Acta Poloniae Pharmaceutica – Drug Research, Vol. 72 No. 6 pp. 1201–1215, 2015 ISSN 0001-6837
Polish Pharmaceutical Society

NATURAL DRUGS

PHYTOCHEMISTRY, ANTIOXIDATIVE AND ANTIDIABETIC EFFECTS OF VARIOUS PARTS OF *EUGENIA CARYOPHYLLATA* THUNB. *IN VITRO*

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Abstract: *Eugenia caryophyllata* Thunb. possesses a wide variety of therapeutic potential and has been recognized as a source of antioxidative and antidiabetic agents. This study was designed to investigate and compare the antioxidative and antidiabetic effects of different parts (bud, leaf, stem and root) of *E. caryophyllata*. Samples were sequentially extracted using solvents of increasing polarity and investigated for 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, total reducing power, inhibition of hemoglobin glycosylation, α-amylase and α-glucosidase activities as markers of *in vitro* antidiabetic effects. Phytochemicals were analyzed using gas chromatography-mass spectrometry (GC-MS) analysis. The ethanol (EtOH) extracts of the bud, leaf and stem exhibited a higher total polyphenol and flavonoid content compared to other extracts, with the EtOH extracts of the bud and leaf exhibiting lower IC_{50} values than the extracts of the other plant parts for all the models used in this study (DPPH bud: 0.17 ± 0.20 mg/mL; leaf: 0.03 ± 0.01 mg/mL; hemoglobin glycosylation: bud: 0.17 ± 0.02 mg/mL; 0.83 ± 0.04 mg/mL; α-amylase: bud: 0.20 ± 0.02 mg/mL; 322.27 ± 73.29 mg/mL; α-glucosidase: bud: 0.03 ± 0.01 mg/mL; 0.74 ± 0.02 mg/mL). A similar result was observed for the reducing potentials of Fe^{3+} to Fe^{2+} by the extract. The GC-MS analysis of these parts indicated several aromatic phenols, acids, carophyllene and long chain aliphatic acids.Conclusively, various solvent extracts from the leaf, bud and stem of *E. caryophyllata* showed higher antioxidative and antidiabetic effects in comparison to common standards used in these assays.

Keywords: α-amylase, α-glucosidase, antidiabetic, antioxidative, *Eugenia caryophyllata*

With the increasing cost of drugs and the accessibility of these drugs to African countries, especially in rural and remote areas, the popularity of using medicinal plants for therapeutic purposes has increased significantly during the past decade. In Africa, about 80% of the population depend almost entirely on traditional medicine or herbal medicine, for their primary health care needs (1, 2). This is not surprising as several modern conventional drugs were originally obtained from plant sources and caused minimal or no side effects compared to synthetic drugs. For instance, metformin, an antidiabetic drug originated from *Galega officinalis* (3), quinine and quinidine, antiarrhythmic drugs were phytochemicals from *Cinchona spp* (4). In addition, the perceived effectiveness of the herbal therapies as well as the availability of these medicinal plants makes them a popular source of medicines. Moreover, the African continent accounts for about 25% of the total number of higher plants in the world, where more than 5400 medicinal plants are reported to have over 16300 medicinal uses (5). Consequently, the World Health Organization (WHO) has encouraged researchers to investigate and validate the folklore uses of plants used in the treatment of various diseases such as diabetes mellitus, hypertension, malaria and microbial infections among others (6).

Eugenia caryophyllata Thunb. (Syn. *Syzygium aromaticum* (Linn.) Merr. & L.M. Perry) or clove is an aromatic plant that belongs to the *Myrtaceae* family (7) and is widely available in Africa, Asia and North America. The bud and leaf are locally used as spice in various food preparations and possess a wide variety of therapeutic potential (8). *E. caryophyllata* parts or extracted oils are traditionally used in the treatment of toothache (9) and also been reported to have a strong antimicrobial effect (10). It is locally utilized in the treatment of asthma in Asia (11), disorders associated with respiratory

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and digestive systems (12) and in diarrheal and sexual disorders (13).

Previous studies reported that *E. caryophyllata* bud and its essential oils possessed strong antioxidative properties and thus terminate lipid peroxidation and other oxidative processes (14, 15). Adefegha and Oboh (16) reported the ability of the bud to inhibit α-amylase and α-glucosidase activities *in vitro*. The insulinotropic effect (17) and hepatoprotective effect against ethanol-induced liver cell injury (18) of the bud has also been reported. More recently, antihyperglycemic, hypolipidemic, hepatoprotective and antioxidative effects of clove powder have also been reported (19). Active principles identified in the bud or its essential oils include eugenol, β-caryophyllene, fatty acids, triterpenes, alcohols, flavonoids and other phenolics (20). It is hypothesized that since *E. caryophyllata* buds have been reported to have antioxidative and antidiabetic effects, other parts of the plant may also have similar activities and possess similar bioactive compounds. However, till now, the majority of scientific investigations had only focused on the buds and its essential oils. No data is available on the validity or potential of extracts of the other parts of the plant. Thus, this study was designed to investigate and compare the antioxidative and antidiabetic effects of various parts (including bud) of *E. carophyllata* using several *in vitro* models. Additionally, phytochemical analysis of the possible bioactive compounds present in most active extracts was also carried out using GC-MS analysis.

MATERIALS AND METHODS

Chemicals and reagents

Ascorbic acid, quercetin, hemoglobin (human lyophilized powder), gallic acid, aluminum chloride, α-amylase from porcine pancreas, α-glucosidase from *Saccharomyces cerevisiae* and 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) were purchased from Sigma-Aldrich through Capital Lab Supplies, New Germany, South Africa. Folin Ciocalteau reagent was purchased from Merck Chemical Company, South Africa. Gentamycin was purchased from EMD Chemicals, San Diego, CA, USA.

Plant material

The bud, leaf, stem and root samples of *E. carophyllata* were identified and authenticated at the herbarium unit of the Biological Science Department, Ahmadu Bello University, Zaria, Nigeria by Mr. Umar Gallah and a voucher specimen number 13209 was deposited accordingly. The plant samples were immediately washed and shade-dried to constant weights for two weeks. The dried samples were ground to a fine powder, and then stored individually in airtight containers to transport to the University of KwaZulu-Natal, Westville campus, Durban, South Africa for subsequent analysis.

Preparation of the plant extracts

Forty (40) grams of each of the fine powdered plant parts were separately defatted with 200 mL of n-hexane. The defatted material was sequentially extracted with ethyl acetate, ethanol and water by soaking for 48 h in 200 mL of the relevant solvent followed by a 2 h orbital shaking at 200 rpm. After filtration through Whatmann filter paper (No. 1), respective solvents were evaporated under vacuum, using a rotary evaporator (Buchi Rotavapor II, Buchi, Germany) at 40^{o}C under reduced pressure to obtain the different solvent extracts with the exception of the aqueous extracts which were dried on a water bath at 45^{o}C. The extracts in each case were weighed, transferred to micro tubes and stored in a refrigerator at 4^{o}C until further analysis.

Estimation of total polyphenol content

The total polyphenol content of each extract was determined (as gallic acid equivalent) according to the method described by McDonald et al. (21) with slight modifications. Briefly, 200 µL of the extract (240 µg/mL) was incubated with 1 mL of 10 times diluted Folin Ciocalteau reagent and 800 µL of 0.7 M Na_2CO_3 for 30 min at room temperature. The absorbance values were then determined at 765 nm in a Shimadzu UV mini 1240 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). All measurements were done in triplicate.

