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J Herbmed Pharmacol. 2023; 12(1): 109-117.

http://www.herbmedpharmacol.com doi: 10.34172/jhp.2023.10

Journal of Herbmed Pharmacology

A phytochemical screening, antioxidant and antibacterial activity analysis in the leaves, stems and roots of *Portulacaria afra*

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ARTICLE INFO

Article Type:
Original Article

Article History:
Received: 15 August 2022
Accepted: 8 October 2022

Keywords:
African traditional medicine
Medicinal plants
Phytochemistry
Plant extracts
Secondary metabolites

ABSTRACT

Introduction: *Portulacaria afra* is a medicinal plant commonly used among African traditional healers to treat skin conditions and dehydration. The aim of this study was to scientifically validate the use of *P. afra* among traditional healers.

Methods: Standard phytochemical colour tests were used to determine the presence of ten phytochemicals, using four solvents of varying polarities (hexane, ethyl acetate, methanol, and water). The antioxidant activity was determined using 1-diphenyl-2-picrylhydrazyl (DPPH) and hydrogen peroxide scavenging assays. An agar-well diffusion assay was used to determine the antibacterial activities of the leaves, stems, and roots of *P. afra* against *Staphylococcus aureus* and *Escherichia coli*.

Results: *P. afra* exhibited a high phytochemical presence in the methanolic extracts, with seven out of the 10 phytochemical groups present. Flavonoids and phlobatannins were absent in all of the plant's extracts. The methanolic root extract exhibited the highest DPPH scavenging activity (IC_{50} = 0.39) whilst the hexane leaf extract (IC_{50} = 14.83) was the only extract to exceed the acceptable upper limit. The scavenging activity of the plant was stronger against hydrogen peroxide than it was against DPPH. The methanolic and hot water stem extracts displayed the largest zone of inhibition (of 20 mm) against *E. coli*. The cold-water and room-temperature water extracts, of all three plant parts, showed no zone of inhibition against either bacterial strain.

Conclusion: *P. afra* has the capacity to be used as a nutritional supplement for its antioxidant properties, while the antibacterial properties may provide relief against *E. coli* infections.

Implication for health policy/practice/research/medical education:
This study contributes new knowledge on the phytochemical and biological activities of *P. afra*. The high phytochemical presence, as well as antioxidant and antibacterial activities exhibited by the plant extracts in this study validate the use of *P. afra* among traditional healers. The plant displays the potential of enhancing human health.

Please cite this paper as: Basson DC, Teffo TK, Risenga IM. A phytochemical screening, antioxidant and antibacterial activity analysis in the leaves, stems and roots of *Portulacaria afra*. J Herbmed Pharmacol. 2023;12(1):109-117. doi: 10.34172/jhp.2023.10.

Introduction

Medicinal plants are defined as plants that have compounds within their structures, which can be extracted and used for medicinal purposes (1). These compounds are more commonly known as phytochemicals, which are synthesised by plants through primary or secondary metabolism. The combination of secondary metabolites in plants is generally responsible for the medicinal properties expressed by plants (2). Plants have the ability to produce an infinite number of these phytochemicals because they are synthesised in response to stressors such as herbivory or competition (3). Many chemicals have been extracted from plants and replicated to produce modern drugs, such as salicin (more commonly known as aspirin), which was extracted from the bark of a white willow tree (4). The use of traditional medicine is extensive and continues to increase. The World Health Organization (WHO) has estimated that 80% of the world population relies on traditional medicine as their primary source of health care (5). Thus, it is important to scientifically validate the use of medicinal plants for treatment. Despite African traditional medicine being regarded as one of the oldest and most diverse uses of medicinal plants (6), it has not been well documented throughout history. An example of

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this is *Portulacaria afra.*

