Nutritional value and bioactive properties of *Opuntia ficus-indica* cladodes

Submitted in fulfilment for the Degree of Master of Applied Sciences in Biotechnology in the Department of Biotechnology and Food Technology, Durban University of Technology, Durban, South Africa

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DECLARATION

I, Mr. Mabotja M.B- 21014410, declare that this study titled: **Nutritional value and bioactive properties of *Opuntia ficus-indica* cladodes,** presents my original work and it has not been submitted in any form to another academic institution. Where use was made of the work of others, it has been duly acknowledged in the text. The research described in this dissertation was carried out in the Department of Biotechnology and Food Technology, Faculty of Applied Sciences, Durban University of Technology, South Africa and the Agricultural Research Council, Roodeplaat, Pretoria, South Africa under the supervision of **Prof T. Kudanga** and **Prof S.O. Amoo**.

Signature of student **Date**

Signature of supervisor **Date**

Signature of co-supervisor **Date**

DEDICATION

To the Mabotja family, “Bakone ba ntšhi dikgolo”.

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LIST OF ABBREVIATIONS

Abs	Absorbance
ANT	Antioxidant activity
ATCC	American type culture collection
BHT	Butylated hydroxytoluene (2,6-Di-*tert*. butyl-*p*-cresol)
CE	Catechin equivalents
DMRT	Duncan"s Multiple Range Test
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DPPH	1,1-Diphenyl-2-picrylhydrazyl
Folin-C	Folin-Ciocalteu
GAE	Gallic acid equivalents
gDW	Gram dry weight
H_2O	Water
HCl	Hydrochloric acid
HPLC	High-performance liquid chromatography
IC_{50}	Half maximal inhibitory concentration
ICP-OES	Inductively coupled plasma - optical emission spectrometry
INT	*p*-Iodonitrotetrazolium chloride
MBC	Minimum bactericidal concentration
MeOH	Methanol
MIC	Minimum inhibitory concentration
NaOH	Sodium hydroxide
PE	Petroleum ether

pNP	*p*-nitrophenol
ROS	Reactive oxygen species
RSA	Radical scavenging activity

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Mabotja, M.B., Amoo, S.O., Venter, S., Du Plooy, C.P., Kudanga, T. 2018. Nutritional value and bioactive property of spineless cactus pear. African Combined Congress. Cape Town, South Africa, 14-18 January 2018

Mabotja, M.B., Amoo, S.O., Venter, S., Du Plooy, C.P., Kudanga, T. 2018. Antibacterial activity of selected spineless cultivars of *Opuntia ficus-indica* against food pathogens. 2nd International Conference for Food Safety and Security. Pretoria, South Africa, 15-17 October 2018

ABSTRACT

Medicinal properties and pharmacological activities of plants have been attributed to their bioactive compounds. However, research has shown that bioactive compound concentrations, biological activities and nutritional profile are influenced by cultivar type.

The aim of the study was to characterize the cladodes of 42 spineless cultivars *of Opuntia ficus*-indica at the Agricultural Research Council in terms of their chemical, nutritional and medicinal properties. The antidiabetic potential of selected extracts was investigated *in vitro* against alpha-glucosidase enzyme. Aqueous methanol extracts were assayed for total phenolic and flavonoid content, and antioxidant activities using 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and β-carotene linoleic acid system assays. Antibacterial activities of different extracts were assessed against two Gram (-) and two Gram (+) bacteria and their minimum inhibitory concentration (MIC) values recorded. Vitamin C, β-carotene and individual phenolic acid contents were analysed using a Shimadzu HPLC (LC-2030C 3D) equipped with a C18 Luna® column. Quantification was achieved by calibration curve plotted using different standards. Mineral elements were quantified using inductively coupled plasma - optical emission spectrometry.

There were variations in the yield depending on the cultivars, however, 50% methanol extracts generally had higher yield as compared to petroleum ether extracts. Significant variation in phytochemical composition, pharmacological activities and nutritional value was observed in the different cultivars studied. The total phenolic content of Berg x Mexican was about 5 times higher than that of Robusta and Montery. Similarly, the flavonoid content of Turpin and Berg x Mexican was about six folds higher than that of Corfu, Montery and Amersfoort. Different cultivars showed different percentage antioxidant activities. Many of the cultivars exhibited antioxidant activity comparable to

that of butylated hydroxytoluene (BHT), a synthetic antioxidant usually used as a food additive to prevent the damage caused by free radicals during oxidation processes.

Alpha glucosidase inhibitory assay revealed a dose dependent activity with IC_{50} values ranging from 0.06 - 1.85 mg/ml and 27 of the cultivars showing IC_{50} values lower than that of acarbose. Noteworthy antibacterial activity was observed against *Bacillus subtilis* and *Escherichia coli* with MIC values below 1 mg/ml. The poorest activity was observed against *Klebsiella pneumoniae*. Petroleum ether extracts generally had the best antibacterial activity when compared to 50% methanol extracts.

Compounds profiling indicated that catechin and gallic acid were found to be present in significant quantities in all the cultivars investigated, whilst the least occurring compound was quercetin. Vitamin C content ranged from a lowest of 8.95 mg/100 g to a highest of 124.10 mg/100 g. β-carotene content ranged from a lowest of 3.9 mg/100 g and highest of 31.4 mg/100 g. Potassium and calcium were the most abundant elements present in the *Opuntia ficus-indica* with a highest of 4980.00 mg/100 g for potassium, whilst iron was found to be the least present with a range of 0.20 to 54.67 mg/100 g.

In conclusion, the observed findings indicate that the spineless cladodes of *Opuntia ficus-indica* cultivars are important sources of nutrients and bioactive properties and can be considered as functional foods. Although no cultivar could be singled out as the best as each pharmacological, phytochemical and nutritional trait was different in each cultivar, the observed findings indicate the need for careful cultivar selection when using spineless cactus pear cultivars for product development to ensure product integrity

Chapter 1 General introduction

1.1 Background

Multipurpose plants play a vital role in our everyday life and serve as an indicator of regional biocultural diversity (Mao *et al*., 2018). The use of such plants is choice-dependent based on an individual's need, such as the choice of use for recreational purpose, as food or for medicinal purpose. However, existing evidence suggest that more value is given to the edible uses of plants in comparison to other uses (Mao *et al*., 2018). Edible, multipurpose plants are especially valued for their constituent health-promoting substances such as proteins, micronutrients, and carbohydrates.

Another important aspect of multipurpose plants is their significant medicinal use, a practice that is as old as human existence, with a majority of the plants used in primary health care and relied upon by an increasing number of people (Kilham, 2008). The World Health Organisation (2013) estimated that about 80% of the world population depends on medicinal plants in meeting their primary health care needs. As a result, there has been a major growth in plant-derived, medicinally useful formulations, drugs as well as other healthcare products all over the world (Wakdikar, 2004).

Opuntia ficus-indica (Figure 1.1), a native plant of Mexico, commonly known as cactus pear, is well known for its multipurpose use as a food source and for medicinal purpose, amongst others. Although mostly cultivated for its fruits in South Africa, its medicinal properties have become a significant consideration (Feugang *et al*., 2006).

Figure 1.1: Spineless cultivars of *Opuntia ficus-indica* grown inside a glasshouse at the Agricultural Research Council – Vegetable and Ornamental Plants (ARC-VOP) (Photo: M.B. Mabotja)

The cactus pear has been used for many years in traditional medicine mainly due to its potential in the treatment of diseases. The plant has a number of pharmacological properties such as antioxidant, antidiabetic, antibacterial, anti-inflammatory, anticancer and stomach ulceration inhibitory properties (Budinsky *et al*., 2001; Kaur *et al*., 2012). The application of *Opuntia ficus-indica* for the treatment or management of indigestion, asthma, burns and wounds has also been documented (Tesoriere *et al*., 2005; Kaur *et al*., 2012; Bauman and Schmidt, 2015). In addition to its use in traditional medicine, cactus pear is an excellent source of natural dietary or mineral elements that can improve overall human health (Stintzing and Carle, 2005; Santos Díaz *et al*., 2017).

Of 300 reported species of *Opuntia*, *Opuntia ficus-indica* is the most economical and most distributed species in the cactus family (Arba *et al*., 2017). Cactus pear is

particularly a climate-smart crop that can be grown under harsh conditions due to its increased or high water use efficiency and low water demand, making it drought-tolerant (Domínguez López, 1995). With these characteristics, it grows naturally in desert or dry regions. It is able to tolerate strong winds and adapt well to high temperatures (Department of Agriculture, Forestry and Fisheries, 2009).

According to the Alien and Invasive Species Regulations in terms of the National Environmental Management: Biodiversity Act (Act No 10 of 2004) of South Africa, spiny cactus pear cultivars have been declared as a weed and are legally disallowed for trading or planting. However, the spineless cultivars are exempted from this legislation, allowing for their trading and planting. Therefore, this study focused on the spineless cultivars of *Opuntia ficus-indica.*

The spineless cladodes are used for various human applications. The young cladodes which are referred to as nopalitos, can be eaten in several ways, fresh or cooked and are also used in the production of creams, body lotions, shampoos and beverages (Stintzing and Carle, 2005; Trivedi and Raval, 2017). The older, mature cladodes serve as fodder for animal feed especially during drought conditions (López-García *et al*., 2001). The cladodes are also used for the production of flours, cakes, and nutraceuticals (Lee *et al.*, 2002).

The medicinal properties and pharmacological activities of plants have been attributed to their bioactive compounds (Tilahun and Welegerima, 2018). Bioactive compounds are defined as substances or phytochemicals with biological activities and are able to control certain metabolic processes, which results in health promoting benefits

(Galanakis, 2016; Angiolillo *et al*., 2015). Due to its strong bioactive constituents, cactus pear has recently gained much interest as a potential functional food, improving overall wellbeing and health benefits beyond the basic nutritional value. Currently, the Agricultural Research Council maintains a cultivar bank that has 42 spineless cultivars of cactus pear, some of which are not found elsewhere. However, research has shown that bioactive compound concentrations, biological activities and nutritional profile are influenced by cultivar type (Abdel-Hameed *et al*., 2014). There is thus a need to profile the available cultivars for the identification and informed selection of the best cultivars for different applications such as fodder, nutraceuticals and product development.

1.2 Aim

To characterize the cladodes of 42 spineless cactus pear cultivars in terms of their phytochemical, nutritional and medicinal properties.

1.3 Objectives

- To investigate the antidiabetic, antibacterial and antioxidant activities of different extracts of spineless *Opuntia ficus-indica* cladodes obtained from selected cultivars.
- To determine phytochemical and nutritional properties of selected cultivars using chromatographic and spectrophotometric techniques.

1.4 Hypothesis

Cladodes obtained from plants grown under the same condition will vary in terms of their phytochemical content, pharmacological properties and nutritional value, depending on cultivar type.

1.5 Thesis overview

Chapter 2: This chapter reviews literature about *Opuntia ficus-indica*, covering the description and history of the species, previous findings and information on the pharmacological, phytochemical properties, nutritional value and the different uses of the plant in different parts of the world.

Chapter 3: This chapter provides an investigation on the pharmacological (antioxidant, antibacterial and α-glucosidase inhibitory activities) and phytochemical (total and individual phenol and flavonoid) properties of selected spineless cultivars of *Opuntia ficus-indica* cladodes.

Chapter 4: In this chapter, selected nutritional traits (vitamin C, β-carotene, mineral elements) of 42 spineless cultivars of *Opuntia ficus-indica* are reported and discussed.

Chapter 5: This chapter provides a summary of key results and outlines possible correlations and interactions observed, providing conclusions and recommendations for future work.

References: Alphabetical list of references cited in this study is provided

Chapter 2 Literature review

2.1 Taxonomy, distribution and description of *Opuntia ficus-indica.*

Opuntia ficus-indica belongs to the family *Cactaceae*, commonly known as cactus family with about 1600 species belonging to 130 genera (Wallace and Gibson, 2002). An estimate of 300 species of the genus *Opuntia* has been documented (Mohamed-Yasseen *et al*., 1996) of which about 104 species are found in Mexico (Bravo-Hollis, 1937). The plant is known in different parts of the world with different common names such as cactus pear, Indian fig or Barbary fig (English), nopal (Spanish), Indianische feige (German), figo da India (Portuguese), Fichi d'India (Italian), chardon d'Inde or figue de Barbarie (French) (Mondragón-Jacobo and Pérez-González, 2001).

Occurring from Canada to Argentina, *Opuntia* is the most widely distributed genus in the Cactus family (Wilson, 2007). It is widely distributed around the entire American hemisphere: Central America, southern United States and the semi-arid regions of the Mexico, Peru, Chile, Brazil, Colombia (Gibson and Nobel, 1986; Smith, 2004; Wilson, 2007; Zorgui *et al*., 2008). The species later spread to countries like Italy, Portugal, Spain, Australia, India and into African countries such as South Africa where it has become part of the natural landscape leading people to believe that cactus pear is native to the country (Barbera, 1995; Casas and Barbera, 2002; Wilson, 2007; Bauman and Schmidt, 2015).

Opuntia species are grown worldwide for different purposes, especially in the semi-arid countries. In 2001, about 1.8 million hectares was used in the cultivation of the plant for fodder throughout the northeast of Brazil and the northern Africa although the plant has been grown for its fruits many years ago throughout Mexico, South Africa,

Morocco, Algeria, Tunisia, USA and in Italy (Mondragón-Jacobo and Pérez-González, 2001; Potgieter, 2006).

Potgieter and Smith (2006) reported that South Africa is the largest source of Cactus pear germplasm in Africa, with more than 70 cultivars of Cactus pear. The genus *Opuntia* was introduced into South Africa through the Cape region in the 17th century and was later distributed throughout the Eastern Cape and the deserted Karoo (Mciteka, 2008).

Opuntia ficus-indica is recognised as a small ground-hugging plant but can also grow to a massive tree with majority of them as shrubs. It is characterised as a perennial plant often typically branched with rounded stem segments called cladodes known commonly as pads, that appear flattened, fleshy and distinctively jointed as shown on Figure 2.1 (Wilson, 2007).

The fleshy stems or cladodes have the adaptability to store water, as well as replacing the leaves with regards to photosynthetic function. They are well articulated and are protected by a thick cuticle to reduce water loss (Feugang *et al*., 2006). The high capacity of the stems to store sufficient amount of water is due to a whitish tissue called parenchyma tissue, which enables the plant to withstand and survive long periods of drought (Nobel, 1992).

Figure 2.1: Cladodes of spineless *Opuntia ficus-indica* (photo: M.B Mabotja)

The stems or cladodes of cactus pear contain a number of varying areoles that produce the spines or hairs and can also result in the production of flowers (Figure 2.2) and later into fruits (Figure 2.3) or new stems. These areoles come in different shapes (elliptic, obovate or circular) and are distributed over the stem (Wilson, 2007). They can produce white, grey or tan to brown hair. The spines produced may come in two forms, either as hair like spines known as glochids or large fixed spines (Granados and Castañeda, 1996). The flowers come in varying colour and may be yellow, orange, red, magenta, pink or whitish (Wilson, 2007).

Figure 2.2: Flowers of *Opuntia ficus-indica* (Photo: M.B Mabotja)

The shape of the flowers varies from cylindrical, club shaped to spherical or more of less like egg shaped and may be spiny or even spineless with varying colours from green, yellow, purple, red, tan and grey (Wilson, 2007). Just like any plant of the *Opuntideae* subfamily, the fruits (Figure 2.3) of *Opuntia ficus-indica* produce seed that are hard and have a funicular envelope surrounding them (Pinkava, 2003, Stuppy, 2002, Anderson, 2001; Benson, 1982).

Infestation by the spiny forms of *Opuntia species* is controlled by biological methods with regards to the regulation against the spiny forms. Initiatives are being made as to growing and increasing the production of the spineless forms of the *Opuntia ficus-indica* which have been introduced in the Mexico for the use as fodder crops since 1914 (Barbera, 1995; De Kock, 1980).

Figure 2.3: Fruits of *Opuntia ficus-indica* (Meyers cultivar) (Photo: H. Fouché)

The use of *Opuntia ficus-indica* as a drought tolerant crop in South Africa has many advantages as it has a good potential for utilization in the dry part of the country. Some of the benefits and advantages of this plant include its high-water use efficiency, high productivity, multiple uses of the plant and its ability to adapt in varied environments (Potgieter and Smith, 2006).

2.2 Phytochemical composition

2.2.1 Phenols and Flavonoids

Polyphenols are a structural class of organic molecules that are found widely in the plant kingdom and are characterized by the presence of phenolic groups, which are the by-product of plant metabolism (Quideau *et al*., 2011). These compounds are

known to be responsible for plants antioxidant, anti-inflammation, anticancer potential and improving the overall human health (El-Mostafa *et al*., 2014; Laughton *et al*., 1991).

Research has indicated that various species of *Opuntia* particularly *Opuntia ficus-indica,* have higher concentrations of phenolic compounds such as kaempferol, taxifolin, quercetin and narcissin (Wilson, 2007; Dib *et al*., 2013; Asteloo-Garcia, 2015; Santos Díaz *et al*., 2017). Other reported phenolic compounds found in *Opuntia ficus-indica* includes feruloyl sucrose, ferulic acid and sinapoyl diglucoside (Osuna-Martínez *et al*., 2014). Some of the chemical structures of the phenolic compounds present in *Opuntia ficus-indica* are shown in Figure 2.4. *Opuntia ficus-indica* also contain some terpenoids and alkaloids (Jiang *et al*., 2003; Lee *et al*., 2003).

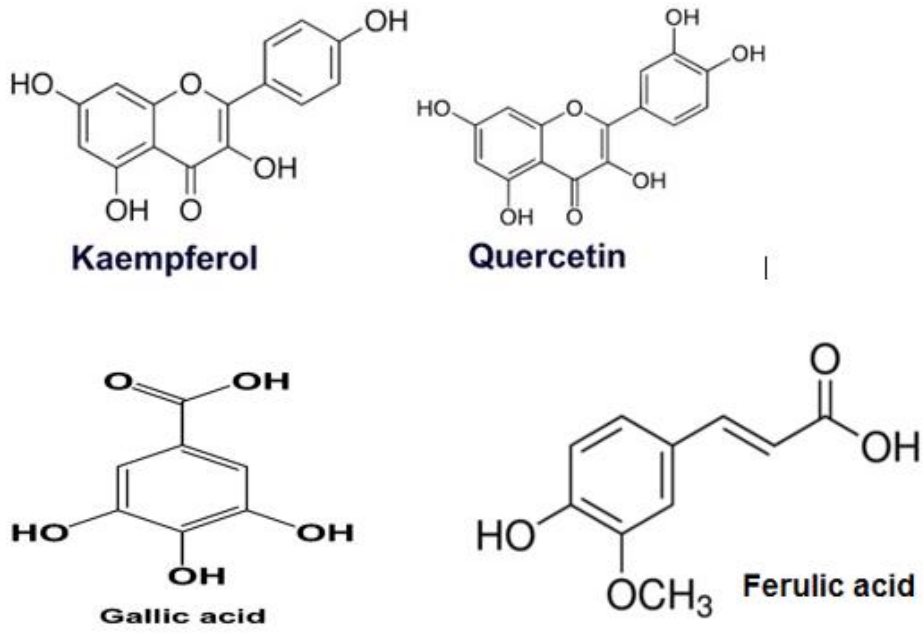

Figure 2.4: Chemical structures of some of the polyphenolic compounds present in *Opuntia ficus-indica* (He *et al*., 2015).