Determination of total flavonoid content

The total flavonoid content of the plant extracts were determined using a method reported by Chang et al. (22) with slight modification. Briefly, 500 µL (240 µg/mL) of each sample was mixed with 500 µL methanol, 50 µL of 10% $AlCl_3$, 50 µL of 1 mol/L potassium acetate and 1.4 mL water, and allowed to incubate at room temperature for 30 min. The absorbance of the reaction mixture was subsequently measured at 415 nm using the spectrophotometer mentioned above. The total flavonoid content was calculated as quercetin equivalent (QE) in µg per mg dry extract.

DPPH radical scavenging activity

The total free radical scavenging activity of the extracts was determined and compared to that of

ascorbic and gallic acids by using a slightly modified method described by Tuba & Gulcin (23). An aliquot of 500 µL of a 0.3 mM solution of DPPH in methanol was added to 1 mL of the extracts at different concentrations (30, 60, 120 and 240 µg/mL). These solutions were mixed and incubated in the dark for 30 min at room temperature. The absorbance was measured at 517 nm against blank samples lacking the free radical scavengers.

Ferric (Fe^{3+}) reducing antioxidant power assay

The ferric reducing antioxidant power method of Oyaizu (24) was used with slight modifications to measure the reducing capacity of the extracts. To perform this assay, 1 mL of each extract (30, 60, 120 and 240 µg/mL) was incubated with 1 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 1% potassium ferricyanide at 50°C for 30 min. After 30 min incubation, the reaction mixtures were acidified with 1 mL of 10% trichloroacetic acid. Thereafter, 1 mL of the acidified sample of this solution was mixed with 1 mL of distilled water and 200 µL of $FeCl_3$ (0.1%). The absorbance of the resulting solution was measured at 700 nm in a spectrophotometer. Increased absorbance of the reaction mixture indicated greater reductive capability of the extracts (15).

Inhibition of hemoglobin glycosylation

Inhibition of non-enzymatic glycosylation of hemoglobin by various extracts was investigated by the modified method of Pal & Dutta (25). Glucose (2%), hemoglobin (0.06%) and gentamycin (0.02%) solutions were prepared in phosphate buffer 0.01 M, pH 7.4. An aliquot of 1 mL of each of the solutions were mixed with 1 mL of different concentration of the extracts (30, 60, 120 and 240 µg/mL) in dimethyl sulfoxide (DMSO). These mixtures were incubated in the dark at room temperature for 72 h. The percentage inhibition of glycosylation of hemoglobin was calculated from the absorbance measured at 520 nm. Gallic acid was used as a standard.

α-Amylase inhibitory effect

The α-amylase inhibitory effect of the extracts was carried out using a modified method of McCue and Shetty (26). Briefly, a 250 µL aliquot of extract at different concentrations (30, 60, 120 and 240 µg/mL) was placed in a tube and 250 µL of 0.02 M sodium phosphate buffer (pH 6.9) containing α-amylase solution was added. This solution was preincubated at 25°C for 10 min, after which 250 µL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added at a time interval of 10 s and then further incubated at 25°C for 10 min. The

Table 1. Percentage yield, total polyphenol and flavonoid contents of various solvent extracts of *E. caryophyllata* parts.

Samples	Yield (%)	Total polyphenols content (mg/g GAE)	Total flavonoids content (mg/g QE)
Bud			
Ethyl acetate	2.75	9.68 ± 0.26^{e}	1.08 ± 0.29^{a}
Ethanol	5.00	27.23 ± 1.45^{h}	10.64 ± 0.59^{e}
Aqueous	1.25	11.17 ± 0.11^{f}	3.19 ± 0.41^{c}
Leaf			
Ethyl acetate	0.85	7.45 ± 0.11^{d}	1.11 ± 0.24^{a}
Ethanol	4.05	13.83 ± 0.22^{g}	5.72 ± 1.59^{d}
Aqueous	1.55	6.94 ± 0.10^{d}	2.17 ± 0.53^{b}
Root			
Ethyl acetate	0.17	2.07 ± 0.08^{c}	0.72 ± 0.18^{a}
Ethanol	0.75	2.09 ± 0.05^{c}	1.64 ± 0.35^{b}
Aqueous	0.55	2.65 ± 0.05^{c}	0.58 ± 0.12^{a}
Stem			
Ethyl acetate	0.62	0.66 ± 0.05^{a}	0.17 ± 0.12^{a}
Ethanol	2.77	6.12 ± 0.11^{d}	2.47 ± 0.12^{b}
Aqueous	1.45	1.40 ± 0.12^{b}	0.33 ± 0.12^{a}

Data are presented as the mean ± SD values of triplicate determinations. $^{a-h}$Different superscripted letters within a column are significantly different from each other (Tukey's-HSD multiple range *post hoc* test, $p < 0.05$).