Portulacaria afra is a succulent evergreen plant, indigenous to southern Africa (7). It is a large shrub or small tree and can grow up to a height of 5 m. The plant is characterised by its small flat leaves and a red stem. The economic importance of *P. afra* is heightened as a result of its wide-ranging uses, such as its carbon sequestration properties, which have been extensively studied. However, the studies on the medicinal properties of the plant are limited. Through previously conducted interviews, it was determined that traditional healers commonly use the leaves topically to treat skin conditions such as rashes and acne (8). Due to the limited phytochemical and pharmacological studies performed on the plant, this study aims to perform a phytochemical screening and determine the antioxidant and antibacterial activities in the stems, roots and leaves of *P. afra*. Four solvents, with varying polarities, were used to create a thorough examination of the plant parts, as the polarity of the solvent affects the yield of the extract (9). This study will contribute new knowledge on the phytochemical profile and biological activities of *P. afra.*

Materials and Methods

Plant material

The whole plant of *P. afra* was collected from the Witkoppen Wildflower nursery (26°03'32.6"S 27°56'15.9"E) in December 2020 (South African summer). The plant was identified and authenticated by researcher Dr Ida Risenga and a voucher specimen was deposited in the institute's herbarium centre (IRM 001). The plant was maintained in the Oppenheimer Life Sciences greenhouse at the University of Witwatersrand for a period of three months. The fresh leaves, stems, and roots of 20 *P. afra* were placed in a hot air dryer for four days at 40°C. The dried plant materials were ground into a fine crude powder. The powdered plant was then enclosed in foil and placed in an airtight container, which was stored in a room-temperature cupboard till tests commenced.

Preparation of the extracts

The crude leaf, stem, and root extracts were prepared using four solvents: 80% methanol, hexane, ethyl acetate, and 100% distilled water at three different temperatures (4°C, 25°C, and 40°C). All crude plant extracts were prepared by placing one gram of the powdered plant into separate bottles and adding 25 mL of a solvent. The methanol, hexane, ethyl acetate, and room-temperature water (25°C) extracts were placed in a mechanical shaker. The hot water extract was placed on a hot plate with a magnetic stirrer. The cold-water extract was placed in the fridge and physically shaken every few hours. All extracts were shaken or stirred for 48 hours. The water extract was centrifuged to decrease its viscosity. All extracts were then filtered through a filter paper into a resealable vial. The supernatants were collected and placed in a vial and stored in the refrigerator until the tests were conducted.

Phytochemical screening

A qualitative analysis was conducted in order to determine the presence or absence of 10 phytochemical groups in the leaves, stems, and roots of *P. afra* using standard colour test methods (10).

Froth test for saponins

In a test tube, 0.5 mL of the crude plant extract was mixed with 5 mL of distilled water. The test tube was then vigorously shaken. Three drops of olive oil were placed inside the test tube and shaken vigorously. The presence of a stable foam indicated the presence of saponins.

$FeCl_3$ test for phenolics

In a test tube, 1 mL of the crude plant extract was mixed with 5 to 10 drops of 10% ferric chloride ($FeCl_3$). The appearance of a green-blue or violet colour was an indication of the presence of phenolic compounds.

Hydrochloric acid test for flavonoids

In a test tube, 1 mL of the crude plant extract was mixed with three drops of hydrochloric acid (HCl). After a yellow colour was observed, a few drops of dilute acid were then added. A colourless solution indicated the presence of flavonoids.

Salkowski's test for glycosides

In a test tube, 0.5 mL of the crude plant extract was mixed with 2 mL of concentrated HCl. A reddish-brown colour indicated the presence of the steroidal aglycone part of the glycoside.

Bromine water test for tannins

In a test tube, 0.5 mL of the crude plant extract was mixed with 10 mL of bromine water. Discolouration of bromine water indicated the presence of tannins.

Test for terpenoids

In a test tube, 1 mL of crude plant extract was mixed with 0.5 mL of chloroform ($CHCl_3$). A few drops of concentrated sulphuric acid (H_2SO_4) were added to the mixture. The formation of a reddish-brown precipitate indicated the presence of terpenoids.

Test for steroids

In a test tube, 2 mL of the crude plant extract was mixed with 2 mL of $CHCl_3$ and 2 mL of concentrated HCl. A reddish-brown ring at the junction indicated the presence of steroids.

Test for coumarins

In a test tube, 1 mL of crude plant extract was reacted with 1 mL of 10% NaOH in a test tube. The formation of a yellow colour indicated the presence of coumarins.

