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Abstract

Background: Existing populations of *Cryptocarya latifolia* (Lauraceae) are rapidly declining as a consequence of their substitutive use for *Ocotea bullata*. The uncontrolled and excessive removal of the bark and roots of this species has led to the death of many of these plants and may eventually result in its depletion in the natural habitat.

Materials and Methods: The secondary metabolites from the leaves and fruits of *C. latifolia* were extracted using solvents of various polarities, isolated using column chromatography and identified using spectroscopic techniques. The *in vitro* free radical scavenging activity (antioxidant capacity) of selected phytochemicals at varied concentrations was determined by the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay. A propagation study of the species was also conducted.

Results: The compounds isolated from the plant were the novel compound, α -pyrone (5-hexyltetrahydro-2H-pyran-2-one) and known compounds quercetin-3-O-rhamnoside, β -sitosterol, copaene and nerolidol. The radical scavenging activity of the isolated compounds indicated moderate to good anti-oxidant activity. Treatment of explants with BAP: NAA at 1.0:0.01 mg L⁻¹ produced the highest percentage of shoots (94%) and longest shoot length (8.06 mm).

Conclusion: This study validates the ethno-medicinal use of the plant and supports the replacement of bark and roots by leaves and fruits for the management and conservation of this declining plant species. The benefits of consuming the fruits are two-fold as they can also contribute to the recommended dietary allowances of most essential elements for the majority of individuals.

Key words: secondary metabolites, antioxidants, bud break, nutrition.

Introduction

Lauraceae is a family comprising 50 genera and 2500-3000 species (Bannister et al., 2012; Cuca et al., 2013). The family consists mainly of aromatic evergreen trees and shrubs distributed worldwide (Miller and Tuck, 2013). The genus *Cryptocarya* is commonly found in some parts of Southern Africa. *Cryptocarya latifolia* (broad-leaved quince) is found in the KwaZulu-Natal and Eastern Cape provinces of South Africa where the plant is believed to possess similar medicinal properties to *Ocotea bullata*, a highly endangered species.

In South Africa, *O. bullata* and now *C. latifolia* (Zschocke and van Staden, 2000), are used by traditional healers for the treatment of various ailments including headaches, morning sickness, pulmonary disease, tuberculosis and bacterial and fungal infections (Drewes et al., 1995; Wang et al., 2009; Sabitha et al., 2009). As a result of its substitutive use for *O. bullata*, *C. latifolia* is massively exploited and is on the list of declining indigenous medicinal plant species in South Africa. Many plants are exploited by traditional healers within a large informal business system (Van Wyk, 1997) since they are collected illegally through unsustainable harvesting techniques, resulting in the depletion of wild populations (Cunningham, 1993).

This study aimed at isolating the phytochemical constituents of the leaves and fruits of *C. latifolia* and comparing their biological activity to that of compounds previously isolated from the bark and root to evaluate the substitutive use of the leaves and fruits in traditional medicine as a conservation strategy. Besides the medicinal significance of *C. latifolia*, it is also of economic importance as a food source due to the fruits being eaten by humans. An elemental analysis of the fruits was also conducted to assess for nutritional value. Because of the conservation status of *C. latifolia* due to over-exploitation, the development of appropriate propagation protocols to enhance its conservation is vital. Plant tissue culture, which is the *in vitro* mass-propagation of plants on nutrient media under aseptic conditions, has become the most widely used technique for rapid propagation of plants in need of conservation (Bhojwani and Razdan, 1986; Chawla, 2002; Rout et al., 2006). Therefore, this study also aimed to develop an efficient shoot multiplication protocol for sustainable mass propagation of *C. latifolia*.

Materials and Methods

General Experimental Procedure

Sample Collection

The leaves and fruits of *C. latifolia* were collected in December, 2012 from the University of KwaZulu-Natal (UKZN)

Botanical Gardens at the Pietermaritzburg campus in the province of Kwazulu- Natal, South Africa while the potted plants of *C. latifolia* were purchased from Tropical Nursery (Durban, South Africa). These were identified by herbarium technician, Mr Edward Khathi, from the Ward Herbarium, School of Life Sciences, UKZN (Westville).