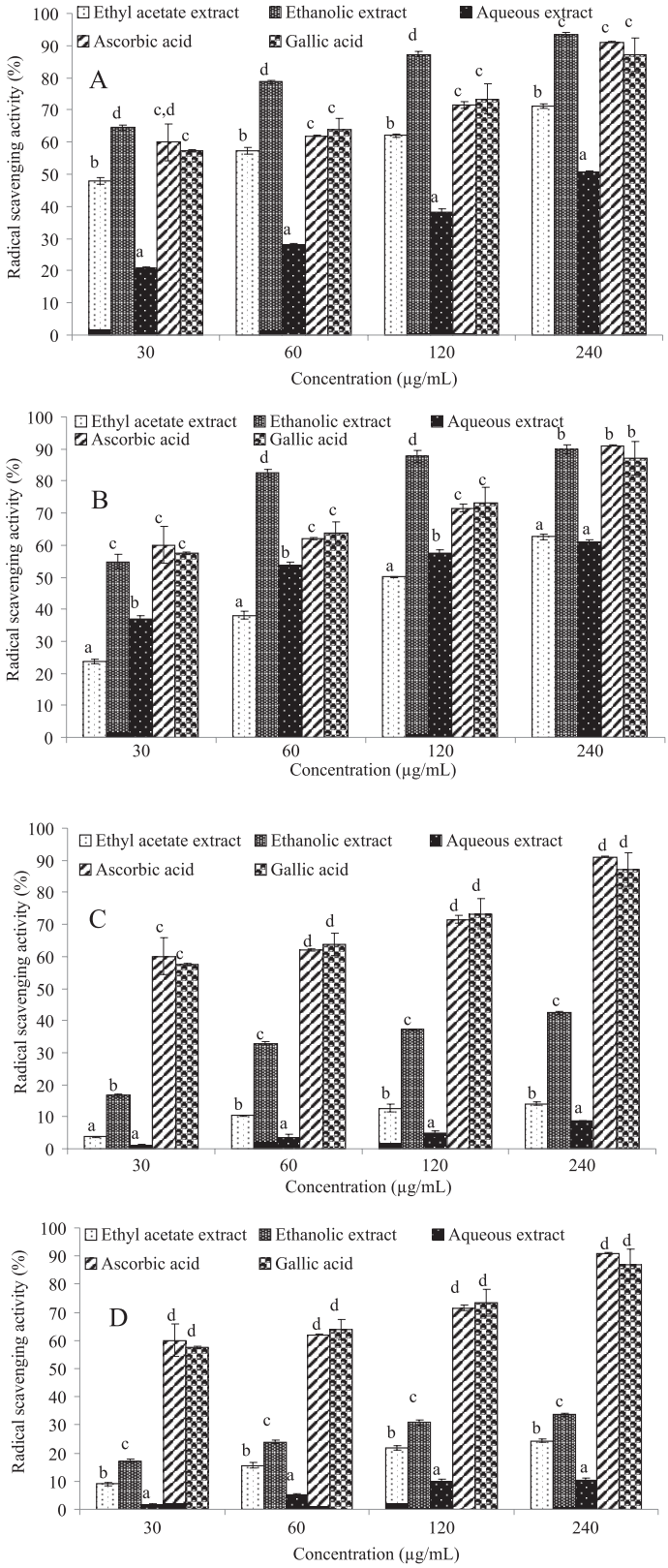

Figure 1. DPPH radical scavenging activity (%) of bud (A), leaf (B), root (C) and stem (D) extracts of *E. caryophyllata*. Data are presented as the mean ± SD of triplicate determinations. [a-d] Different letters presented over the bars for a given concentration of each extract are significantly different from each other (Tukey's HSD multiple range *post hoc* test, $p < 0.05$).

reaction was terminated after incubation by adding 1 mL of dinitrosalicylic acid (DNS) reagent. The tube was then boiled for 10 min and cooled to room temperature. The reaction mixture was diluted with 5 mL of distilled water and the absorbance was measured at 540 nm using a Shimadzu UV mini 1240 spectrophotometer. A control was prepared using the same procedure, replacing the extract with distilled water.

α-Glucosidase inhibitory effect

The inhibitory effect of the plant extracts on α-glucosidase activity was determined according to the method described by Kim et al. (27) using α-glucosidase from *Saccharomyces cerevisiae*. The substrate solution p-nitrophenyl glucopyranoside (pNPG) was prepared in 20 mM phosphate buffer, pH 6.9. An aliquot of 500 µL of α-glucosidase was then preincubated with 250 µL of the different concentrations of the extracts (30, 60, 120 and 240 µg/mL) for 10 min. Thereafter, 250 µL of 5.0 mM pNPG was dissolved in 20 mM phosphate buffer (pH 6.9) as a substrate to start the reaction. The reaction mixture was incubated at 37°C for 30 min. The α-glucosidase activity was determined by measuring the yellow colored p-nitrophenol released from pNPG at 405 nm. The results of the DPPH, inhibition of hemoglobin glycosylation, α-amylase and α-glucosidase assays were expressed as a percentage of the control (blank) according to the following formula:

$$\% \text{ Inhibition} = [(\text{Abs. of control} - \text{Abs. of extract})/\text{Abs. of control}] \times 100$$

Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC_{50}) were calculated from the data as well.

Gas chromatography-mass spectroscopic (GC-MS) analysis

Based on the results of antioxidative and antidiabetic studies, the most active extracts (EtOH bud, leaf and stem) were subjected to GC-MS analysis. The GC-MS analysis was conducted with an Agilent Technology 6890 gas chromatograph cou-

Table 2. IC_{50} values of various solvent extracts of *E. caryophyllata* parts in different antioxidative and antidiabetic models.

Samples	IC_{50} (mg/mL)			
	DPPH scavenging activity	Non-enzymatic glycosylation of hemoglobin	α-Amylase inhibitory effect	α-Glucosidase inhibitory effect
Bud				
Ethyl acetate	0.06 ± 0.01[a]	535.62 ± 372.42[b]	320.36 ± 167.03[c]	0.68 ± 0.03[a]
Ethanol	0.02 ± 0.01[a]	0.17 ± 0.02[a]	0.20 ± 0.02[a]	0.03 ± 0.01[a]
Aqueous	0.62 ± 0.14[b]	559.96 ± 377.16[b]	386.36 ± 97.28[c]	5260.41 ± 54.99[f]
Leaf				
Ethyl acetate	0.16 ± 0.03[a]	301.92 ± 195.09[b]	505.79 ± 32.95[d]	414.04 ± 75.44[d]
Ethanol	0.03 ± 0.01[a]	0.83 ± 0.04[a]	322.27 ± 73.29[c]	0.74 ± 0.02[b]
Aqueous	0.07 ± 0.02[a]	594.56 ± 234.39[b]	983.49 ± 62.64[e]	596.30 ± 7.32[e]
Root				
Ethyl acetate	296.73 ± 117.28[d]	ND	ND	ND
Ethanol	3.66 ± 1.16[c]	ND	ND	ND
Aqueous	222980.33 ± 815.45[e]	ND	ND	ND
Stem				
Ethyl acetate	230.68 ± 99.66[d]	1019.48 ± 75.97[c]	1110.51 ± 108.53[e]	89149.67 ± 107.99[h]
Ethanol	0.99 ± 0.32[b]	1.03 ± 0.42[a]	349.53 ± 282.83[c]	6.89±0.11[c]
Aqueous	527647.61 ± 684.74[f]	650.06 ± 276.14[b]	2561.78 ± 109.69[f]	83528.18 ± 1001.29[g]
Ascorbic acid	0.03 ± 0.02[a]	ND	ND	ND
Gallic acid	0.05 ± 0.01[a]	0.20 ± 0.01[a]	ND	ND
Acarbose	ND	ND	4.91 ± 0.80[b]	0.34 ± 0.02[b]

Data are presented as the mean ± SD values of triplicate determinations. [a-h] Different superscript letters presented within a column for a given parameter are significantly different from each other (Tukey's-HSD multiple range *post hoc* test, $p < 0.05$). ND = Not determined.

pled with an Agilent 5973 Mass Selective Detector and driven by Agilent Chemstation software. Compounds were identified by direct comparison of the retention times and mass spectral data with those in the National Institute of Standards and Technology (NIST) library.

Statistical analysis

All data are presented as the mean ± SD of triplicates determination. Data were analyzed by using a statistical software package (SPSS for Windows, version 18, IBM Corporation, NY, USA) using Tukey's HSD multiple range *post-hoc* test. Values were considered significantly different at $p < 0.05$.

RESULTS

The yield recovered from different solvent extracts of various parts of *E. carophyllata* indicated that higher yields are obtained from the bud and leaf extracts compared to roots and stem (Table 1). Furthermore, different parts showed variable amounts of polyphenols and flavonoid contents. EtOH extracts of various parts of the plant possessed a significantly ($p < 0.05$) higher total polyphenol and flavonoid content with the bud, leaf and stem containing the highest (Table 1). It was also observed that the aqueous extracts from the bud and stem showed a higher polyphenolic and flavonoid content compared to the ethyl acetate extracts. In the leaf, the aqueous extract had lower polyphenolic content than the ethyl acetate extract and in the root, the aqueous extract exhibited a lower flavonoid content compared to the ethyl acetate extract.