Test for phlobatannins

In a test tube, 1 mL crude plant extract was mixed with a few drops of concentrated HCl. The appearance of red colour indicated the presence of phlobatannins

Test for volatile oils

In a test tube, 1 mL of the crude plant extract was added to 0.2 mL solution of 1% NaOH. The presence of a precipitate indicated the presence of volatile oils.

Antioxidant scavenging activity

Two antioxidant assays were conducted in this study to determine the antioxidant activities of the crude plant extracts. These assays included 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity (11) and a hydrogen peroxide test (12), which are described below.

1-Diphenyl-2-picrylhydrazyl scavenging assay

The stable radical DPPH was used to determine the scavenging activity and hence, the antioxidant activity of the plant parts of *P. afra*. The crude plant extract was placed in capped test tubes at varying concentrations (10-50 µL) and mixed with 700 µL of the DPPH work solution. Methanol 80% was used to create a mixture with a volume of 1 mL. The test tubes were incubated in a dark cupboard, at room temperature for 45 minutes. Following the incubation period, the mixtures were placed into cuvettes, which were placed within a spectrophotometer (Genesys 10S UV-VIS). The absorbance was measured at a wavelength of 517 nm. The test was then triplicated while the percentage of inhibition was calculated using the following formula below.

$$\%\text{Inhibition of DPPH radical} = ([A_{br}-A_{ar}]/A_{br}) \times 100$$

Where A_{br} is the absorbance of the control and A_{ar} is the absorbance of the sample. The concentrations were then plotted against percentage inhibition values, in order to calculate IC_{50}.

Hydrogen peroxide scavenging activity

A 40mM solution of 30% aqueous hydrogen peroxide was prepared in a phosphate buffer (pH 7.4). The crude plant extracts were placed in capped test tubes at varying concentrations (10-50 µL) and mixed with 600 µL of the hydrogen peroxide solution. The test tubes were incubated in a dark cupboard, at room temperature for 10 minutes. The absorbance of the mixtures was determined using a spectrometer at a wavelength of 230 nm. The blank used was the phosphate buffer without the hydrogen peroxide solution. This test was then triplicated. The different absorbance values were recorded while the percentage of hydrogen peroxide scavenging activities was calculated using the following formula:

$$\%\text{ Scavenged } [H_2O_2] = ([A_{br}-A_{ar}]/A_{br}) \times 100$$

Where A_{br} is the absorbance of the control and A_{ar} is the absorbance of the sample. The concentrations were then plotted against percentage inhibition values to calculate the IC_{50}.

Determination of antibacterial properties

To determine whether *P. afra* displayed anti-microbial properties, the agar-well diffusion assay was performed (13). The plant extracts were tested against gram-positive *Staphylococcus aureus* (ATCC 25923) and gram-negative *Escherichia coli* (ATCC 25922).

Agar-well diffusion assay

Petri dishes were sterilised and autoclaved before the introduction of the respective agar medium. This experiment used the recommended Mueller-Hinton and Baird Parker agar. After solidification of the media, sterile cotton swabs were used to evenly inoculate the entire surface of the agar medium with the respective bacteria strain. *S. aureus* was inoculated on the Baird Parker agar plates and *E. coli* was inoculated onto the Mueller-Hinton agar plates. Six wells were then bored in the media using a 6 mm sterile borer. The various plant extracts (100 µL) were then impregnated into five of the wells, whereas the sixth well was impregnated with the negative control, dimethyl sulfoxide (DMSO). The plates were then sealed and placed in the fridge for 30 minutes to allow for efficient diffusion. The plates were then transferred to the incubator set at 37°C for 24 hours. Following the incubation period, the zones of inhibition were then measured in millimetres using a ruler. The presence of an inhibitory zone surrounding the well indicated a positive antibacterial activity result.

Results

Phytochemical screening

A qualitative study was conducted and made use of visual cues and colour changes to detect whether phytochemical groups were present or not in the crude plant extract. The intensity of the visual difference was then used to determine the expected amount of the phytochemical groups (absent, present, moderate presence, or high presence) as shown in Table 1.