The collected leaves and fruits were separately air dried at room temperature to constant weight in a drying room. Thereafter, they were separately crushed using a domestic blender (Russell Hobbs) and kept in plastic containers in a refrigerator at 4 °C for further analysis. The potted plants were transferred to a shade house facility and watered twice daily by an automatic sprinkler for 3 min. In addition, the plants were treated with systemic fungicides and nutrient mixtures weekly.

Characterisation and Quantification Methods

NMR spectra (1D and 2D) were recorded in deuterated MeOH (CD₃OD) and deuterated chloroform (CDCl₃) at room temperature using a Bruker Avance^{III} 400 MHz and 600 MHz spectrometer with tetramethylsilane (TMS) as an internal standard. IR spectra were recorded using a Perkin Elmer Universal ATR Spectrometer. UV spectra were recorded using the UV-Vis-NIR Shimadzu UV-3600 spectrometer with MeOH as a solvent. GC-MS data were recorded on an automated GC-MS (split-less mode) equipped with a DB-5SIL MS fused silica capillary column (30 m x 0.25 mm i.d., 0.25 µm film thickness). Helium (0.70 mL min⁻¹) was used as a carrier gas and acetonitrile (ACN) was used to dissolve the sample. 1 µL of each sample solution was injected into the GC-MS. The injector was kept at 250 °C whilst the transfer line was at 280 °C. The column temperature was held at 60 °C for 2 min, and then ramped to 280 °C at 20 °C min⁻¹ where it was held for 10 min. The MS was operated in the EI mode at 70 eV. Melting points (uncorrected) were recorded on an Ernst Leitz Wetzlar micro-hot stage melting point apparatus. All chemicals used were supplied by Merck and Sigma Chemical Companies and were of analytical-reagent grade. All spectral data obtained were compared with those reported in literature for identification of known compounds.

Phytochemical Analysis

Ground leaves (560.6 g) and fruits (50.35 g) were sequentially extracted with hexane, dichloromethane (DCM) and methanol (MeOH) for 72 hr each on an orbital shaker set at 120 rpm. Crude extracts were filtered using Whatman No. 1 filter paper then concentrated under reduced pressure using a rotary evaporator. The crude extracts were transferred into beakers and further dried in a drying room for 48 hr. The beakers were then sealed with plastic paraffin film (Parafilm) and stored in a refrigerator at 4 °C for further analysis.

Crude extracts were subjected to column chromatography using suitably sized columns and silica gel (kieselgel 60, 0.063-0.200 mm, 70-230 mesh ASTM). Fractions collected were monitored by TLC (Merck silica gel 60, 20 x 20 cm F254 aluminium sheets), visualized using anisaldehyde spray reagent (97: 2: 1; MeOH: conc. H₂SO₄: anisaldehyde) and analyzed under UV (254 nm). For the crude methanol extract from leaves, partitioning was performed in a separating funnel before isolation. The aqueous methanol extract was partitioned for 12 hr in triplicate with equal volumes of DCM. The collected DCM fractions were combined, concentrated using a rotary evaporator and subjected to column chromatography. The aqueous MeOH extract was subjected to the same procedure using ethyl acetate.

The ethyl acetate fraction from the MeOH extract of the leaves was separated using a hexane: ethyl acetate solvent system starting with 100% hexane which was gradually increased by 10% to 100% ethyl acetate in hexane. Ten fractions containing 50 mL each were collected from every solvent system. The fractions collected at 100% ethyl acetate, which had the same TLC profiles, were combined to give fraction A. This fraction was rechromatographed using an ethyl acetate: methanol (80:20) solvent system and purified to yield compound **1** (72 mg).

The same solvent system (hexane: ethyl acetate) was used to separate the DCM fraction (main) from the methanol extract of the leaves using column chromatography. Fractions with the same TLC profiles were combined and purified. Compound **2** (28 mg), a white crystalline solid, was isolated in fraction 16 (90:10, hexane: ethyl acetate) and oily substance, compound **3** (158 mg) was isolated in fractions 35-49.

The crude hexane and DCM extracts from the leaves showed similar spots on the TLC plate therefore these extracts were combined then subjected to column chromatography. The mass of the combined extracts was 21.04 g. Initially elution was effected using 100% hexane which was stepped by 5% to 100% ethyl acetate. Ten fractions of 50 mL were collected for each solvent system. Two fractions (33 and 34) yielded compound **4** (17.8 mg). Fractions 31-32 were combined and rechromatographed to give an oily compound, compound **5** (450 mg), which eluted with a hexane: ethyl acetate (95:5) solvent system.

