak and Gryglewski (1988) [18] with some modifications. All the solutions were prepared in 100 mM phosphate buffer (pH 7.4). To 1 mL of nitroblue tetrazolium (NBT, 156 µM), 1 mL of reduced nicotinamide adenine dinucleotide (NADH, 468 µM) and 3 mL of test solution at 3.9, 7.81, 15.625, 31.25, 62.5, 125, 250 and 500 µg/mL concentrations of MBA was mixed. The reaction was initiated by adding 100 µL of phenazine methosulphate (PMS, 60µM). The reaction mixture was incubated at 25º C for 5 min followed by measurement of absorbance at 560 nm using UV/VIS/NIR Spectrophotometer (Lambda 750, Perkin Elmer, Singapore). Ascorbic acid was used as the reference standard. The per cent of inhibition was calculated by using the equation

Per cent inhibition = (1- AT/AC) x 100

AC= Absorbance of control, AT= Absorbance of extracts/ standard

2.8 Nitric oxide anion free radical scavenging activity

The nitric oxide scavenging activity of MBA was measured according to modified method of Sreejayan and Rao (1997). To 2 mL of different concentrations of extract (0.95, 1.95, 3.9, 7.81, 15.625, 31.25, 62.5 and 125 µg/mL), 0.5 mL of 5mM sodium nitroprusside solution in phosphate buffered saline (PBS) (pH 7.4) was added and incubated for 2 hours at room temperature. After incubation, added 1.2 mL of Griess Reagent (equal volume of one per cent sulphanilamide in 5 per cent orthophosphoric acid and 0.1 per cent naphthyl ethylene diamine dihydrochloride in distilled water) to the reaction mixture. The absorbance was read at 546 nm against PBS blank and compared with vitamin C standard using UV/VIS/NIR Spectrophotometer (Lambda 750, Perkin Elmer, Singapore).

Nitric oxide scavenging activity (per cent) = (1-AT/ AC) x 100

AC= Absorbance of control, AT= Absorbance of extracts/ standard

2.9 Total antioxidant activity

The total antioxidant activity (TAA) was measured by modified method of Preito *et al*. (1999). 0.3 mL of extract (3.9, 7.81, 15.625, 31.25, 62.5, 125, 250 and 500 µg/mL concentrations of MBA was combined with 3 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing reaction solution were incubated at 95ºC for 90 min. The absorbance of solution was measured at 695 nm using UV/VIS/NIR Spectrophotometer (Lambda 750, Perkin Elmer, Singapore) against blank after cooling at room temperature. Ascorbic acid was used as the reference standard.

2.10 Statistical analysis

All antioxidant assays were triplicated and the data of was expressed as mean ± SE. All the statistical analysis was conducted using SPSS software version 21.0. Analysis of variance (ANOVA) in a completely randomized design and Duncan's multiple range tests were used to compare any significant differences between extract concentrations. Independent sample t-test was used to compare significant differences between standard vitamin C and MBA activity. The Half Maximal Inhibitory Concentration (IC_{50}) values of methanol extract of *A. hirsutus* were calculated using the online software "Very Simple IC_{50} Tool Kit".

3. Results

3.1 Extract preparation

The yield obtained from methanol extract of *B. alba* whole plant was four per cent with reference to dry starting material.

3.2 Qualitative phytochemical analysis

The methanol extract of *B. alba* detected flavonoids, cardiac glycosides, phenols, saponins and diterpenes upon analysing phytochemical constituents using biochemical tests

Table 1: Phytochemical analysis of methanol extracts of *A. hirsutus*

Test	*MBA*
Steroids	-
Salkowski's test	
Alkaloids	
Dragendorff's test	+
Mayer's test	-
Wagner's test	-
Hager's test	-
Glycosides	
Sodium hydroxide test	+
Benedict's test	-
Cardiac glycosides	
Killer Killiani test	+
Tannins	
Ferric chloride test	+
Gelatin test	-
Flavonoids	
Lead Acetate test	+
Ferric chloride test	+
Diterpene detection test	+
Triterpenes	
Salkowski's test	-
Liberman B.T.	+
Saponins	
Foam test	+
Phenolic compounds	+

3.3 GC-HRMS analysis of MBA

Chromatograms obtained on phytochemical analysis of MBA using GC-HRMS are given in Figure 03. Phytoconstituents obtained on GC-HRMS analysis of MBA are listed in table 02.

