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ORIGINAL ARTICLE

Phytochemical profile and free radical nitric oxide (NO) scavenging activity of *Averrhoa bilimbi* L. fruit extract

Jagadish Kumar Suluvoy[1] · V. M. Berlin Grace[1]

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Abstract *Averrhoa bilimbi* L. belongs to family Oxalidaceae. Traditionally, people use this plant (root, bark, leaves and fruits) for treating several illnesses include itches, boils, syphilis, whooping cough, hypertension, fever and inflammation. The aim of the study was to evaluate the nitric oxide (NO) scavenging activity and GC–MS analysis of *A. bilimbi* L. fruit extract. *Averrhoa bilimbi* L. fruits were collected for the preliminary phytochemical analysis, antioxidant scavenging activity and biologically important compounds were identified by GC–MS analysis. The preliminary phytochemicals, GC–MS, total phenolic content and NO scavenging activity of the plant were analysed. In the present investigation, the *A. bilimbi* L. fruit extract has major phytochemicals. Among the 151 compounds identified in GC–MS, 15 compounds are found to have diverse biological activity. We also observed that the *A. bilimbi* L. fruit extract has high level of total phenolic compounds at a concentration of 209.25 GAE mg/g. Presence of phenolic compound apparently explains the antioxidant activity of the plant. Antioxidant activity of *A. bilimbi* L. fruit extract is proven from its high level of NO scavenging activity of potent IC50 value of 108.10. From the above study, it is apparent that the *A. bilimbi* L. fruit extract is a rich source of phytochemicals (natural products) with biological activity. The GC–MS report on this fruit proves that natural products have pharmacologically and biologically active compounds. A high phenolic content is observed in our study. *A. bilimbi* L. fruit extract is also found to have NO scavenging activity in our study.

Keywords *Averrhoa bilimbi L.* · GC–MS · Nitric oxide · Phenol · Phytochemical · Antioxidant

Introduction

Everyday 50,000 premature deaths are caused due to infectious diseases (Singh et al. 1992; Robin et al. 1998). In accordance with the World Health Organization (WHO) 2014 diseases like malaria, dengue, leishmaniasis, Lyme disease, tuberculosis, schistosomiasis, and yellow fever, carried by mosquitoes, flies, ticks, water snails and air infect one billion people and more than one million people will die. Pathogens and diseases become drug resistant and the best alternate approach are plants to eliminate diseases and therapeutic complications (Fabricant and Farnsworth 2001). From time immemorial plants are used as medicine to treat diseases. Before the discovery of allopathy humans depended on Ayurveda and homeopathy medicine which are completely based on plants and herbs. These herbs and plant materials act as medicine to cure diseases (Nostro et al. 2000). Tribal people depend on the rich diversity of forest to overcome the health care needs. Forests have excellent vegetation (flora) with high quality of medicinal value (Kadhirvel et al. 2010). Phytochemicals are the non-nutrient compounds with beneficial health effects leading to pharmacological importance and are used in medication (Nisa et al. 2011). Fruits play major role in human diet due to their bioactive compounds, natural sugars and organic acids with relatively high antioxidant activity (Rechkemmer 2001) and are a rich source of vitamins (A, B6, C, E, niacin, and thiamine) dietary fibre and minerals

✉ V. M. Berlin Grace
berlsdg@gmail.com; berlin@karunya.edu

Jagadish Kumar Suluvoy
jagadishbabu77@gmail.com

[1] Department of Biotechnology, Karunya University, Karunya Nagar, Coimbatore, Tamil Nadu, India

(Wargovich 2000). *Averrhoa bilimbi* L. is a long-lived green plant which gives edible fruits, belonging to the family Oxalidaceae–Oxalis and grows 16–33ft (5–10 m) in height, with short trunk dividing into number of upright branches. It is found throughout Malaysia, Indonesia, Myanmar, Bangladesh, Srilanka and common in Southeast Asian countries (Rahman et al. 2014). In India it is available mostly in Kerala regions, particularly the Kani tribal traditional healers in Thodu hills region (Kerala) use the raw leaves and fruits of *A. bilimbi* L. plant for ailments in circulatory system (Xavier et al. 2014) and the local name is Irumban puli or Pulingi. The parts like bark, leaves, seeds, flowers, fruits, roots and the entire *A. bilimbi* L. plant is used as alternative medicine to treat numerous diseases majorly as anti-diabetic agent (Kumar et al. 2013). Traditionally it is used in medication to cure cough, cold, boils, itches, syphilis, whooping cough, rheumatism and hypertension (Sabiha et al. 2012). *A. bilimbi* L. shows antimicrobial activity against gram positive and gram negative bacteria (Karon et al. 2011), antifungal activity (Nazmul et al. 2011), cytotoxic activity (Das et al. 2011), anti-diabetic activity (Pushparaj et al. 2000) and the leaves of *A. bilimbi* L. could increase the serum insulin level (Patel et al. 2012) in diabetes mellitus. Administration of *A. bilimbi* L. fruit (toxicity studies) extract 1 g/kg bw did not affect the mice (Savithri et al. 2009). However, in spite of having of such a great traditional medicinal use, the knowledge on its phytochemical is limited. Few studies of phytochemicals on the *A. bilimbi* L. fruit extract have shown contradictory data on the presence of alkaloids, tannins, glycosides, saponins and steroids (Sabiha et al. 2012). This study is therefore designed to analyse the active phytochemicals present in the fruits of *A. bilimbi* L. by biochemical tests and GC–MS which may be useful for exploring its ethno-pharmacological significance and to validate scientifically its medicinal properties. Several previous studies have shown the free radical scavenging activity of *A. bilimbi* L. fruit extract through DPPH scavenging activity (Asna and Noriham 2014; Sabiha et al. 2012). Oxidative stress has major action in human anatomy, physiology and diseases like cardiovascular diseases, diabetes, inflammatory conditions, ageing and cancer (Joyce 1987). Nitric oxide (NO) plays a major role in several in vivo diseases like neuronal signalling, smooth muscle relaxation, regulation of cell-mediated toxicity and inhibition of platelet aggregation (Hagerman et al. 1998). Surplus NO is reported to direct DNA fragmentation, cell damage and neuronal cell death. NO will not affect the DNA and proteins directly but NO is very unstable in aerobic condition and produces NO_2, N_3O_4, N_2O_4 intermediates which are genotoxic, affecting the DNA repair proteins and also deaminate DNA bases (Umamaheswari and Chatterjee 2008). Hence it is essential to reduce the levels of NO in human body. Besides to the reactive oxygen species (ROS) NO was found to be elevated in inflammation (Baygutalp et al. 2015), colon cancer (Erdman et al. 2009) and pathological conditions like gastrointestinal disorders (Cho 2001). The traditional usage of the fruits of *A. bilimbi* L. for anti-inflammation, anti-diabetic and anti-hypertensive was highlighted in a report on Malaysian medicinal plants (Harun et al. 2015). *A. bilimbi* L. is a rich source of vitamin C, A, B1 and 100 g of edible portion was found to have moisture, 94.2–94.7 g; fibre, 0.6 g; ash, 0.31–0.40; protein, 0.61 g; calcium, 3.4 g; iron, 1.01 mg; riboflavin, 0.32 mg; thiamine, 0.010 mg; ascorbic acid, 15.5 mg (Zakaria et al. 2007). Ascorbic acid has been used as standard drug for the estimation of nitric oxide scavenging activity (singh et al. 2012). As the *A. bilimbi* L. fruit extract has also shown a good antioxidant potential against DPPH (Chauhan and Kapfo 2013), we made an attempt to analyse its in vitro NO scavenging activity.