The ability of various solvent extracts to scavenge the DPPH radical were investigated and compared with ascorbic acid and gallic acid. The results are presented in Figure 1. It is evident from the results that the EtOH extracts from various parts of *E. caryophyllata* exhibited lower IC_{50} values compared to other solvent extracts. The bud (A) and leaf (B) EtOH extracts demonstrated significantly ($p < 0.05$) lower IC_{50} values of 0.02 ± 0.01 mg/mL and 0.03 ± 0.01 mg/mL, respectively, compared to other

Table 3. Phytochemicals identified in the EtOH extracts of the bud, leaf and stem by GC-MS

Extracts	Compounds	Retention time (min)	Mass [a.m.u.]
Bud			
1	Eugenol	6.67	164 $[M]^+$
3	Caryophyllene	7.21	204 $[M]^+$
4	2-Acetyl-4(2-propenyl)anisole	7.72	206 $[M]^+$
5	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	11.33, 13.16	279 $[M + H]^+$
6	n-Hexadecanoic acid	10.04-10.22	256 $[M]^+$
7	9,12-Octadecadienal	11.12, 14.40	264 $[M]^+$
8	n-Octadecanoic acid	11.21	284 $[M]^+$
9	9,12-Octadecadienoic acid	11.50	280 $[M]^+$
10	Heneicosanoic acid	11.96	326 $[M]^+$
Leaf			
1	Eugenol	6.67	164 $[M]^+$
3	Caryophyllene	7.22	204 $[M]^+$
4	2-Acetyl-4(2-propenyl)anisole	7.72	206 $[M]^+$
5	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	13.12	279 $[M + H]^+$
Stem			
1	Eugenol	6.66[a]	164 $[M]^+$
2	2-Methoxy-3-(2-propenyl)phenol	6.66[a]	164 $[M]^+$
3	Caryophyllene	7.72	204 $[M]^+$

[a] isomers co-eluted at the same retention time

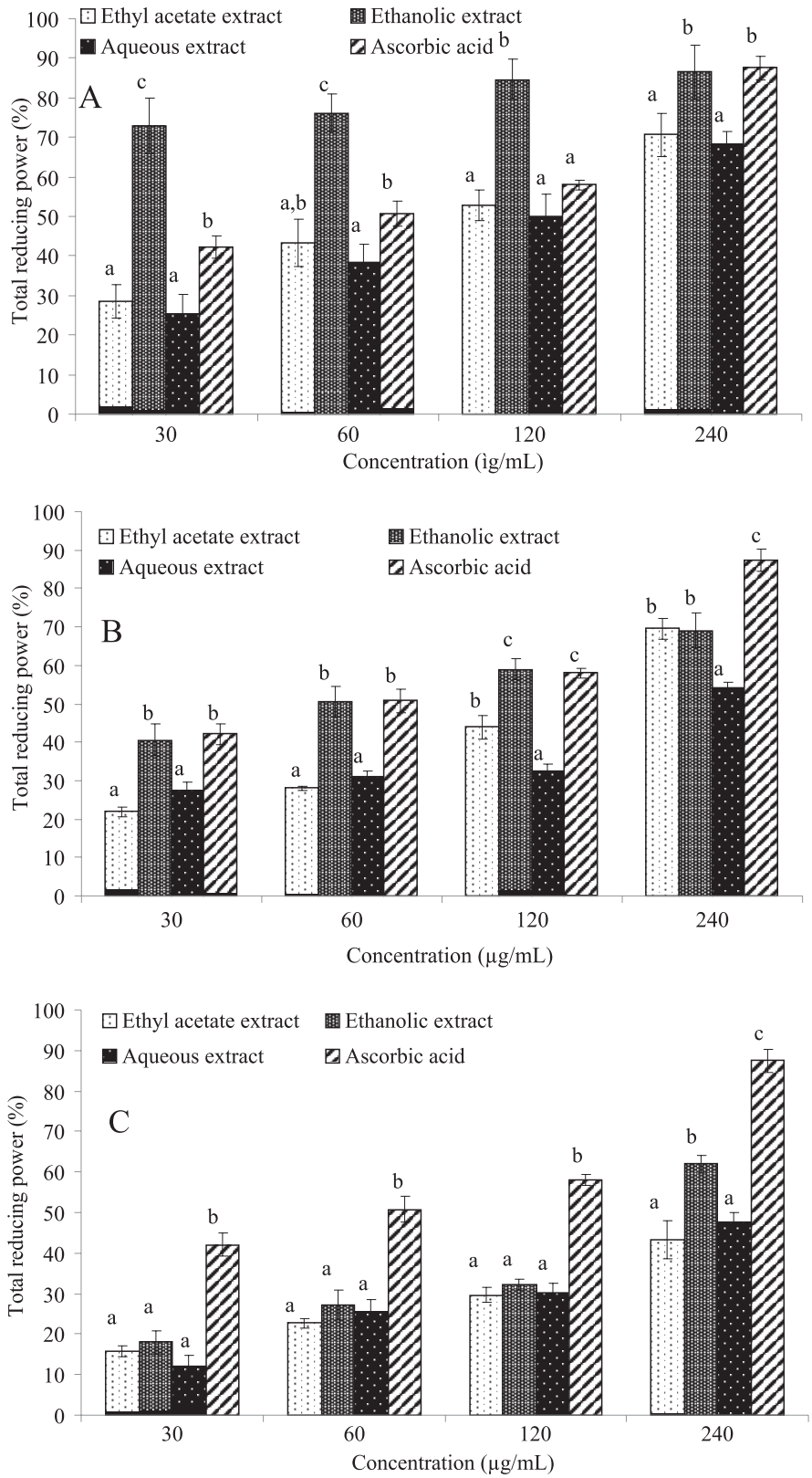

Figure 2. Total reducing power (relative to gallic acid) of bud (A), leaf (B) and stem (C) extracts of *E. caryophyllata*. Data are presented as the mean ± SD of triplicate determinations. [a-c] Different letters presented over the bars for a given concentration of each extract are significantly different from each other (Tukey's-HSD multiple range *post hoc* test, $p < 0.05$)