The cladodes of *Opuntia ficus-indica* have abundant amounts of nicotiflorin, which is a flavonoid glucoside that is mainly associated with anti-inflammatory and neuroprotective activities (El-Mostafa *et al*., 2014; Li *et al*., 2006). Li *et al*. (2006) documented the strong analgesic, anti-anaphylactic and anti-hypertensive effects of nicotiflorin. Huang *et al*. (2007) reported the protective effect of nicotiflorin against energy metabolism failure and oxidative stress in ischemic brains of rats. Although most studies show that gallic acid is most abundant in the flowering part of *Opuntia*, it is also present in the cladodes (El-Mostafa *et al*., 2014). According to El- Mostafa *et al*. (2014), gallic acid has high antioxidant activity that helps in the reduction of DNA damage and buffering of the free radicals. Moreover, gallic acid has been reported to have cytotoxic activity against tumour cells from several cancer origins such as prostate, leukaemia and lung cancer (Yen *et al*, 2002; You and Park, 2010; El-Mostafa *et al*., 2014).

Table 2.1: Some polyphenolic compounds found in *Opuntia ficus-indica* cladodes (El-Mostafa *et al*., 2014)

Polyphenolic compounds	Amount (mg/100 g dry weight)
Gallic acid	0.64–2.37
Coumaric	14.08–16.18
Salicylic acid	0.58–3.54
Ferulic acid	0.56–34.77
Nicotiflorin	2.89–146.5
Isorhamnetin-3-*O*-glucoside	4.59–32.21
Isoquercetin	2.29–39.67
Rutin	2.36–26.17
3, 4-dihydroxybenzoic	0.06–5.02

4-hydroxybenzoic	0.5–4.72
Narcissin	14.69–137.1

2.3 Pharmacological activity

The increase in resistance of microorganisms to currently available antibiotics has resulted in existing drugs being ineffective against several microbial related infections and diseases. Epidemiological studies have shown the benefits of functional foods in reducing the risk of health problems due to their pharmacological activities (Masondo *et al.*, 2016). The pharmacological activities of *Opuntia ficus-indica* are presented on Table 2.2.

Table 2. 2: Pharmacological properties of *Opuntia ficus-indica*

Scientific name	Plant part tested	Extraction solvent	Bioactivity	Report on activity	References
Opuntia ficus-indica	Cladodes	Ethanol	Antioxidant activity	A dose dependant inhibition of free-radical scavenging activity and linoleic acid oxidation in a thiocyanate assay system was observed	Lee *et al.*, 2002.
	Cladodes	Methanol	Anti-inflammatory	β-sitosterol, a phytosterol extracted from the cladodes was found to be the active anti-inflammatory compound	Park *et al*., 2001
	Cladodes Fruits	Methanol and ethyl acetate	Anti-leishmanial activity	The extracts exhibited significant anti-leishmanial activity against *Leishmania donovani* and *Leishmania major*	Bargougui *et al*., 2014
	Cladodes	Water	Anti-ulcer activity	*Opuntia ficus-indica* cladodes induced a cytoprotective effect in ethanol-induced ulcer by breaking up epithelial cells and stimulating increase in mucus production	Galati *et al*., 2001
	Fruit	Aqueous methanol	Anti-cancer activity	The extracts inhibited rapid increase in cancer cells and tumour growth in experimental nude mice ovarian cancer model	Sreekanth *et al*., 2007
	Fruits	None	Antidiabetic activity	Treatment of alloxan-induced diabetic rats with cactus juice reduced abnormal levels of cholesterol, glucose and liver glycogen etc.	Hassan *et al*., 2012

Cladodes	Water	Antiviral activity	Several virus DNA and RNA replication was inhibited after administering horses and mice with cactus extracts	Ahmad *et al*., 1996
Fruits	Methanol	Neuroprotective activity	The extracts induced a neuroprotective action against N-methyl-d-aspartate, kainate and oxygen-glucose deprivation induced neuronal injury in cultured mouse cortical cells	Kim *et al*., 2006
Cladodes	Methanol	Antibacterial activity	Strong antibacterial activity with lowest MBC's ranging from 0.8 to 5.8 mg/ml	Sánchez *et al*., 2014
Cladodes	Water	Anti-hyperlipidemic and hypercholesterolemic activity	There was a reduction in triglyceride plasma, low density lipoprotein and cholesterol levels in rats fed with *Opuntia ficus-indica* cladodes for 30 days	Galati *et al*., 2003

2.3.1 Antioxidant activity

An antioxidant is any substance that increases the degradation of reactive oxygen species (Brieger *et al*., 2012). Reactive oxygen species (ROS) have a number of biological effects and play an important role in many pathological processes and in the pathogenesis of several diseases (Brieger *et al*., 2012). As stated by Liu (2003), the antioxidant activity of fruits and vegetables may be due to the interactions between the additive and synergistic effects of the phytochemicals within the plants.

Most of the different cultivars of *Opuntia ficus-indica* have been reported to contain several antioxidants such as ascorbic acid, carotenoids, taurine, cysteine, reduced glutathionine and flavonoids, such as kaempferol, quercetin and isorhamnetin (du Toit *et al*., 2018; Tesoriere *et al*., 2005).

The antioxidant properties of cactus pear betalains have been documented (Feugang *et al*., 2006). In determining vitamin and antioxidant contents of *Opuntia spp.* cladodes and fruits, the authors observed that higher ascorbic acid, total carotenoids, vitamin E, thiamine and riboflavin concentrations than those in banana, pear, grape and apple.

A study by Osuna-Martínez *et al.* (2014) indicated that *Opuntia ficus-indica* fruits contained significantly higher ascorbic acid than the average amount found in most fruits, such as peaches and plums. By increasing antioxidant defence systems, the cladodes of *Opuntia ficus-indica* indicates a potential hepatoprotective effect against aflatoxicosis in mice (Brahmi *et al.*, 2011). The protective effect of the cladode extracts against oxidative damage is mainly attributed to different antioxidants including vitamin

E, ascorbic acid, carotenoids, flavonoids and phenolic acids (Osuna-Martínez *et al*., 2014; Stintzing *et al.*, 2001).

Avila-Nava *et al*. (2014) conducted a study on the antioxidant activity of *Opuntia ficus-indica* cladodes which indicated an increased blood and plasma antioxidant activity in participants who consumed *Opuntia ficus-indica* as opposed to those who did not. From the study, ten people were subjected to food that were poor in antioxidants for three days and their blood samples taken. After that, 300 g/day of *Opuntia ficus-indica* cladodes was added to the meals for 3 days and samples taken. The results from the blood samples obtained from the participants showed more antioxidant activity after the consumption of *Opuntia ficus-indica*.

The antioxidant activity of *Opuntia ficus-indica* ethanol extracts has been documented (Lee *et al*., 2002). A dose dependant inhibition of free-radical scavenging activity and linoleic acid oxidation in a thiocyanate assay system was observed (Lee *et al*., 2002). Both hydrolysed and unhydrolyzed extracts of *Opuntia ficus-indica* cladodes exhibited a concentration-dependent radical scavenging activity against two of the most important reactive species, superoxide and hypochlorous acid (Avila-Nava *et al.*, 2014). By using Fentons reaction mixture reaction, *Opuntia ficus-indica* ethanol extracts, indicated a protective effect on plasmid DNA against strand breakage caused by hydroxyl radicals (Lee *et al*., 2002).

2.3.2 Anti-inflammatory activity

Park *et al*. (2001) reported the anti-inflammatory activity of *Opuntia ficus-indica*. In the study, it was discovered that β-sitosterol was the active anti-inflammatory principle from the stem extract. Using writhing and hot plate test, the analgesic activity of both

fruit and stem extracts of *Opuntia ficus-indica* were evaluated (Osuna-Martínez *et al*., 2014). A study by Loro *et al*. (1999) in evaluating the anti-inflammatory activity of *Opuntia ficus-indica* showed the anti-inflammatory activity to be dose dependent when tested in rats.

Wiese *et al*. (2004) reported that hangover severity can be due to the inflammation and disruption of lipid metabolism. Osuna-Martínez *et al*. (2014) showed that alcohol hangover is mainly due to the activation of inflammation and reported the positive effect of *Opuntia ficus-indica* on alcohol hangover. *Opuntia ficus-indica* plant extract reduced hangover symptoms by inhibiting production of inflammatory mediators (Osuna-Martínez *et al*., 2014). Blood and urine samples obtained from participants who consumed *Opuntia ficus-indica* capsules before alcohol intake, indicated a reduction in hangover symptoms including dry mouth and nausea (Wiese *et al*., 2004).

2.3.3 Anti-leishmanial activity

Leishmaniases is a parasitic disease that is caused by two species of protozoan *Leishmania*, affecting millions of people worldwide (Bargougui *et al*., 2014). Alvar *et al*. (2004) reported two major forms of leishmaniasis depending on the host resistance and the species, one form being visceral leishmaniasis, which is caused by *Leishmania donovani* and the other being cutaneous leishmaniasis caused by *Leishmania major*.

In a year, about 0.5 million cases of visceral leishmaniasis and 1.5 million cases of cutaneous leishmaniasis are reported worldwide (Alvar *et al*., 2004). Treatment of leishmaniases can have toxic effects and sometimes results in drug resistance (Bargougui *et al*., 2014). New approaches have been applied for the treatment and

management of the disease. One of such approaches is the use of plant extracts (Bargougui *et al*., 2014). Ethyl acetate and methanolic extracts of *Opuntia ficus-indica* cladodes and fruits showed significant antileishmanial activity against *Leishmania donovani* and *Leishmania major* (Bargougui *et al*., 2014).

2.3.4 Anti-ulcer activity

Galati *et al*. (2001) reported the use of *Opuntia ficus-indica* cladodes in the treatment of gastric ulcer in Sicilian folk medicine and studied the effect of administration of freeze-dried cladodes on ethanol-induced ulcer in rats. From the results, administration of the cladodes showed a protective effect. Pre-treatment in the rats revealed the protective action against the ethanol-induced ulcer (Galati *et al*., 2003). Kaur *et al*. (2012) stated that the freeze-dried *Opuntia ficus-indica* cladodes maintained a cellular composition or cytoarchitecture of the gastric. The mucilage found in *Opuntia ficus-indica* cladodes carry a vital role in preventing the gastric mucosa from being penetrated by necrotizing agents (Kaur *et al*., 2012). In addition to mucilage, the cladodes also contain pectin which can affect the regeneration of gastrointestinal mucosa (Kaur *et al*., 2012). By breaking up the epithelial cells and stimulating an increase in mucus production, *Opuntia ficus-indica* cladodes induces a cytoprotective effect in ethanol-induced ulcer (Galati *et al*., 2001).

2.3.5 Anti-cancer activity

Feugang *et al*. (2006) and Kaur *et al*. (2012) reported that cactus pear fruit extract has the potential to prevent tumour growth in experimental nude mice ovarian cancer model *in vivo* and also has a benefit of inhibiting the rapid increase of ovarian, cervical and bladder cancer cells *in vitro*. The study revealed that the inhibition of rapid increase in cancer cells was either dose- or time-dependent *in vitro* (Sreekanth *et al*.,

2007). An observation on the unaffected body weight of the mice during experimentation gave an indication that cactus extracts does not have any significant toxic effect in animals (Feugang *et al*., 2006).

2.3.6 Antidiabetic activity

Several *Opuntia* spp. including *Opuntia ficus-indica* have been well documented on their potential use for treatment of diabetes (López, 1995; Bauman and Schmidt, 2015; Trivedi and Raval, 2017). The hypoglycaemic effect of *Opuntia* spp., including *Opuntia ficus-indica* has been documented (Trejo-González *et al*., 1996; Ibañez-Camacho *et al*., 1979; 1983; Frati-Munari *et al*., 1988; 1990). *Opuntia* species plant extracts in combination with insulin reduce blood glucose and glycated haemoglobin to normal (Feugang *et al*., 2006). Another study suggested a decrease in serum glucose concentrations when using cactus seed oil as a diet supplement in rats (Ennouri *et al.*, 2005). After inducing diabetes in rats using alloxan, abnormal levels of several biochemical parameters including cholesterol, glucose, urea, aminotransferase, alkaline phosphatase, haemoglobin and liver glycogen etc., were observed (Kaur *et al*., 2006). By treating the alloxan-induced diabetic rats with high doses of cactus juice, all the levels of the above biochemical parameters can be restored to normal levels (Hassan *et al*., 2012).

2.3.7 Antiviral activity

Administration of cactus stem extracts on horses, mice and humans inhibited the replication of several virus's DNA and RNA including influenza, pseudorabies, herpes simplex virus type 2 and HIV-1 (Ahmad *et al*., 1996). Moreover, extracts of *Opuntia*

ficus-indica cladode inactivated extracellular viruses but with no certainty or information regarding the active compounds in the cactus extract that is responsible for the inhibition (Ahmad *et al*., 1996)

2.3.8 Neuroprotective activity

The neuroprotective effect of *Opuntia ficus-indica* in cultured rat cortical cells has been reported (Dok-Go *et al*., 2003). The flavonoids, quercetin, quercetin 3-methyl ether and dihydroquercetin were found to be responsible for the neuroprotective action (Kaur *et al*., 2012). Quercetin, a flavonoid present in *Opuntia ficus-indica* was responsible for the neuroprotective action of methanolic extracts of *Opuntia ficus-indica* against N-methyl-d-aspartate, kainate and oxygen-glucose deprivation induced neuronal injury in cultured mouse cortical cells (Kim *et al*., 2006).

2.3.9 Antibacterial activity

The antimicrobial activity of 8 cultivars of *Opuntia ficus-indica* cladodes methanol extracts against *Campylobacter jejuni*, *Vibrio cholera*, and *Clostridium perfringens* was determined (Sánchez *et al*., 2014). The study showed all the cultivars to exhibit antibacterial activity against the microorganisms with the lowest MBC's ranging from 0.8 to 5.8 mg/ml. Kim *et al*, (2002; 2005) also reported antibacterial activity of *Opuntia ficus-indica* methanol extracts against *Enterococcus faecium, Escherichia coli*, *Salmonella* spp., *Pseudomonas aeruginosa* and *Staphylococcus aureus.*

Another study was done on the effect of *Opuntia ficus-indica* on the growth of *Campylobacter*, which is one of the most common bacteria causing human

gastroenteritis and diarrhoeal diseases (El-Mostafa *et al*., 2014). The study showed that *Opuntia ficus-indica* had antibacterial effect on the growth of two strains namely, *Campylobacter jejuni* and *Campylobacter coli* (El-Mostafa *et al*., 2014). Methanol extracts of *Opuntia ficus-indica* demonstrated strong antimicrobial effect with minimum bactericidal concentration (MBC) of 3.0 mg/ml against *Vibrio cholera* when compared to ethanol and water extracts (Sánchez *et al*., 2010)

2.3.10 Anti-hyperlipidemic and hypercholesterolemic activity

Research has indicated that *Opuntia* spp. including *Opuntia ficus-indica*, can lower the cholesterol level in the human blood and alloxan induced diabetic rabbits and mice (Feugang *et al*., 2006; You *et al*., 2010; Becerra-Jiménez *et al.*, 2012; Osuna-Martínez *et al*., 2014). There was a reduction in triglyceride plasma, low density lipoprotein and cholesterol levels in rats fed with *Opuntia ficus-indica* cladodes for 30 days (Galati *et al*., 2003). The anti-hyperlipidemic and anti-hypercholesterolemic activity of the cactus pear cladodes may be attributed to the high fibre content of the plant (Osuna-Martínez *et al*., 2014). Cactus pear seed oil has been shown to induce a decrease in plasma total cholesterol and low-density lipoprotein cholesterol in rats (Ennouri *et al*., 2005).

2.4 Nutritional content

Most studies concerning the nutritional content of cactus were focused mainly on the fruit than the vegetative part or cladodes. According to Sáenz (2000), cactus pear fruits had the same nutritional content or value as other fruits such as peach, apple, apricot and melon except that cactus pear fruits had higher total soluble solids. Another distinguishable factor of the fruit from other tropical fruits is the significant amount of magnesium, calcium and technologically interesting fibres (Stintzing *et al*., 2001). The

cactus pear fruit contains reducing sugars with about 53% being glucose and the other part being fructose (Rodríguez *et al*., 1996).

Cladodes, on the other hand, have been reported to contribute high levels of water to the diet and very rich in fibre content compared to other fruits and vegetables such as spinach, broccoli, eggplant, melon, grapes, apricot and radish (Schmidt-Hebbel *et al.,* 1992; Ayadi *et al*., 2009). In an investigation of the *Opuntia ficus-indica* plant in food value, the cladodes of *Opuntia ficus-indica* have significantly high water content of about 90.1 g/100 g, carbohydrate at 5.6 g/100 g, protein at 1.7 g/100 g, low fat content of 0.3 g/100 g and higher fibre content of 3.5 g/100g (FAO, 2013; Muñoz de Chávez *et al*., 1995).

Studies have shown that the chemical composition of cladodes can be influenced by age or maturity stage, the harvesting season, the type of species or cultivar and environmental conditions (Santos Díaz *et al*., 2017). For example, younger cladodes of *Opuntia ficus-indica* have better nutritional quality than older cladodes (Rodríguez-Felix and Cantwell, 1988). This is because the younger cladodes have a less thick cuticle and there is less expansion of the water storage parenchyma as compared to older cladodes (Rodríguez-Felix and Cantwell, 1988).

Young stems had moderate amounts of carotenoids and vitamin C at 30 µg and 11 mg/100 g, respectively (McConn and Nakata, 2004). Young cladodes or nopalitos are said to contain the same nutritional value as that of lettuce and spinach, although young cladodes are advantageous as they can still grow in unfavourable conditions such as higher temperature and with insufficient water, which are conditions that leafy vegetables cannot withstand (Cantwell, 1999).

