# Antidiabetic, anti-inflammatory, anticholinesterase and cytotoxicity determination of two *Carpobrotus* species

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**Abstract**

*Carpobrotus edulis* (L.) N. E. Br., commonly grown in many parts of the world, and *C. dimidiatus* (Haw.) L. Bolus are highly used medicinal plants in South Africa for treating diabetes, wounds, inflammation and other closely related diseases. This study was aimed at evaluating the antidiabetic, anti-inflammatory, and acetylcholinesterase inhibitory activities as well as cytotoxicity effects (using RAW 264.7, Vero kidney and HepG2 liver cells) of different solvent (water, 70% acetone, and 50% methanol) extracts obtained from the leaves of *C. edulis* and *C. dimidiatus*. Antidiabetic and anti-inflammatory activities were evaluated using alpha-glucosidase and 15-lipoxygenase (LOX) enzyme inhibition assays, respectively. In addition, the phenolic content of the two plant species was quantified. Water extract of *C. edulis* leaves exhibited noteworthy alpha-glucosidase inhibitory activity with an $IC_{50}$ value of 5 µg/ml. Acetone and water extracts of *C. edulis* showed promising 15-LOX inhibition activity with $IC_{50}$ values of 22.3 and 59.8 µg/ml, respectively. These same extracts demonstrated strong free radical scavenging activity as reflected by their $EC_{50}$ values of 5.6 and 6.2 µg/ml, respectively. *Carpobrotus edulis* leaves had a significantly high total phenolic and flavonoid contents when compared to *C. dimidiatus*. Furthermore, all extracts exhibited very low cytotoxicity against all the tested cells. The current findings offer supporting evidence for the use of these plant species in the development of natural products to treat diabetes, among other ailments. These plant species can be used in the production of natural supplements or functional food for the management of pain, diabetes and free radical induced disorders.

**Keywords:** Alpha-glucosidase; Acetylcholinesterase; Antioxidant; Cytotoxicity; Diabetes; Inflammation.

# 1. Introduction

Diabetes has become one of the largest global health emergencies of the 21st century (International Diabetes Federation, 2017). Diabetes mellitus is a metabolic disorder, characterized by high blood glucose level (hyperglycaemia) due to defects in insulin secretion from the pancreas. It gives rise to macro-vascular complications of diabetes such as blindness, renal failure and nervous damage while the micro-vascular complications include atherosclerosis, strokes, coronary heart disease and many more, leading to death (Seetaloo et al., 2019). About 425 million people (aged 20 -79 years) are said to be living with diabetes worldwide and the prediction is that by 2045, this number will be approximately 629 million (International Diabetes Federation, 2017). Studies have indicated that inflammation is involved in the pathophysiology of diabetes (Donath and Shoelson, 2011; Pollack et al., 2016) with some authors suggesting the targeting of inflammation in diabetes treatment (Donath, 2014).

Many of those suffering from diabetes in developing countries do not readily have access to, or are unable to afford orthodox therapy for diabetes management. The undesirable side effects such as liver problems, lactic acidosis, flatulence, diarrhoea and abdominal discomfort (Stein et al., 2013; Suzuki et al., 2009) associated with the current drugs used in treating diabetes present another challenge in the management of diabetes. Acarbose, a commercially available enzyme inhibitor for type II diabetes, is reported to cause various side effects such as abdominal distention, flatulence and possibly diarrhoea (Abirami et al., 2014). Hypoglycaemic drugs such as biguanides and sulfoylureas are accompanied by unpleasant side effects such as severe hypoglycemia, lactic acidosis, peripheral edema and abdominal discomfort (Ghorbani, 2013). The presence of such side effects can negatively affect the compliance of patients with treatment regimen, leading to further diabetic complications (Ho et al., 2006). Thus, many researchers have advocated the search for less costly but more potent

antidiabetic agents that are especially of natural origin with minimal or no negative side effects. While stating the case for more research evaluations, the World Health Organization (WHO) acknowledged the role of traditional and complementary/alternative medicine in disease prevention, treatment of non-communicable diseases and improvement of the quality of life for persons living with chronic diseases (WHO, 2001).

Alpha-glucosidase is a vital enzyme essential for cleavage of maltose to glucose for the absorption into the blood stream in the small intestine (Ryu et al., 2011). Alpha-glucosidase inhibitors could regulate abnormally high levels of plasma glucose after carbohydrate ingestion (Ryu et al., 2011). In the search for alternative potent drugs, medicinal plants possessing alpha-glucosidase inhibitors have thus received more attention (Seetaloo et al., 2019).