Materials and methods

Chemicals and reagents

All the chemicals used for this experiment were analytical grade purchased from Hi-Media, and SD Fine Chemicals.

Selection and authentication of fruit

Averrhoa bilimbi L. fruit samples were collected from Palakkad district, Kerala, during February to March (2015). The fruits of *A. bilimbi* L. are used as a source of food and medicine by tribes and settler communities of the local people. The authentication of the fruit was done by the Botanical Survey of India (BSI) Coimbatore, Tamilnadu, India. The authentication number given by the BSI is BSI/SRC/5/23/2015/Tech.

Extraction of fruit material

The fruits of the *A. bilimbi* L. were collected, air dried and made into fine powder by the mortar and pestle. Extraction from the fruits was done according to the method described by Singh et al. (2012). The powder (25 g) was used for the extraction with 250 ml of methanol (95% v/v) in a soxhlet apparatus. The remaining methanol was evaporated using rotary evaporator. The obtained thick semi-solid crude extract was stored at 2–4 °C for further use.

Phytochemical screening of *A. bilimbi* L. fruits

The *A. bilimbi* L. fruit (methanol) extract was analysed for the presence of alkaloid, carbohydrate, glycosides, phenols, flavonoids, saponins, steroids and tannins using the respective biochemical tests as follows.

Test for alkaloids

Two millilitre of 1% HCl was mixed with 0.1 gm of crude extract and heated slightly. After cooling Wagner's reagent and Mayer's reagent were added to it. The presence of buff-coloured precipitate indicated the presence of alkaloids (Sofowora 1993).

Test for carbohydrates

Benedict's reagents was mixed with the 0.1 gm of crude extract and slightly boiled, appearance of reddish brown precipitate indicated the presence of the carbohydrates (Harborne 1973).

Test for flavonoids

The appearance of pink scarlet colour when 0.1 gm of crude extract was mixed with few drops of concentrated HCl and Mg pellets indicated the presence of flavonoids (Odebiyi and Sofowora 1978).

Test for phenols

Two millilitre of 2% ferric chloride was mixed with the 0.1 gm of crude extract and the presence of blue-green or black coloration indicated the presence of phenols (Yadav and Agarwala 2011).

Test for saponins

Saponin presence was detected by the frothing test. Briefly 0.1 gm of crude extract was mixed well in water and shaken, the appearance of foam indicated the preliminary evidence for the presence of saponins (Kumar et al. 2009).

Test for steroid (Liebermann test)

0.1 gm of crude extract was mixed with 2 ml H_2SO_4 and slowly added to 2 ml of acetic anhydride. The colour change from violet to green or blue indicated the presence of steroids (Edeoga et al. 2005).

Test for tannins

0.1gm of crude extract of *A. bilimbi* L. fruit was mixed in distilled water and filtered. Few drops of ferric chloride solution were added to the filtrate. The green or blue-green precipitate indicated the presence of tannins *(*Trease and Evans 2002).

GC–MS analysis on *A. bilimbi* L. fruit extract

Averrhoa bilimbi L. methanolic fruit extract was subjected to gas chromatography–mass spectroscopy (GC–MS) analysis. The Thermo GC-Trace Ultra VER: 5.0 (Bremen, Germany) and Mass Spectroscopy (MS) MS DSQ II electron ionization mode with ionization energy of 70 eV were used. The temperature of the column was set to 80–250 °C at 8 °C/min rate. Temperature of 280 and 290 °C were set for the GC injector and MS transfer, respectively. Helium was used as a carrier gas at a flow rate of 1.0 ml/min. The sample volume of 1 µl was used for analysis. By the retention time and mass fragmentation patterns, the major compounds present in the fruit extract were analysed. The National Institute of Standards and Technology (NIST) and Wiley 9.0 library was used (Sakthivel and Guruvayoorappan 2013) for the detection of compounds.