The influence of harvesting season on the chemical composition of *Opuntia ficus-indica* was observed (Retamal *et al*., 1987b). The highest content of moisture, free reducing sugars, starch and CP were detected in spring (92.5%; 103 mg/g DW; 226 mg/g DW; 14.8% respectively) (Retamal *et al*., 1987b). At the end of the season, ash content, crude fibre and calorific content presented the highest values (29.8%; 36 mg/g DW, 144 888 KJ/kg, respectively) (Retamal *et al*., 1987b).

2.4.1 Dietary fibre

Intake of enough dietary fibre reduces the risk of diseases such as diabetes, obesity, bowel cancer, heart diseases and gallstones (Ahmad, 1995; Horn, 1997; Ayadi *et al.,* 2009). It is also beneficial for the treatment of gastrointestinal disorders, illnesses associated with low dietary fibre intake, reduction of cholesterol levels in the blood, anti-hypercholesterolemic and anti-hyperlipidemic effects (Osuna-Martínez *et al*., 2014). Dietary fibres also have functional properties that can be used in the formulation of foods, resulting in texture modification and enhancement of food stability during production and storage. Both the nutritional value and functional properties of dietary fibres are important in the potential development of a wide range of fibre-enriched foods (e.g. meat products, dairy products, drinks, sauces and bakery products) (Ayadi *et al*., 2009).

Opuntia ficus-indica cladodes have been reported to be a very good source of dietary fibre, which may assist in weight loss (Santos Díaz *et al*., 2017). The dietary fibre in the cladodes of *Opuntia ficus-indica* is composed of chemical compounds that are

resistant to digestive enzymes like pectin, cellulose, lignin and gums (Martinez-Rodríguez *et al*., 1993).

2.4.2 Fatty acids

Osuna-Martínez *et al*. (2014) and Dib *et al*. (2013) reported the presence of fatty acids in *Opuntia ficus-indica*. In addition to palmitic and linoleic acids being the most abundant, cladodes also contain oleic and linolenic acids which together with palmitic and linoleic acids make up to 90% of the total fatty acid content in cactus cladodes (El-Mostafa *et al.,* 2014; Andreu-Coll *et al*., 2019).

Although previous studies suggest the cactus fruit to be rich in fatty acids as compared to the other parts of the plant, a recent study indicated high levels of saturated fatty acids in young cladodes, higher than those of cactus fruits and old cladodes (Andreu-Coll *et al*., 2019). The high levels of fatty acids reported in *Opuntia ficus-indica* may contribute in the management of various health conditions such as cancer, diabetes mellitus, cardiovascular diseases and obesity (Andreu-Coll *et al*., 2019)

2.4.3 Vitamins

Ascorbic acid, vitamin B and α-tocopherol were reported to be the main vitamins present in the *Opuntia ficus-indica* although with varying amounts in different parts of the plant (Slimen *et al.*, 2016). The fruits are rich in betalains, vitamin K and ascorbic acid while the skin of the fruit in particular is rich in vitamin E (El-Mostafa *et al.*, 2014). *Opuntia ficus-indica* fruit contains about 180 to 300 mg/kg fresh weight of vitamin C, which is higher than that found in other common fruits like apple, banana and grape (El-Mostafa *et al*., 2014; Piga, 2004).

Although in trace amounts, the cladode is the only part of the plant that contains vitamin B particularly vitamin B1 (thiamine), vitamin B2 (riboflavin) and vitamin B3 (niacin) in which one report indicated quantities of 0.14, 0.60 and 0.46 mg/100 g fresh weight, respectively (El-Mostafa *et al.,* 2014; Slimen *et al.*, 2016). Cladodes of *Opuntia ficus-indica* contain carotenoids with varying quantities depending on the age of the plant (Jaramillo-Flores *et al*., 2003). Three of the identified carotenoids in young cladodes includes β-carotene, lutein and α-cryptoxanthin (Jaramillo-Flores *et al*.,2003; Hadj Sadok *et al*., 2008).

2.4.4 Mineral elements

Studies show that *Opuntia ficus-indica* is rich in nutritional elements including sulphur, iron, sodium, magnesium, calcium, potassium and phosphorus (Sawaya *et al*., 1983; Medina *et al*., 2007; Mciteka, 2008; Bauman and Schmidt, 2015). However, potassium and calcium were reported to be the major minerals present in higher amounts in the cladodes (Table 2.3) (El-Mostafa *et al.*, 2014).

Table 2.3: Mineral element contents of *Opuntia ficus-indica* according to El-Mostafa *et al.* (2014), expressed as mg/100 g

Mineral element	Amount (mg/100 g dry weight)
Potassium	2.35-55.20
Calcium	5.64-17.95
Magnesium	8.80
Phosphorus	0.15- 2.59
Sodium	0.3-0.4
Manganese	0.19- 0.29

Iron	0.09
Zinc	0.08

Potassium (K) was present in remarkably high amount in *Opuntia ficus-indica* cladodes (El-Mostafa *et al.*, 2014). Potassium is essential for water balance and osmotic pressure regulation, muscle contraction, acid-base balance and electrolyte balance (Maynard *et al.*, 1979; El-Mostafa *et al.*, 2014; Asteloo-Garcia, 2015; Santos Díaz *et al*., 2017).

Although the calcium level in cladodes is generally high, multiple studies indicate that in older cladodes the calcium binds to oxalates and is available in form of calcium oxalate and cannot be easily absorbed by the human digestive system (Gibson and Nobel, 1986; Wallace and Gibson, 2002). However, in younger cladodes or nopalitos, the calcium oxalate levels are moderately low, and calcium can easily be accessible (Mondragón-Jacobo and Pérez-González, 2001). This explains the rationale for the consumption of the younger cladodes as salads or vegetables and older cladodes being used in other applications, as the calcium oxalate content increases with age (Wallace and Gibson, 2002). Calcium is involved in ATP and phospholipids hydrolysis and is responsible for bone and teeth formation, blood coagulation and transmission of nerve impulses (McDonald *et al.,* 1991; El-Mostafa *et al.*, 2014; Asteloo-Garcia, 2015; Santos Díaz *et al*., 2017). Other studies show that other mineral element content in *Opuntia ficus-indica* cladodes vary significantly with the age of the plant species (Nobel and Hartsock, 1983; Retamal *et al*., 1987b; Gregory and Felker, 1992).

2.4.5 Amino acids

Cladodes of *Opuntia ficus-indica* contains several amino acids although the major ones detected were glutamine, leucine, lysine, valine, arginine, phenylalanine and isoleucine (El-Mostafa *et al*., 2014) Some amino acids such as carnosine, citrulline, ornithine, proline, taurine, and glycine were only detected in trace amounts in the cladodes (Table 2.4).

Table 2.4: Amino acid content of young *Opuntia ficus-indica* cladodes expressed as g/100 g (El-Mostafa *et al*., 2014).

Amino acid	Amount (mg/100 g dry weight)
Alanine	1.25
Arginine	5.01
Asparagine	3.13
Asparaginic acid	4.38
Glutamic acid	5.43
Glutamine	36.12
Cysteine	1.04
Histidine	4.18
Isoleucine	3.97
Leucine	2.71
Lysine	5.22
Methionine	2.92
Phenylalanine	3.55
Threonine	4.18
Tyrosine	1.46
Tryptophan	1.04

Valine	7.72
α-Aminobutyric acid	Trace
Carnosine	Trace
Citrulline	Trace
Ornithine	Trace
Proline	Trace
Taurine	Trace
Glycine	Trace

2.5 Application, uses and products of *Opuntia* spp.

Different kinds of method and practices have been applied in the processing and preservation of cactus fruit and cladodes as shown on Table 2.5 (du Toit *et al.,* 2018; Sáenz ,2000). Some of the traditional foods made from cactus pear include juices, syrups, jams, nectars, pickles, liquors, and flours (Figure 2.5). A number of functional compounds can also be extracted and used to formulate or enrich new foods. These include natural additive gums and colorants with potential for the food, pharmaceutical and cosmetic industries (Stintzing and Carle 2005; Lee *et al.*, 2003; Ayadi *et al*., 2009). Fibre-rich products can be made to help control diabetes and obesity. The plant is also the host of the cochineal insect and thus its basis for producing a highly valued natural colorant.

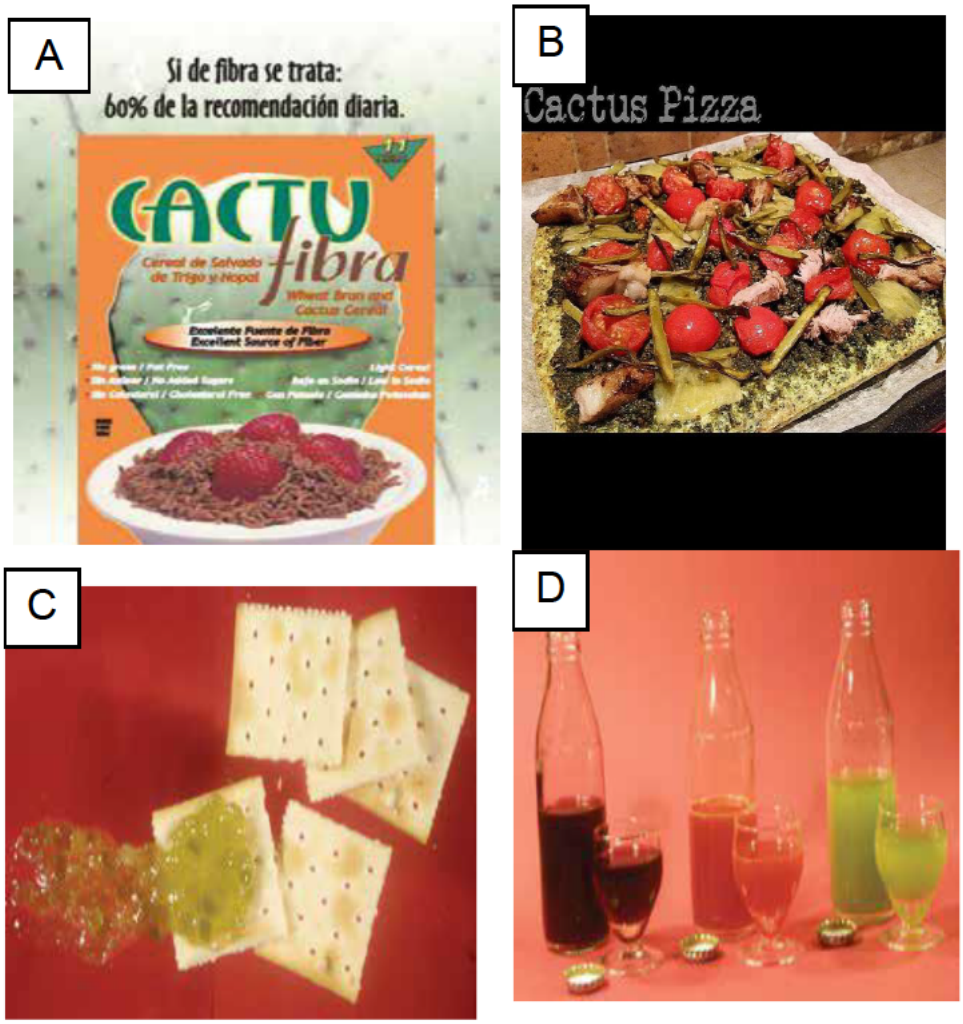

Figure 2.5: Food products made from *Opuntia ficus-indica* (A-cereals, B- pizza, C-jam and biscuits and D- juice) (Sáenz, 1996).

Table 2.5: Several new opportunities for production of cactus products based on different plant parts (Sáenz *et al*., 1996; du Toit *et al*., 2018)

Plant parts	Application and potential use
Cladodes	Food and beverage industry (alcoholic and non-alcoholic beverages. Livestock feed industry (feed supplements) Cosmetic industry (lotions and creams) Construction industry (binding products) Pharmaceutical (capsules and tablets)

	Natural additives (colourants)
	Agriculture (organic material and erosion control)
	Energy (biogas and fuelwood
	Tourism industry (artisan crafts)
Fruits/pulp and seed	Food and beverage industry (alcoholic and non-alcoholic beverages.
	Oil production
	Commercial food production
Flowers	Pharmaceutical (capsules and tablets)
	Ornamental purpose

2.5.1 *Opuntia* in traditional medicine

Parts of the *Opuntia* species particularly *Opuntia ficus-indica* have been around and used traditionally by humans for thousands of years (Smith, 1967). Although most studies have reported the consumption of the cactus fruit as a beverage or as food, other parts of the plant including the cladodes have also been used as medicine and products commercially produced in the form of capsules, pills, powder and drinks (Figure 2.6).

Figure 2.6: Medicinal products made from cladodes (Sahelian, 2016; http://wax.ricagroup.com/liposoluble_wax/*Opuntia*-oil/and https://www.amazon.com/Tadin-Aloe-Vera-Cactus-Bags/dp/B005F5KCYE)

The cladodes of *Opuntia ficus-indica* have been prepared and used in folk medicine for treatment of burns, wounds, oedema and indigestion (Kaur *et al*., 2012). In some instances, the cladodes were believed to regulate weight, blood sugar and used in the facilitation of childbirth (Casas and Barbera, 2002). Infusions of the fruits, flowers and cladodes have been used in folk medicine for treatment of fatigue (Feugang *et al*., 2006). In India, decoctions made from *Opuntia dillenii* cladodes are used orally for the treatment of snakebites (Kalita *et al*., 2014).

Preparations from the fruits and cladodes are taken as laxatives and the use of the cladodes is believed to kill pain and promote healing and thus used as a dressing for bone fractures (Stintzing and Carle, 2005; Meiswinkel *et al*., 2004). Glochids or hair like spines from the plant were rubbed into warts and moles to assist in their removal (Train *et al*., 1941). The cladodes are used in the treatment of whooping cough, as "anti-infective agents" and in the treatment of gastric ulcer (Galati *et al*., 2001, Park *et al*., 2001). Though usually well-tolerated and generally considered non-toxic when taken orally, prickly pear preparations have been reported to cause mild diarrhoea, nausea, increased stool volume, increased stool frequency, abdominal fullness, and headache (Rodriguez-Fragoso *et al*., 2008).

2.5.2 *Opuntia* use as forage

Due to its capability to adapt and grow in arid and semi-arid or dry places, *Opuntia* can help in the stabilization of agricultural industry as it can prevent losses during droughts, help in the reduction of over grazing, serving as an alternative feed for livestock and reducing poverty in poor areas and generating income (Rodrigues *et al.,* 2016). *Opuntia ficus-indica* has been used as a source of forage in dry seasons due to its good palatability, high humidity, water content and in-vitro digestibility (Suñigiga, 1980; Silva and Santos, 2007).

Several cases of using *Opuntia* as a forage for sheep, dairy goats and dairy cows were reported (Silva and Santos, 2007; Ben Salem and Smith, 2008; Rekik *et al*., 2010; Vilela *et al*., 2010; Andrade-Montemayor *et al*., 2011; Costa *et al*., 2009; 2012).

The cladodes can be processed in various ways for application in livestock feed (López-García *et al*., 2001). Although not preferred, direct feeding of the cladodes may be harmful to the animals due to the presence of spines. Different methods may be

applied for the removal of the spines including removal of the cladodes edge, which is the region with most spines or burning the cladodes with propane burner and superficial burning (Mondragón-Jacobo and Pérez-González *et al*., 2001). By applying different burning methods, the spines are removed, and the cladodes can be consumed either directly or mixed with grazing (Mondragón-Jacobo and Pérez-González *et al*., 2001).

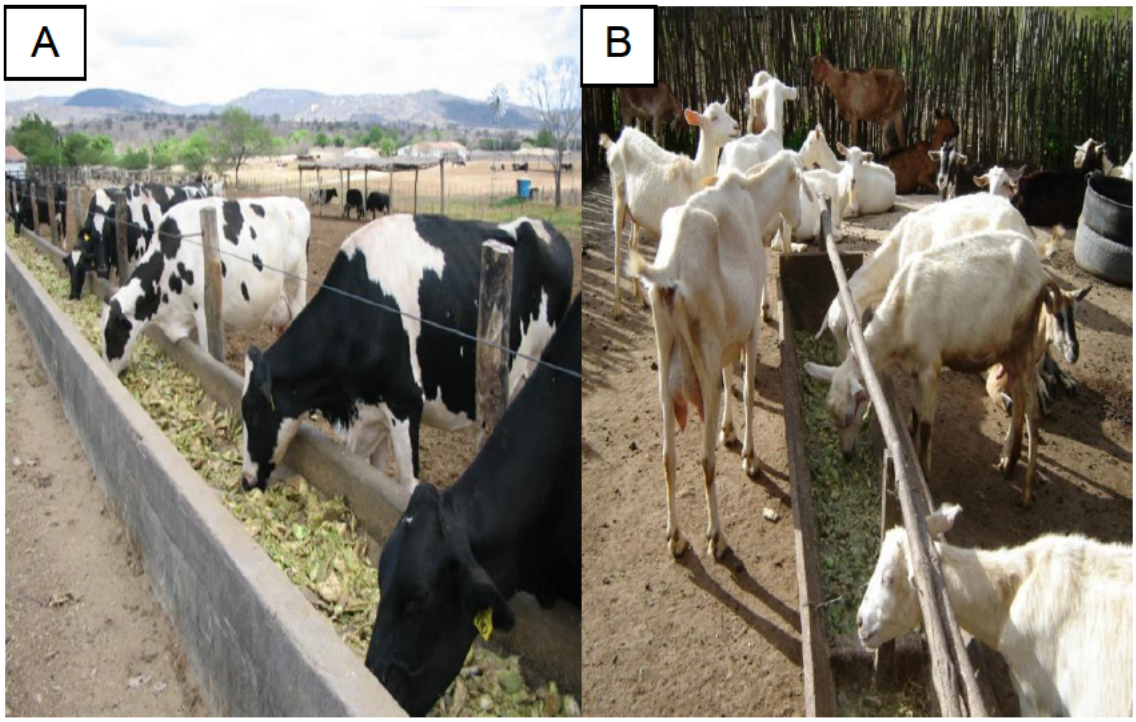

Figure 2.7: Cladodes used as fodder for cattle (A) and goats (B) (Dubeux, 2011)

During drought, when there is insufficient water and unavailability of other feed alternatives, spineless cactus cladodes can be fed in any form as shown in Figure 2.7, because livestock particularly sheep and cattle can survive on it for a long period (Casas and Barbera, 2002). Although the survival of livestock on cladodes alone has been documented, it has been indicated that for optimal utilization, the cladodes should be supplemented with other sources of livestock feed (Casas and Barbera,

2002). Cactus pear is very deficient in proteins and as such, the best supplements for animal feed should be a protein rich supplement. According to De Kock (1980), the most suitable supplement for spineless cactus meal appears to be alfalfa meal or alfalfa hay with the ration of 100 g of alfalfa in summer and 200 g in winter per sheep. Any other legume hay with reasonably high protein content can be used instead of alfalfa. Spineless cactus pads can also be used as supplementary feed on Karoo veld. The cladodes are beneficial during dry seasons due to its high-water content (Casas and Barbera, 2002)

2.5.3 Effect of *Opuntia ficus-indica* cladodes on dough characteristics and cake making

The influence of *Opuntia ficus-indica* cladodes powder on dough characteristics was determined (Ayadi *et al*., 2009). Fortification of wheat flours with *Opuntia ficus-indica* cladode powder as a source of dietary fibre indicated increased water absorption capacity of the flour and consequently the dough properties (Ayadi *et al*., 2009). An improve in texture of baking products can also be achieved by using cladodes powder flour because of its high water holding capacity (Lopez-Cervantes *et al*., 2011). Another study indicated improved nutritional content (dietery fiber, ash, potassium, magnesium and calcium) when cladodes powder flour was used as compared to wheat flour (Msaddak *et al*., 2015). A physio-chemical analysis of some Tunisian spiny (*O. ficus-indica* f. *amylocea)* and spineless cultivar cladodes (*O. ficus-indica* f. *inermis)* indicated richness of bio-molecules which can be of potential value by inclusion in food formulation to improve nutritional, technological and stability of formulated food (Ayadi *et al*., 2009). The use of cladodes powder flour in cake making results in a favourable greener colour and a darker crust of the cake as shown on Figure 2.8, which is

advantageous in that no additional synthetic colour compounds will have to be added (Ayadi *et al*., 2009).