*Carpobrotus edulis* (L.) N. E. Br. and *C. dimidiatus* (Haw.) L. Bolus (family: Aizoaceae) are among commercially important southern African medicinal plants used in traditional medicine to treat diabetes, wound, tuberculosis, high blood pressure, sore throat, dysentery, digestive ailments and toothaches (Semenya et al., 2012; Van Wyk, et al., 2002; Watt and Breyer-Brandwijk, 1962). As indicated in previous studies (Ibtissem et al., 2012; Martins et al., 2011; Omoruyi et al., 2012; Van Der Watt and Pretorius, 2001), *Carpobrotus* species possess compounds with antioxidant and antibacterial properties. *Carpobrotus edulis* ethanol-water extract was also shown to demonstrate inhibitory effect on protein glycation (Hafsa et. al., 2016). Guided by their ethnomedicinal uses, the aim of the current study was to assess the antidiabetic, anti-inflammatory and safety of these two commonly consumed *Carpobrotus* species. In addition, the phytochemical content of both plant species was evaluated.

## 2. Materials and methods

*2.1. Plant collection, identification and extract preparation*

Leaves of *C. edulis* and *C. dimidiatus* were collected from the Agricultural Research Council – Vegetables and Ornamental Plant (ARC-VOP) Research Station, Pretoria and Botanical Gardens of the University of KwaZulu-Natal (UKZN), Pietermaritzburg, South Africa, respectively. After identification by the Curator, their voucher specimens (Mulaudzi RB# 200 and A. Aremu #16) were deposited in the Bews herbarium, UKZN, Pietermaritzburg. Plant materials were oven-dried at 50 °C, powdered and kept at ambient temperature.

The dried, powdered plant materials were extracted non-sequentially using 50% methanol, 70% acetone and distilled water with sonication in a water bath for 1 h. The extracts were filtered through a Whitman No. 1 filter paper. The organic solvent extracts were concentrated under reduced pressure at 30 °C. Water extracts were collected into glass jars and freeze-dried. The concentrated organic extracts were transferred into sample vials and dried at room temperature under a stream of cold air. The dried extracts were kept in airtight glass sample vials at 10 °C in the dark until required for the different assays.

Plant materials for total phenolic content quantification were extracted as described by Makkar (1999) with modification. Dried plant samples (0.2 g) were extracted with 10 ml of 50% methanol by sonication in a water bath containing cold water for 20 min. The extracts were centrifuged at approximately 3,000 U/min for 5 min and the supernatants collected were placed on ice for immediate analysis.

*2.2. Alpha-glucosidase inhibitory activity*

The alpha-glucosidase inhibitory activity of the plant extracts was determined using the method described by Tao et al. (2013) with slight modifications in a 96-well microtiter plate as detailed by Rengasamy et al. (2013). The concentration of *p*-nitrophenol (*p*NP) released was

quantified using an Opsys MR 96-well microplate reader at 405 nm. Acarbose, dissolved in dimethylsulfoxide, was used as a positive control while phosphate buffer served as a negative control. The determinations were carried out in triplicate. The percentage (%) inhibition was calculated by using the following equation:

$$\% \text{ Inhibition} = (A_{control} - A_{sample})/A_{control} \times 100$$

where $A_{control}$ is the absorbance of the control and $A_{sample}$ is the absorbance of the sample.

The $IC_{50}$, which is the half-maximal inhibitory concentration of the sample required to inhibit the enzyme was determined for each sample.

*2.3. Anti-inflammatory activity*

The anti-inflammatory activity was determined using the 15-lipoxygenase (LOX) enzyme inhibition assay as described by Pinto et al. (2007) with slight modifications. The assay is based upon the formation of a complex colour that absorbs maximally at 560 nm. In brief, 20 µl of the plant extract was added to 50 µl of 15-LOX (100 µg/ml, Sigma-Aldrich) in well of a 96-well microtitre plate and incubated for 5 min at 25 °C. After incubation, 50 µl of the substrate (10 µl linoleic acid in 30 µl absolute ethanol and made up to 40 ml with borate buffer) was added to the mixture and the microtitre plate was further incubated for 20 min at 25 °C. After the second incubation period, 100 µl of FOX reagent (a mixture of 90 mL absolute methanol and 10 ml distilled water, 166.66 µl concentrated sulphuric acid, 7.6 mg xylenol and 2.8 mg ferrous sulphate) was added to each well and the microtitre plate was incubated again at 25 °C for 25 min. After incubation, the absorbance readings at 560 nm were recorded. Quercetin was used as a positive control. The experiment was done in triplicate and repeated three times.

*2.4. Acetylcholinesterase inhibitory activity*

Inhibition of acetylcholinesterase enzyme (AChE) by plant extracts was evaluated using a colorimetric method which measures the increase in yellow color produced from thiocholine when it reacts with the dithiobis nitrobenzoate ion (Ellman et al., 1961). The detail of the procedure was outlined by Fawole et al. (2010). Galanthamine was used as a positive control. The experiment was conducted in triplicate. Percentage inhibition by the extracts and galanthamine were calculated using the equation below:

$$\text{Inhibition (\%)} = \left[1 - \left(\frac{\text{Sample reaction rate}}{\text{Blank reaction rate}}\right)\right] \times 100$$

*2.5. Antioxidant activity*

*2.5.1. β-Carotene–linoleic acid model assay*

The antioxidant potential of *C. edulis* and *C. dimidiatus* extracts to inhibit or reduce *β*-carotene–linoleic acid oxidation was evaluated using the method of Amarowicz et al. (2004) with slight modifications as previously outlined by Amoo et al. (2012). Butylated hydroxytoluene (BHT) and methanol were used as positive and negative controls, respectively. All samples (extracts and positive control) were evaluated in triplicate.