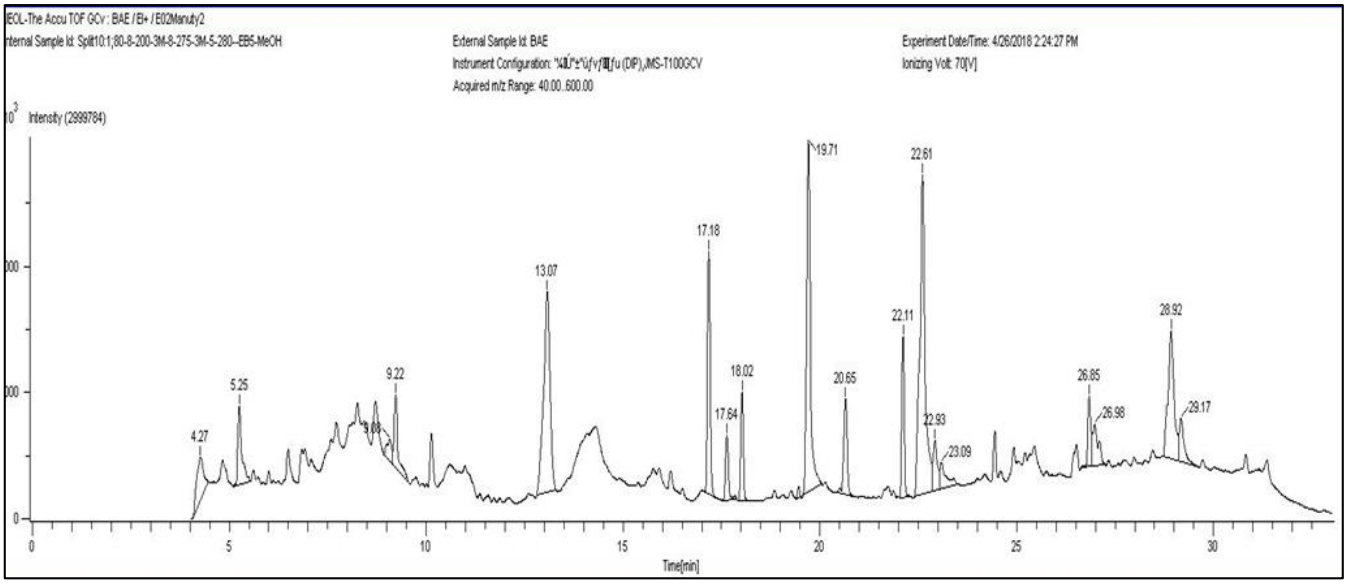

Fig 3: Gas chromatography- high resolution mass spectroscopy (GC-HRMS) chromatogram of MBA

Table 2: Gas chromatography- high resolution mass spectroscopy (GC-HRMS) analysis of phytochemicals in MBA.

Sl No.	RT(min)	Name of compound	Molecular formula	MW (g/mole)	Class	Probability %
1	5.24	2-Pyrrolidinone	C_4H_7NO	85	Pyrrolidone	51
2	6.49	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP)	$C_6H_8O_4$	144	Flavonoid fraction	75.9
3	7.68	6-Acetyl-β-d-mannose	$C_8H_{14}O_7$	222	Amino sugars	16.3
4	7.95	Acetin	$C_5H_{10}O_4$	134	Glyceride	35.8