Figure 2.8: Cake made from cladodes flour (Ayadi *et al*., 2009)

2.5.4 Other uses

The use of Opuntia ficus-indica as a windbreak and for fencing is common around small villages and farms in Morocco and Algeria (Barbera, 1995). The plants used as fence are still utilized for their fruits and as a source of fodder during dry periods (Barbera, 1995). Furthermore, the cladodes have been used in the production of shampoos, conditioners, face and body lotions (Figure 2.9), soaps, hair gels, sun protectors, and bioethanol (Stintzing and Carle, 2005; Trivedi and Raval, 2017).

Figure 2.9: Shampoos and lotions made from cladodes (Shatzman, 2018)

Chapter 3 Pharmacological and phytochemical investigation of *Opuntia ficus-indica* cladodes

3.1 Introduction

An increase in new and re-emerging pathogens, coupled with drug resistance, insufficient therapies and negative side effects of some currently used drugs, has become a major concern worldwide (Cragg *et al*., 1997). There is thus a need to find alternative, reliable therapies or solutions to the current concerns in drug research and development (Eloff, 2019). Natural products have proven to be great sources of active ingredients in drug development, showing pronounced structural diversities and biological activities when compared to their synthetic counterparts.

Plants have long been used in the past as traditional medicine and are regarded as a source of many important biologically active molecules of medicinal value (Amoo *et al*., 2009). These natural compounds can be lead compounds, allowing the design and rational planning of new drugs and the discovery of new therapeutic properties not yet attributed to known compounds (Rates, 2001).

Medicinal plants are considered as reliable sources of antimicrobial agents as they are easily accessible, perceived to be relatively safe and have less side effects (Welegerima *et al*., 2018). Bioactive components found in different parts of plants are responsible for the medicinal properties and pharmacological activities such as anthelminthic, antioxidant, antimicrobial, anti-inflammatory and anticholinesterase properties (Tilahun and Welegerima, 2018; Fawole *et al.*, 2010).

Opuntia ficus-indica has been long used in traditional or folk medicine and is considered a potential source of useful phytochemicals such as caretonoids, flavonoids, phenols and ascorbic acid (Tilahun and Welegerima, 2018). Extracts of

this species possess wide pharmacological activities such as anti-inflammatory, hypoglycemic, antimicrobial, anticancer, stomach ulceration inhibition and antioxidant properties (Tilahun and Welegerima, 2018).

The first step in the pharmaceuticals or manufacturing of phytochemical-rich products involves the use of solvents to extract such compounds of importance in plant materials and investigation of the properties responsible for prevention and treatment of diseases (Tilahun and Welegerima, 2018). Dual chemical-biological screening approach needs to be employed for the discovery of important plant-derived bioactive compounds (Hostettman and Marston, 2002). The Agricultural Research Council, South Africa, maintains a cultivar bank that has 42 spineless cultivars of *Opuntia ficus-indica*, some of which are not found elsewhere and with no or insufficient studies on their pharmacological and phytochemical properties. As indicated by Abdel-Hameed *et al*. (2014), bioactive compound concentrations and biological activities are influenced by cultivar type. It is then important to carefully study the cultivar influence of the available cultivars and identify some of the best cultivars with regards to their pharmacological and phytochemical properties. The aim of this chapter was to investigate the pharmacological potential and phytochemical content of cladodes obtained from 42 cultivars of spineless *Opuntia ficus-indica*.

3.2 Materials and methods

3.2.1 Plant material collection and preparation

Cladodes from 42 different cultivars of *Opuntia ficus-indica* were collected from the Agricultural Research Council, Vegetables and Ornamental Plant, Roodeplaat campus, Pretoria, South Africa, where they were grown in a glasshouse (25°36’1”S

28°21’42”E). The harvested cladodes (about one year old) were washed with distilled water to remove soil and any other foreign matter. The cladodes were weighed, cut into small pieces and oven-dried at 50 °C in the dark. After oven drying, the material was re-weighed and grounded to fine powders using a pulverised mill.

Twenty cultivars were selected based on their total phenolic and flavonoid content (seven of the highest, six intermediate and seven of the lowest content) and further profiled for their individual phenolic content and antibacterial activity determination.

3.2.2 Total phenolic and flavonoid content determination

The extraction procedure was carried out as described by Amoo *et al.* (2012). An amount of 0.2 g plant material was extracted by sonication in an ultrasonic bath containing ice-cold water for 30 min using 10 ml of 50% methanol. After sonication, the mixture was centrifuged at 1073.3 x g for 2 min at 25^{o}C.

Total phenolic content was determined using the Folin-Ciocalteu method described by Makkar (2003). Fifty microliters of the extract were pipetted into a reaction tube followed by the addition of 450 µl distilled water and 250 µl of Folin-Ciocalteu reagents. A volume of 1250 µl (2% w/v) sodium carbonate was added into the reaction tube. The mixture was vortexed before incubating for 40 min at room temperature. The absorbance was recorded using an Analytik Jena spectrophotometer (Specord 210 plus, Analytik Jena, Germany) at 725 nm. The assay was done in triplicates and a calibration curve was prepared using gallic acid as standards. Results were expressed in mg gallic acids equivalents (GAE) per gram dry weight (gDW)

Flavonoid content was determined using aluminium chloride method as described by Marinova *et al*. (2005). The sample (250 µl) was pipetted into a reaction tube and 1 ml of distilled water added. A volume of 75 µl (5% w/v) sodium nitrite was added to the reaction tube. After 5 min, 75 µl of aluminium chloride (10% w/v) was added followed by the addition of 0.5 ml (1 M) NaOH and 0.6 ml of distilled H2O. The mixture was vortexed, and absorbance measured using an Analytik Jena spectrophotometer (Specord 210 plus, Analytik Jena, Germany) at 510 nm. The assay was done in triplicates and a calibration curve was prepared using catechin as standards. Results were expressed in mg catechin equivalents (CE) per gram dry weight (gDW).

3.2.3 Individual phenolic content

Following the methods by Stalikas (2007), 0.2 g of the plant material was extracted using 2.5 ml of 50% methanol and 2.5 ml of 2 M HCl and incubated in a water bath at 70 °C for 10 min. After incubation, 5 ml of absolute methanol was then added and sonicated in an ultrasonic bath containing ice-cold water for 5 min. The mixture was filtered using Whatman No. 41 filter paper.

An aliquot of 1 ml of the extracted sample was transferred into a vial for high performance liquid chromatography (HPLC) analysis using a Shimadzu HPLC (LC-2030C 3D, Shimadzu Corporation, Kyoto, Japan) equipped with a C_{18} Luna® column (150 ×4.6mm, 5µ) at 25 °C. Gallic acid, caffeic acid, vanillic acid, *p*-coumaric acid and *trans*-ferullic acid were used as phenolic standards while catechin, quercetin and rutin were used as flavonoid standards. As guided by Penã-Neira (2000) and Seal (2016), with modifications, conditions for HPLC determination of phenolic acids and flavonoids are described in Table 3.1.

Table 3.1: High Pressure Liquid Chromatography (HPLC) conditions for analysis of individual phenolic acids and flavonoids

Parameter	Phenolic acids	Flavonoids
Mobile phase	Acetic acid: Acetonitrile: Water (2: 30: 68)	Methanol: Distilled water: Orthophosphoric acid (60: 40: 0.2%)
Flow rate	1 ml/min	1 ml/min
Pump mode	Isocratic	Isocratic
Injection Volume	20 µl	20 µl
Wavelength	280-360 nm	270
Column temperature	25 °C	25 °C

3.2.4 Radical scavenging activity

Samples were extracted using the extraction method described previously by Amoo *et al*. (2012) with slight modifications. An amount of 20 g dried powdered cladode from each cultivar was extracted with 300 ml of 50% methanol in a non-sequential manner. The extraction was done by sonication in an ultrasonic bath containing ice-cold water for 1 h before filtration using Whatman No. 41 filter paper. The extract volume was then condensed on a rotary evaporator at 40 °C before air-drying.

The antioxidant activity was determined using the DPPH method as described by Sharma and Bhat (2009) with modifications as described by Amoo *et al*. (2011). The crude extracts (10 mg of each) were dissolved in 1 ml of 50% methanol as a stock solution. Thirty microliters of the 50% methanol extracts at different concentrations were diluted with methanol to a final volume of 750 µl. Equal volume of DPPH solution was then added to make a total volume of 1500 µl. Ascorbic acid was used as a positive control. The mixture was incubated at room temperature for 40 min before recording absorbance at 517 nm using a UV spectrophotometer. The following

equation (1) was used for the calculation of percentage free radical scavenging activity (RSA) as determined by the discolouration of DPPH solution:

$$RSA\ (\%) = \left\{ 1 - \left(\frac{Abs_{517\ nm}\ Sample - Abs_{517\ nm}\ Blank}{Abs_{517\ nm}\ Neg\ Control} \right) \right\} \times 100 \qquad (1)$$

Where $Abs_{517\ nm}$ *Sample* is the absorbance of the sample mixture, $Abs_{517\ nm}$ *Neg Control* is the absorbance of the negative control (methanol and DPPH) and $Abs_{517\ nm}$ *Blank* is the absorbance of the blank (50% methanol in place of DPPH)

3.2.5 Total antioxidant determination

Extraction procedure was carried out as described in subsection 3.2.4. Antioxidant activity using β-carotene linoleic acid assay was determined using a method described by Amarowicz *et al*. (2003) with slight modifications detailed by Amoo *et al*. (2011). β-carotene (5 mg) was dissolved in 1 ml of chloroform. Hundred microliters of linoleic acid and 1 ml of Tween 20 were then added to the mixture. The volume was made up to 250 ml using distilled water and the emulsion mixed thoroughly.

Aliquot (2.4 ml) of the β-carotene emulsion was dispensed into reaction tubes in triplicate and then 100 µl of sample extract added at a predetermined concentration. Butylated hydroxytoluene was prepared as a positive control at concentration of 6.25 mg/ml. Fifty percent methanol in place of sample was used as a negative control.

Absorbance was measured at 470 nm immediately and then a second absorbance reading at 470 nm done after incubation in a water bath at 50 °C for 1 h. β-carotene bleaching rate was calculated using equation (2):

$$Bleaching\ rate\ (R) = \left\{ln\left(\frac{A_{t=0}}{A_{t=t}}\right)\right\} \times \frac{1}{t} \quad (2)$$

Where $A_{t=0}$ is the absorbance of the emulsion at 0 min and $A_{t=t}$ is the absorbance of the emulsion at 60 min. The bleaching rate was used to calculate the percentage antioxidant activity (ANT) expressed as percentage inhibition of the rate of β-carotene bleaching using equation (3),

$$\%\ ANT = \left(\frac{R_{\ control} - R_{\ sample}}{R_{\ control}}\right) \times 100 \quad (3)$$

Where $R_{\ control}$ and $R_{\ sample}$ is the average β-carotene bleaching rates for the control and plant extract or BHT, respectively.

3.2.6 Alpha-glucosidase inhibitory activity

Extraction procedure was carried out as described in subsection 3.2.4. Alpha-glucosidase inhibitory activity was determined using a method described by Li *et al*. (2019) with slight modifications. Yeast alpha-glucosidase (0.5 unit/ml) was dissolved in 0.1 M potassium phosphate buffer (pH 6.8) and the substrate (5 mM *p*-nitrophenyl-α-D-glucopyranoside) prepared in the same buffer (pH 6.8). Different concentrations of the samples were prepared using dimethyl sulfoxide (DMSO). Sample wells contained 20 µl sample, 100 µl buffer and 20 µl enzyme. Sample blank wells contained 20 µl sample, 100 µl buffer and 20 µl DMSO. Negative control wells contained 20 µl DMSO, 100 µl buffer and 20 µl enzyme. The plates were pre-incubated at 37 °C for 5 min and then 20 µl of the substrate added to initialise the reaction. The plates were incubated at 37 °C for 30 min. After incubation, the reaction was stopped by addition of 80 µl of 0.2 M sodium carbonate (prepared in the same potassium phosphate buffer). The tests were performed in triplicates and acarbose used as a positive control. The amount of *p*-nitrophenol (pNP) released was quantified using a 96-well

microplate reader at 405 nm. The alpha-glucosidase inhibitory rate (%) was calculated using equation (4)

$$\%\ inhibition\ rate = [1 - \left(\frac{Abs_{\ sample} - Abs_{\ sample\ blank}}{Abs_{\ negative\ control}} \right) \times 100 \qquad (4)$$

Where *Abs* $_{sample}$ is the absorbance of the sample mixture, *Abs* $_{sample\ blank}$ is the absorbance of Sample blank (20 µl sample, 100 µl buffer and 20 µl DMSO) and *Abs* $_{negative\ control}$ is the absorbance of the negative control (20 µl DMSO, 100 µl buffer and 20 µl enzyme). The IC_{50}, which is the concentration of the extract required to inhibit 50% of the alpha-glucosidase was determined for each extract using GraphPad Prism software (version 4.03). The data were log-transformed, normalized and fitted into a nonlinear regression for IC_{50} determination.

3.2.7 Antibacterial activity

Petroleum ether (100%, analytical grade) and 50% methanol were used as extraction solvents for antibacterial assay. Extraction procedure was carried out as described in subsection 3.2.4.

Antibacterial activity was determined using a serial micro-plate dilution assay developed by Eloff (1998). The petroleum ether and 50% methanol extracts were tested against two Gram-positive; *Staphylococcus aureus* (ATCC 9144)*, Bacillus subtilis* (ATCC 6051) and two Gram-negative; *Escherichia coli* (ATCC 8739) and *Klebsiella pneumonia* (ATCC 13883) bacteria. The bacterial cultures were maintained on Mueller Hinton agar medium in petri dishes. An inoculum of each microorganism was grown in Mueller Hinton broth and incubated at 37 °C for 24 h.

Equal volumes (100 µl) of distilled water and plant extract were transferred into first row wells and two-fold serially diluted down through the 96-well plates to prepare extracts with different concentrations. The excess 100 µl in the last well (well H) was discarded. Hundred microliters of the bacterial solution were then added to all the wells and ciproflaxin used as a positive control. For negative control, 50% methanol and petroleum ether were used. The plates were covered with parafilm and incubated for 24 h at 37 °C. After incubation, 40 µl of *p*-iodonitrotetrazolium chloride (INT) was added and minimum inhibitory concentration (MIC) values determined using the lowest concentration where there was no colour change as bacterial growth was indicated by pink colour whilst bacterial inhibition was indicated by no colour change after addition of INT.

3.2.8 Statistical analysis

Total phenolic and flavonoid contents, Individual phenolic acids, antioxidant, and antidiabetic data were subjected to one-way analysis of variance (ANOVA) using Statsoft (Statistica 8) software. The mean values were compared using Duncan's Multiple Range Test and significant difference was based on $P = 0.05$. All results were reported as mean ± standard error of triplicate analyses.

3.3 Results and discussions

3.3.1 Yield of plant extracts

Table 3.2 shows the percentage yield of different extracts of the studied cultivars of *Opuntia ficus-indica* cladodes. Fifty percent methanol extracts generally had higher yield as compared to petroleum ether extracts. Moreover, there were variations in the

yield depending on the cultivars. The extract yields of the highest cultivars were up to three-fold and ten-fold of the lowest yields for methanol and petroleum ether extracts, respectively.

A possible reason for the higher yields may be due to the extraction of more polar compounds than non-polar compounds in the plant material extracted. As was described by Truong *et al*. (2019), polarity of the extraction solvents could cause a wide variation in the level of bioactive compounds in the extract. Truong *et al*. (2019) further attributed the higher yields of methanol extracts on the fact that extraction efficiency favours highly polar solvents.

Table 3.2: Percentage yield obtained from different extracts of *Opuntia ficus-indica* cultivars

	Extract yield (% w/w)	
Cultivar	50% Methanol	Petroleum ether
Algerian	21.30	0.33
American giant	6.75	0.76
Amersfoort	15.95	0.80
Arbiter	11.97	0.89
Berg x mexican	21.10	0.32
Blue Motto	18.63	0.41
Corfu	8.57	0.25
Cross x	22.04	0.88
Direkteur	15.03	0.24
Ficus indice	17.38	0.18
Fresno	13.46	0.47
Fusicaulis	13.16	0.52
Gymno Carpo	20.22	0.21
Malta	25.38	0.95
Messina	14.74	0.86
Mexican	10.36	0.22
Meyers	23.83	0.88
Montery	17.83	0.63
Murado	17.78	0.22
Muscatei	20.17	**1.88**
Nepgen	20.27	0.73
Nudosa	19.66	0.19
Ofer	15.24	0.19
Polypoly	14.28	0.14

Postmasburg	13.00	0.48
R1 251	22.50	0.55
R1 259	13.63	0.22
R1 260	18.86	0.21
Robusta	15.79	0.78
Robusta x Castilo	17.59	0.57
Roedtan	9.29	0.23
Rossa	25.14	0.79
Santa Rossa	**26.10**	0.58
Schagen	12.41	0.69
Sharsheet	19.39	0.97
Sicilian Indian fig	11.81	0.18
Skinner Court	15.13	0.35
Tormentosa	11.93	0.20
Turpin	22.05	0.23
Van A5	9.65	0.37
Vryherd	10.13	0.65
Zastron	13.04	0.46

*Bold values indicating the highest recorded yield.