*2.5.2. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay*

The free radical scavenging activity of the plant materials was determined using the DPPH assay as described by Karioti et al. (2004) with modifications (Fawole et al., 2010). The reaction mixtures were incubated at room temperature for 30 min in the dark. Decrease in the purple colouration of the reaction mixtures was read at 517 nm using a UV–visible spectrophotometer. The negative control was methanol while ascorbic acid was used as a

positive control. The determination was carried out in triplicate. The $EC_{50}$ value, which is the half-maximal concentration of the sample required to scavenge 50% of DPPH free radical was determined for each sample. The antioxidant activity index for each extract was thereafter calculated as outlined by Scherer and Godoy (2009).

### *2.6. Total phenolic content determination*

The total phenolic concentration in each plant sample was determined using the Folin Ciocalteu (Folin C) method as described by Makkar (1999). Gallic acid was used as a standard for plotting the calibration curve. Each sample was assayed in triplicate. Total phenolic concentration was expressed as gallic acid equivalents (GAE) per g dry weight.

### *2.7. Flavonoid content quantification*

The flavonoid content was evaluated using aluminium chloride colorimetric method described by Zhishen et al. (1999). Catechin was used as a standard for plotting the calibration curve. Each sample quantification was done in triplicate. The amount of flavonoids in each plant material was expressed as catechin equivalents (CE) per g dry weight.

### *2.8. Condensed tannin content quantification*

The butanol-HCl colorimetric method (Makkar 1999) was used to quantify condensed tannin content in each plant material. Each sample determination was done in triplicate. Condensed tannin content (% per dry matter) was calculated as leucocyanidin equivalents (LUE) using the formula indicated by Porter et al. (1986):

Condensed tannin = ($A_{550\ nm}$ × 78.26 × dilution factor)/(% dry matter)

where $A_{550\ nm}$ is the absorbance of the sample at 550 nm.

*2.9. Cytotoxicity assay*

*2.9.1. Cell culture*

Three cell lines were used for the cytotoxicity experiments, which are the Vero (African green monkey kidney), $HepG_2$, and RAW 264.7 murine macrophage cell lines obtained from the American Type Culture Collection (Rockville, MD, USA). The cells were cultured in plastic culture flasks in Dulbecco's Modified Eagle's Medium (DMEM) containing L-glutamine supplemented with 10% foetal calf serum (FCS) and 1% PSF (penicillin/streptomycin/fungizone) solution under 5% $CO_2$ at 37 °C, and were split twice a week. Cells were seeded in 96 well-microtitre plates and incubated for 24-48 h.

*2.9.2. Cell viability and cytotoxicity assessments*

To determine the viability of the cell lines, a cytotoxicity assay was performed following cell culture as previously described by Mosmann (1983) with slight modifications as outlined by Adebayo et al. (2015). The percentage cell viability was calculated with reference to the control (cells without extracts as 100% viability). The cytotoxicity of the extracts against the cells was assessed by the MTT reduction assay as previously described (Mosmann, 1983) and detailed by Adebayo et al. (2015).

*2.10. Data analysis*

Data obtained were subjected to statistical analysis using student *t*-test and analysis of variance to determine significant differences ($p \leq 0.05$) between the mean values. Data analysis was accomplished using SigmaPlot (version 8.0) and SPSS (version 16.0) software programs, respectively.

## 3. Results and discussion

Enzyme inhibition responses for extracts from both plant species are presented in Table 1. Water extract of *C. edulis* leaves exhibited noteworthy $IC_{50}$ value of 0.005 mg/ml against alpha-glucosidase enzyme as compared to water extracts of *C. dimidiatus* with $IC_{50}$ value 0.652 mg/ml. When compared to the positive control acarbose, *C. edulis* water extract and *C. dimidiatus* methanol extract demonstrated a stronger alpha-glucosidase inhibitory activity. It was particularly interesting to observe a noteworthy inhibitory effect with water extracts against alpha-glucosidase enzyme given that the majority of the traditional remedies are prepared as water extracts. This finding shows that these two plant species could be a good source of alpha-glucosidase inhibitor.