Estimation of phenols in *A. bilimbi* L. fruit extract

The total phenolic compounds were estimated using the Folin–Ciocalteu reagent (Slinkard and Singleton 1977). Briefly 0.1 ml of *A. bilimbi* L. fruit extract of different concentrations (50, 100, 150, 200, and 250 µg/ml) were mixed with 2 ml of 10% Folin–Ciocalteu reagent and 3 ml of 7% Na_2CO_3 was added. This was incubated for 30 min at room temperature and the absorbance was measured using UV-spectrophotometer at 760 nm. Gallic acid was used as standard and all the results were performed in triplicates. The total phenol concentration is expressed in mg gallic acid equivalent (GAE).

Nitric oxide (NO) scavenging activity of *A. bilimbi* L. fruit extract

The nitric oxide (NO) scavenging activity of the *A. bilimbi* L. fruit extract was expressed in percentage inhibition (Vaijanathappa et al. 2008). Briefly 3 ml of 10 mM sodium nitroprusside (0.5 mM PBS pH 7.4) was mixed with 1 ml

of *A. bilimbi* L. fruit extract at different concentrations (25, 50, 75, 100, 125, and 150 µg/ml) and incubated at 25 °C for 150 min. Then 0.5 ml of the reaction mixture was removed and 1 ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) was added and again incubated for 5 min at 25 °C. After adding 1 ml of naphthyl ethylene diamine dichloride (0.1 w/v), the entire reaction mixture was allowed to stand for 30 min at room temperature. The absorbance was measured at 540 nm. Similar procedure was repeated for the standard ascorbic acid at different concentrations (25, 50, 75, 100, 125, 150 µg/ml). The same reaction mixture with the methanol served as control (without extract and standard)

$$\%\text{Inhibition} = (A_0 - A_1)/A_0 \times 100,$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

Results

Phytochemical analysis

Preliminary phytochemical tests revealed the presence of alkaloids, carbohydrates, phenols, flavonoids, saponins and tannins (Table 1). The presence of more phenols was observed in the preliminary screening. The test for sterols answered negative in our study.

Phytochemical compounds identified by GC–MS analysis

By comparing with the National Institute of Standards and Technology (NIST) and Wiley 9.0 library, the major compounds are identified and listed in Table 2. The GC–MS chromatogram is shown in Fig. 1. Among the 151 compounds identified, 15 compounds are found to have various biological activity which were reported from other studies as mentioned in Table 3. Furthermore, the GC–MS analysis reported the presence of various phenol, flavonoid, lipid, alkaloid and acid compounds which were shown in basic phytochemical screening test.

Table 1 The phytochemicals present in *A. bilimbi* L. fruit extract. It reveals the presence of alkaloids, carbohydrates, phenols, flavonoids, saponins, tannins. (++ indicates more amount) and the absence of steroids (−)

S. no	Test	Results
1	Alkaloids	+
2	Carbohydrates	+
3	Phenols	++
4	Flavonoids	+
5	Saponins	+
6	Steroids	−
7	Tannins	+

Total phenolic content

The total phenolic content of the *A. bilimbi* L. fruit extract was expressed as gallic acid equivalent in milligram per gram (GAE mg/g) of methanolic fruit extract. The optical density values and its straight line equation ($y = mx + c$) of standard gallic acid is shown in Fig. 2. The total phenolic content for 250 µg/ml is found to be 209.25 GAE mg/g.

Nitric oxide (NO) scavenging activity

The *A. bilimbi* L. fruit extract showed an increased nitric oxide scavenging activity with increase of concentration of the extract. Ascorbic acid is used as a standard for determining the IC_{50} value. Decreased OD values were observed when the concentration of fruit extract increased. The percentage of inhibition is shown in Table 4 and the regression curve for the standard ascorbic acid and *A. bilimbi* L. extract is shown in Fig. 3, respectively. The IC50 value of *A. bilimbi* L. fruit extract and standard ascorbic acid was found to be 108.10 and 85.01 which is shown in Table 4.

Discussion

Traditionally 6000 plants are used in Indian folk and herbal medication and 3000 plants are in documented medicine used against diseases (Rajshekharan 2002). Their medicinal value is due to the presence of phytochemicals. Phytochemicals are also called as natural products, plant constituents, and secondary metabolites which have medicinal properties to which they belong and the mechanism of action was not known up to the extent. These phytochemicals have great potentialities in drug discovery for various diseases (Justin et al. 2014). The phytochemicals like alkaloid, carbohydrate, glycosides, phenols, flavonoids, saponins, steroids, and tannins compounds are remedy to cure diseases and fight against different kinds of pathogens, as medicine (Hassan et al. 2004). In the current investigation, we have revealed the presence of phytochemicals (alkaloids, carbohydrate, phenols, flavonoids, saponins and tannins) in the *A. bilimbi L.* fruit extract. Our result on phytochemical presence is consistent with an earlier study (Hasanuzzaman et al. 2013). Moreover, there may be a region-wise difference in the presence of phytochemicals in any plant. Gas chromatographic–mass spectrometry (GC–MS) is a ubiquitous analytical technique of choice in toxicology, environmental research, food