The methanolic extracts of all three plant parts were able to extract the most phytochemical groups; seven out of the 10 phytochemical groups were detected in each plant part, namely: saponins, glycosides, phenolics, terpenoids, steroids, coumarins, and volatile oils. However, methanolic extracts were unable to extract tannins. The weakest extraction was exhibited by the room-temperature water and hexane extracts. There was a higher number of phytochemical groups extracted by the hot water in comparison to the cold water and room temperature water.

The presence of coumarins was high in the leaves and stem, whilst only the methanolic extract was able to detect

Table 1. Colour screening results for the methanol, cold water, room-temperature water, hot water, ethyl acetate, and hexane extracts of the stems, leave, and roots of *Portulacaria afra*

Plant Part	Phytochemical	Solvent					
		Methanol	Cold water	Room temperature water	Hot water	Ethyl acetate	Hexane
Stems	Saponins	++	+	-	++	+	++
	Flavonoids	-	-	-	-	-	-
	Glycosides	++	-	-	+	-	-
	Phenolics	+	-	-	-	-	-
	Tannins	-	+	+	+	++	-
	Terpenoids	+	-	-	-	-	-
	Steroids	+	-	-	-	-	-
	Coumarins	+++	+	+	+	++	-
	Phlobatannins	-	-	-	-	-	-
	Volatile oils	++	-	-	+	+	+
Leaves	Saponins	+	-	-	-	+	++
	Flavonoids	-	-	-	-	-	-
	Glycosides	++	+	-	+	-	-
	Phenolics	+	+	+	+	+++	-
	Tannins	-	+	+	+	+++	+
	Terpenoids	+	+	-	-	-	+
	Steroids	++	-	-	+	-	-
	Coumarins	++	+++	++	+++	-	-
	Phlobatannins	-	-	-	-	-	-
	Volatile oils	+	+	-	++	+	+
Roots	Saponins	+	++	++	++	-	-
	Flavonoids	-	-	-	-	-	-
	Glycosides	++	+++	+	++	-	-
	Phenolics	+	-	-	-	+++	-
	Tannins	-	-	-	-	-	+
	Terpenoids	+++	-	-	-	+	-
	Steroids	+	-	-	-	-	+
	Coumarins	+	-	-	-	-	-
	Phlobatannins	-	-	-	-	-	-
	Volatile oils	+	-	++	+	-	++

(-): Absent; (+): Present; (++): Moderate presence; (+++): High presence.

coumarins in the roots. Ethyl acetate extracts of the leaves and roots showed a high amount of phenolics. The plant showed no presence of flavonoids or phlobatannins in either plant part. The leaves had the highest number of phytochemical groups present in all solvents among the plant parts.

Antioxidant activity

In this study, the radicals DPPH and H_2O_2 were used to determine the scavenging activities of various crude plant extracts of *P. afra*. IC_{50} value was calculated for each extract and plotted in the following graphs. One-way ANOVA tests were used to determine the statistical significance between the different extracts for each plant part. Tukey's test was used as the post-hoc test.

In the DPPH assay, the hexane leaf extract (IC_{50}= 14.83 mg/mL) was the only extract to exceed the acceptable upper limit of 10 (Figure 1A). Ethyl acetate experienced the lowest IC_{50} value (2,65 mg/mL).

The stems displayed the lowest IC_{50} value against DPPH in the ethyl acetate extracts (Figure 1B). The methanol, cold water, and room temperature extracts exhibited IC_{50} values in the acceptable upper range.

The methanolic root and the ethyl acetate root extracts displayed the highest antioxidant activity with IC_{50s} of 0.39 mg/mL and 0.31 mg/mL, respectively. The root extracts outperformed the stems and the leaves counterparts in five out of the six extracts – with the exception of the hot

water extracts.

In the H_2O_2 assay, the highest scavenging activity was exhibited by the hot water leaf extract (0.217 mg/mL). The extracts showed high antioxidant activity in the leaves (Figure 2A).

All of the leaf extracts exhibited IC_{50} values below 10. The results between the different extract types were calculated as being statistically significant. The cold-water stem extract exceeded the IC_{50} threshold at 11.01 mg/mL. The hot water stem extract exhibited the lowest IC_{50} value of 0,46 mg/mL (Figure 2B).