Inflammation is a common risk factor in the pathogenesis of conditions such as infections and type 2 diabetes mellitus (Donath and Shoelson, 2011; Donath, 2014). Therefore, anti-inflammatory activity of *Carpobrotu*s species was evaluated using 15-LOX model of inhibition activity. In general, extracts obtained from *C. edulis* demonstrated a significantly strong LOX-17 inhibitory activity in comparison to extracts from *C. dimidiatus* (Table 1). The 70% acetone and water extracts of *C. edulis* particularly showed highly promising 15-LOX inhibition with $IC_{50}$ value less than 100 µg/ml. Nevertheless, the inhibitory activity shown by *C. dimidiatus* extracts indicates the potential of this plant species in managing inflammatory conditions. In a previous study (Fawole et al., 2010), *C. dimidiatus* leaf extracts exhibited

higher cyclooxygenase-2 (COX-2) than COX-1 enzyme inhibition. Thus, this plant species may potentially contain compound(s) that are dual inhibitors of COX and LOX enzymes. The search for, or discovery of dual inhibitors is deemed to be a better alternative to the use of non-steroidal anti-inflammatory drugs (Viji and Helen, 2008).

In diabetic neuropathy, it is known that cerebral uptake of glucose is significantly affected and the brain suffers from hypoglycemic episodes as has been explained by Biessels et al. (1994) and Galanopoulas et al. (1988). The AChE activity recorded with the extracts from the two *Carpobrotus* species was very low. However, *C. edulis* extracts exhibited significantly high AChE inhibition activity when compared to *C. dimidiatus* extracts.

Reactive free radicals can result in degradation of protein, lipid peroxidation, and oxidation of DNA, which have been considered to be linked with many chronic diseases, such as diabetes, cancers, and atherosclerosis (Jayathilake et al., 2016). Compounds that can scavenge free radicals have great potential in ameliorating these disease processes (Houghton et al., 2007). Oxidative stress in diabetes may partially be reduced by antioxidants and it has been suggested to reduce the long-term complications in diabetic patients (Sabu and Kuttan, 2002). Two *Carpobrotus* species that are used to treat diabetes were thus tested for free radical scavenging activity using DPPH assay. This assay is widely used to test the ability of compounds or extracts to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity (Kirtikar and Basu, 2006; Shekhar and Anju, 2014). The β-carotene-linoleic model assay, which is based on inhibition of lipid peroxidation, was also used to determine the sample antioxidant activity. The 70% acetone and water extracts of *C. edulis* demonstrated significantly high free radical scavenging as indicated by their $EC_{50}$ values with correspondingly high antioxidant activity indices when compared to other extracts (Table 2). All the extracts (except *C. dimidiatus* 70% acetone) obtained from the two plant species demonstrated high antioxidant activity (> 70%) in the lipid peroxidation assay (Table 2). Potent

antioxidant and anti-inflammatory activities of these *Carpobrotus* species can be considered suitable for the prevention and/or lessening of diabetes complication.

Significantly high concentrations of total phenolic content (81.5 mg GAE/g dry matter) and flavonoids (41.2 mg CE/g dry weight) were detected in *C. edulis* as compared to *C. dimidiatus* (Fig. 1). However, condensed tannin content was similar for the two *Carpobrotus* species. Research has indicated that the consumption of phenolic rich foods or beverages can prevent diseases such as cancer, inflammation and diabetes (Chalise et al., 2010). Previously, Martins et al. (2011) isolated catechin and epicatechin from *C. edulis*. Phenolic compounds are well known for their antioxidant activity and LOX enzyme inhibitory activity (Adebayo et al., 2015; Albano et al., 2012). Tannins are associated with the ability to form complexes with proteins, such as digestive enzymes and fungal or viral toxins (Bruneton, 1995). Tannins also have a vasoconstriction effect and reduce fluid loss from wounds and burns, thereby enhancing tissue regeneration (Bruneton, 1995; Van Wyk et al., 2009). Thus, the observed activities in the two *Carpobrotus* species might be due to the presence of the phenolic constituents. Moreover, *Carpobrotus* species are known to possess hyperoside, which contributes to anti-inflammatory properties (Bruneton, 1995).

Studies by several researchers (Dalla Nora et al., 2010; Ping et al., 2012; Ribeiro et al., 2016) revealed that some of the plants used as food or in traditional medicine have cytotoxic and genotoxic effects. Figures 2 and 3 show the cell viability of RAW 264.7, Vero kidney and HepG2 cells after exposure to *C. edulis* and *C. dimidiatus* extracts at 250, 100 and 50 µg/ml. As the concentration decreased, there was an increase in cell viability in majority of the cases (Fig. 2). A crude extract is generally considered to have very strong *in vitro* cytotoxic activity if the $LC_{50}$ is < 20 µg/ml (Boik, 2001). All extracts had very low cytotoxicity activity against all tested cells. The methanol extract of *C. dimidiatus* exhibited lowest cytotoxicity effect with $LC_{50}$ >1000 µg/ml against RAW 264.7 and Vero kidney cells (Table 3). It was interesting to

observe moderate to low $IC_{50}$ values (ranging from 297.51 to >1000 µg/ml) of water extracts against RAW 264.7 and Vero kidney cell. Hafsa et al. (2016) reported that *C. edulis* exhibited a cytotoxic effect against human colon cancer cell line HCT116 with a significant decrease in cell viability after 24 h of incubation. *C. dimidiatus* displayed no toxic effects on the brine shrimp and had no mutagenic potential towards TA 98 and TA 100 (Hurinanthan, 2009). In the cytotoxicity assay using the cell line K562, the extract had a stimulatory effect in a concentration dose dependent manner (Hurinanthan, 2009). However, other cytotoxicity tests against different cell lines need to be conducted to confirm the safety of the plant.