Table 2 Compounds identified by GC–MS in the *A. bilimbi* L. fruit extract

S. no	Compound	Empirical formula	Empirical weight	Probability	Area %
1	*N*-Methoxy-*N*-methylacetamide	$C_4H_9NO_2$	103	26.21	2.96
2	Propane nitrile, 3-(methylthio)-(CAS)	$C_4H_7N_S$	101	12.73	2.96
3	d-Mannitol	$C_6H_{14}O_6$	182	7.33	2.96
4	d-Glycero-D-manno-heptitol	$C_7H_{16}O_7$	212	4	2.96
5	Propionic acid, 2-mercapto-, allyl ester	$C_6H_{10}O_2S$	146	3.69	2.96
6	Boronic acid, ethyl-, bis(2-mercaptoethyl ester)	$C_{16}H_{15}BO_2S_2$	194	3.69	2.96
7	N1-Methyluracil	$C_5H_6N_2O_2$	126	13.48	3.26
8	D-alanine, *N*-propargyloxycarbonyl-, isohexyl ester	$C_{13}H_{21}NO_4$	255	7.91	3.26
9	Uracil, 1-*n*-methyl	$C_5H_6N_2O_2$	126	6.06	3.26
10	1,5-Bis(dimethylpiperidyl)-2,2-dimethylpentane	$C_{21}H_{42}N_2$	322	5.82	3.26
11	D-alanine, *N*-propargyloxycarbonyl-, decyl ester	$C_{17}H_{29}NO_4$	311	5.14	3.26
12	2,2-Diethyl-*N*-ethylpyrrolidine	$C_{19}H_{33}NO_4$	155	4.74	3.26
13	L-alanine, n-propargyloxycarbonyl-, dodecyl ester	$C_{19}H_{33}NO_4$	339	3.63	3.26
14	N-Cyano-3-oxobutanamide	$C_5H_6N_2O_2$	126	3.21	3.26
15	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	$C_6H_8O_4$	144	90.5	2.05
16	4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl(CAS)	$C_6H_8O_4$	144	90.5	2.05
17	(2R*, 3R*)-2-butyl-3-hydroxy-3-phenylpropionoic acid ethyl ester	$C_{15}H_{22}O_3$	250	0.34	2.05
18	2-n-Propylthiane	$C_8H_{16}S$	144	0.26	2.05
19	5-Hydroxymethylfurfural	$C_6H_6O_3$	126	75.99	12.5
20	2-Furancarboxaldehyde, 5-(hydroxymethyl)- (CAS)	$C_6H_6O_3$	126	75.99	12.5
21	5-(hydroxymethyl)-2-Furanecarboxaldehyde	$C_6H_6O_3$	126	20.71	12.5
22	5-Hydroxymethyl-2-furaldehyde	$C_6H_6O_3$	126	75.99	12.5
23	Thienylethanal	C_6H_6OS	126	1.4	12.5
24	5-(hydroxymethyl)-2-(dimethoxymethyl)Furan	$C_8H_{12}O_4$	172	65.9	2.41
25	Oxiraneethanol, á-(1-ethoxyethoxy)-, [2R-[2R@[r@(R@)]]]	$C_9H_{14}O_3$	170	8.04	2.41
26	4-Methoxymethoxy-4-methyl-hex-2-ynal	$C_9H_{14}O_3$	170	8.04	2.41
27	2-(methoxycarbonylmethylidene)-5-Hydroxymethyltetrahydrofuran	$C_8H_{12}O_4$	172	1.85	2.41
28	4-Hydroxylamino-6-methylpyrimidin-2(1H)-one	$C_5H_7N_3O_2$	141	1.56	2.41
29	Methyl 4-chloro-2,2-dimethyl-4-pentenoate	$C_8H_{13}ClO_2$	176	1.1	2.41
30	3-(hydroxymethyl)-9-Oxabicyclo [3.3.1] nonan-3-ol	$C_9H_{16}O_3$	172	0.82	2.41
31	Methanal, (5-methyl-3-isoxazolyl)amino-, oxime	$C_5H_7N_3O_2$	141	0.72	2.41
32	Ethyl 4-hydroxy-3-methylbut-2-enoate	$C_7H_{12}O_3$	144	87.65	1.1
33	(E)-ethyl-4-hydroxy-3-methylcrotonate	$C_7H_{12}O_3$	144	2.38	1.1
34	Acetic acid, 2-methylhex-3-yl ester	$C_9H_{18}O_2$	158	0.53	1.1
35	2,4,4-Trimethyl-2-pentyl-3-oxa-zolidinyloxy	$C_{11}H_{22}NO_2$	200	0.45	1.1
36	4,5-Dihydro-2-methyl-5-(nitrimino)-1H-tetrazole	$C_2H_4N_6O_2$	144	0.27	1.1
37	1-Naphthyl n-propyl carbamate	$C_{14}H_{15}NO_2$	229	0.26	1.1
38	Glutaric acid, 2,4-dichlorobenzyl hexadecyl ester	$C_{28}H_{44}Cl_2O_4$	514	0.25	1.1
39	Glutaric acid, 3,4-difluorobenzyl nonyl ester	$C_{21}H_{30}F_2O_4$	384	0.25	1.1
40	Glutaric acid, 3-heptyl hexyl ester	$C_{18}H_{34}O_4$	314	0.22	1.1
41	Glutaric acid, decyl 2,5-difluorobenzyl ester	$C_{22}H_{32}F_2O_4$	398	0.22	1.1
42	(−)-Hygroline	$C_8H_{17}NO$	143	19.21	5.12
43	DL-Proline, 5-oxo-, methyl ester	$C_6H_9NO_3$	143	8.17	5.12
44	(Z)-2-Pentenal	C_5H_8O	84	7.22	5.12
45	Methyl pyroglutamate	$C_6H_9NO_3$	143	6.38	5.12
46	L-Proline, 5-oxo-, methyl ester (CAS	$C_6H_9NO_3$	143	6.38	5.12
47	DL-Proline, 5-oxo-, methyl ester	$C_6H_9NO_3$	143	8.17	5.12
48	(+)-Sedridine [2-(2-hydroxypropyl) piperdine]	$C_8H_{17}NO$	143	6.13	5.12
49	rac-5-oxopyrrolidine-2-carbonsaure-methylester	$C_6H_9NO_3$	143	4.45	5.12