The methanol extract (4.56 g) from the fruits was subjected to column chromatography using a hexane: ethyl acetate solvent system, starting with 100% hexane that was stepped by 10% to 100% ethyl acetate. Ten fractions of 50 mL each were collected from every solvent system. The fractions collected at 100% ethyl acetate yielded compound **1** (38.8 mg).

The crude hexane and DCM extracts from the fruits showed similar TLC profiles therefore they were combined. The column was eluted with 100% hexane which was stepped by 5% to 100% ethyl acetate. Compound **2** (12 mg) was eluted with a hexane: ethyl acetate (85:5) solvent system.

Anti-Oxidant Activity

The antioxidant activity of the isolated compounds was determined using the DPPH radical scavenging assay as described by Murthy et al. (2012) with little modification. A volume of 150 µL of a methanolic solution of each compound at varying concentrations (240, 120, 60, 30 and 15 µg mL⁻¹) was mixed with 500 µL of methanolic DPPH solution (0.1 mM). These solutions were incubated in the dark for 30 min at room temperature thereafter the absorbance was recorded at 517 nm against the blank (MeOH) with the aid of a UV spectrophotometer. Ascorbic acid was used as the standard and a methanolic DPPH solution without phytochemicals was used as the control. The percentage scavenging activity was calculated according to the equation below:

$$\% \text{ scavenging activity} = \frac{Ac - As}{Ac} \times 100$$

Where Ac = Absorbance of Control, As = Absorbance of Sample.

Elemental Analysis

The analytical reagents used were of analytical reagent grade and were supplied by Merck and Sigma Chemical Companies. Double distilled water was used throughout the experiments. All the glassware used were washed with 6.0 M HNO₃ and rinsed with double distilled water to remove contaminants.

Digestion of fruits was achieved with the aid of a CEM MARS microwave digester (Model No. Mars6) by means of Teflon (TFM) lined vessels (HF 50). In order to get accurate results, five replicate digestions were done. Approximately 0.5 g of the fruit samples were accurately weighed and transferred into the vessels, to which, 10 mL of 70% HNO₃ was added and sealed for digestion. The power was set at 1600 W and the temperature was held at 210 °C for 30 min. After the digestion was completed, the power was reduced gradually until cooled. The digests were then transferred to 50 mL volumetric flasks and diluted with double distilled water to the graduation mark. These were then analysed for As, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Se and Zn by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES). The accuracy of the elemental analysis was achieved by use of the Certified Reference Material (CRM), *lyophilized brown bread* (BCR 191), from the Community Bureau of Reference of the Commission of the European Communities. The emission lines were selected according to minimal spectral interferences.

Biotechnological Study

Stem segments of *C. latifolia* (8-10 cm) containing nodes were excised from the mother plants in the shadehouse. They were rinsed using tap water to remove dust particles and one-third of the leaf sections were removed. Thereafter, individual nodal segments were decontaminated in a laminar flow hood using various sterilants (70% ethanol, 1.75% NaOCl and Tween20[®]), antibiotic (Ampicillin[®]) and fungicides (Celest[®] and Heritage[®]). Subsequently, the cut ends of nodal segments were trimmed with a sterile blade and placed onto nutrient media comprising full strength Murashige and Skoog (1962) basal salt medium, sucrose (30 g L⁻¹), agar (10 g L⁻¹) and supplemented with various concentrations and combinations of plant growth regulators (PGRs). Prior to the addition of agar, the pH of all media was adjusted to 5.8. PGR-free media was used as a control. The media was autoclaved at 121 °C and 1.2 kg cm⁻² for 20 min. One nodal segment per culture tube was used. Sixteen explants were used for each treatment. The cultured explants were placed in a growth room at 25 °C, 16:8 hour light:dark photoperiod under diffuse white light at 55 μmol m⁻² s⁻¹ and observed every three days for bud break (BB).

Statistical Analysis

Percentage shoot length and shoot numbers were analysed using one way ANOVA and Tukey's Post-Hoc (HSD) test using the Statistical package for the Social Sciences (SPSS) (version 21).