The IC_{50} values for the root extracts were consistently low. The statistical analysis determined that there was no statistical significance between the root extract types (Figure 2C). Four out of the six extract types showed that the leaves outperformed the stems and roots counterparts. The cold-water extracts had the highest IC_{50} value among all three plant parts. All IC_{50} values for the leaves, stem,

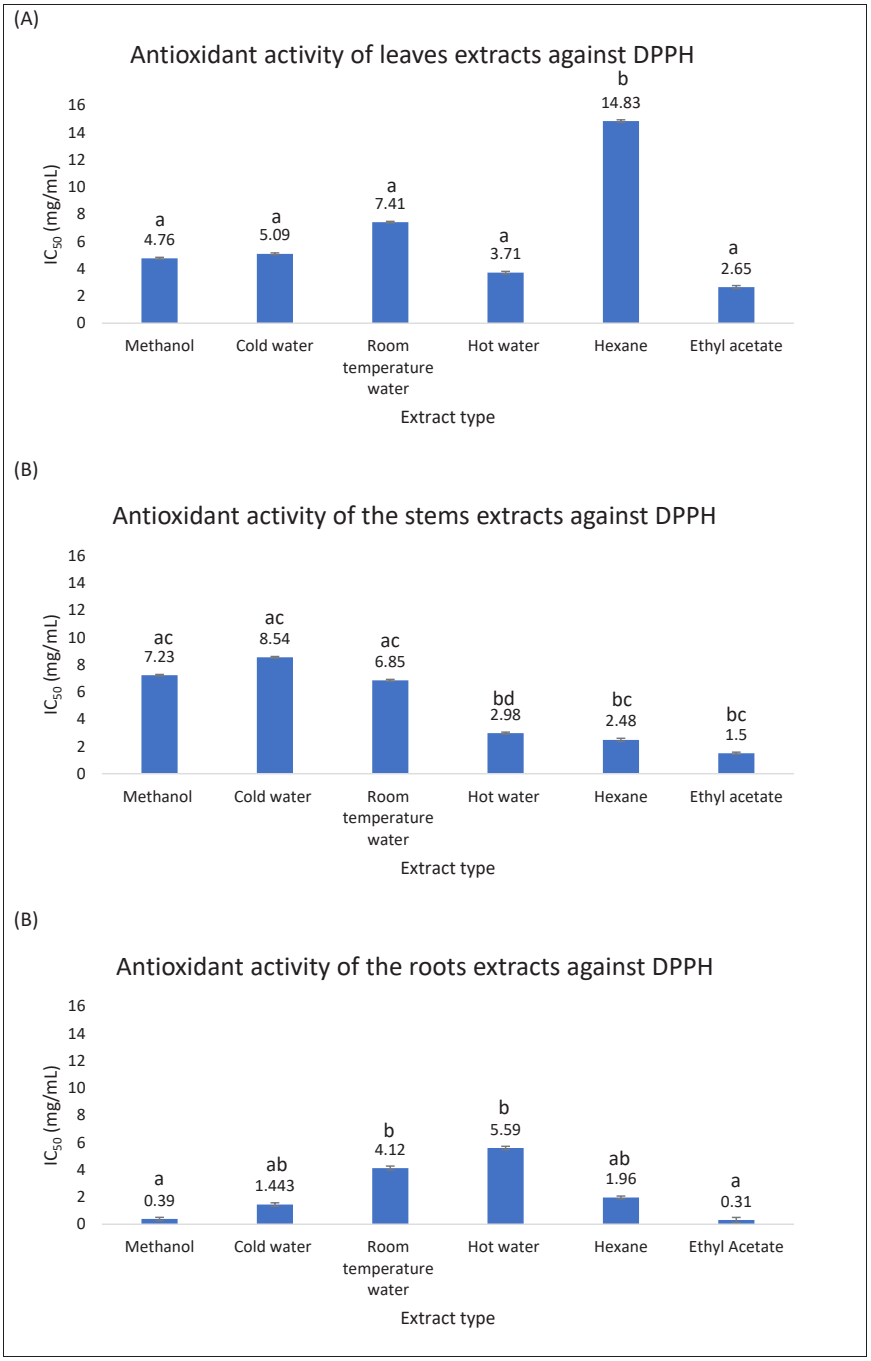


Figure 1. IC_{50} values of the leaves (A), stems (B) and roots (C) extracts of *Portulacaria afra* using methanol, cold water, room temperature water, hot water, hexane, and ethyl acetate against 1-diphenyl-2-picrylhydrazyl (DPPH). Different small letters (a, b, c, and d) between the different groups indicate a statistical difference between extracts type ($P < 0.05$).