3.3.2 Total phenolic and flavonoid content

Figure 3.1 shows the total phenolic and flavonoid content of 42 cultivars of spineless *Opuntia ficus-indica* cladodes. Significant variation was observed in the different cultivars studied. The total phenolic content of the highest cultivars was about 5 times higher than those with the lowest total phenolic content and similarly, the flavonoid content of the highest cultivars was about six folds higher than those with the lowest flavonoid content.

A study on the total phenolic content of four cultivars of cactus pear by Chougui *et al*. (2013) reported that the total phenolic content varied amongst the different cultivars, which corroborate the findings of this study. In addition, a comparative study indicated higher flavonol and phenolic content in two South African cultivars compared to some Sicilian and Egyptian cultivars (Moussa-Ayoub *et al*., 2014). The observed findings in this study suggest higher (approximately 10 ×) total phenolic contents (4.92- 8.63 mg

GAE/g) in cactus pear cladodes than that observed in the cactus pear fruits (0.47- 0.67 mg GAE/g) as reported by Chougui *et al*. (2013). Total phenolic content of 8.26 mg GAE/g was reported in spineless *Opuntia ficus-indica* by Ayadi *et al.* (2009), which is in line with the total phenolic content reported in some of the cultivars in this study.

Phenolic compounds are one of the major groups of plant compounds reported to play a significant role in the antioxidant potential of various plants (Amoo, 2009; Abdel-Hameed *et al*., 2014). The antioxidant activity of phenolic compounds is attributed to its redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators (Abdel-Hameed *et al*., 2014).

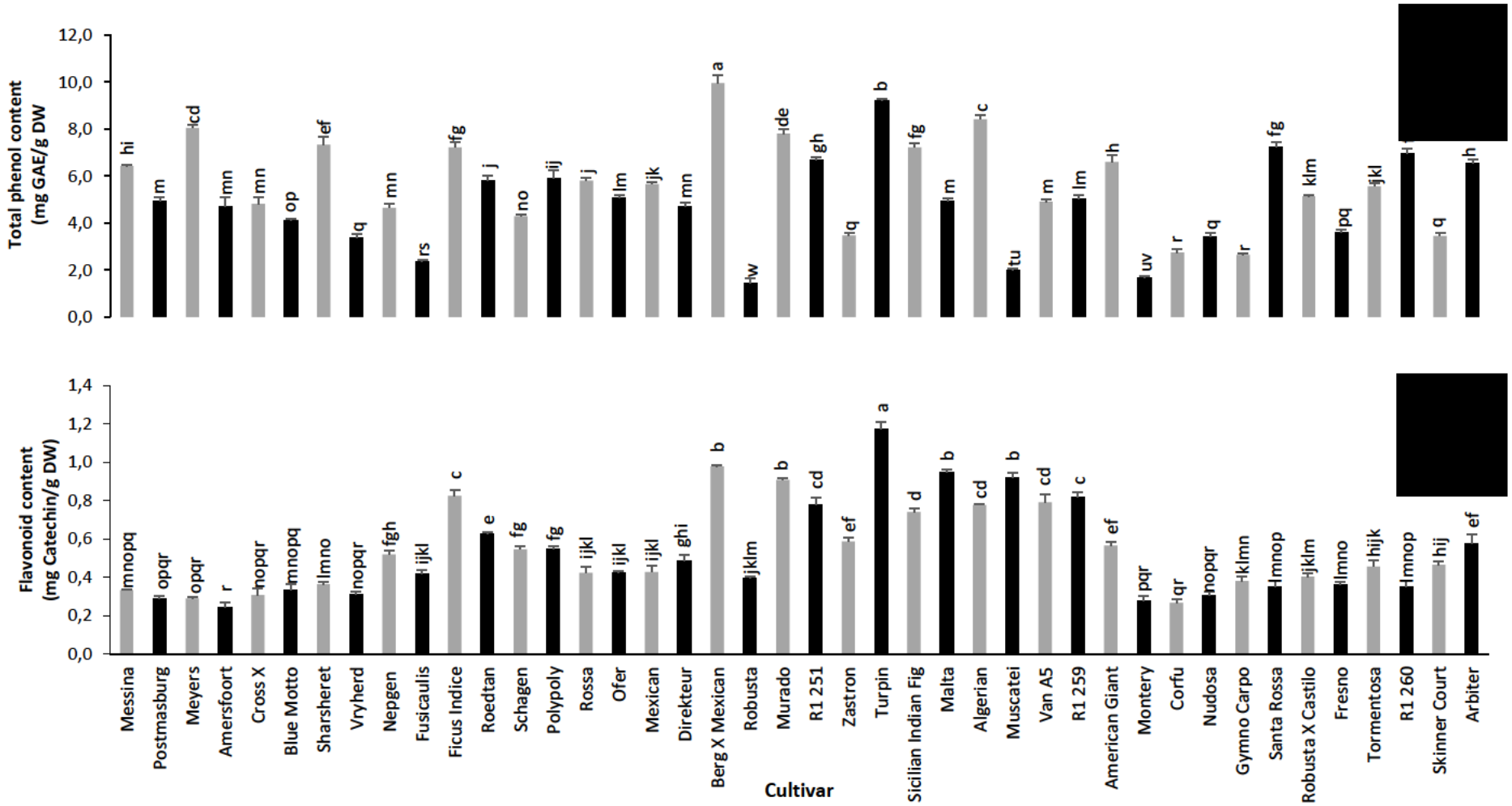

Figure 3.1: Total phenolic and flavonoid content of 42 cultivars of spineless *Opuntia ficus-indica* cladodes. A. Total phenolic content. B. Flavonoid content. Bars bearing different letters indicate significant differences (P = 0.05) according to Duncan's Multiple Range Test (DMRT). Values are means ± standard error (n=3)

3.3.3 DPPH radical scavenging activity

When dissolved in methanol, DPPH is characterised by a deep purple colour, which in the presence of an antioxidant becomes bleached or colourless. This means that antioxidants can quench the DPPH free radicals in a process of donating hydrogen atoms or electron transfer via a free radical attack on the DPPH molecules (Amarowicz *et al*., 2004).

Figure 3.2 shows the free radical scavenging activity of the different cultivars of spineless *Opuntia ficus-indica* cladodes at a concentration of 10 mg/ml. Statistical variation was observed in the radical scavenging activity of the different cultivars. Among the cultivars, the highest radical scavenging activity was observed in the cultivar Polypoly, which was about seven folds higher than that recorded in the other cultivars labelled to have the lowest percentage including Messina, Postmasburg, Meyers, Amersfoort, Fresno, Gymno Carpo, Blue Motto and Corfu.

The antioxidant activity of the cultivars may be attributed to the type and presence of glycosides and phenolic compounds (Aruwa *et al*., 2018). According to Gallegos-Infante *et al.* (2009), low radical scavenging activity found in the cladodes is mainly due to monohydroxylated phenolic compounds with low activity. It is likely that the cultivars with low antioxidant activity have a high composition of monohydroxylated phenolic compounds.

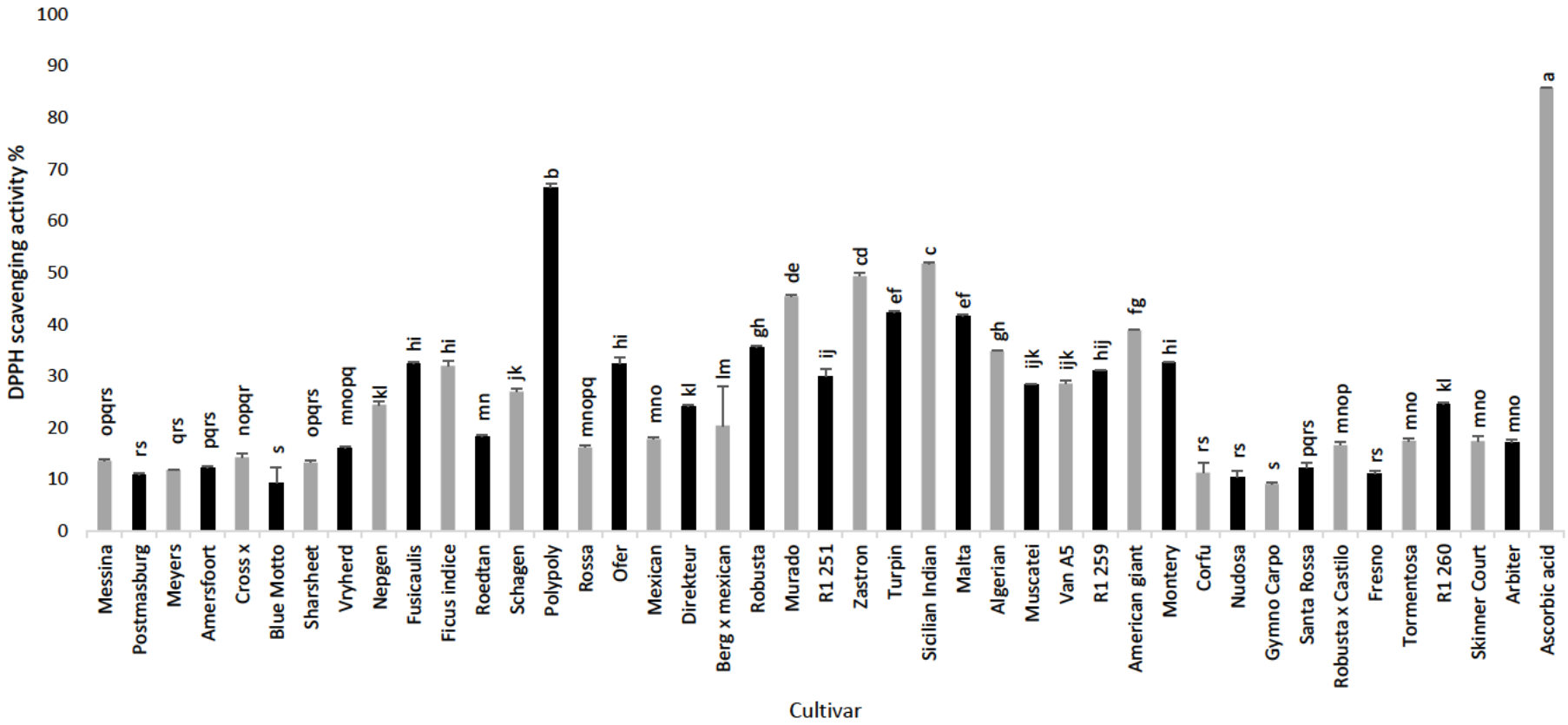

Figure 3.2: DPPH radical scavenging activity of different cultivars of spineless *Opuntia ficus-indica* cladodes. Bars with different letters indicate significant differences (P = 0.05) according to DMRT. Values are means ± standard error (n=3)

3.3.4 β-Carotene linoleic acid assay

This assay is characterised by decolourisation of β-carotene in absentia of an antioxidant (Amarowicz *et al*., 2004). The loss of orange colour by the carotenoids occurs when β-carotene molecules lose their conjugation in a process of oxidation, during which a hydrogen atom removed from the linoleic acid forms a pentadienyl free radical that attacks highly unsaturated β-carotene molecules to regain a hydrogen atom (Amarowicz *et al*., 2004). According to Amoo *et al*. (2012), this assay measures antioxidant activity towards linoleic acid by simulating the oxidation of membrane lipid constituents.

Using β-carotene-linoleic acid assay, antioxidant activities of 42 different cultivars of spineless *Opuntia ficus-indica* cladodes are shown in Figure 3.3. The antioxidant activity of the different cultivars based on the β-carotene bleaching rate ranged from 43 to 80%. The lowest percentage was recorded in cultivar Muscatei (43%) which was about half of the activity recorded with BHT (73%). The highest percentage antioxidant activity (80%) was recorded in Sicilian Indian Fig and American giant. Although different cultivars showed different percentage antioxidant activities, many of the cultivars exhibited antioxidant activity comparable to that of BHT. BHT is a synthetic antioxidant usually used as a food additive to prevent the damage caused by free radicals during oxidation processes (Yehye *et al*., 2015). The observed results suggest that selected spineless cultivars of *Opuntia ficus-indica* cladodes can be used in the food and cosmetic industry as some of the cultivars exhibit antioxidant activities that are similar to, or even slightly greater than BHT antioxidant activity.

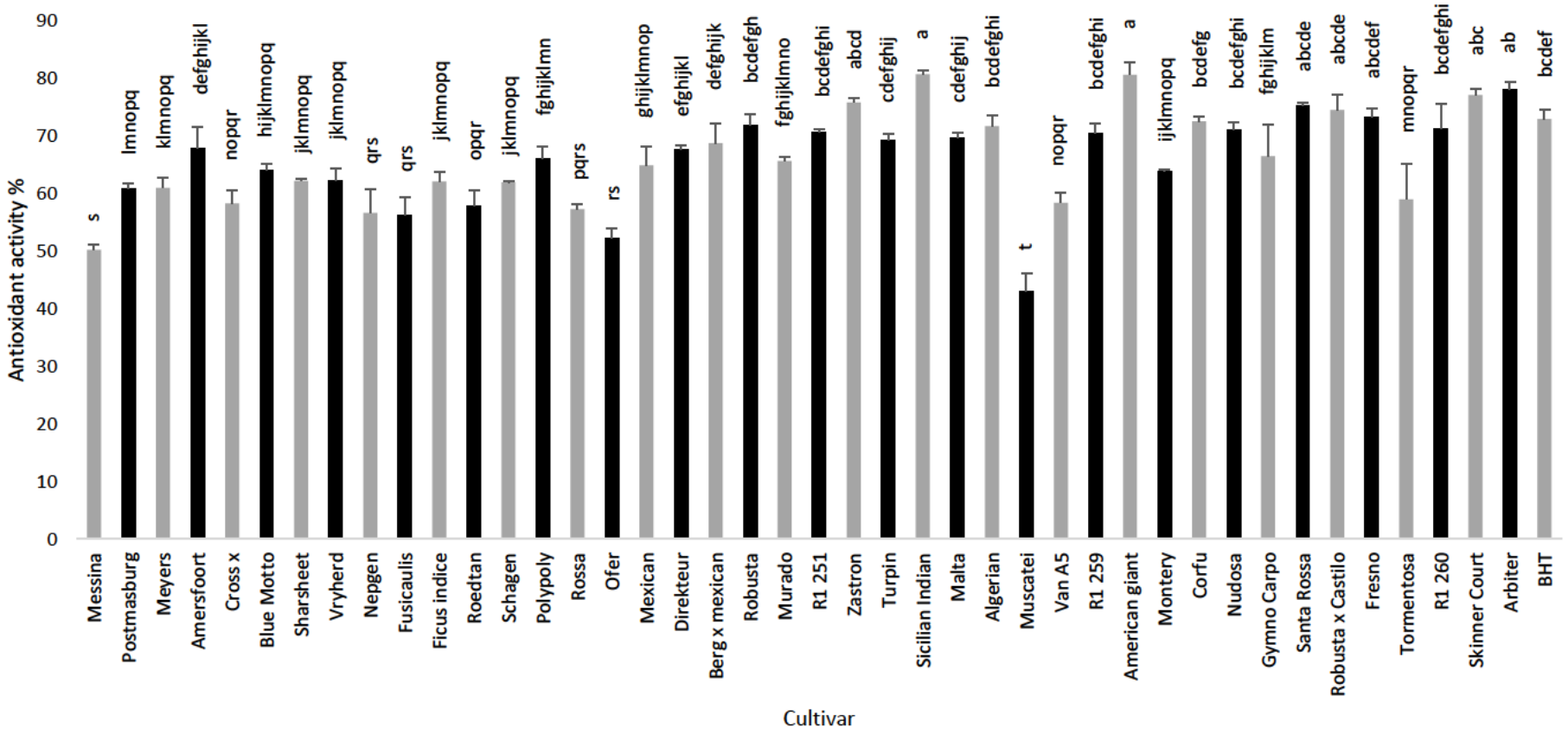

Figure 3.3: Antioxidant activity (%) of different cultivars of spineless *Opuntia ficus-indica* cladodes in β-carotene-linoleic acid model system at 10 mg/ml. Bars bearing different letters indicate significant differences (P = 0.05) according to DMRT. Values are means ± standard error (n=3)

3.3.5 Alpha-glucosidase inhibitory activity

Table 3.3 presents the alpha-glucosidase inhibitory activity of 42 cultivars of spineless *Opuntia ficus-indica* cladodes. The activities were dose-dependent with IC_{50} values ranging from 0.06 to 1.85 mg/ml. It is interesting to note that of the 42 cultivars investigated, 27 cultivars showed IC_{50} values lower than that of acarbose (1.07 ± 0.06 mg/ml).

Alpha-glucosidase is a known key enzyme in carbohydrate digestion and considered as a therapeutic target for the modulation of postprandial hyperglycemia, a common abnormality in type-2 diabetes (Vadivelan *et al*., 2019). Thus, alpha-glucosidase inhibitors can be effective in the management of hyperglycemia by delaying the effects of postprandial hyperglycemia (Rengasamy *et al*., 2013).

The traditional use of *Opuntia ficus-indica* in folk medicine for the treatment of diabetes has been reported by various authors (López, 1995; Bauman and Schmidt, 2015). A study by Lee *et al*. (2016) indicated a dose-dependent inhibition of the alpha-glucosidase by *Opuntia ficus-indica* var. *saboten*. However, to the best of our knowledge, no reports have been made on the alpha-glucosidase inhibitory activity of the cultivars investigated in this study. The observed results indicate the potential use of spineless *Opuntia ficus-indica* cladodes in the treatment or management of diabetes through alpha-glucosidase enzyme inhibition. *In vivo* studies need to be conducted to investigate the effects of these cultivars on glucose adsorption in the human digestive tract.