Results and Discussion

Phytochemical Analysis

Two known compounds, quecetin-3-O-rhamnoside (**1**) and β-Sitosterol (**2**) were isolated as a yellow amorphous solid and white crystalline solid both from the leaves and fruits of the plant, respectively. Their structures were identified by comparison of their spectral data with that from literature (De-Eknamkul and Potduang, 2003; Ma et al., 2005; Park et al., 2011). Compounds **3** and **5** were isolated with a mass of 158 mg and 450 mg, respectively. These compounds were the major components of the essential oil from *C. latifolia* leaves. Their structural identification was by GC-MS according to the fragmentation patterns and by comparison with the National Institute of Standard and Technology (NIST 05, 2005) database and literature (Klopell et al., 2007; Xie et al., 2012). Compound **3** was identified as copaene while compound **5** was identified as nerolidol.

Compound **4** (Figure 1), a yellow amorphous solid with a mass of 17.4 mg, was elucidated using a combination of different spectroscopic methods; UV (CH₃OH) 223 nm; IR ν_{max} (cm⁻¹) 2923 (C-H), 1714 (C=O), 1167 (C-O); ¹H-NMR (CDCl₃, 400 MHz) δ ppm; 3.99 (2H, dd, J = 2.42, 5.86 Hz, H-6), 2.33 (2H, t, J = 0.68 Hz, H-3), 1.69 (2H, m, H-4), 1.59 (1H, m, H-5), 1.35 to 1.25 (m, H-7, H-8, H-9, H-10 and H-11), 0.91 (3H, t, J = 7.42 Hz, H-12); ¹³C-NMR (CDCl₃, 400 MHz) δ ppm; 173 (C-2), 33.8 (C-3), 24.3 (C-4), 38.6 (C-5), 66.7 (C-6), 29.6 (C-7), 23.7 (C-8), 28.7 (C-9), 30.3 (C-10), 22.8 (C-11), 10.8 (C-12); GC-MS m/z; 185 (M⁺ + 1), 129, 99.0.

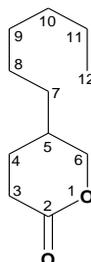


Figure 1: Structure of α-Pyrone (**4**) isolated from *C. latifolia* leaves

The $^1\text{H-NMR}$ spectrum of compound **4** showed a resonance at δ_{H} 3.99 (2H, dd, $J = 2.42, 5.86$ Hz, H-6) and a multiplet resonating at δ_{H} 1.59 (1H, H-5). The proximity and position of these protons was confirmed by the COSY experiment. The triplet at δ_{H} 2.33 (2H, t, $J = 0.68$ Hz, H-3) correlated with the carbon resonance at δ_{C} 33.8 as confirmed by the HSQC experiment and with the carbon at δ_{C} 24.3 (C-4) in the HMBC experiment. The resonance at δ_{H} 1.69 (2H, m, H-4) was assigned to this position due to HSQC, COSY and HMBC correlations. A single methyl resonance at δ_{H} 0.91 (3H, t, $J = 7.42$ Hz, H-12) and five methylene resonances between δ_{H} 1.35 to 1.25 (H-7, H-8, H-9, H-10 and H-11) indicated the presence of a hydrocarbon side chain. The $^{13}\text{C-NMR}$ spectrum of compound **4** showed 11 carbon signals. The quaternary carbon resonance at δ_{C} 173 (C-2) as indicated by the DEPT (90 & 135) spectra was due to the carbonyl functional group. The DEPT (90 & 135) spectra also confirmed the presence of a methine group at δ_{C} 38.6 (C-5). The IR spectrum of compound **4** showed a band at 2923 cm^{-1} due to the C-H stretching vibration, a sharp band at 1714 cm^{-1} due to the presence of the carbonyl group ($-\text{C}=\text{O}$) and a band at 1167 cm^{-1} confirming the presence of a C-O bond. The UV spectrum showed a peak at 223 nm characteristic of lactone absorption. The GC-MS spectrum showed molecular ion peak at m/z 185 ($\text{M}^+ + 1$) therefore, compound **4** was identified as α -Pyrone (5-hexyltetrahydro-2H-pyran-2-one), a novel compound with molecular formula of $\text{C}_{11}\text{H}_{20}\text{O}_2$.