5	9.2	2-methoxy, 4-vinylphenol	$C_9H_{10}O_2$	150	Phenol	35
6	10.1	Methyl pyroglutamate	$C_6H_9NO_3$	143	Amino acid ester	23.7
7	10.19	2-Propyl phenol	$C_9H_{12}O$	136	Phenol	46.3
8	13.6	3-Deoxy-d-mannoic-lactone	$C_6H_{10}O_5$	162	Cyclic ester	56.4
9	13.08	Levoglucosan	$C_6H_{10}O_5$	162	Hexoses	23.3
10	17.64	Phytol	$C_{20}H_{40}0$	296	Diterpens	32.6
11	19.71	Palmitic acid	$C_{16}H_{32}O_2$	256	Fatty acid	78.7
12	20.65	β-Carbolines	$C_{11}H_8N_2$	168	β-Carbolines	61.5
13	22.11	Phytol	$C_{20}H_{40}O$	296	Diterpens	57.6
14	22.61	Lenolenic acid	$C_{18}H_{30}O_2$	278	Lenolenic acid	15.8
15	24.44	Octanoic acid,2-dimethylaminoethyl-ester	$C_{12}H_{25}NO_2$	215	Fatty acid	24.5
16	25.45	2-Cyclohexylpiperidine	$C_{11}H_{21}N$	167	Piperidines	12.5
17	26.85	Palmitic acid β-monoglyceride	$C_{19}H_{38}$	330	Palmitic acid	50.4
18	26.98	Hexadecanoic acid, 3-[(trimethylsilyl)oxy] propyl ester	$C_{22}H_{46}O_3i$	386	Glyceride	22.5
19	28.92	Cholesterol	$C_{27}H_{46}O$	386	Sterol	38.6
20	29.17	Hexadecanoic acid,(3-bromoprop-2-ynyl)ester	$C_{19}H_{33}BrO_2$	372	Glyceride	39.4

3.4 DPPH radical scavenging potential

The per cent inhibition of DPPH radical by MBA at concentrations ranging from 3.9 to 500 µg/mL are presented in table 03. There was rise in percent inhibition of DPPH free radical for *B. alba* presenting significant ($p < 0.05$) concentration dependent DPPH radical scavenging potential which was significantly lower than that of vitamin C. The Inhibitory Concentration 50 (IC_{50}) calculated for MBA was found to be 22.51 ± 7.81 µg/mL.

Table 3: DPPH free radical scavenging assay: the per cent inhibition of DPPH free radical by MBA.

Concentrations (µg/mL)	% inhibition of DPPH free radical	
	MBA	Vitamin C standard
3.9	29.56 ± 1.27^{aA}	49.05 ± 4.30^{aB}
7.81	33.81 ± 1.44^{aA}	52.55 ± 2.94^{aB}
15.625	36.51 ± 1.42^{aA}	63.96 ± 1.55^{aB}
31.25	48.12 ± 1.36^{bA}	74.43 ± 2.27^{bB}
62.5	50.09 ± 1.41^{bA}	75.66 ± 2.64^{bB}
125	54.08 ± 1.00^{cA}	76.81 ± 2.71^{bB}
250	56.54 ± 1.50^{dA}	77.22 ± 3.30^{bB}
500	58.87 ± 0.93^{eA}	78.00 ± 2.80^{bB}
IC_{50}	22.51 ± 7.81^{A}	14.65 ± 0.64^{A}

Values are expressed as mean ± SE (n = 6) Means bearing the different superscript (a - d in column, A-B in rows) vary significantly at $p < 0.05$.

3.5 Superoxide anion free radical scavenging activity

The per cent superoxide radical inhibition by MBA at concentrations ranging from 3.9 to 500 µg/mL are presented in table 04. *B. alba* showed a significant ($p < 0.05$) concentration dependent superoxide free radical scavenging activity which indicated no significant difference ($p > 0.05$) with vitamin C at higher concentrations. The IC_{50} calculated for MBA was 105.54 ± 8.53 µg/mL and it was significantly higher ($p < 0.05$) than that of vitamin C IC_{50} at 4.13±5.73µg/mL.

Table 4: Superoxide anion free radical scavenging assay: the per cent inhibition of superoxide anion radical by MBA.