Table 2 continued

S. no	Compound	Empirical formula	Empirical weight	Probability	Area %
50	L-Proline, 5-oxo-, methyl ester	$C_6H_9NO_3$	143	6.38	5.12
51	Cyclohexanone, 2,3,4-trihydroxy-6-methyl-, [2S-(2à,3á,4á,6à)]	$C_7H_{12}O_4$	160	10.49	1.05
52	Guanosine (CAS)	$C_{10}H_{13}N_5O_5$	283	7.83	1.05
53	2-Amino-9-(3,4-dihydroxy-5-hydroxymethy l-tetrahydro-furan-2-yl)-3,9-dihydro-puri	$C_{10}H_{13}N_5O_5$	283	7.22	1.05
54	(2s,3r,4r,6r)-2,3,4-trihydroxy-6-methylcyclohexanon	$C_7H_{12}O_4$	160	10.49	1.05
55	Xanthosine (CAS)	$C_{10}H_{12}N_4O_6$	284	5.67	1.05
56	Guanosine (CAS)	$C_{10}H_{13}N_5O_5$	283	7.83	1.05
57	à-D-Galactopyranoside, methyl 3,6-anhydro- (CAS)	$C_7H_{12}O_5$	176	5.23	1.05
58	2-Deoxy-D-galactose	$C_6H_{12}O_5$	164	4.83	1.05
59	D-fructose, 1,3,6-trideoxy-3,6-epithio- (CAS)	$C_6H_{10}O_3S$	162	3.79	1.05
60	2-Cyclohexylpiperidine	$C_{11}H_{21}N$	167	47.66	0.83
61	à-Pyrrolidone, 5-[3-hydroxybutyl]-	$C8H_{15}NO_2$	157	47.66	0.83
62	L-Serine, O-(phenylmethyl)- (CAS)	$C_{10}H_{13}NO_3$	195	7.52	0.83
63	2-[p-chlorobenzyl]Piperidine	$C_{12}H_{16}ClN$	209	3.2	0.83
64	Formyl glutamine	$C_9H_{14}N_2O_5$	230	2.95	0.83
65	4-[Dichloromethyl]-2-[[2-[1-methyl-2-pyrrolidinyl]ethyl]a mino-6-trichloromethylpyrimidine	$C_{13}H_{17}C_{l5}N_4$	404	2.26	0.83
66	Tridecanedioic acid (CAS)	$C_{13}H_{24}O_4$	244	1.92	0.83
67	à-Methyl-l-sorboside	$C_7H_{14}O_6$	194	88	3.97
68	Methyl-à-d-fructopyranoside	$C_7H_{14}O_6$	194	7.92	3.97
69	2-Methylacetophenone-dioxolane	$C_{11}H_{14}O_2$	178	0.51	3.97
70	D-glucose (CAS)	$C_6H_{12}O_6$	180	0.2	3.97
71	á-D-Glucopyranose, 4-O-á-D-galactopyranosyl	$C_{12}H_{22}O_{11}$	342	0.19	3.97
72	Isopropyl-á-D-thiogalactopyranoside	$C_9H_{18}O_5S$	238	0.15	3.97
73	4′-Methylphenyl-1C-sulfonyl-á-d-galactoside	$C_{13}H_{18}O_7S$	318	0.15	3.97
74	Ethyl-1-thio-á-d-glucopyranoside	$C_8H_{16}O_5S$	224	0.14	3.97
75	Galactopyranodise, 1-deoxy-1-undecylthio	$C_{17}H_{34}O_5S$	350	0.1	3.97
76	2-Octenoic acid, 4,5,7-trhydroxy	$C_8H_{14}O_5$	190	9.79	1.26
77	Desulphosinigrin	$C_{10}H_{17}NO_6S$	279	8.65	1.26
78	2-Octenoic acid, 4,5,7-trhydroxy	$C_8H_{14}O5$	190	8.32	1.26
79	2-d,2-pentadecyl-1,3-dioxepane	$C_{20}H_{39}DO_2$	312	6.7	1.26
80	2-Acetylamino-3-hydroxy-propionic acid	$C_5H_9NO_4$	147	5.4	1.26
81	2-acetylamino-3-hydroxy-propionic acid	$C_5H_9NO_4$	147	4.56	1.26
82	2-Hydroxyhexadecyl butanoate	$C_{20}H_{40}O_3$	328	3.58	1.26
83	2-[(*N*,*N*-Dimethylamino)methyl]-4-fluorophenol	$C_9H_{12}FNO$	169	38.97	0.49
84	1-Isobutyl-7,7-dimethyl-hexahydro-isobenzofuran-3a-ol	$C_{14}H_{26}O_2$	226	5.55	0.49
85	Hydrazinecarboxamide, 2-(2-methylcyclohexylidene)-	$C_8H_{15}N_3O$	169	38.97	0.49
86	1,3-Diethyl-1,3,3a,5,6,6a-hexahydrocyclopenta[c]thiophen -4-one	$C_{11}H_{18}OS$	198	3.92	0.49
87	2-Furoic acid, bromomethyldimethylsilyl ester	$C_8H_{11}BrO_3Si$	262	3.16	0.49
88	2-Furancarboxylic acid, tert-butyldimethylsilyl ester	$C_{11}H_{18}O_3Si$	226	2.35	0.49
89	Hydrazinecarboxamide, 2-(2-methylcyclohexylidene)(CAS)	$C_8H_{15}N_3O$	169	4.25	0.49
90	3-Furoic acid, benzyldimethylsilyl ester	$C_{14}H_{16}O_3Si$	260	2.08	0.49
91	2-Furoic acid, (3-cyanopropyl)dimethylsilyl ester	$C_{11}H_{15}NO_3Si$	237	2.93	0.84
92	Chimanine D	$C_{12}H_{11}NO$	185	39.01	0.55
93	Methyl 5-(*N*-Hydroxy)carboximidamido-2-thiophenecarboxylate	$C_7H_8N_2O_3S$	200	29.88	0.55
94	Octadecanoic acid, 2,3-dihydroxypropyl ester (CAS)	$C_{21}H_{42}O_4$	358	6.81	0.55
95	2-[5-(2-Hydroxy-propyl)-tetrahydrofuran-2-yl]-propionic acid, t-butyl ester	$C_{14}H_{26}O_4$	258	1.7	0.55
96	à-D-Glucopyranoside	$C_{20}H_{34}O_9$	418	1.17	0.55
97	1-allyl-2,3-5,6-tetra-o-acetyl-mannofura noside	$C_{17}H_{24}O_9$	372	1.12	0.55