## 4. Conclusion

The present study demonstrated the medicinal potential of the two *Carpobrotus* species. The current findings clearly indicated their antidiabetic, anti-inflammatory and antioxidant properties, albeit using *in vitro* assays. The current findings provide a rationale for the use of these plant species in traditional medicine. Extracts of these plants can be developed into functional food for the amelioration or prevention of chronic diseases such as diabetes and chronic inflammation. However, the use of animal models or *in vivo* assays are needed to establish the safety and efficacy of these species in traditional medicine.

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**Table 1**

Anticholinesterase (AChE), alpha-glucosidase and anti-inflammatory inhibitory properties of two *Carpobrotus* species

| Plant species | Extract | AChE inhibition (%) at 1.0 mg/ml | Alpha-glucosidase inhibition ($IC_{50,}$ mg/ml) | 15-LOX inhibition activity ($IC_{50}$, µg/ml) |
|---|---|---|---|---|
| *Carpobrotus edulis* | 50% methanol | 28.8 ± 3.07 a | ND | 120.2 ± 17.42 b |
| | 70% acetone | 34.0 ± 2.88 a | ND | 22.3 ± 4.11 a |
| | Water | 20.0 ± 0.08 b | 0.005 ± 0.000 a | 59.8 ± 5.50 a |
| *Carpobrotus dimidiatus* | 50% methanol | 3.4 ± 1.46 c | 0.144 ± 0.017 a | 1532.9 ± 12.93 e |
| | 70% acetone | 5.8 ± 1.1 c | ND | 528.7 ± 2.08 c |
| | Water | 0 c | 0.652 ± 0.085 b | 700.0 ± 0.01 d |
| Galanthamine (at 1 µM) | | 82.8 ± 0.29 | | |
| Acarbose | | | 0.429 ± 0.012 | |
| Quercetin | | | | 60.35 ± 5.26 |

ND = not determined, LOX = lipoxygenase. In each column, values with different letters are significantly ($p \leq 0.05$) different based on Duncan's Multiple Range Test.

**Table 2**

Antioxidant activity of different extracts from leaves of two *Carpobrotus* species.

| Plant species | Extract | Antioxidant activity (%) at 2.0 mg/ml | Free radical scavenging activity | | Antioxidant activity index |
|---|---|---|---|---|---|
| | | | Activity (%) at 83.33 µg/ml | $EC_{50}$ (µg/ml) | |
| *Carpobrotus edulis* | 50% methanol | 89.3 ± 1.57 a | 92.8 ± 0.31 a | 10.6 ± 0.52 b | 1.9 |
| | 70% acetone | 81.8 ± 4.13 b | 93.5 ± 0.58 a | 5.6 ± 0.58 a | 3.5 |
| | Water | 78.3 ± 1.94 b | 85.9 ± 0.31 c | 6.2 ± 0.12 a | 3.2 |
| *Carpobrotus dimidiatus* | 50% methanol | 76.4 ± 1.81 b | 10.5 ± 0.51 e | ND | ND |
| | 70% acetone | 45.1 ± 0.83 c | 90.5 ± 0.31 b | 9.2 ± 0.28 b | 2.1 |
| | Water | 77.9 ± 1.79 b | 22.2 ± 0.34 d | ND | ND |
| BHT | | 97.7 ± 0.58 | | | |
| Ascorbic acid | | | | 2.1 ± 0.22 | |

ND = not determined, BHT = butylated hydroxytoluene. In each column, values with different letter(s) are significantly ($p \leq 0.05$) different based on Duncan's Multiple Range Test

**Table 3**

Cytotoxicity of *Carpobrotus edulis* and *C. dimidiatus* leaf extracts.

| Plant species | Extracts | Cell lines $LC_{50}$ (µg/mL) | | |
|---|---|---|---|---|
| | | RAW 264.7 | Vero kidney | HepG2 liver |
| *Carpobrotus edulis* | 50% Methanol | 224.16 ± 10.85 | 191.33 ± 2.43 | 89.98 ± 10.29 |
| | 70% acetone | 173.74 ± 47.69 | 157.39 ± 23.02 | 849.86 ± 7.13 |
| | Water | 300.63 ± 2.78 | 309.27 ± 19.71 | 297.51 ± 11.03 |
| *Carpobrotus* | 50% Methanol | >1000 | >1000 | 826.15±40.56 |
| *dimidiatus* | 70% acetone | 244.70 ± 9.82 | 176.93 ± 1.91 | 160.86 ± 39.93 |
| | Water | 420.57 ± 16.20 | >1000 | 69.58 ± 24.47 |
| Doxorubicin | | 5.53 ± 0.51 | 1.02 ± 0.25 | 0.125 ± 0.11 |