Concentrations (µg/mL)	% inhibition of superoxide anion free radical	
	MBA	Vitamin C standard
3.9	24.14 ± 2.47^{aB}	72.78 ± 2.48^{aA}
7.81	28.70 ± 1.64^{aB}	73.86 ± 2.53^{aA}
15.625	46.77 ± 1.67^{bB}	74.62 ± 2.43^{aA}
31.25	62.03 ± 1.51^{cC}	75.61 ± 2.37^{aB}
62.5	72.10 ± 1.43^{dB}	75.66 ± 2.35^{aB}
125	78.09 ± 1.63^{eB}	76.08 ± 1.98^{aB}
250	79.60 ± 1.84^{eB}	76.10 ± 2.00^{aB}
500	80.59 ± 2.28^{eB}	76.37 ± 2.74^{aB}
IC_{50}	105.54 ± 8.53^{A}	4.13 ± 5.73^{B}

Values are expressed as mean ± SE (n = 6) Means bearing the different superscript (a - b in column, A-B in rows) vary significantly at $p < 0.05$.

3.6 Nitric oxide anion free radical scavenging activity

The per cent nitric oxide radical inhibition by MBA at concentrations ranging from 0.95 to 125 µg/mL are presented in table 05. *B. alba* exhibited significant ($p < 0.05$) concentration dependent nitric oxide free radical scavenging potential which was significantly ($p < 0.05$) lower than that of vitamin C. The IC_{50} calculated for MBA was found to be 43.123 ± 7.36 µg/mL and had no significance ($p > 0.05$) with

the IC_{50} calculated for vitamin C which was 53.22 ±9.77 µg/mL.

Table 5: Nitric oxide anion free radical scavenging assay: the per cent inhibition of nitric oxide anion radical by MBA.

Concentrations (µg/mL)	% inhibition of Nitric oxide anion free radical	
	MBA	Vitamin C standard
0.95	17.36 ± 4.26^{aB}	23.84 ± 4.49^{aA}
1.95	22.39 ± 3.54^{bB}	30.83 ± 3.55^{bA}
3.9	30.85 ± 3.55^{cB}	39.10 ± 3.07^{cA}
7.81	36.91 ± 3.39^{B}	45.37 ± 2.85^{dA}
15.625	37.37 ± 3.40^{cB}	46.78 ± 2.60^{dA}
31.25	38.28 ± 3.62^{cB}	48.73 ± 2.80^{dA}
62.5	37.67 ± 3.79^{cB}	47.90 ± 2.85^{dA}
125	40.81 ± 3.09^{cB}	49.95 ± 2.82^{dA}
IC_{50}	43.123 ± 7.36^{A}	53.22 ± 9.77^{A}

Values are expressed as mean ± SE (n = 6) Means bearing the different superscript (a - d in column, A-B in rows) vary significantly at $p < 0.05$.

3.6 Total antioxidant assay

The absorbance values obtained on total antioxidant assay of MBA at concentrations ranging from 3.9 to 500 µg/mL are presented in table 06. There was significant ($p < 0.05$) increase in absorbance with increase in concentrations for *B. alba* and vitamin C had non-significant rise in absorbance with increase in concentrations. MBA exhibited significantly lower activity in total antioxidant assay compared to vitamin C.

Table 6: Total antioxidant assay: the absorbance of MBA on total antioxidant assay.

Concentrations (µg/mL)	Absorbance	
	MBA	Vitamin C standard
3.9	0.058 ± 0.001^{aA}	0.103 ± 0.008^{aB}
7.81	0.059 ± 0.001^{aA}	0.138 ± 0.013^{aB}
15.625	0.060 ± 0.001^{aA}	0.203 ± 0.029^{aB}
31.25	0.063 ± 0.002^{aA}	0.383 ± 0.062^{aB}
62.5	0.073 ± 0.004^{bA}	0.732 ± 0.138^{bB}
125	0.080 ± 0.003^{cA}	1.339 ± 0.224^{bB}
250	0.104 ± 0.004^{dA}	3.000 ± 0.585^{cB}
500	0.243 ± 0.023^{eA}	6.061 ± 0.768^{dB}

Values are expressed as mean ± SE (n = 6) Means bearing the different superscript (a - e in column, A-B in rows) vary significantly at $p < 0.05$.