Antioxidant Activity

The DPPH radical scavenging assay is the most widely used method for testing the ability of plant extracts to scavenge free radicals generated from the DPPH reagent (Dahech et al., 2013). Figure 2 shows the anti-oxidant capacity of the various compounds isolated from *C. latifolia* using the DPPH free radical scavenging assay. The results show that the anti-oxidant capacity of the compounds is dependent on concentration similar to the standard (ascorbic acid).

All the compounds except β -sitosterol (**2**) exhibited good anti-oxidant activity. Therefore, β -sitosterol was omitted from the figure and further discussions. Quercetin-3-*O*-rhamnoside (**1**) showed percentage scavenging inhibition between 92-98% at all concentrations tested. Similarly, 5-hexyltetrahydro-2H-pyran-2-one (**4**) was also found to have 91% inhibition at a higher concentration ($240\text{ }\mu\text{g mL}^{-1}$), however, at a lower concentration ($15\text{ }\mu\text{g mL}^{-1}$) it showed 73% inhibition, indicating good oxidative effect at high concentrations. Copaene (**3**) was also found to exhibit anti-oxidant activity, though it was lower than the other compounds tested. Similarly, nerolidol (**5**) has free radical scavenging activity with highest inhibition at $240\text{ }\mu\text{g mL}^{-1}$. This assay confirms the radical scavenging abilities of the compounds isolated from the leaves of *C. latifolia* supports its use in reducing oxidative stress in the body.

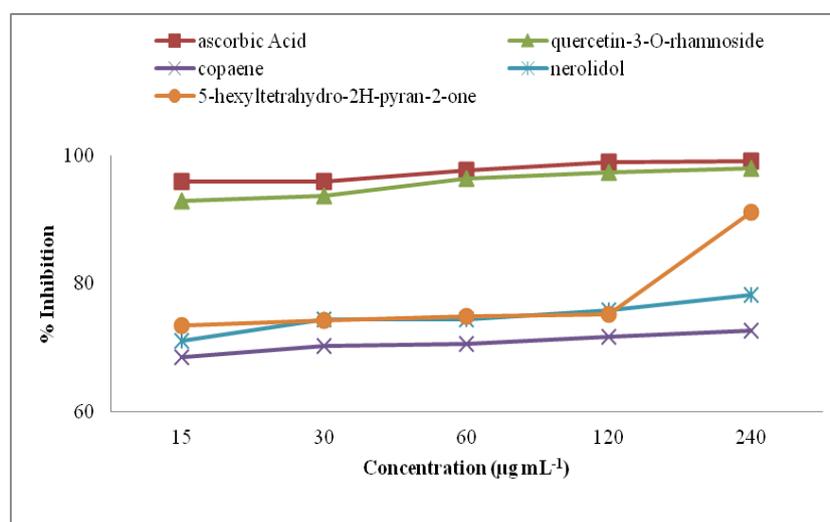


Figure 2: Anti-oxidant activity of compounds.

Medicinal Significance of Isolated Compounds

Quercetin-3-*O*-rhamnoside is known to have anti-influenza and anti-diarrheal activity (Choi et al., 2009) and it is used for the treatment of stage I hypertension as reported by Edwards et al (2007). β -sitosterol is reported to modify the level of cholesterol concentration in cancer membranes, thereby inhibiting the viability of cancer-cell growth in the stomach, lungs and breast (Sosińska et al., 2013). Nerolidol is used as a topical skin penetration enhancer for the delivery of therapeutic drugs. Nerolidol also possesses anti-fungal, anti-malarial and anti-ulcer activity and is cytotoxic on renal cell adenocarcinoma (Klopell et al., 2007; Sperotto et al., 2013). Copaene is reported to possess anti-hepatotoxic activity (Vinholes et al., 2013).

Elemental Analysis

The accuracy of the method for elemental analysis was measured by comparing results obtained with certified results (Table 1). The values for Fe, Mn, Cu and Zn are certified whilst those for As, Mg, and Ca are suggestive therefore no uncertainties are provided for these elements. All the values were found to be in agreement with the CRM values thereby validating the method. The concentration of elements in *C. latifolia* fruits were found to be in increasing order of Pb < Cr < Se < Ni < Cu < Zn < Mn < Fe < Mg < Ca. Elements that were not detected were As, Cd, and Co because their concentrations were below the detection limit of the instrument.

Table 1: Comparison of measured and certified values in the certified reference material (*lyophilized brown bread* (BCR 191).