**Figure legend**

Figure 1: Total phenolic, flavonoids and condensed tannin contents of two *Carpobrotus* spp. Values are reported as mean ± SEM (standard error). GAE = gallic acid equivalents; catechin equivalents (CE); LUE = leucocyanidins equivalents. *** = 0.01, ns= non-significant (student's *t*-test)

Figure 2: The effect of *Carpobrotus edulis* leaf extracts on cell viability of three cell lines A) RAW 264.7, B) Vero and C) HepG2 cell viability. Values are reported as mean ± SEM (standard error). Positive control (doxorubicin) against RAW 264-7 cell at 4, 2, 1 µM showed 77.96 ± 2.12; 80.95 ± 5.50; 87.06 ± 4.95 percentage cell viability respectively; Vero cells at same concentration showed 13.07 ± 1.36; 52.07 ± 7.03; 52.07 ± 7.03 percentage cell viability; HepG2 cells at same concentration showed 5.04 ± 1.17; 12.78 ± 11.70; 49.25 ± 3.30 percentage cell viability.

Figure 3: The effect of *Carpobrotus dimidiatus* leaf extracts on cell viability of three cell lines A) RAW 264-7, B) Vero and C) HepG2. Values are reported as mean ± SEM (standard error). Positive control (doxorubicin) against RAW 264-7 cell at 4, 2, 1 µM showed 77.96 ± 2.12; 80.95 ± 5.50; 87.06 ± 4.95 percentage cell viability respectively; Vero cells at same concentration showed 13.07 ± 1.36; 52.07 ± 7.03; 52.07 ± 7.03 percentage cell viability; HepG2 cells at same concentration showed 5.04 ± 1.17; 12.78 ± 11.70; 49.25 ± 3.30 percentage cell viability.

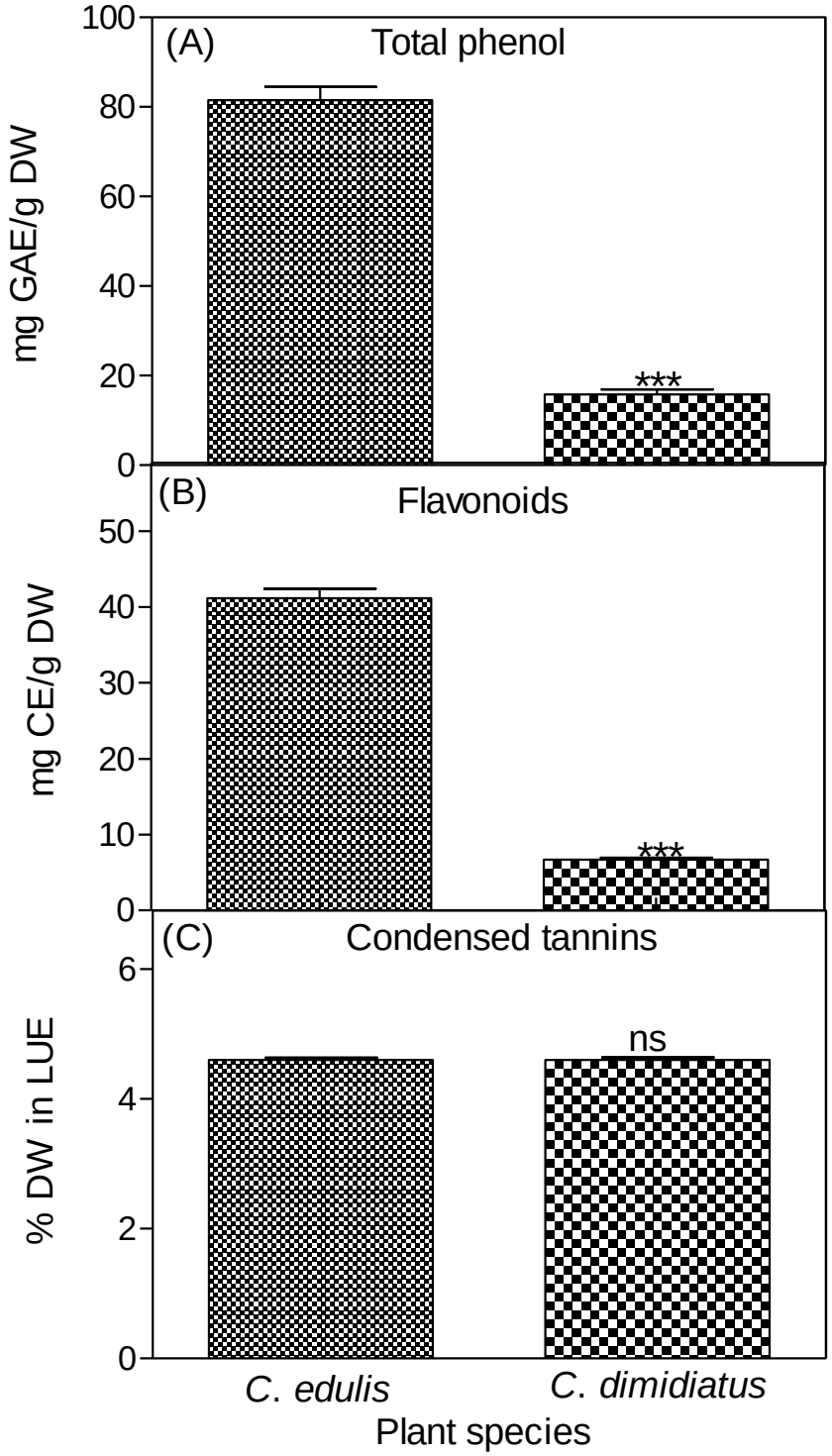
(A)
Total phenol
mg GAE/g DW
100
80
60
40
20
0
***
(B)
Flavonoids
mg CE/g DW
50
40
30
20
10
0
***
(C)
Condensed tannins
% DW in LUE
6
4
2
0
ns
C. edulis
C. dimidiatus
Plant species