4. Discussion

Antioxidants protects various biological systems against oxidative stress produced by reactive oxygen species (ROS) or free radicals. Reactive oxygen species are the products of normal aerobic cellular metabolism produced by polymorphonuclear leukocytes, macrophages and peroxisomes internally, while exogenous sources include tobacco smoke, organic solvents, certain pollutants and pesticides. Small amount of ROS leads to detrimental changes in cell function via many alterations like, lipid peroxidation, oxidative DNA damage and enzyme inactivation. This oxidative damage is responsible for diseases like cancer, rheumatoid arthritis, ulcerative colitis, cardiovascular and neurological degenerative diseases (Buettner, 1993; Halliwell, 1999; Mak *et al*., 1983) [3, 5, 10].

In the present investigation, methanol extract of *B. alba* whole plant (MBA) showed presence of flavonoids, cardiac glycosides, phenols, saponins and diterpenes on qualitative phytochemical analysis. Similar results showing presence of flavonoids, terpenoids, fixed oils and fats, mucilage, carbohydrates, proteins, steroids and alkaloids in methanol extract of *B. alba* aerial part were obtained by Anandarajagopal *et al*. (2011) [1]. Reshmi *et al*. (2012) [17] found presence of phenols and flavonoids in *B. alba* fruit extract which were responsible for strong antioxidant activity.

Methanol extract of *B. alba* on GC-HRMS analysis revealed 20 compounds out of which 2-pyrrolidinone; 2,3-dihydro-3,5-dihydroxy-6-methyl-4H- pyran-4-one (DDMP); acetin; 2-methoxy, 4-vinylphenol; 2-propyl phenol; 3-deoxy-d-mannoic-lactone; β-carbolines; phytol; palmitic acid; β-monoglyceride; cholesterol and hexadecanoic acid,(3-bromoprop-2-ynyl)ester were major. Most of them belongs to class of pyrans, phytol, flavonoids, phenols, glycerides, esters, fatty acids, palmitic acid, diterpens and sterols. Liu (2015) performed GC-MS analysis of *B. Alba* seeds oil and identified 19 compounds including hexadecanoic acid, linoleic acid, palmitic acid and stearic acid. Baskaran *et al*. (2015) [2] also found similar results on GC-MS analysis of *B. alba* leaves extract.

Vadivel and Gopalakrishnan (2011) [25] stated that 2-methoxy-4-vinylphenol, a phenolic compound was having antimicrobial, antioxidant, anti-inflammatory and analgesic activity. Authors also stated that 2, 3-dihydro-3, 5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) had antimicrobial and anti-inflammatory activity. Thangam *et al*. (2013) [24] stated that 2-pyrrolidinone has potent anticancer effect. Phytol has shown potent anticancer effect via induction of apoptosis by ROS production (Thakor *et al*., 2017) [23]. Zhang *et al*. (2004) [27] stated that palmitic acid had anticancer effect. Bivalent β-carbolines had shown potent cytotoxic effect against cancer cell lies (Shi *et al*., 2013) [20]. In present study, MBA also showed presence of 2-methoxy-4-vinylphenol, DDMP, 2-pyrrolidinone, phytol, palmitic acid, β-carbolines compounds which might be responsible for anticancer and antioxidant activity.

In DPPH scavenging assay, DPPH is reduced to respective stable hydrazine by hydrogen atom supplied from antioxidant and the reaction is indicated by colour change from purple to yellow. Phadungkit *et al.* (2012) [13] conducted DPPH assay of *B. alba* Lin. ethanol extract and found IC_{50} value 102.99 ± 4.37 µg/mL at dose ranging from 50 to 1000 µg/mL against ascorbic acid standard. Author also stated that phenols found on phytochemical screening were responsible for antioxidant activity.