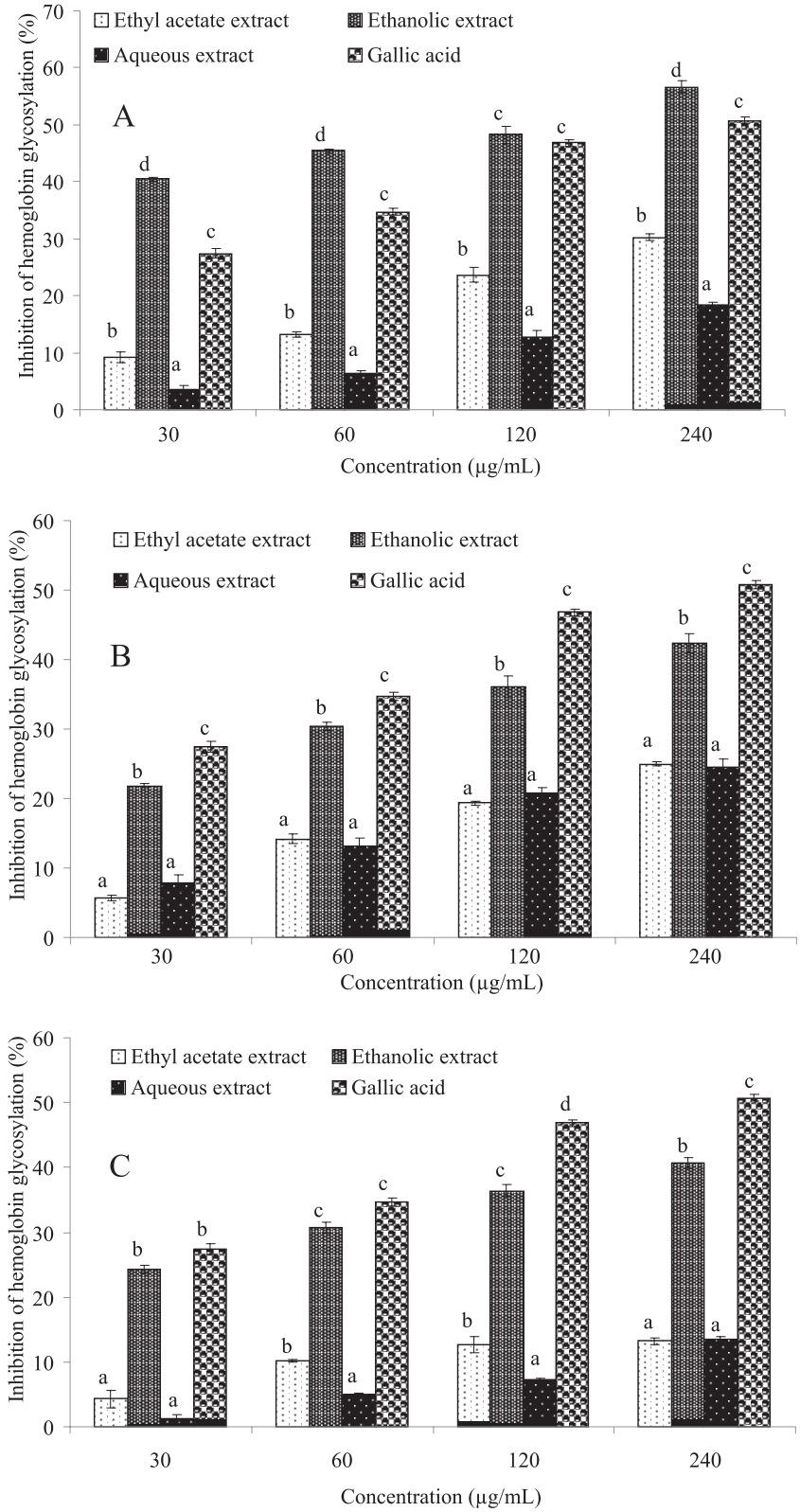

Figure 3. Inhibition of hemoglobin glycosylation (%) of bud (A), leaf (B) and stem (C) extracts of *E. caryophyllata*. Data are presented as the mean ± SD of triplicate determinations. [a-d] Different letters presented over the bars for a given concentration of each extract are significantly different from each other (Tukey's HSD multiple range *post hoc* test, $p < 0.05$)0

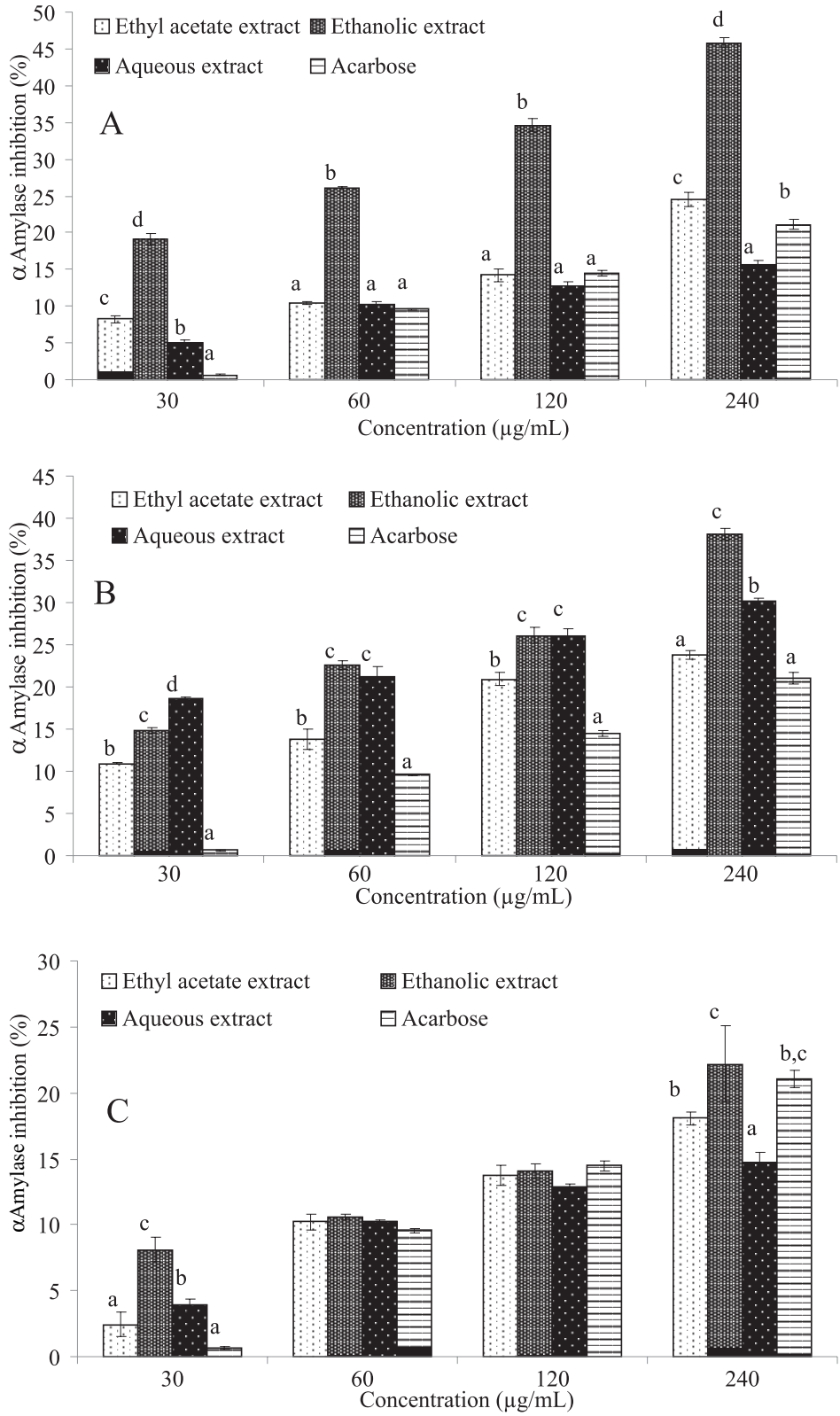

Figure 4. α-Amylase inhibition (%) of bud (A), leaf (B) and stem (C) extracts of *E. caryophyllata*. Data are presented as the mean ± SD of triplicate determinations. [a-d] Different letters presented over the bars for a given concentration of each extract are significantly different from each other (Tukey's HSD multiple range post hoc test, $p < 0.05$)