Elements	Concentration*	
	Measured**	Certified**
Fe	40.6 ± 1.2 µg g ⁻¹	40.7 ± 2.3 µg g ⁻¹
Mn	20.3 ± 0.6 µg g ⁻¹	20.3 ± 0.7 µg g ⁻¹
Cu	2.6 ± 0.1 µg g ⁻¹	2.6 ± 0.1 µg g ⁻¹
Zn	19.5 ± 0.4 µg g ⁻¹	19.5 ± 0.5 µg g ⁻¹
As	23.1 mg g ⁻¹	23.0 mg g ⁻¹
Mg	0.50 mg g ⁻¹	0.51 mg g ⁻¹
Ca	0.41 mg g ⁻¹	0.40 mg g ⁻¹

*Based on dry mass, **Mean ± S.D, at 95% confidence interval, n = 4.

The elemental distribution in the fruit was compared to Dietary Reference Intakes (DRIs) (Table 2). The table shows the contribution of 50 g dry mass (DM) of *C. latifolia* fruits to the nutritional requirements of most individuals for most elements.

People from rural provinces tend to depend on staple crops such as wheat and rice for their nutritional needs because they cannot afford to purchase or cultivate vegetables and fruits, as a result, malnutrition is common. In South Africa, rural families live near wild fruit-bearing trees; they can consume the wild fruits along with these staple crops to obtain a balanced diet. Table 2 shows that consumption of 50 g of *C. latifolia* fruits contributes about 8% towards the RDA for Ca. Calcium is an essential element which helps strengthen bones and its deficiency can lead to poor blood clotting and osteoporosis (Dorozhkin and Epple, 2002). The fruits of *C. latifolia* can be utilized to supplement the body with the required amount of Ca needed for a healthy life, especially for people that consider their source of Ca from milk unfavourable due to lactose intolerance.

Table 2: Dietary Reference Intake (DRI) – Recommended Dietary Allowance (RDA) and Tolerable Upper Intake Levels (UL) of elements for most individuals – compared to average concentration of elements (n = 4) in the fruits.

Element	Average Concentration (mg/50 g, DM)	DRI (mg/day)		Estimated Contribution to RDA (%)
		RDA	UL	
Ca	82.9	1000-1300	2500	6.4-8.3
Cr	0.02	0.024-0.035	ND*	57-83
Cu	1.40	0.9	8.00	156
Fe	6.00	8-18	45.0	33-75
Mg	55.9	310-320	350	17-18
Mn	3.70	1.6-2.3	9.00	161-231
Ni	0.43	ND	1.00	ND
Pb	0.01	ND	ND	ND
Se	0.12	55	400	0.2
Zn	3.18	8-11	34.0	29-40

* ND- Not determined.

In 2005, the Fe status of children under the age of 1-9 years from rural and informal urban areas of South Africa was 16% (Labadarios, 2007). Consumption of *C. latifolia* fruits can contribute to alleviating this problem as 50 g of the fruits contributes about 33-75% towards the RDA for this element (Table 2). Zinc being the only metal present in all classes of enzymes is distributed all over the human body and it helps to enhance learning (Takeda, 2001). Consumption of 50 g of *C. latifolia* fruits may contribute 29-40% towards the RDA for this element. Manganese was found to exceed the RDA for this element but not the Tolerable Upper Intake level (UL) therefore it is likely to pose no risk of adverse effects. Similarly, the elements Cr, Ni, Pb and Se were all found to conform to the RDAs and could contribute to the RDAs for these elements.

The present study on the fruits of *C. latifolia* has demonstrated that the fruits are a good source of important dietary elements, which are needed for normal growth and development, especially to vulnerable communities who have closer access to this plant.

Biotechnological Study

The results showed that explants treated with PGRs (cytokinins and auxins) had a better shoot response compared to the control (Figure 3). This indicates the influence of PGRs in shoot induction and has been reported in other studies (Hudson et al., 2002; Renukdas et al., 2010). However, the number of explants forming shoots in BB1, BB2 and BB3 were lower (41%, 71% and 52%, respectively) than the number of explants treated with combination PGRs i.e. BB4, BB5 and BB6 (76%, 94% and 82%, respectively). This revealed the greater effects of combining PGRs in promoting shoot development which was also reported in other