Table 2 continued

S. no	Compound	Empirical formula	Empirical weight	Probability	Area %
98	Mannofuranoside, 1-allyl-2,3-5,6-tetra-O-acetyl	$C_{17}H_{24}O_9$	372	1.12	0.55
99	9-Hexadecenoic acid, methyl ester, (Z)- (CAS)	$C_{17}H_{32}O_2$	268	37.1	0.79
100	Methyl hexadec-9-enoate	$C_{17}H_{32}O_2$	268	26.94	0.79
101	Pentadecanoic acid, 14-methyl-, methyl ester (CAS)	$C_{17}H_{34}O_2$	270	13.97	5.54
102	Hexadecanoic acid (CAS)	$C_{16}H_{32}O_2$	270	54.59	5.54
103	l-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652	14.07	5.22
104	9-Octadecenoic acid (Z)- (CAS)	$C_{18}H_{34}O_2$	282	4.71	5.22
105	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	3.8	5.22
106	Elaidinsaeure methyl ester	$C_{19}H_{36}O_2$	296	7.69	14.89
107	Methylelaidate	$C_{19}H_{36}O_2$	296	19.42	14.89
108	cis-vaccenic acid	$C_{18}H_{34}O_2$	282	16.52	12.28
109	Oleic acid	$C_{18}H_{34}O_2$	282	4.9	12.28
110	Heptadecene-(8)-carbonic acid-(1)	$C_{18}H_{34}O_2$	282	3.16	12.28
111	Octadecanoic acid, 2-(2-hydroxyethoxy)ethyl ester	$C_{22}H_{44}O_4$	372	4.87	0.57
112	Octadecanoic acid (CAS)	$C_{18}H_{36}O_2$	284	58.57	0.57
113	Tricosane	$C_{23}H_{48}$	324	11.2	0.83
114	Eicosane	$C_{20}H_{42}$	282	7.46	0.83
115	Pentacosane	$C_{25}H_{52}$	352	7.17	0.83
116	Heneicosane	$C_{21}H_{44}$	296	6.33	0.83
117	Hexatriacontane	$C_{36}H_{74}$	506	6.09	0.83
118	Nonadecane	$C_{19}H_{40}$	268	5.38	0.83
119	Docosane	$C_{22}H_{46}$	310	5.38	0.83
120	Pentatriacontane	$C_{35}H_{72}$	492	5.17	0.83
121	Triacontane	$C_{30}H_{62}$	422	4.36	0.83
122	1-Heptacosanol	$C_{27}H_{56}O$	396	6.62	1.94
123	n-Tetracosanol-1	$C_{24}H_{50}O$	354	5.6	1.94
124	1-Heneicosanol	$C_{21}H_{44}O$	312	4.94	1.94
125	Z-12-Pentacosene	$C_{25}H_{50}$	350	4.75	1.94
126	9-Hexacosene	$C_{26}H_{52}$	364	3.54	1.94
127	10-Heneicosene (c,t)	$C_{21}H_{42}$	294	3.27	1.94
128	9-Tricosene, (Z)-	$C_{23}H_{46}$	322	3.27	1.94
129	n-Nonadecanol-1	$C_{19}H_{40}O$	284	2.89	1.94
130	1-Heneicosyl formate	$C_{22}H_{44}O_2$	340	2.27	1.94
131	Octacosane (CAS)	$C_{28}H_{58}$	394	5.4	1.38
132	9-Octadecenamide	$C_{18}H_{35}NO$	281	18.5	0.51
133	cis-13-Eicosenoic acid	$C_{20}H_{38}O_2$	309	17.07	0.51
134	2-Hexadecanol	$C_{16}H_{34}O$	242	8.48	0.74
135	17-Pentatriacontene	$C_{35}H_70$	490	5.81	0.74
136	cis-10-nonadecenoic acid	$C_{19}H_{36}O_2$	296	3.73	0.74
137	cis-11-Eicosenoic acid	$C_{20}H_{38}O_2$	310	3.44	0.74
138	Erucic acid	$C_{22}H_{42}O_2$	338	3.04	0.74
139	1-[(4′á)-3′-Ethylenedioxy-18′-norkaur-15′-en-17′-yl]pyrroli dine	$C_{25}H_{39}NO_2$	385	51.45	4.63
140	3á-(Peroxymethyl)-5-vinyl-A,B-bisnor-5á-cholestane	$C_{28}H_{48}O_2$	416	15.13	4.63
141	2-Allyl-6-(1,1-dimethylpropyl)-3-n-pentadecylphenol	$C_{29}H_{50}O$	414	9.17	4.63
142	(2S,3S)-2,3-Isopropylidenedioxy-4-tosyloxybutan-1-yl tetrahydropyran ether	$C_{19}H_{28}O_7S$	400	6.1	4.63
143	3-O-(trimethylsilyl)-5,7,4′-tri-O-methylkaempferol	$C_{21}H_{24}O_6SI$	400	1.4	4.63
144	*N*,*N*-Diethyl-1,3-dihydro-1-oxo-3,3-diphenyl-5-isobenzo-f urancarboxamide	$C_{25}H_{23}NO_3$	385	0.59	4.63
145	13-Docosenamide	$C_{22}H_{43}NO$	337	60.05	6.91