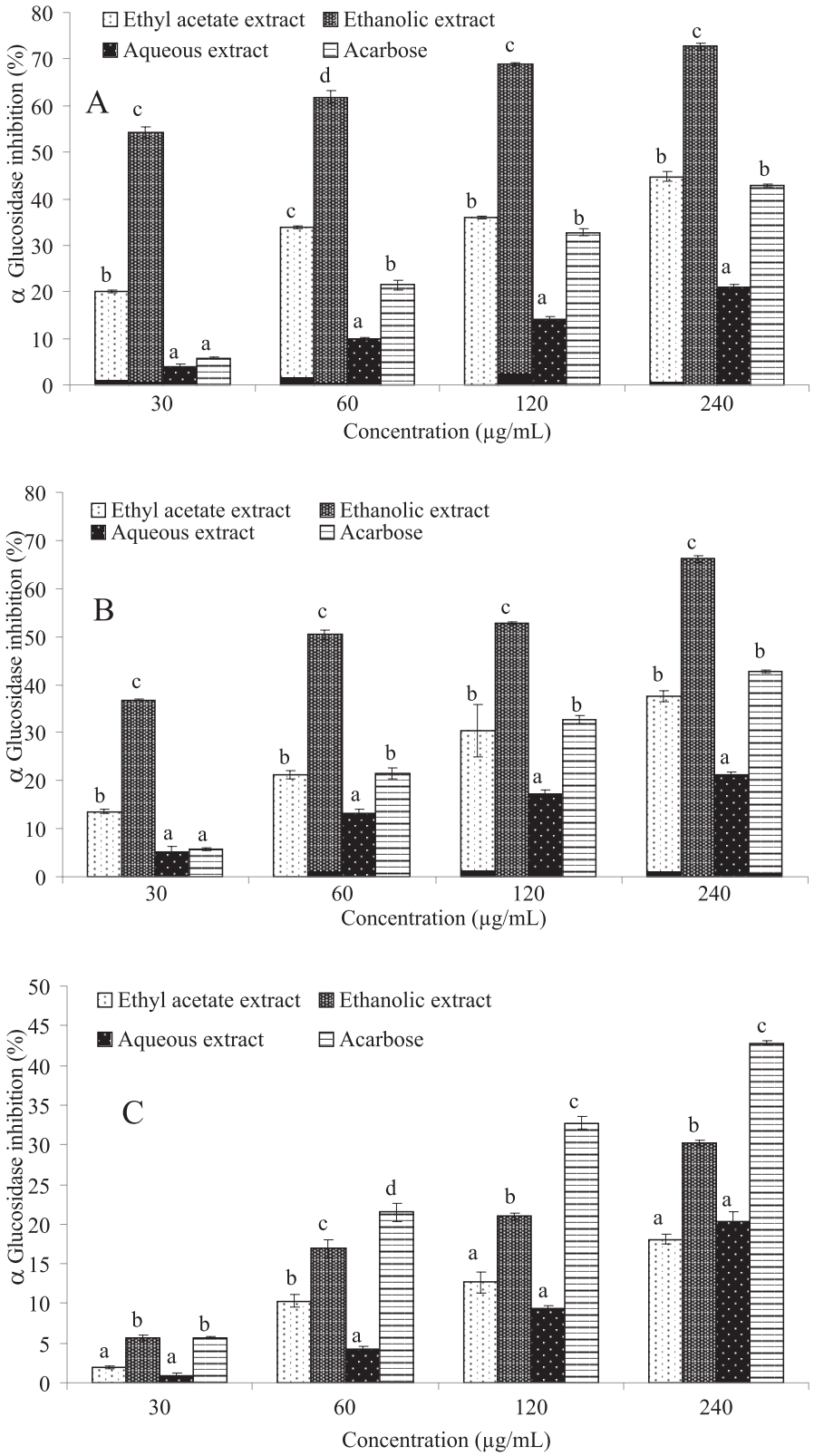

Figure 5. α-Glucosidase inhibition (%) of bud (A), leaf (B) and stem (C) extracts of *E. caryophyllata*. Data are presented as mean ± SD of triplicate determinations. [a-d] Different letters presented over the bars for a given concentration of each extract are significantly different from each other (Tukey's HSD multiple range *post hoc* test, $p < 0.05$)

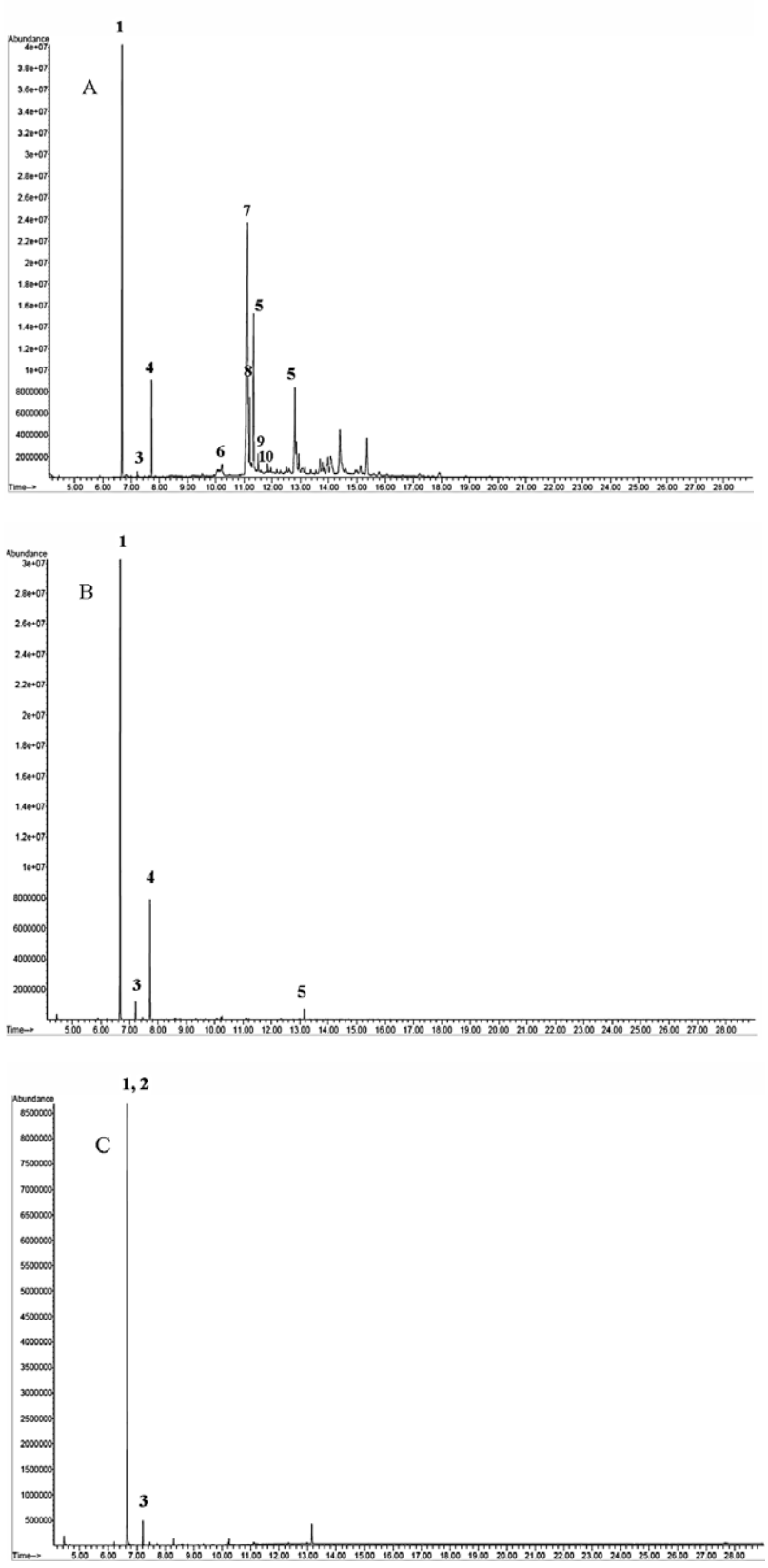

Figure 6. GC-MS chromatograms of ethanolic extracts of bud (A), leaf (B) and stem (C) extracts of *E. caryophyllata*