Fig. 1

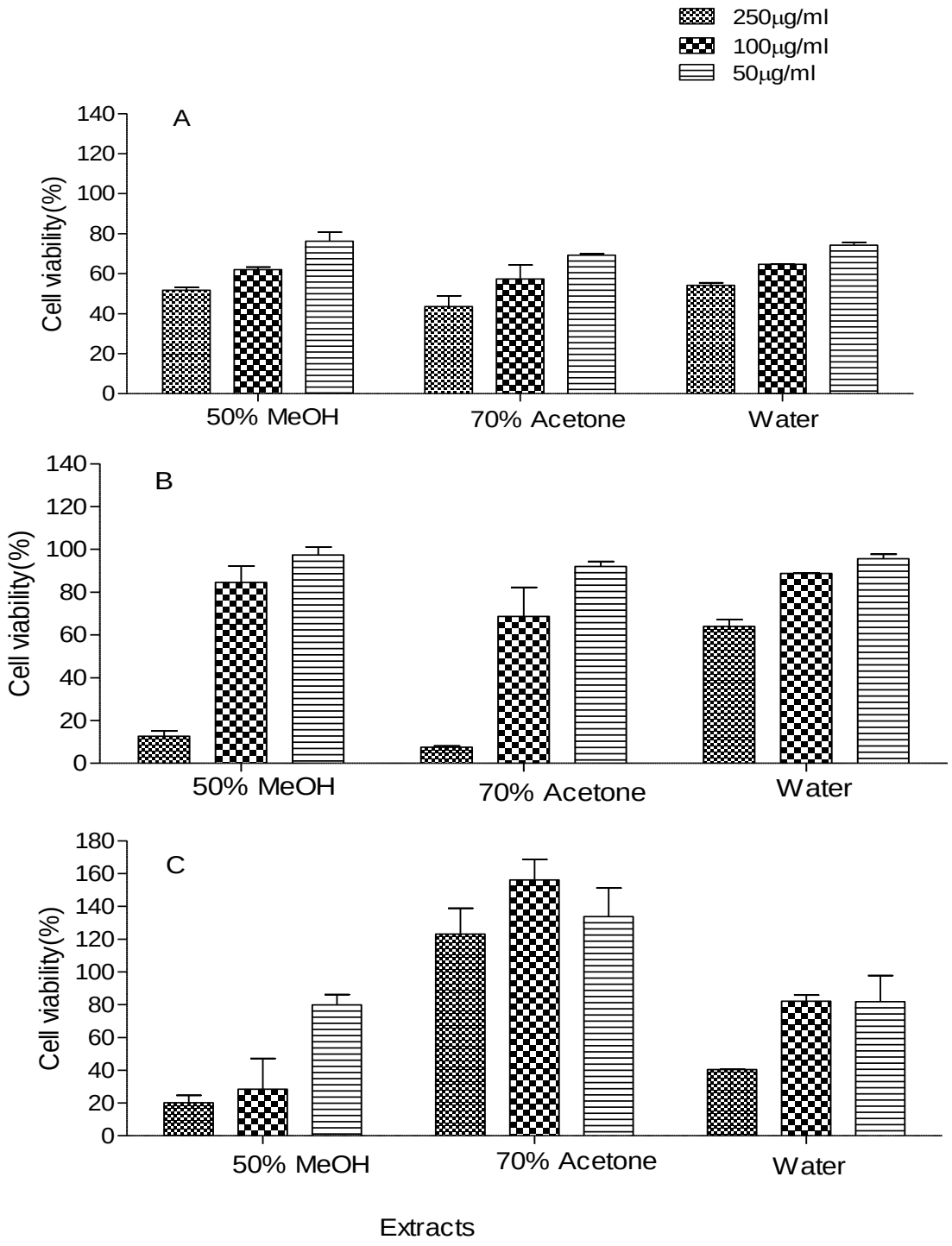
250µg/ml
100µg/ml
50µg/ml
A
B
C
Cell viability(%)
0
20
40
60
80
100
120
140
160
180
50% MeOH
70% Acetone
Water
Extracts