Table 3.3: Alpha-glucosidase inhibitory activity of different cultivars of spineless *Opuntia ficus-indica* cladodes

Cultivar	IC_{50} (mg/ml)	Cultivar	IC_{50} (mg/ml)
Algerian	0.52 ± 0.002^{b}	Nudosa	1.43 ± 0.017^{lm}
American Giant	1.11 ± 0.075^{fgh}	Ofer	0.09 ± 0.003^{a}
Amersfoort	0.10 ± 0.001^{a}	Polypoly	0.08 ± 0.001^{a}
Arbiter	0.96 ± 0.021^{e}	Postmasburg	0.06 ± 0.000^{a}
Berg X Mexican	0.08 ± 0.000^{a}	R1 251	0.89 ± 0.004^{de}
Blue Motto	0.09 ± 0.000^{a}	R1 259	1.50 ± 0.002^{m}
Corfu	1.85 ± 0.165^{n}	R1 260	$1.21 \pm 0.024^{h\,j}$
Cross X	0.07 ± 0.003^{a}	Robusta	0.10 ± 0.000^{a}
Direkteur	0.10 ± 0.002^{a}	Robusta X Castilo	1.19 ± 0.003^{ghi}
Ficus Indice	0.07 ± 0.004^{a}	Roedtan	0.07 ± 0.002^{a}
Fresno	1.35 ± 0.088^{kl}	Rossa	0.11 ± 0.004^{a}
Fusicaulis	0.10 ± 0.000^{a}	Santa Rossa	1.30 ± 0.007^{ijk}
Gymno Carpo	1.37 ± 0.007^{kl}	Schagen	0.12 ± 0.001^{a}
Malta	0.85 ± 0.008^{d}	Sharsheret	0.09 ± 0.001^{a}
Messina	0.08 ± 0.000^{a}	Sicilian Indian Fig	1.12 ± 0.013^{fgh}
Mexican	0.13 ± 0.003^{a}	Skinner Court	1.30 ± 0.007^{jk}
Meyers	0.11 ± 0.000^{a}	Tormentosa	1.11 ± 0.120^{fgh}
Montery	1.09 ± 0.001^{fgh}	Turpin	1.09 ± 0.004^{fg}
Murado	0.12 ± 0.001^{a}	Van A5	0.66 ± 0.025^{c}
Muscatei	1.44 ± 0.003^{lm}	Vryherd	0.10 ± 0.000^{a}
Nepgen	0.08 ± 0.000^{a}	Zastron	0.74 ± 0.004^{c}
		Acarbose	1.07 ± 0.058^{f}

Mean values with different letters indicate significant differences (*P* =0.05) according to DMRT. Values are means ± standard error (n=3).

3.3.6 Individual phenolic compounds

Table 3.4 shows the individual phenolic compounds of 20 selected cultivars of spineless *Opuntia ficus-indica* cladodes. Overall, catechin and gallic acid were present in significant quantities in all the cultivars investigated, whilst the least present compound was quercetin. The highest catechin content of 682.87 mg/100 g was recorded in the cultivar Tormentosa whilst the highest gallic acid content of 572.41 mg/100 g was recorded in Sicilian Indian Fig.

Interestingly, all the cultivars except Turpin and Sicilian Indian Fig, showed presence of all the individual phenolic acids investigated. Quercetin was not detected in the two mentioned cultivars (Turpin and Sicilian Indian Fig). It is interesting to note that Turpin and Sicilian Indian Fig are some of the top cultivars that showed best pharmacological activities. Some of the cultivars had low amounts of phenolic acids and flavonoids yet show potential pharmacological activities. A possible reason may be that phenolic acids are affected by extraction solvents and processing methods used on the plant material (Aruwa *et al*., 2019).

Another study by Kim *et al*. (2016) suggested that flavonoids, particularly narcissin and isorhamnetin 3-O-β-D-glucopyranoside, were the most abundant in *Opuntia ficus-indica* plant. More studies on the plants phenolic constituents are essential to determine which of the individual phenolic acids are responsible for each biological activity.

Table 3.4: Quantities of some phenolic acids present in 20 selected cultivars of spineless *Opuntia ficus-indica* cladodes

Cultivar name	mg/100 g dry weight							
	Gallic acid	Caffeic acid	Vanillic acid	p-coumaric acid	trans-ferullic acid	Catechin	Rutin	Quercetin
Algerian	389.41 ± 0.811^{e}	**75.14 ± 1.221^{a}**	3.40 ± 0.162^{o}	4.26 ± 0.046^{e}	37.10 ± 0.800^{c}	454.57 ± 1.810^{g}	83.03 ± 0.621^{c}	3.71 ± 0.042^{e}
American Giant	251.00 ± 2.092^{h}	24.73 ± 0.131^{g}	6.16 ± 0.031^{k}	4.89 ± 0.019^{d}	4.24 ± 0.153^{m}	580.14 ± 19.546^{bc}	22.69 ± 0.435^{g}	1.112 ± 0.03^{gh}
Berg X Mexican	289.82 ± 2.952^{g}	26.93 ± 0.142^{f}	7.74 ± 0.032^{j}	2.61 ± 0.021^{g}	23.63 ± 0.381^{e}	357.00 ± 2.823^{i}	45.21 ± 0.412^{d}	**9.42 ± 0.150^{a}**
Blue Motto	248.86 ± 1.592^{h}	4.14 ± 0.123^{m}	5.51 ± 0.082^{l}	1.01 ± 0.052^{m}	15.98 ± 1.542^{fgh}	252.72 ± 4.863^{k}	26.36 ± 0.370^{g}	1.78 ± 0.032^{f}
Corfu	197.23 ± 5.913^{l}	15.57 ± 0.092^{i}	12.98 ± 0.055^{f}	4.18 ± 0.051^{e}	15.81 ± 0.277^{fgh}	597.18 ± 2.706^{b}	4.54 ± 0.032^{ij}	1.83 ± 0.034^{f}
Direkteur	241.56 ± 1.212^{hi}	15.61 ± 0.241^{i}	13.77 ± 0.604^{e}	2.23 ± 0.044^{j}	5.83 ± 0.278^{l}	221.88 ± 2.282^{kl}	8.38 ± 0.346^{i}	7.72 ± 0.121^{b}
Fresno	202.13 ± 4.021^{l}	14.11 ± 0.043^{i}	4.62 ± 0.142^{m}	2.46 ± 0.053^{ghi}	15.16 ± 0.252^{ghi}	560.08 ± 9.412^{cd}	1.86 ± 0.343^{j}	0.69 ± 0.022^{h}
Gymno Carpo	220.21 ±1.272^{jk}	11.52 ± 0.038^{j}	17.47 ± 0.048^{c}	**7.75 ± 0.038^{a}**	9.67 ± 0.141^{k}	579.49 ± 13.301^{bc}	37.59 ± 1.069^{e}	1.52 ± 0.122^{fg}
Malta	435.33 ± 4.931^{cd}	15.36 ± 0.287^{i}	9.96 ± 0.161^{h}	2.40 ± 0.111^{hij}	37.66 ± 0.603^{c}	561.85 ± 7.051^{bcd}	82.73 ± 0.632^{c}	5.9 6 ± 0.101^{c}
Mexican	254.23 ± 2.700^{h}	14.87 ± 0.073^{i}	11.68 ± 0.172^{g}	2.96 ± 0.072^{f}	14.67 ± 0.613^{hi}	532.86 ± 20.512^{de}	7.93 ± 0.157^{i}	3.75 ± 0.030^{e}
Murado	321.40 ± 0.242^{f}	35.88 ± 0.601^{e}	14.22 ± 0.042^{e}	1.30 ± 0.021^{l}	22.18 ± 0.040^{e}	205.92 ± 6.812^{m}	34.90 ± 0.511^{e}	6.48 ± 0.151^{c}
Nudosa	206.38 ± 2.638^{kl}	10.04 ± 0.059^{k}	5.31 ± 0.061^{l}	5.31 ± 0.032^{c}	28.05 ± 0.051^{d}	588.48 ± 2.062^{bc}	14.71 ± 0.904^{h}	1.05 ± 0.012^{gh}
Ofer	422.67 ± 7.313^{d}	44.48 ± 0.821^{c}	9.27 ± 0.087^{i}	1.26 ± 0.021^{l}	17.27 ± 0.580^{f}	295.12 ± 1.980^{j}	13.55 ± 0.359^{h}	6.26 ± 0.150^{c}

Polypoly	444.76 ± 9.438^{c}	19.83 ± 0.182^{h}	8.12 ± 0.179^{j}	0.89 ± 0.060^{m}	13.82 ± 0.651^{ij}	388.60 ± 19.611^{hi}	15.12 ± 4.992^{h}	7.92 ± 0.843^{b}
R1 251	**572.41 ± 16.164^{a}**	43.72 ± 0.480^{c}	15.02 ± 0.378^{d}	2.57 ± 0.140^{gh}	**58.59 ± 0.361^{a}**	581.23 ± 3.811^{bc}	**206.23 ± 1.242^{a}**	1.02 ± 0.012^{gh}
R1 259	297.11 ± 3.743^{g}	38.42 ± 0.812^{d}	8.04 ± 0.252^{j}	5.86 ± 0.143^{b}	16.41 ± 0.301^{fg}	488.89 ± 10.501^{f}	14.75 ± 0.376^{h}	3.76 ± 0.034^{e}
Roedtan	437.65 ± 3.160^{cd}	24.74 ± 0.562^{g}	7.60 ± 0.104^{j}	1.34 ± 0.092^{l}	14.64 ± 0.433^{hi}	505.72 ± 5.300^{ef}	12.62 ± 0.891^{h}	4.74 ± 0.034^{d}
Sicilian Indian Fig	491.00 ± 1.003^{b}	36.81 ± 1.153^{e}	**20.49 ± 0.202^{a}**	2.31 ± 0.046^{ij}	**57.25 ± 0.323^{a}**	359.48 ± 8.782^{i}	132.66 ± 1.851^{b}	ND
Tormentosa	229.77 ± 7.857^{ij}	7.16 ± 0.082^{l}	4.02 ± 0.051^{n}	2.04 ± 0.022^{k}	12.41 ± 0.221^{j}	**682.87 ± 20.977^{a}**	30.51 ± 1.231^{f}	0.48 ± 0.033^{hi}
Turpin	375.87 ± 0.911^{e}	59.74 ± 0.121^{b}	19.07 ± 0.116^{b}	2.44 ± 0.051^{ghi}	48.31 ± 0.391^{b}	414.66 ± 13.292^{h}	83.22 ± 0.462^{c}	ND

*ND= Not detected. Mean values with different letters indicate significant differences (*P* =0.05) according to DMRT. Values are means ± standard error (n=3). *Bold values indicate the highest recorded quantities.

3.3.7 Antibacterial activity

The antibacterial activities of extracts of selected cultivars are presented in Table 3.5. It is important to note that in this study, only extracts with MIC values below 1 mg/ml were considered active (Ríos and Recio, 2005). The lower the MIC values, the stronger the antibacterial activity. In general, noteworthy antibacterial activity was observed against *B. subtilis* and *E. coli* with MIC values below 1 mg/ml. The poorest activity was observed against *K. pneumoniae,* with R1 251 cultivar having the lowest MIC value of 1.56 mg/ml.

Petroleum ether extracts generally had the best antibacterial activity when compared to 50% methanol extracts. The best MIC value was recorded in petroleum ether extract of Malta cultivar (0.39 mg/ml) against *B. subtilis* and *E. coli*, and 0.78 mg/ml against *S. aureus*.

Research has indicated the antibacterial potential of *Opuntia* spp. and different plants parts; however, limited information is available on the antibacterial activity of *Opuntia ficus-indica* cladodes, particularly of those in the southern African region (Aruwa *et al*., 2019).

Studies suggest that the antimicrobial potential of plants against microbial pathogens is attributed to their phenolic composition (Mwinga *et al*., 2019; Melgar *et al*., 2017). Bari *et al*. (2012) indicated distinctive antibacterial activity of methanolic extracts as compared to other extracts of *Opuntia monacantha*. However, in this study, 50% methanol extracts did not show good activity against the selected microorganisms

when compared to petroleum ether extracts. This may be attributed to the difference in polarity of the two solvents.

According to Matu and Van Staden (2003), low antibacterial activity recorded against Gram-negative bacteria may be due to the thick murein layer in their structure preventing the entry of inhibitors. Rabe and Van Staden (1997) also stated that Gram-negative bacteria are more resistant than Gram-positive ones. Moreover, poor activity in some of the extracts may be due to low concentrations of active compounds that are responsible for the plant's antimicrobial activity (Rabe and Van Staden, 1997).

Foodborne pathogenic bacteria such as *S. aureus*, *B. subtilis* and *E. coli* have biofilm forming abilities causing food deterioration or spoilage and constitute a global health problem (Takó *et al*., 2020; Logan, 2012). Many of the pathogenic bacteria have become resistant to antibiotics and as such, there has been an increase in research activities to find alternative new and strong antimicrobial agents particularly from plants (Welegerima *et al*., 2018).

The strong antibacterial activity of some of the spineless cladodes documented in this study suggest the potential use of cladodes against food spoilage microorganisms. However, this requires careful cultivar selection as some cultivars show better MIC values than others.

Table 3.5: Antibacterial activity of 20 selected cultivars of spineless *Opuntia ficus-indica* cladodes

	Bacillus subtilis (Gram +)		*Staphylococcus aureus* (Gram +)		*Escherichia coli* (Gram -)		*Klebsiella Pneumoniae* (Gram -)	
Cultivar	Extracts (MIC) (mg/ml)							
	MeOH	P.E	MeOH	P.E	MeOH	P.E	MeOH	P.E
Algerian	6.25	1.56	> 6.25	3.13	> 6.25	3.13	3.13	3.13
American Giant	> 6.25	3.13	3.13	3.13	3.13	> 6.25	> 6.25	> 6.25
Berg X Mexican	6.25	1.56	> 6.25	3.13	> 6.25	1.56	3.13	> 6.25
Blue Motto	> 6.25	1.56	> 6.25	> 6.25	3.13	1.56	6.25	> 6.25
Corfu	3.13	> 6.25	3.13	> 6.25	3.13	> 6.25	> 6.25	> 6.25
Direkteur	> 6.25	1.56	3.13	1.56	6.25	1.56	> 6.25	> 6.25
Fresno	> 6.25	> 6.25	> 6.25	1.56	> 6.25	1.56	6.25	> 6.25
Gymno Carpo	6.25	3.13	> 6.25	1.56	> 6.25	> 6.25	3.13	> 6.25
Malta	3.13	**0.39**	6.25	**0.78**	> 6.25	**0.39**	> 6.25	3.13
Mexican	6.25	1.56	> 6.25	1.56	1.56	1.56	6.25	> 6.25
Murado	> 6.25	**0.78**	> 6.25	1.56	> 6.25	1.56	> 6.25	> 6.25
Nudosa	3.13	6.25	6.25	1.56	> 6.25	> 6.25	3.13	> 6.25
Ofer	6.25	1.56	3.13	1.56	6.25	1.56	3.13	> 6.25
Polypoly	1.56	1.56	> 6.25	1.56	3.13	1.56	3.13	> 6.25
R1 251	6.25	1.56	3.13	1.56	1.56	**0.78**	3.13	1.56
R1 259	6.25	3.13	6.25	3.13	> 6.25	> 6.25	> 6.25	> 6.25
Roedtan	> 6.25	**0.78**	> 6.25	1.56	6.25	**0.78**	6.25	> 6.25
Sicilian Indian Fig	> 6.25	**0.39**	> 6.25	1.56	> 6.25	**0.78**	> 6.25	> 6.25
Tormentosa	> 6.25	3.13	> 6.25	1.56	6.25	1.56	6.25	6.25
Turpin	> 6.25	**0.78**	3.13	1.56	> 6.25	**0.78**	> 6.25	> 6.25
Ciproflaxin	0.10		0.10		0.05		0.10	

*Bold values indicate MIC values considered very active (< 1 mg/ml). PE- Petroleum ether, MeOH- Methanol. Ciproflaxin used as positive control.

3.4 Conclusion

Strong antidiabetic activity coupled with the observed antioxidant and antibacterial activities, although varied with cultivars, indicate the potential of using cladodes as functional food and in applications against food spoilage in place of synthetic compounds. No cultivar could be singled out as the best as each pharmacological and phytochemical trait was different in each cultivar. However, from this work it is clear that cladodes of spineless *Opuntia ficus-indica* cultivars can be used in the formulation

of phytochemical-rich products and other pharmaceutical products. To the author's knowledge, this is the first time some of the pharmacological activities are reported on the investigated cultivars and further studies need to be carried out to explore other potential pharmacological activities and bioactive constituents responsible for the medicinal value of these cultivars.

Chapter 4 Nutritional value of *Opuntia ficus-indica* cladodes

4.1 Introduction

Although steps aimed at achieving zero hunger are being taken, sustainability and improved nutrition are important elements to consider when addressing food security (Stephens *et al*., 2018). According to the 2015-2016 global statistics, malnutrition prevalence has been on a rise from 10.8% in 2015 to 11.0% in 2016, affecting 794 to 815 million people with the most affected resident in the African countries (Prosekov and Ivanova, 2018). As a result, there has been an increase in death rate worldwide due to diet-related diseases with about 5 million children under 5 years of age dying annually because of malnutrition-related diseases (Schönfeldt and Pretorius, 2011; Mc Carthy *et al*., 2018).

Malnutrition, which is a result of diets that are poor in energy, protein and micronutrients including vitamins and mineral elements, has been defined as all types of poor nutrition that is linked to poor diets resulting in both under and over nutrition (Gumede, 2018). Findings from a survey revealed that in the United States alone, more than half of the population were not meeting the recommended dietary allowance for micronutrients such as magnesium, vitamin A and vitamin C (Kim *et al*., 2016).

Opuntia commonly known as cactus pear has a long history of use as a source of fruits and vegetables (Bouzoubaâ *et al*., 2014). Of the genus, *Opuntia ficus-indica* is the most economical, distributed and consumed species with about 50 000 ha in Mexico alone, linked to a production of about 300 000 megaton of cultivated cactus pear for

fresh produce (Yahia and Mondragon-Jacobo, 2011). The consumption of the fruits, known as prickly pear, and the stems, known as cladodes, is common around different parts of the world (Ayadi *et al*., 2009; De Santiago *et al*., 2018). Both the fruits and the cladodes are well known for their nutritional value and the cladodes in particular are associated with high fibre content including pectin, mucilage, cellulose and hemicellulose, which assist in the metabolism of glucose and lipids (De Santiago *et al*., 2018).

The cladodes are consumed either fresh or cooked and in the form of several products available, such as juice and sauces made from the cladodes (De Santiago *et al*., 2018; Trivedi and Raval, 2017). The high water use efficiency and ability to grow and withstand harsh conditions has made cladodes to be an excellent alternative food source for humans and drought-resilient feed resource for animal consumption during drought conditions (Aruwa *et al*., 2018; López-García *et al*., 2001, López, 1995). In this regard, the importance of the plant is seen in arid and semi-arid environments where accessibility of most vegetables is insufficient (Morales *et al*., 2012).

Different cultivars of *Opuntia ficus-indica* exist, characterized by cladodes, fruits and flowers of various shapes, size and colour. However, studies suggest that the compounds profile and nutritional properties of cladodes of *Opuntia* cladodes can change with species type, plant age, environment and cultivar type (Astello-García *et al*., 2015; Contreras-Padilla *et al*., 2011; Guevara-Figueroa *et al*., 2010).