Reshmi *et al*. (2012) [17] studied antioxidant activity of betacyanin, extracted from *B. alba* fruit after acidified methanol treatment. Betacyanin significantly inhibited DPPH radical activity with IC_{50} values 60.82 mg/mL. The DPPH radical scavenging effect at 1, 10 and 50 mg/mL was 19.1, 45 and 54 per cent respectively. Authors also stated that presence of phenols and flavonoids in *B. Alba* fruit extract were responsible for strong antioxidant activity. Similarly in present study, there was rise in per cent inhibition for MBA with significant ($p < 0.05$) concentration dependent increase in DPPH radical scavenging activity which was significantly lower than that of vitamin C standard. The Inhibitory Concentration 50 (IC_{50}) calculated was 22.51 ± 7.81 µg/mL whereas the IC_{50} calculated for vitamin C standard was 14.65± 0.64 µg/mL.

Superoxide radicals produced from dissolved oxygen by phenazine methosulphate (PMS) - nicotinamide adenine dinucleotide (NADH) coupling can be measured with their

power to reduce nitroblue tetrazolium (NBT). Reaction shows colour change on consumption of superoxide anion and superoxide radicals scavenging activity is measured by Spectrophotometer. Utilization of antioxidant by superoxide decreases absorbance depending on its concentration. In present study, at higher concentrations MBA showed no significant difference ($p > 0.05$) with vitamin C superoxide free radical scavenging activity. The IC_{50} calculated for MBA was 105.54 ± 8.53 µg/mL whereas the IC_{50} calculated for vitamin C standard was 4.13 ± 5.73µg/mL. Results were in accordance with Sasikumar and Kalaisezhiyen (2014) who achieved superoxide radical scavenging activity for methanol extract of *Kedrostis foetidissima* with IC_{50} value 3.2 mg/mL for concentrations ranging from 2-10 mg/mL.

Nitric oxide has a many important physiological effects such as neural signal transduction, control of blood pressure, platelet function, antitumor and antimicrobial activity. Sodium nitroprusside at physiological pH in aqueous solution abruptly generates nitric oxide which react with oxygen to produce nitrite ions which can be measured using Griess reagent. Nitric oxide scavengers compete with oxygen, resulting into reduced nitrite ions production. *B. alba* exhibited significant concentration dependent nitric oxide free radical scavenging potential comparable to that of vitamin C standard. The IC_{50} calculated for MBA was 43.123 ± 7.36 µg/mL whereas the IC_{50} calculated for vitamin C standard was 53.22 ±9.77 µg/mL. Results were agreeable with Reni, (2018) who acquired marked NO scavenging activity of methanol extracts of *E. variegata* and *M. pudica* with IC_{50} values 28.14 ± 3.42 and 23.05 ± 8.37 µg/mL respectively against ascorbic acid standard (IC_{50} value: 76.27 ± 3.93 µg/mL)

The total antioxidant capacity is assessed by assay depending on the reduction of Molybdenum Mo (VI) to Mo (V) by antioxidants and production of green coloured phosphate Mo (V) complex at acidic pH. This complex's intensity is calculated using spectrophotometer at 695 nm. In present study MBA showed significant ($p < 0.05$) increase in absorbance with increase in concentrations indicating potent total antioxidant activity against vitamin C standard. Results were in accordance with Islam *et al*. (2013) [7] who obtained strong concentration dependant increase in absorbance for methanol extract of *Syzygium fruticosum* (Roxb.).

5. Conclusion

The results of the present study confirmed the *in vitro* antioxidant activity of the methanol extract of whole plant of *Basella alba* using DPPH, superoxide, nitric oxide (NO) free radical scavenging assays and total antioxidant assay. The presence of flavonoids, phenols and diterepens in GC-HRMS analysis and in phytochemical screening are indicative of their possible role in antioxidant activity.

6. Acknowledgements

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