studies (Shaik et al., 2010). The results also showed that the number of shoots formed per explant was not dependent on the different concentrations of the combination PGRs because significant differences were not observed amongst these treatments. According to Shokri et al. (2012) the concentration of cytokinin (BAP) or auxin (NAA) at higher or lower amounts influences shoot development. Similarly Rout et al. (2006) recommended the use of the cytokinin BAP at moderate concentrations to induce shoots. It was recorded that BB5 resulted in the highest percentage of explants forming shoots (94%) compared to the other treatments and the control. The highest average number of shoots forming per explant is only one and this was found for treatments BB2, BB4, BB5 and BB6 (data not shown). But the best shoot length was achieved in BB5 (Table 3). Thus, BB5 could be a suitable treatment for *in vitro* bud break in *C. latifolia*; however, multiplication of the shoots using various PGR combinations and concentrations needs further investigation.

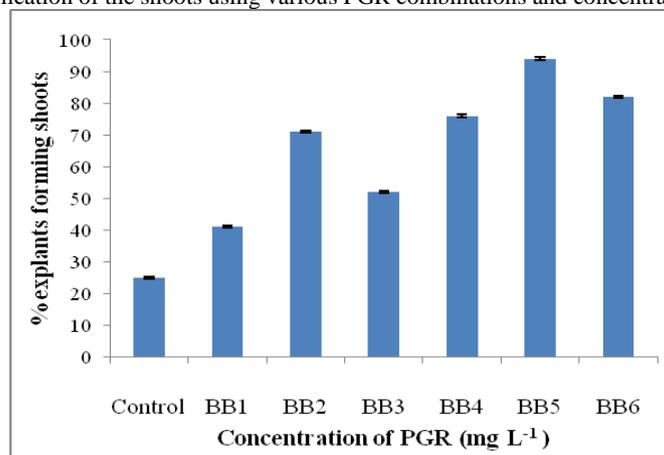


Figure 3: Effect of various combinations and concentrations of cytokinins and auxins on bud break in *C. latifolia* after four weeks of culture. Bars above each column represent mean \pm SD.

Where;

BB1: Bud Break treatment with 0.5 mg L⁻¹ BAP

BB2: Bud Break treatment with 1.0 mg L⁻¹ BAP

BB3: Bud Break treatment with 2.0 mg L⁻¹ BAP

BB4: Bud Break treatment with 0.5 mg L⁻¹ BAP: 0.01 mg L⁻¹ NAA

BB5: Bud Break treatment with 1.0 mg L⁻¹ BAP: 0.01 mg L⁻¹ NAA

BB6: Bud Break treatment with 2.0 mg L⁻¹ BAP: 0.01 mg L⁻¹ NAA

Table 3: Effect of various combinations and concentrations of cytokinins and auxins on shoot length in *C. latifolia*

Bud break (BB) treatment	Average shoot length per explant (mm)
Control	0.25 ^a \pm 0.6
BB1	1.63 ^{ab} \pm 2.1
BB2	2.34 ^{ab} \pm 2.3
BB3	2.06 ^{ab} \pm 2.6
BB4	3.44 ^b \pm 3.0
BB5	8.06 ^c \pm 3.7
BB6	3.16 ^b \pm 2.3

Different letters in a column are significantly different at $P < 0.05$. Standard deviations are included after each mean value.

Conclusion

The study investigated the phytochemical constituents in the leaves and fruits of *C. latifolia*. The compounds isolated were quercetin-3-*O*-rhamnoside (1), β -sitosterol (2) copaeene (3), α -pyrone (4) and nerolidol (5). The radical scavenging activities of the isolated compounds indicate that all compounds, except β -sitosterol, exhibit good anti-oxidant activity compared to ascorbic acid. The analytical study conducted on the fruits of *C. latifolia* indicates that they are a good source of important dietary elements and may contribute significantly to the diet. The biotechnological study, showed bud break success in nodal explants using various media formulations, however, the combination of BAP:NAA at 1.0:0.01 mg/L in full strength MS medium (4.4 g/L) supplemented with sucrose (30 g/L) and solidified with agar (10 g/L) produced the most explants (94%) that formed new shoots. This study has provided a bud break protocol that can be used to generate *C. latifolia* shoots for medicinal or conservation purposes, it validates the ethno-medicinal use of the plant and supports the replacement of bark and roots by leaves and fruits for the management and conservation of this declining plant species.

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