Significant research has been conducted on the morphology and uses of *Opuntia ficus-indica*. However, there is insufficient data on the nutritional value of spineless

cladode cultivars of *Opuntia ficus-indica*, particularly of the 42 spineless cultivars used in this study. In this chapter, the nutritional value of 42 spineless cultivars of *Opuntia ficus-indica* cladodes and their potential use as functional food is investigated.

4.2 Materials and methods

4.2.1 Plant material collection and preparation

Plant material used in this chapter was collected and prepared as described in chapter 3, subsection 3.2.1.

4.2.2 Extraction procedures

4.2.2.1 Vitamin C content

Extraction was done according to Odriozola-serrano *et al.* (2007) with modifications. An amount of 0.2 g of the plant material was extracted by sonication in an ultrasonic bath containing ice-cold water for 12 min using 10 ml of 4.5% metaphosphoric acid. The mixture was then filtered using Whatman No. 41 filter paper.

4.2.2.2 β-carotene content

Extraction was done according to Biehler *et al.* (2010) with slight modifications. A volume of 5 ml methanol was added to 0.2 g of the sample and vortexed for 10 seconds, followed by the addition of 15 ml of hexane: acetone (1:1). The mixture was vortexed for 10 seconds before sonicating in an ultrasonic bath containing ice-cold water for 15 min. Saturated sodium chloride solution (5 ml) was added to the mixture, vortexed for 10 seconds and centrifuged at 1073.3 x g for 2 minutes at 25 °C. The upper layer of hexane extract was transferred into a 10 ml volumetric flask and made up to the mark with hexane.

4.2.2.3 Mineral elements

Digestion was done according to Ang and Lee (2005). Samples (0.5 g of each cultivar) were weighed and 9 ml of 65% nitric acid and 37% hydrochloric acid added to the sample before boiling for 30 min in a water bath at 95 ºC until the sample had dissolved.

4.2.3 Determination of vitamin C content

Vitamin C content was determined using a method described by Odriozola-serrano *et al.* (2007) with modifications. One millilitre of the sample was transferred into a vial and analysed using a Shimadzu HPLC (LC-2030C 3D, Shimadzu Corporation, Kyoto, Japan) equipped with a C18 Luna® column (150 ×4.6 mm, 5 µ) at 25 °C. Water: acetonitrile: formic acid (99: 0.9: 0.1 v/v/v) were used as mobile phase at a flow rate of 1 ml/min in isocratic mode, with an injection volume of 20 µl and wavelength of 254 nm. Identification and quantification of vitamin C was achieved by plotting a calibration curve using pure ascorbic acid as a standard.

4.2.4 Determination of β-carotene content

β-carotene content was determined using a method described by Biehler *et al*. (2010) with modifications. An aliquot of 1 ml from the extraction procedure was transferred into a vial and analysed using a Shimadzu HPLC (LC-2030C 3D, Shimadzu Corporation, Kyoto, Japan) equipped with a C18 Luna® column (150 × 4.6 mm, 5 µ) at 35 °C. Acetonitrile: DCM: Methanol (70:20:10, v/v/v) was used as mobile phase at a flow rate of 1 ml/min in isocratic mode, with an injection volume of 20 µl and

wavelength of 450 nm. Identification and quantification of β-carotene was achieved by plotting a calibration curve using pure β-carotene as a standard.

4.2.5 Determination of mineral elements

The mineral elements were quantified using an inductively coupled plasma - optical emission spectrometry (ICP-OES) (ICPE-9820, Shimadzu Corporation, Kyoto, Japan) as described by Ang and Lee (2005). The following mineral elements were analysed: calcium, iron, magnesium, manganese, potassium, sodium, phosphorus and zinc.

4.2.6 Statistical analysis

One-way analysis of variance was done using Statsoft (Statistica 8) software. The mean values were further separated by Duncan's multiple range test based on $P = 0.05$. Data were reported as mean ± standard error of triplicate analyses.

4.3 Results and discussions

4.3.1 Vitamin C and β-carotene content

In addition to their significant role in metabolism, vitamins play a major role as food additives and beneficial medicinal agents (Kim *et al*., 2016). Some of the vitamins reported in cactus pear include vitamin C and carotenoids (Aruwa *et al*., 2018; du Toit *et al.*, 2018; Kuti, 2004). These vitamins are essential for different functions in the human body such as the role of vitamin A for vision, reproduction, embryogenic development, and immune function and vitamin C role in normal metabolism and immune function. Both vitamins A and C are characterized by their high antioxidant

properties and are thus regarded as nutritional or dietary antioxidants (Kim *et al*., 2016).

Vitamin C and β-carotene contents of 42 cultivars of *Opuntia ficus-indica* cladodes are shown in Figure 4.1 and Figure 4.2, respectively. Significant variation was observed in the vitamin C and β-carotene content of the different cultivars. Of the 42 cultivars, vitamin C content ranged from a lowest of 8.95 mg/100 g in Ofer to a highest (almost 14 times the lowest content) in Malta cultivar. Sixteen cultivars (Schagen, Rossa, Ofer, Direkteur, Robusta, R1251, Zastron, Turpin, Sicilian Indian fig, Van A5, Montery, Corfu, Fresno, Tormentosa, R1260 and Arbiter) had vitamin C content below 10 mg/100 g.

Interestingly, the observed results imply that the vitamin C content in the spineless cultivars is somewhat high and within the daily recommended intake. About 8 mg/100 g of vitamin C content is adequate to prevent signs of scurvy (associated with vitamin C deficiencies) in infants (FAO/WHO, 2004). This means that the cultivars such as Ofer with a lowest vitamin C content of (8.95 mg/100 g) can still be used in diets to eliminate vitamin C deficiencies. Although it is believed that vitamin C content in cactus pear fruits is significantly high as compared to the cladodes (Feugang *et al*., 2006), it is noteworthy that the vegetative parts are considered as an excellent and reliable source of vitamin C as the supply of vegetables is extended for longer periods than fruits (FAO/WHO, 2004).

Among the 42 cultivars examined in this study, β-carotene content ranged from a lowest of 3.9 mg/100 g in Murado cultivar to a highest of 31.4 mg/100 g recorded in Cross X (Figure 4.2). A study by Kuti (2004) on the fruit of different varieties of *Opuntia* revealed higher ascorbic acid content as compared to carotenoid content. Likewise, in this study the cladodes generally contain higher vitamin C content as compared to the β-carotene content of the examined cultivars of *Opuntia ficus-indica*.

The nutritional value of nopalitos or young cladodes is comparable to that of some vegetables such as lettuce and spinach (FAO, 2013). β-carotene content of the spineless cultivars used in this study were about 10 folds higher than most leafy vegetables such as spinach, amaranth and coriander (Pritwani and Mathur, 2017). In addition to their significant nutritional value and medicinal properties, an advantage of using cladodes as a substitute vegetable is that they can be produced fast and can be easily grown under relatively harsh conditions such as high temperatures and insufficient water (FAO, 2013).

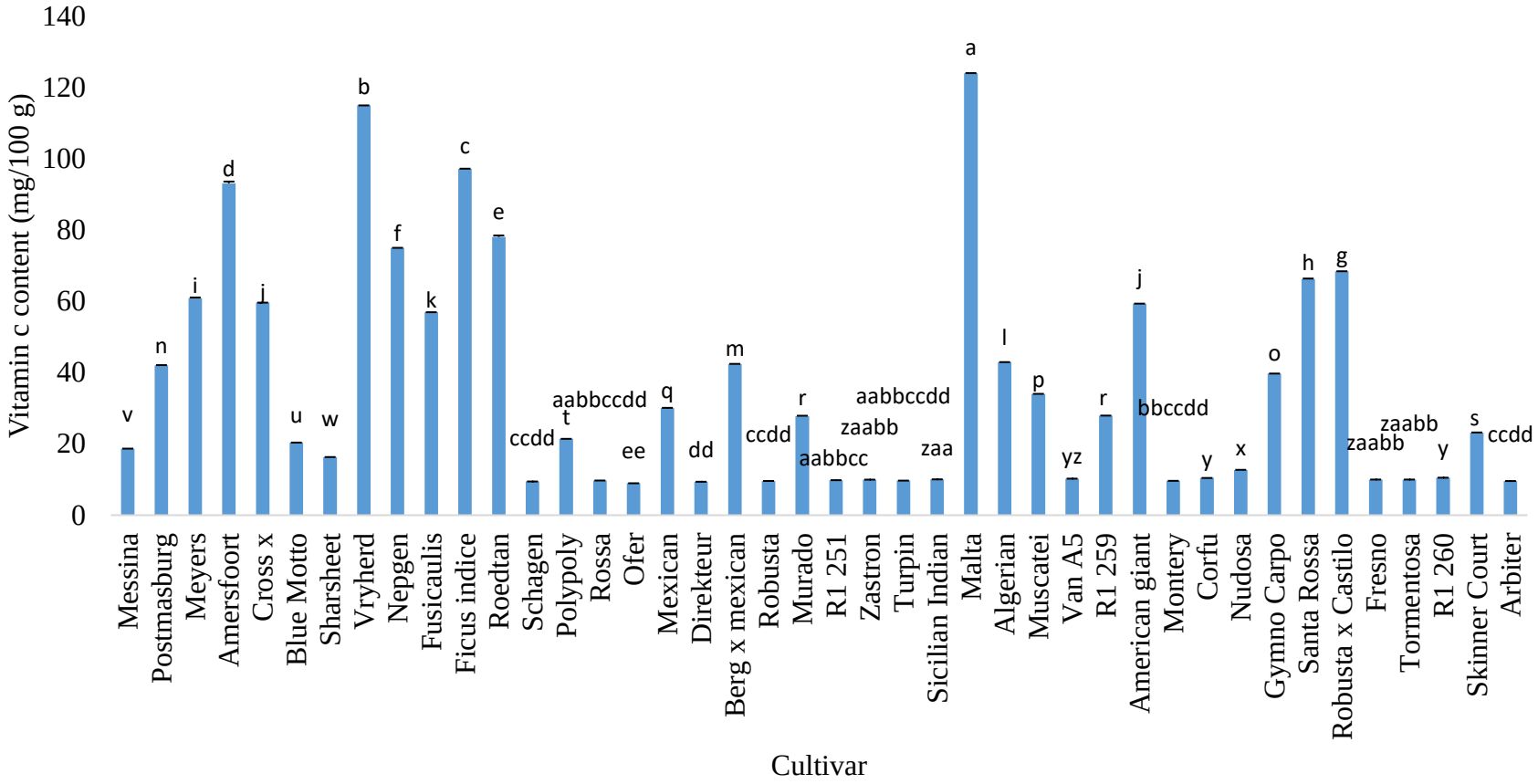

Figure 4.1: Vitamin C content of 42 spineless cultivars of *Opuntia ficus-indica* cladodes. Bars with different letters are significantly different (*P* = 0.05) according to Duncan's multiple range test. Values are means ± standard error (n=3)

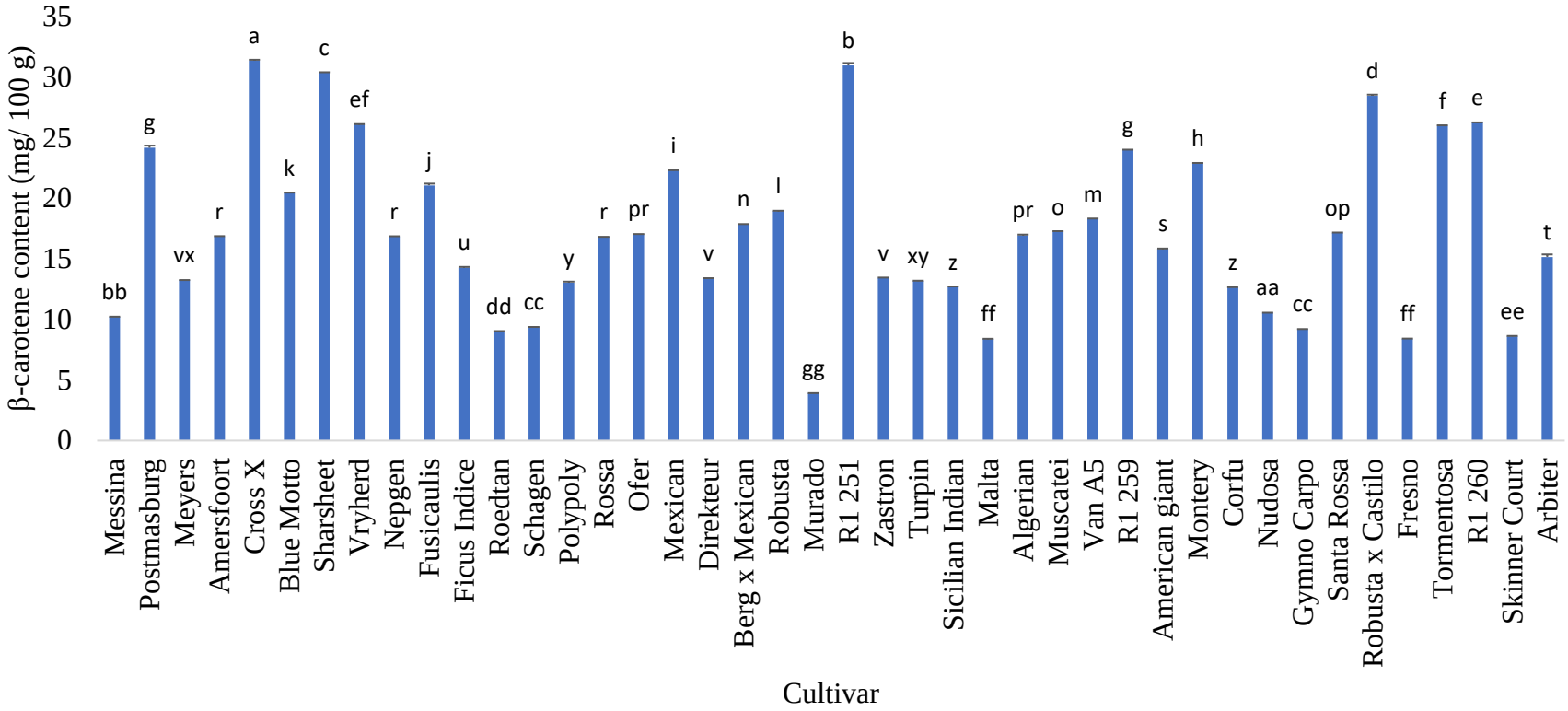

Figure 4.2: β-carotene content of 42 spineless cultivars of *Opuntia ficus-indica* cladodes. Bars with different letters are significantly different (*P* = 0.05) according to Duncan's multiple range test. Values are means ± standard error (n=3)

4.3.3 Mineral elements

Significant differences in the mineral element content amongst the 42 varieties of spineless *Opuntia ficus-indica* were observed (Table 4.1). Potassium and calcium were the most abundant elements present in the *Opuntia ficus-indica* with a highest of 4980.00 mg/100 g recorded in Direkteur and 4146.67 mg/100 g in Muscatei, respectively, whilst iron was found to be the least present with a highest of 54.67 mg/100 g recorded in cultivar R1251. The highest magnesium content was 674.67 mg/100 g recorded in Turpin, although without significant differences in comparison to R1251, Robusta x Castilo, Corfu, Algerian and Nepgen, while the highest zinc content was recorded in Montery (28.40 mg/100 g). Furthermore, Fausicaulis cultivar had the highest manganese and phosphorus with 220 and 402 mg/100 g, respectively, whilst the highest sodium content of 113 mg/100 g was recorded in cultivar Ficus Indice. The spineless cladodes seem to have higher mineral contents as compared to other cactus pear plant parts such as the fruit as reported by Kunyanga *et al*. (2014). The accumulation of mineral elements is greater in cladodes due to the higher transpiration rate in cladodes as compared to the fruits (Bañuelos *et al*., 2012).

Nutritional elements are essential for human health and metabolism (Abdel-Hameed *et al*., 2014). Intake of potassium has been associated with lowering of blood pressure (Kim *et al*., 2016). Méndez *et al*. (2015) reported that potassium was found to be the most abundant mineral element in cladodes of *Opuntia ficus-indica* and *Opuntia dillenii*, which is in line with the findings in this study.

Similarly, calcium was also previously reported to be present in higher quantities in cactus pear (Ben Salem *et al*., 2005). Calcium is associated with promotion of bone

health, neuromuscular function, blood clotting and reduction of osteoporosis risks (Kim *et al*., 2016; Moyo *et al*., 2018).

Zinc and iron deficiency remain a major concern in Africa and the rest of the world (Moyo *et al*., 2018). Zinc is important for cellular, immune and antioxidant function whereas iron is associated with haemoglobin formation and transportation of oxygen (Kim *et al*., 2016). Although zinc and iron are generally low in most vegetables (Kim *et al*., 2016) and in the examined cultivars in this study, the findings suggest the use of the selected spineless cultivars of cactus pear in the supplementation of nutritional mineral elements.

Table 4.2 indicates a selection of the top twenty cultivars with high concentration for each nutritional. Overall, Nepgen was observed to have the highest frequency (9 of 10) of almost all the nutritional components except the presence of zinc. The cultivars Nepgen, Schagen, Corfu, Nudosa and Robusta x Castillo had a higher frequency of macro elements, whilst Robusta, Corfu, Robusta x Castillo and Skinner court had higher frequency of micro elements studied.