Fig. 2

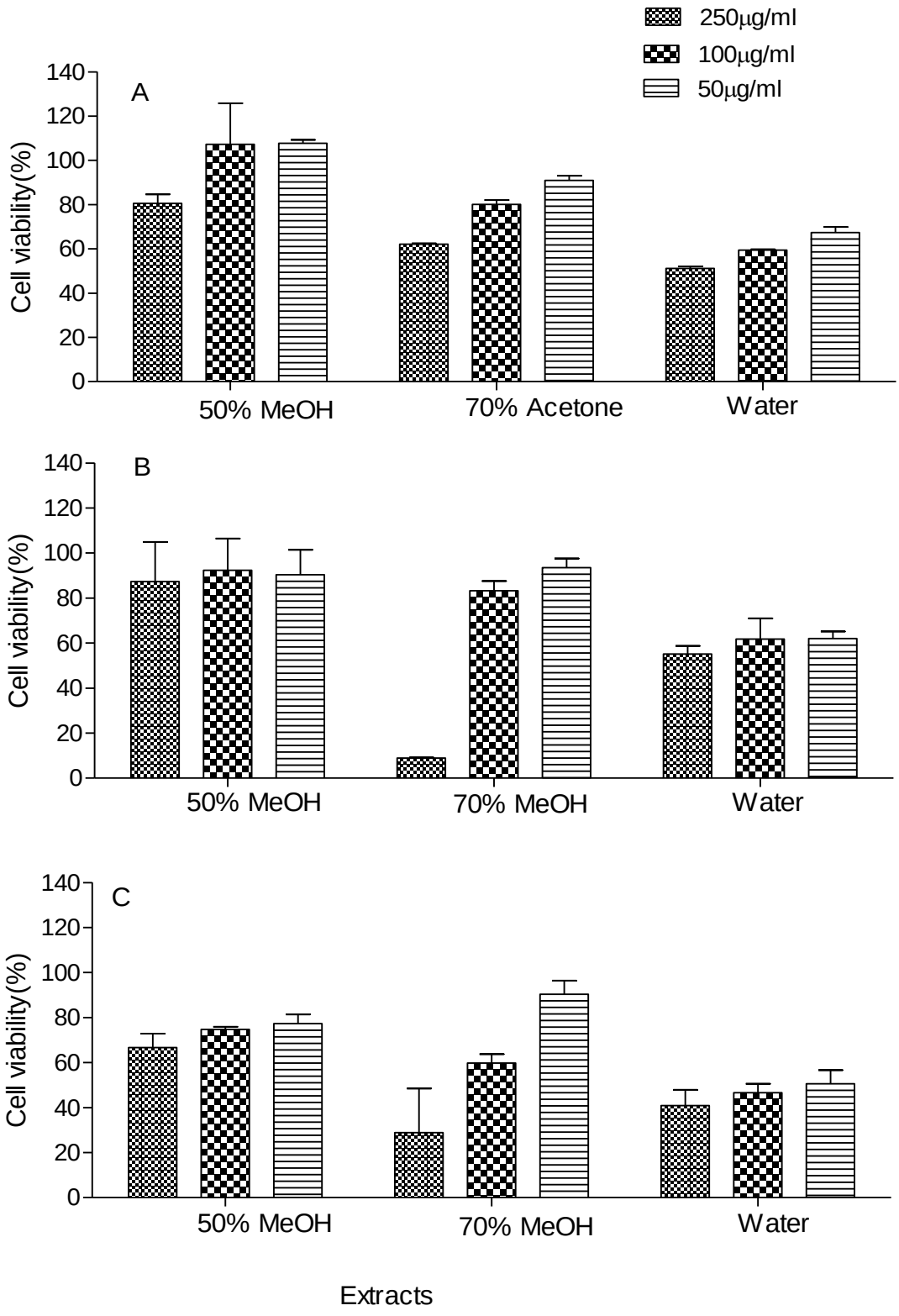
250µg/ml
100µg/ml
50µg/ml
A
B
C
Cell viability(%)
0
20
40
60
80
100
120
140
50% MeOH
70% Acetone
70% MeOH
Water
Extracts

Fig. 3