Table 2 continued

S. no	Compound	Empirical formula	Empirical weight	Probability	Area %
146	Squalene	$C_{30}H_{50}$	410	10.15	0.63
147	trans-Geranylgeraniol	$C_{20}H_{34}O$	290	4.62	0.63
148	Methyl trisporate C	$C_{19}H_{28}O_4$	320	6.92	2.03
149	2-Cyclohexene-1-carboxylic acid	$C_{19}H_{28}O_4$	3200	6.92	2.03
150	Thalmiculimine	$C_{37}H_{38}N_2O_7$	622	3.1	2.03
151	Bis[3-(3,5-di-tert-butyl-4-hydroxyphenyl)propyl] maleate	$C_{38}H_{56}O_6$	608	2.43	2.03

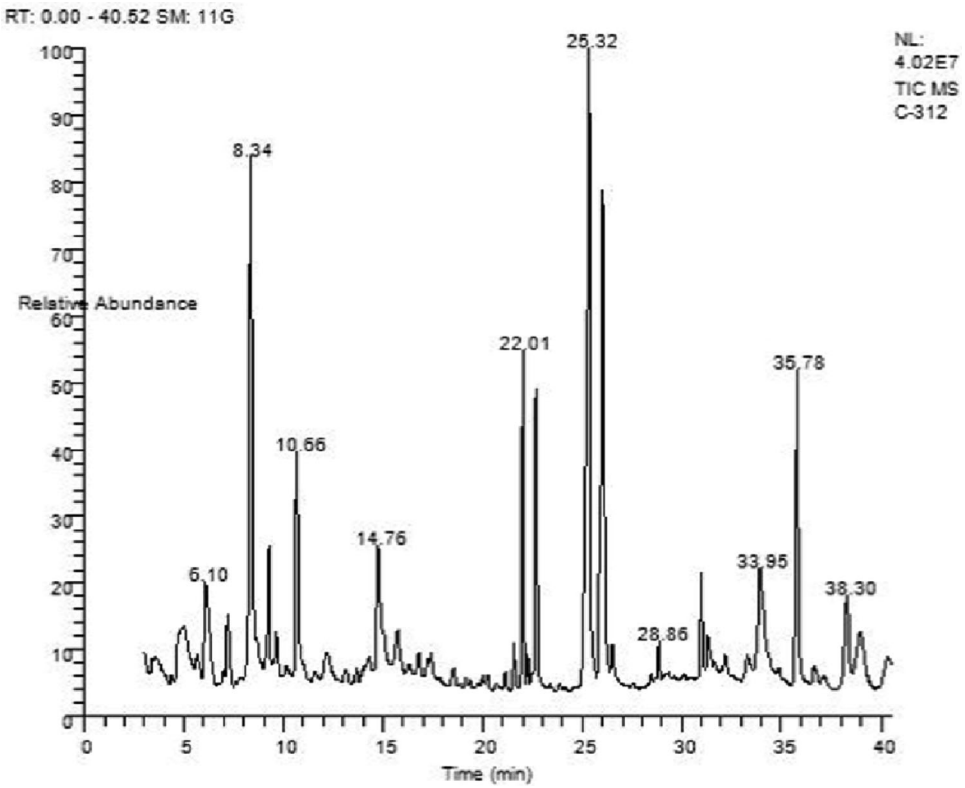

Fig. 1 GC–MS chromatogram of *A. bilimbi* L. fruit extract performed in the THERMO GC—TRACE ULTRA VER: 5.0, THERMO MS DSQ II machine. Non-polar column DB 5-MS capillary standard, helium gas as a carrier, with an injection volume of 1 µl was used

science and forensic research. *A. bilimbi L.* fruit extract was separated by GC and the compounds were identified by the MS by the NIST and Wiley 9.0 libraries. GC–MS analysis revealed the presence of major biologically active compounds (4H-pyran-4-one, 2,3-dihydro3,5-dihydroxy-6-methyl, hexadecanoic acid, squalene, erucic acid, oleic acid, chimanine D, boronic acid, 5-hydroxymethyl furfural, 2-deoxy-D-galactose, mannitol, desulphosinigrin, methyl pyroglutamate) having medicinal important as given in Table 3. We have not identified any steroid compounds in GC–MS report which correlates well with the results of phytochemical screening. Total phenolic compounds present in the *A. bilimbi* L. fruit extract were determined by Folin–Ciocalteu method. We have also observed a high level of total phenolic compounds in the *A. bilimbi* L. fruit extract at a concentration of 209.25 GAE mg/g. Presence of phenolic compound apparently explains the antioxidant nature of the plant (Awika et al. 2003) due to its hydroxyl group which have the scavenging activity (Hatano et al. 1989). More and more phenolic compounds are used in foods to improve the nutritional quality (Kahkonen et al. 1999). The presence of benzenoid ring (hydrophobic) and hydrogen bonding in phenolic hydroxyl groups will help in interacting with the proteins, accounting for its potent nature to act as antioxidants (Parr and Bolwel 2002). Free radicals possess high reactive nature; they attack nearest stable molecules like lipids, proteins, DNA and carbohydrates by sneaking their electrons (Patil et al. 2013).