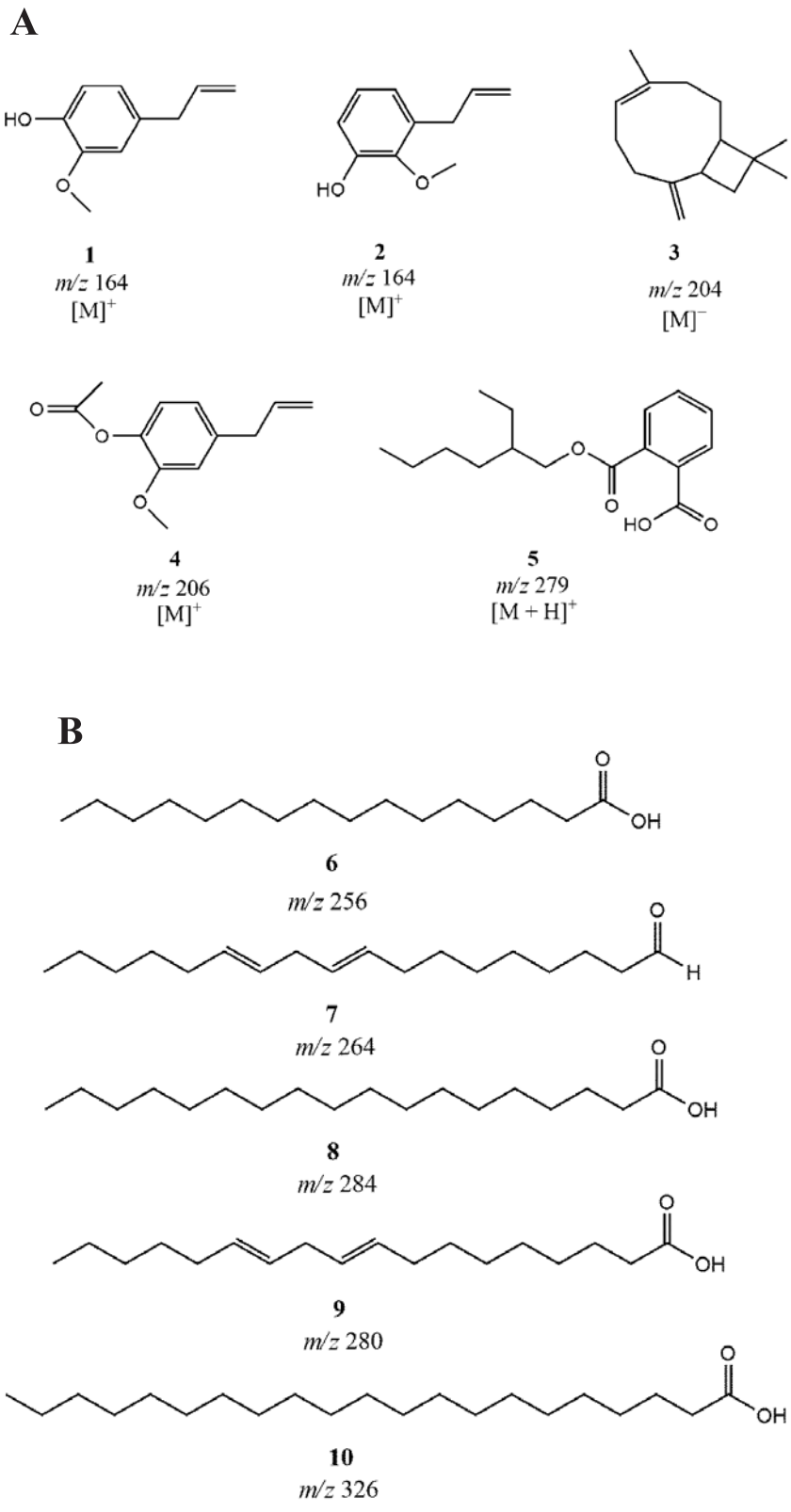

Figure 7. Structures of identified compounds from bud, leaf and stem of *E. caryophyllata* [Aromatic phenols, acids and caryophyllene (**A**), long chain aliphatic acids (**B**)]

solvent extracts (Table 2). The effect was comparable to that of ascorbic acid (0.03 ± 0.02 mg/mL) and gallic acid (0.05 ± 0.01 mg/mL).

Furthermore, our results indicated that the extracts of all the parts with the exception of the root, possessed the ability to reduce Fe^{3+} to Fe^{2+} (in terms of percentage gallic acid equivalent), which is comparable to that of ascorbic acid (Fig. 2). The EtOH extracts demonstrated a significantly ($p < 0.05$) higher activity compared to other solvent extracts in a dose-dependent manner. Bud (A), leaf (B) and stem (C) EtOH extracts showed higher reducing power compared to other solvent extracts. Similarly, the highest activity was exhibited by the EtOH extract of the bud, which is significantly higher compared to ascorbic acid. The extent of the reducing power (Fe^{3+} to Fe^{2+}) by different parts of *E. caryophyllata* is in the order: bud > leaf > stem > root.

In addition, the EtOH extracts of the bud (A), leaf (B) and stem (C) exhibited significantly ($p < 0.05$) higher inhibitory effects toward hemoglobin glycosylation (Fig. 3). The IC_{50} values recorded were 0.17 ± 0.020, 0.83 ± 0.04 and 1.03 ± 0.42 mg/mL for the bud, leaf and stem, respectively (Table 2). Similarly, various extracts from the root showed no inhibitory effect towards hemoglobin glycosylation. The leaf and stem EtOH extracts recorded higher IC_{50} values than the bud EtOH extracts although not significantly different ($p < 0.05$) in comparison to gallic acid (IC_{50} values: 0.20 ± 0.01 mg/mL).

Figure 4 shows the α-amylase inhibitory effect of various extracts from *E. caryophyllata* parts. Only the bud EtOH extract exhibited a significantly ($p < 0.05$) lower IC_{50} value (0.20 ± 0.02 mg/mL) compared to acarbose (IC_{50} value: 4.91 ± 0.80 mg/mL). The inhibitory effects observed on the leaf and stem EtOH extracts as well as the bud ethyl acetate and aqueous extracts did not differ significantly. The IC_{50} values demonstrated by these extracts were significantly higher compared to acarbose (4.91 ± 0.80 mg/mL). In addition, ethyl acetate extracts from various parts showed lower IC_{50} values compared to the aqueous extracts (Table 2). No activity was recorded with various solvent extracts from the root.