Table 4.1: Quantification of mineral elements (mg /100 g) of 42 spineless cultivars of *Opuntia ficus-indica* cladodes

	Ca	Fe	K	Mg	Mn	Na	P	Zn
Algerian	3066.67 ± 13.333^{h}	4.70 ± 0.012^{l}	3866.67 ± 17.642^{f}	**667.33 ± 4.064^{ab}**	55.80 ± 0.120^{aa}	85.87 ± 0.133^{f}	225.33 ± 3.713^{n}	18.81 ± 0.074^{g}
American giant	2660.00 ± 0.000^{l}	5.99 ± 0.131^{j}	2253.33 ± 6.669^{uv}	601.33 ± 1.756^{gh}	90.47 ± 0.242^{q}	65.27 ± 0.132^{w}	320.67 ± 0.241^{d}	12.06 ± 0.002^{qr}
Amersfoort	1408.67 ± 4.667^{gg}	1.39 ± 0.033^{v}	2240.00 ± 11.551^{uv}	562.00 ± 1.152^{n}	56.60 ± 0.121^{aa}	82.13 ± 0.592^{hi}	186.80 ± 4.002^{rs}	11.45 ± 0.161^{tu}
Arbiter	2420.00 ± 11.547^{o}	0.20 ± 0.044^{y}	3620.00 ± 30.545^{j}	580.67 ± 0.673^{lm}	73.27 ± 0.241^{v}	70.67 ± 0.132^{t}	362.67 ± 2.907^{b}	17.89 ± 0.114^{h}
Berg x Mexican	1655.33 ± 8.969^{cc}	2.29 ± 0.045^{rst}	2886.67 ± 6.667^{q}	608.00 ± 3.056^{fg}	50.60 ± 0.122^{bb}	68.53 ± 0.273^{uv}	157.13 ± 1.602^{u}	10.84 ± 0.181^{wxy}
Blue Motto	2186.67 ± 6.667^{s}	1.33 ± 0.067^{v}	2966.67 ± 33.331^{p}	498.00 ± 0.001^{r}	160.33 ± 0.642^{f}	54.73 ± 0.345^{x}	258.00 ± 5.256^{j}	17.87 ± 0.043^{h}

Corfu	2560.00 ± 11.552^{n}	9.57 ± 0.056^{e}	3953.33 ± 17.643^{e}	**670.67 ± 0.667^{ab}**	115.73 ± 0.131^{k}	85.73 ± 0.482^{f}	272.67 ± 0.118^{h}	17.88 ± 0.011^{h}
Cross X	1576.67 ± 4.671^{ee}	2.85 ± 0.101^{p}	1840.00 ± 23.862^{y}	596.67 ± 1.760^{hij}	92.73 ± 0.641^{p}	97.47 ± 1.332^{c}	262.00 ± 0.672^{ij}	12.50 ± 0.091^{p}
Direkteur	3840.00 ± 11.547^{b}	1.56 ± 0.062^{uv}	**4980.00 ± 30.552^{a}**	572.00 ± 5.291^{m}	183.47 ± 0.444^{c}	72.80 ± 0.232^{s}	255.33 ± 0.484^{jk}	20.60 ± 0.182^{e}
Ficus Indice	1820.00 ± 2.311^{z}	6.49 ± 0.089^{i}	4226.67 ± 24.043^{b}	600.67 ± 0.667^{gh}	75.20 ± 0.401^{u}	**113.40 ± 0.811^{a}**	248.00 ± 0.122^{kl}	17.37 ± 0.110^{i}
Fresno	1716.67 ± 7.692^{bb}	4.17 ± 0.144^{n}	3073.33 ± 26.667^{no}	558.67 ± 9.961^{n}	79.20 ± 0.353^{t}	80.47 ± 0.578^{jk}	222.67 ± 1.764^{no}	12.26 ± 0.171^{pq}
Fusicaulis	2706.67 ± 6.672^{k}	0.91 ± 0.045^{w}	3993.33 ± 17.642^{de}	582.00 ± 1.151^{l}	**220.00 ± 0.002^{a}**	74.27 ±0.289^{pqr}	**402.00 ± 2.913^{a}**	27.67 ± 0.132^{b}
Gymno Carpo	3173.33 ± 6.671^{f}	4.45 ± 0.082^{m}	3406.67 ± 6.667^{l}	638.00 ± 1.145^{d}	30.07 ± 0.243^{ee}	86.67 ± 0.640^{f}	135.87 ± 4.901^{v}	8.68 ± 0.081^{aa}

Malta	2073.33 ± 6.672^{v}	5.04 ± 0.042^{k}	3213.33 ± 17.640^{m}	532.00 ± 6.111^{o}	28.60 ± 0.122^{ff}	81.07 ± 0.131^{ij}	354.00 ± 2.312^{b}	18.57 ± 0.089^{g}
Messina	1182.00 ± 2.311^{j}	8.31 ± 0.089^{f}	2206.67 ± 6.667^{v}	649.33 ± 0.667^{c}	56.73 ± 0.067^{aa}	99.00 ± 0.201^{b}	190.67 ± 1.145^{qrs}	11.83 ± 0.045^{rs}
Mexican	2106.67 ± 6.671^{u}	2.75 ± 0.089^{pq}	3520.00 ± 11.555^{k}	662.00 ± 1.155^{b}	156.00 ± 0.640^{g}	64.73 ± 0.442^{w}	235.33 ± 2.445^{m}	14.76 ± 0.142^{m}
Meyers	1608.67 ± 4.372^{dd}	1.71 ± 0.067^{u}	2146.67 ± 17.644^{w}	586.67 ± 6.667^{jkl}	120.47 ± 0.180^{j}	76.60 ± 0.200^{n}	216.00 ± 4.668^{op}	11.57 ± 0.062st
Montery	2706.67 ± 6.667^{k}	1.31 ± 0.052^{v}	2433.33 ± 13.334^{s}	600.00 ± 1.153^{gh}	194.47 ± 0.840^{b}	83.93 ± 0.241^{g}	303.33 ± 0.584^{e}	**28.40 ± 0.053^{a}**
Murado	2206.67 ± 6.667^{s}	2.49 ± 0.082^{qr}	3686.67 ± 6.671^{i}	592.67 ± 4.671^{hijk}	44.07 ± 0.292^{cc}	77.07 ± 0.350^{mm}	184.40 ± 3.530^{s}	10.71 ± 0.079^{xy}
Muscatei	**4146.67 ± 17.638^{a}**	5.01 ± 0.075^{k}	3833.33 ± 6.672^{fg}	588.67 ± 4.371^{ijkl}	44.27 ± 0.132^{cc}	83.87 ± 0.522^{g}	144.00 ± 4.161^{v}	11.10 ± 0.044^{uvw}

Nepgen	2633.33 ± 6.672^{m}	37.20 ± 0.204^{b}	3700.00 ± 11.552^{i}	**670.67 ± 0.668^{ab}**	162.60 ± 0.611^{e}	83.20 ± 0.402^{gh}	269.33 ± 5.033^{hi}	14.19 ± 0.193^{n}
Nudosa	3140.00 ± 0.001^{g}	3.57 ± 0.067^{o}	3793.33 ± 17.640^{gh}	634.00 ± 5.031^{d}	121.87 ± 0.182^{i}	76.53 ± 0.182^{n}	290.67 ± 0.671^{f}	21.27 ± 0.094^{d}
Ofer	2286.67 ± 6.672^{q}	0.74 ± 0.006^{wx}	3060.00 ± 11.551^{o}	524.67 ± 4.665^{op}	66.53 ± 0.235^{y}	67.27 ± 0.346^{v}	359.33 ± 0.121^{b}	16.25 ± 0.122^{k}
Polypoly	1504.00 ± 1.146^{ff}	2.65 ± 0.087^{pq}	3760.00 ± 20.002^{h}	508.00 ± 4.161^{q}	111.33 ± 0.286^{l}	79.53 ± 0.271^{kl}	196.73 ± 4.372^{q}	14.19 ± 0.070^{n}
Postmasburg	1860.67 ± 5.462^{y}	4.94 ± 0.052^{kl}	1711.33 ± 5.211^{z}	623.33 ± 3.331^{e}	121.13 ± 0.352^{ij}	65.07 ± 0.367^{w}	169.60 ± 3.331^{t}	18.79 ± 0.078^{g}
R1251	1344.00 ± 2.311^{hh}	**54.67 ± 0.267^{a}**	1858.67 ± 5.212^{y}	**669.33 ± 1.761^{ab}**	67.00 ± 0.234^{y}	69.73 ± 0.243^{tu}	157.80 ± 1.148^{u}	9.38 ±0.052^{z}
R1259	3626.67 ± 17.643^{c}	11.13 ± 0.113^{d}	3986.67 ± 6.665^{e}	516.67 ± 1.334^{pq}	69.13 ± 0.132^{x}	74.53 ± 0.371^{pq}	282.00 ± 1.145^{fg}	19.96 ± 0.054^{f}

R1260		1484.00 ± 4.622^{ff}	12.41 ± 0.044^{c}	1303.33 ± 11.622^{aa}	592.67 ± 0.667^{hijk}	56.47 ± 0.131^{aa}	98.33 ± 0.332^{bc}	157.80 ± 6.002^{u}	11.31 ± 0.143^{tuv}
Robusta		1260.00 ± 5.032^{ii}	2.92 ± 0.042^{p}	2286.67 ± 6.666^{u}	623.33 ± 5.462^{e}	159.67 ± 0.478^{f}	78.67 ± 0.755^{l}	212.67 ± 1.051^{p}	15.81 ± 0.125^{l}
Robusta x Castilo		2873.33 ± 6.668^{i}	4.05 ± 0.071^{n}	4240.00 ± 11.552^{b}	**677.33 ± 1.757^{a}**	100.67 ± 0.479^{o}	90.40 ± 0.500^{e}	301.33 ± 2.912^{e}	20.53 ± 0.043^{e}
Roedtan		1322.00 ± 0.001^{hh}	1.42 ± 0.024^{v}	2240.00 ± 11.553^{uv}	518.00 ± 0.002^{p}	40.67 ± 0.133^{dd}	72.80 ± 0.119^{rs}	244.00 ± 1.031^{lm}	10.56 ± 0.072^{y}
Rossa		1780.67 ± 1.332^{aa}	0.59 ± 0.025^{x}	2373.33 ± 13.334^{t}	600.00 ± 1.152^{gh}	87.27 ± 0.132^{r}	75.13 ± 0.807^{op}	275.33 ± 3.329^{gh}	18.48 ± 0.146^{g}
Santa Rossa		1614.67 ± 0.672^{dd}	0.84 ± 0.022^{wx}	1943.33 ± 11.616^{x}	594.67 ± 2.910^{hijk}	92.73 ± 0.132^{p}	64.00 ± 0.231^{w}	142.27 ± 0.642^{v}	8.57 ± 0.092^{aa}
Schagen		3520.00 ± 0.002^{d}	2.37 ± 0.101^{rs}	4040.00 ± 23.087^{d}	601.33 ± 1.332^{gh}	58.80 ± 0.310^{z}	69.47 ± 0.590^{tu}	269.33 ± 5.811^{hi}	18.72 ± 0.202^{g}

Sharsheet	2560.00 ± 11.551^{n}	0.20 ± 0.056^{y}	2980.00 ± 11.548^{p}	597.33 ± 1.761$^{gh\ j}$	176.40 ± 0.122^{d}	76.00 ± 0.373^{no}	335.33 ± 1.762^{c}	16.71 ± 0.111^{j}
Sicilian Indian	2580.00 ± 0.004^{n}	2.85 ± 0.022^{p}	3680.00 ± 23.091^{i}	558.67 ± 2.402^{n}	109.80 ± 0.418^{m}	73.87 ± 0.292^{pqrs}	286.67 ± 2.913^{f}	14.86 ± 0.042^{m}
Skinner Court	3833.33 ± 13.327^{b}	7.15 ± 0.087^{h}	3413.33 ± 13.332^{l}	584.67 ± 1.331^{kl}	126.07 ± 0.289^{h}	95.07 ± 0.528^{d}	242.67 ± 2.401^{lm}	23.87 ± 0.092^{c}
Tormentosa	2360.00 ± 11.546^{p}	2.07 ± 0.046^{t}	3113.33 ± 17.641^{n}	598.67 ± 1.333^{ghi}	102.40 ± 0.354^{n}	76.73 ± 0.180^{n}	300.00 ± 6.362^{e}	23.73 ± 0.182^{c}
Turpin	3460.00 ± 11.551^{e}	4.93 ± 0.023^{kl}	4133.33 ± 17.642^{c}	**674.67 ± 4.368^{a}**	67.53 ±0.074^{y}	77.27 ± 0.351^{mn}	195.13 ± 1.152^{qr}	11.89 ± 0.091^{rs}
Van A5	2246.67 ± 13.332^{r}	7.65 ± 0.132^{g}	2860.00 ± 11.548^{q}	614.00 ± 1.151^{ef}	71.67 ± 0.411^{w}	72.80 ± 0.420^{s}	329.33 ± 0.668^{c}	11.01 ± 0.135^{vwx}
Vryherd	2140.00 ± 11.546^{t}	2.87 ± 0.08^{p}	2686.67 ± 6.673^{r}	600.00 ± 1.150^{gh}	120.67 ± 0.524^{j}	73.33 ± 0.733^{qrs}	226.67 ± 1.762^{n}	13.19 ± 0.122^{o}

Zastron	2773.33 ± 6.667^{j}	2.17 ± 0.100st	3600.00 ± 34.637^{j}	619.33 ± 3.531^{e}	86.20 ± 0.232^{s}	78.40 ± 0.503^{lm}	158.33 ± 0.671^{u}	11.37 ± 0.236^{tu}

Means within the same column followed by different letter(s) are significantly different (P = 0.05) according to Duncan's multiple range test. Values are means ± standard error (n=3).

Table 4.2: Frequency of top 20 cultivars with high macro and microelements

Cultivar	**Macro elements**					**Micro elements**					**Vitamin C** (≥27 mg/100 g)	**Beta-carotene** (≥16.90 mg/100 g	Total frequency
	K (≥3400 mg/100 g	Ca (≥2300 mg/100 g	Mg (≥600 mg/100 g	P (≥255 mg/100 g	Frequency	Na (≥77 mg/100 g	Fe (≥2.9 mg/100 g	Mn (≥92 mg/100 g	Zn (≥15.8 mg/100 g	Frequency			
Algerian	×	×	×		**3**	×	×		×	**3**	×	×	**8**
American giant		×	×	×	**3**		×	×		**2**		×	**6**
Amersfoort					**0**	×				**1**	×	×	**3**
Arbiter	×	×		×	**3**				×	**1**			**4**
Berg x mexican			×		**1**					**0**		×	**2**
Blue Motto				×	**1**			×	×	**2**		×	**4**
Corfu	×	×	×	×	**4**	×	×	×	×	**4**			**8**
Cross x				×	**1**	×		×		**2**		×	**4**
Direkteur	×	×		×	**3**			×	×	**2**	×	×	**7**
Ficus indice	×		×		**2**	×	×		×	**3**	×		**6**
Fresno					**0**	×	×			**2**		×	**3**
Fusicaulis	×			×	**2**			×	×	**2**	×		**5**

Gymno Carpo	×	×	×		**3**	×	×			**2**	×	×	**7**
Malta				×	**1**	×	×		×	**3**	×	×	**6**
Messina		×	×		**2**	×	×			**2**	×	×	**6**
Mexican	×		×		**2**			×		**1**			**3**
Meyers					**0**			×		**1**	×		**2**
Montery		×	×	×	**3**	×		×	×	**3**			**6**
Murado	×				**1**	×				**1**		×	**3**
Muscatei	×	×			**2**	×	×			**2**		×	**5**
Nepgen	×	×	×	×	**4**	×	×	×		**3**	×	×	**9**
Nudosa	×	×	×	×	**4**		×	×	×	**3**	×		**8**
Ofer		×		×	**2**				×	**1**	×	×	**5**
Polypoly	×				**1**	×		×		**2**			**3**
Postmasburg			×		**1**		×	×	×	**3**	×		**5**
R1 251			×		**1**		×			**1**			**2**
R1 259	×	×		×	**3**		×		×	**2**	×		**6**
R1 260					**0**	×	×			**2**			**2**
Robusta			×		**1**	×	×	×	×	**4**	×		**6**
Robusta X Castilo	×	×	×	×	**4**	×	×	×	×	**4**			**8**
Roedtan					**0**					**0**			**0**

Rossa			×	×	**2**			×	×	**2**		×	**5**
Santa Rossa					**0**			×		**1**	×	×	**3**
Schagen	×	×	×	×	**4**				×	**1**			**5**
Sharsheet				×	**1**			×	×	**2**	×	×	**5**
Sicilian Indian fig	×	×		×	**3**			×		**1**	×		**5**
Skinner Court	×	×			**2**	×	×	×	×	**4**			**6**
Tormentosa		×		×	**2**			×	×	**2**		×	**5**
Turpin	×	×	×		**3**	×	×			**2**			**5**
Van A5			×	×	**2**		×			**1**	×	×	**5**
Vryherd					**0**			×		**1**	×		**2**
Zastron	×	×	×		**3**	×				**1**			**4**

4.4 Conclusion

The observed findings indicate that the spineless cladodes of *Opuntia ficus-indica* cultivars are important sources of nutrients and can be considered as functional foods with a potential use in the battle against malnutrition or deficiency of micronutrients including vitamin C and nutritional mineral elements such as calcium, zinc and iron. No cultivar can be singled out as the best cultivar since the examined nutritional traits varied amongst each cultivar. However, based on the summary on Table 4.2, Nepgen can be suggested to be a cultivar of interest based on nutritional value since it had the highest frequency of nutritional components among the top 20 cultivars. The observed findings indicate the need for careful cultivar selection when using spineless cactus pear cultivars for product development to ensure product integrity.

Chapter 5 General Conclusion

Multipurpose plants such as *Opuntia ficus-indica* play an important role in our everyday lives. These plants can serve as food, medicine or for recreational purposes. In this study the phytochemical, pharmacological and nutritional values of different cultivars of spineless *Opuntia ficus-indica* cladodes were evaluated. Young cladodes from about one-year old plants were used in this study based on different review articles on *Opuntia ficus-indica* indicating that young cladodes or nopalitos are commonly used as human food and as a strong medicinal source.

Overall, significant variations in the phytochemical, pharmacological and nutritional value of different cultivars of *Opuntia ficus-indica* were observed. With regard to the pharmacological and phytochemical properties, strong antidiabetic activity coupled with the observed antioxidant and antibacterial activities indicate the potential of using cladodes as a functional food and in applications against food spoilage in place of synthetic compounds. The cladodes can potentially be used in the formulation of phytochemical-rich products and other pharmaceutical products.

An evaluation of the nutritional value of the different cultivars indicate that the spineless cladodes are rich sources of nutrients and can be considered as functional foods in the battle against malnutrition, including micronutrient deficiency. In view of its strong antidiabetic activity, *Opuntia ficus-indica* spineless cladodes show great potential for use in formulation of beneficial food products targeted at health- or diet-conscious individuals. In addition, some of the major phenolic compounds present in the spineless cladodes have been proven to play an important role in the prevention and treatment of chronic diseases.

In general, no cultivar could be singled out as the overall best as each pharmacological, phytochemical and nutritional trait was different in each cultivar. To our knowledge, this is the first time some of the pharmacological activities and nutritional traits are reported on the investigated cultivars and as such future studies need to be carried out to explore other potential pharmacological activities and bioactive constituents responsible for the medicinal value of these cultivars.

Being a climate-smart crop, its low resource input during cultivation coupled with its multipurpose advantages, places it as an underutilized crop that requires more attention. The observed findings indicate the need for careful cultivar selection when using spineless cactus pear cultivars for product development to ensure product integrity.

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