Table 3 Major compounds identified by GC–MS in the *A. bilimbi* L. fruit extract, reported to have biological activity and cited in PUBMED

S. no	Compound	Activity
1	Hexadecanoic acid, ethyl ester	Antioxidant, hypocholesterolemic nematicide, pesticide, anti-androgenic flavour, hemolytic, 5-alpha reductase inhibitor (Kumar et al. 2010)
2	Squalene	Chemo-preventive against colon cancer (Rao and Harold 1998)
3	Erucic acid	X-linked adrenoleukodystrophy (Rizzo et al. 1989)
4	Oleic acid	Reduce blood pressure (Teres et al. 2008)
5	Chimanine D	Antileishmanial (Fournet et al. 1993)
6	Boronic acid	Potential pharmaceutical agent (selective reduction of aldehydes, enzyme inhibitors, asymmetric synthesis of amino acids) (Yang 2003)
7	5-Hydroxymethyl furfural	Against sickle cell anaemia (Lin et al. 2008)
8	Mannitol	Used for acute traumatic brain injury (Wakai et al. 2013)
9	Desulphosinigrin	Antibacterial (Sabreen et al. 2015)
10	Methyl Pyroglutamate	Antibiotic preparation. Smith 1997 in the book Alkaloids: Chemical and Biological Perspectives Chapter 4: Pyroglutamate as a Chiral Template for the Synthesis of Alkaloids

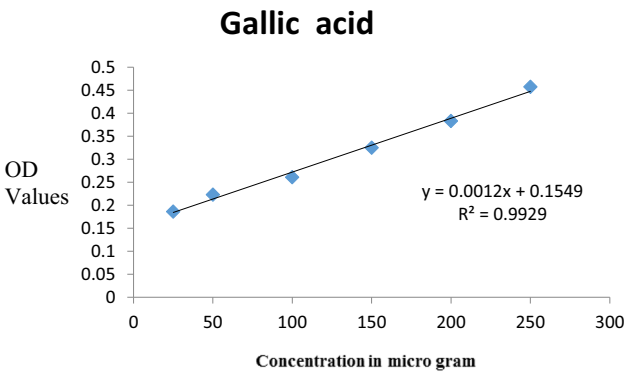

Fig. 2 The total phenolic content of *A. bilimbi* L. fruit extract for 250 µg/ml is 209.25 GAE mg/g

Different forms of free radicals are reactive oxygen species (ROS) and reactive nitrogen species (RNS). Antioxidants are the molecules that scavenge free radicals. They safeguard the cell components from the free radicals (Shenoy and Shirwaikar 2002) by scavenging the free radicals by scavenging the ROS and RNS (Rozina et al. 2012). NO is one of the abundant free radicals categorized under RNS. It is a highly reactive nitrogen species formed during inflammations, capable of damaging proteins, lipids and DNA (Valko et al. 2007). Synthetic antioxidants like butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT) and tertiary butyl hydroquinone are used in food supplements. They are used to treat numerous human diseases, but these compounds have toxic effects (Kombo 2000). Plants are the natural sources for the antioxidants they possess high quantity and quality of antioxidants which can scavenge the free radicals (Wang et al. 1996). In the present investigation, the antioxidant activity for *A. bilimbi* L. fruit extract is proven from its high level of NO scavenging activity similar to the standard ascorbic acid used in our study. The IC50 value of nitric oxide is 85.01, whereas the IC50 value of ascorbic acid is 108.10. Furthermore, boronic acid identified in our GC–MS analysis was reported to have nitric oxide scavenging activity (Yang et al. 2003). This may be one of the reasons for the significant nitric oxide scavenging activity observed in this study for *A. bilimbi* L. fruit extract.

Table 4 Percentage inhibition of *A. bilimbi* L. fruit extract on nitric oxide and its comparison with that of standard ascorbic acid. The IC_{50} of ascorbic acid is 85.01 and IC_{50} of *A. bilimbi* L. extract is 108.10

S. no	Concentration (µg)	Ascorbic acid % inhibition on nitric oxide	*A. bilimbi* L. fruit extract % inhibition on nitric oxide
1	25	21.10 ± 0.84	2.95 ± 0.88
2	50	33.67 ± 0.87	22.9 ± 1.90
3	75	47.15 ± 1.89	32.70 ± 1.60
4	100	56.74 ± 1.22	46.23 ± 1.56
5	125	63.55 ± 4.67	58.84 ± 1.84
6	150	85.14 ± 1.17	71.09 ± 2.67
IC_{50}		85.01	108.10

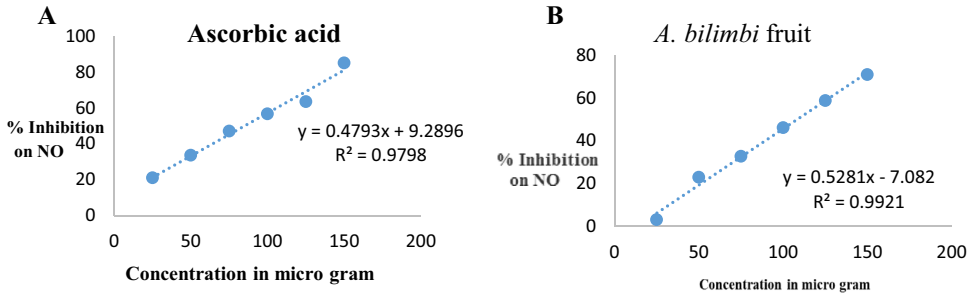

Fig. 3 **A** Percentage inhibition of standard (ascorbic acid) at different concentrations on NO **B** Percentage inhibition of *A. bilimbi* L. fruit extract at different concentrations on NO

Conclusion

From the above study, it is apparent that the *A. bilimbi* L. fruit extract is a rich source of phytochemicals (natural products) with biological activity. The GC–MS report on this fruit proves that natural products have pharmacologically and biologically active compounds. A high phenolic content is observed in our study. *A. bilimbi* L. fruit extract is also found to have NO scavenging activity in our study.

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Compliance with ethical standards

Conflict of interest The author declares that there is no conflict of interest.

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