Similarly, all the solvent extracts with the exception of the root extracts demonstrated dose-dependent inhibition of α-glucosidase (Fig. 5). Significantly ($p < 0.05$) lower IC_{50} values were exhibited by the EtOH extracts of the bud (0.03 ± 0.01 mg/mL), leaf (0.74 ± 0.02 mg/mL), and stem (6.89 ± 0.11 mg/mL) and by the ethyl acetate extract (0.68 ± 0.03 mg/mL) of the bud compared to other solvent extracts (Table 2). The IC_{50} values demonstrated by different parts are in the order of bud > leaf > stem. The ethyl acetate extract of the stem showed the least α-glucosidase inhibitory effect, having the highest IC_{50} value.

Based on the results obtained, the EtOH extracts of the bud, leaf and stem showed consistently higher activity compared to other solvent extracts and was thus subjected to GC-MS analysis to determine the phytochemicals present in this extract. From the results obtained, several peaks were observed in the chromatograms (Fig. 6). Peaks detected were compared with the data available in the NIST library and the compounds detected correspond to aromatic phenols (**1, 2**), caryophyllene (**3**), aromatics containing ether, ester and acid moieties (**4, 5**) and long chain aliphatic acids (**6**-**10**) (Table 3; Fig. 7). Eugenol (**1**) and caryophyllene (**3**) were detected in all the extracts analyzed and 2-acetyl-4-(2-propenyl)anisole (**4**) and 1,2-benzenedicarboxylic acid mono (2-ethylhexyl) ester (**5**) were detected in the EtOH extracts of the bud and leaf. The long chain aliphatic acids (**6**-**10**) were present in the EtOH extract of the bud alone.

DISCUSSION

The present study investigated and compared, for the first time, the antioxidative and antidiabetic effects of various solvent extracts of *E. caryophyllata* parts *in vitro*. This is the first report of the potential of plant parts other than the buds (leaf, root and stem) as antidiabetic and antioxidative agents. From the results of this study, it is evident that EtOH extracts have higher yields and contain a higher total polyphenol and flavonoid content in comparison to other solvent extracts. This is consistent with previous findings that ethanol is the best solvent for the extraction of a maximum yield of polyphenols compared to other solvents (28). The amount of total polyphenols and flavonoid content was in the order of bud > leaf > stem > root (Table 1). A possible explanation could be linked to several factors including genetic and environmental factors (nature of the soil, high temperature and rainfall) in addition to growth or maturation stages (29, 30). Although no correlation analysis was carried out in this study, previous studies strongly correlate antioxidative effect to total polyphenol contents (31).

Methods adapted to assess the antioxidative effect of various parts of *E. caryophyllata* include among others, the DPPH radical scavenging assay, a widely used method for assessing the antioxidant status of compounds or plant products. In addition,

calculated IC_{50} values were used to demonstrate the extent of scavenging power for different parts of the plant. The lower the IC_{50} values the higher the scavenging activity. More importantly, the consistently lower IC_{50} value exhibited by the EtOH extracts, comparable to standard antioxidants (ascorbic acid and gallic acid) (Fig. 2) suggest that the extracts possess compounds with high radical-quenching ability that could terminate free radical activities. This is consistent with previous studies (32-34).

The ferric reducing power which reflects electron donating capacity of various extracts has been used to assess the antioxidative status of several natural products. In this study, the EtOH extracts from the bud, leaf and stem demonstrated higher activity and therefore possessed phytochemicals that cause the reduction of Fe^{3+} to Fe^{2+}, which is monitored by measuring the formation of Perlis prussian blue at 700 nm.

To further explore the antioxidative potential of various extracts, their ability to inhibit glycosylation of hemoglobin was determined. Glycosylation is a term used to describe the non-enzymatic reaction between reducing sugars and proteins (hemoglobin, albumin) and usually contributes enormously to the formation of advanced glycation end products (35). Consequently, it is evident from the results obtained that the EtOH extracts depicted lower IC_{50} values, comparable to that of standard antioxidants used, indicating higher radical scavenging and anti-glycosylation activity (Table 2). This could be linked to the active principles present and the differences observed could be due to variation and concentration of the phytochemicals present in each part.

Moreover, it is an established fact that α-amylase and α-glucosidase inhibitors from natural sources play a significant role in diabetic management and control. This is achieved *via* a decrease in postprandial hyperglycemia through inhibition of α-amylase and α-glucosidase actions (16). However, for effective control of postprandial hyperglycemia, moderate α-amylase inhibition and potent α-glucosidase inhibition provide better options for controlling the availability of dietary glucose for absorption in the intestinal tract (36). This is due to adverse effects associated with strong α-amylase inhibition such as abdominal distension, flatulence, bowel necrosis and diarrhoea (37). In this study, various solvent extracts demonstrated mild α-amylase inhibition and potent α-glucosidase inhibition, indicating a potential role as an anti-diabetic agent. The inhibitory effects of *E. caryophyllata* bud reported by Adefegha and Oboh (16) correspond with the results of this study. Furthermore, various solvent extracts from the leaf and stem could be good substitutes for the bud as potential antidiabetic agents, as the bud is being used locally in most parts of the world.

Phytochemical analysis of the most active parts resulted in the identification of compounds with potential medicinal usage (20). For example, eugenol (**1**) has already been implicated with a wide array of therapeutic application such as antioxidative, antidiabetic and antimicrobial effects. Interestingly, eugenol (**1**) and caryophyllene (**3**) were present in all parts while others like 2-acetyl-4(2-propenyl) anisole (**4**) appear in the bud and leaf but not the stem. The availability of eugenol (**1**) in most parts of *E. caryophyllata* has already been reported (38-40). Additionally, long chain aliphatic acids (**6**-**10**), also detected in the bud could synergistically or independently contribute to the observed higher activities of this part compared to others. Furthermore, the hydroxyl group present in compounds **1** and **2** could directly or indirectly be the key feature that contributed to the higher antioxidative and antidiabetic effects depicted by the bud, leaf and stem extracts (Fig. 7). The low reduction potentials of phenolics, hydroxyls and other related compounds inactivate and terminate the initiation and propagation of chain reactions associated with oxidative damage (41). In a similar way, phenolics and hydroxyls were reported to interfere with some surface amino acid side chains in both α-amylase and α-glucosidase structures (42). This causes some conformational changes on the enzyme structure, thereby decreasing their actions and causing reduction on blood glucose levels and subsequently reduced postprandial hyperglycemia.

CONCLUSIONS

In conclusion, various solvent extracts from the bud, leaf and stem of *E. caryophyllata* possessed antioxidative as well as antidiabetic effects *in vitro* while the root extracts showed very low or no significant effects in the same assays. The effects of the leaf extracts were comparable to that of the bud and could therefore serve as a good substitute for various culinary and medicinal potentials of the bud. Hence, it is recommended that bioassay-guided fractionation of the EtOH extracts could be done in order to fully investigate the *in vivo* antidiabetic and antioxidative effects of this extract.

Declaration of interest

There is no conflict of interest within this article.

Acknowledgments

This study was supported by a competitive research grant from the Research Office, University of KwaZulu-Natal (UKZN), Durban; an incentive grant for rated researchers and a grant support for women and young researchers from the National Research Foundation (NRF), Pretoria, South Africa. The first author was awarded a Ph.D. study fellowship by the Ahmadu Bello University, Zaria, Nigeria.

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Received: 17. 09. 2014