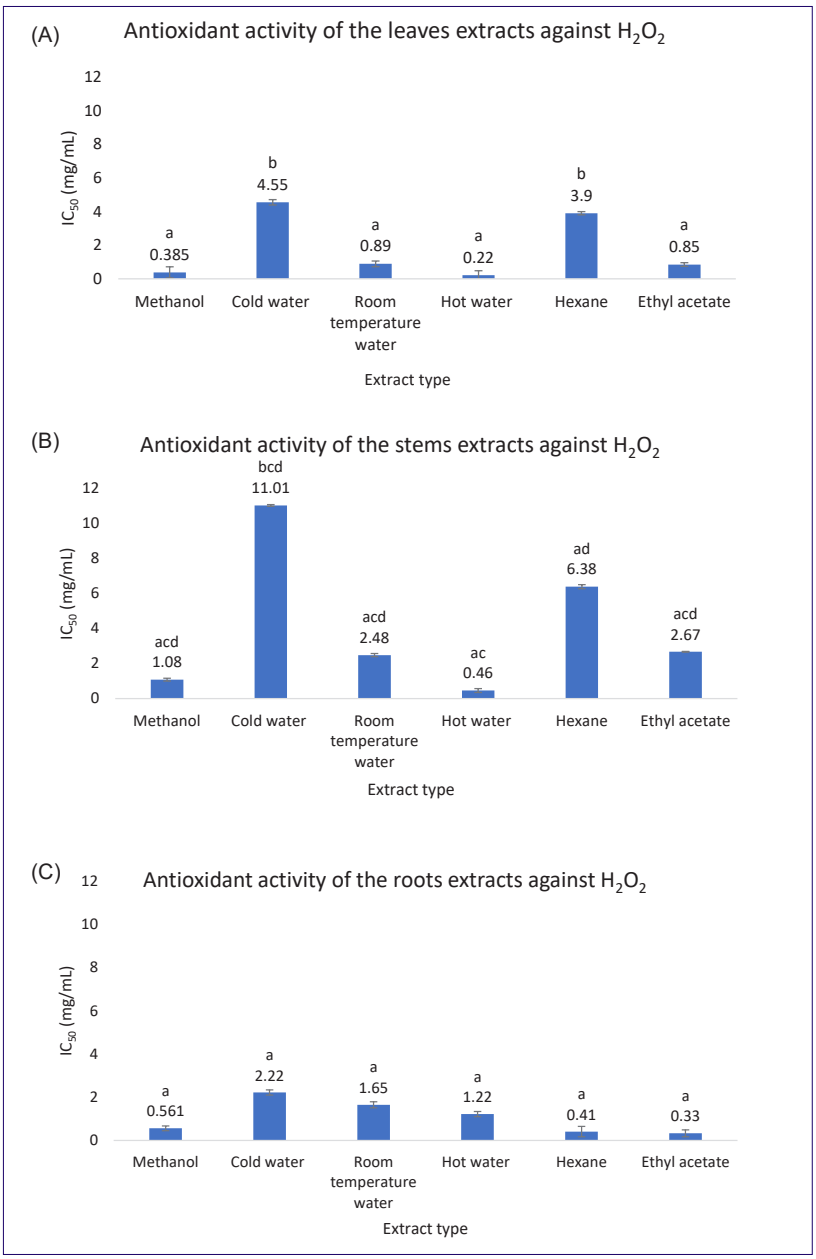


Figure 2. IC_{50} values of the leaves (A), stems (B) and roots (C) extracts of *Portulacaria afra* using methanol, cold water, room temperature water, hot water, hexane, and ethyl acetate against H_2O_2. Different small letters (a, b, c, and d) between the different groups indicate a statistical significance between the extract type (P<0.05).

and roots were below 5mg/mL, except for the hexane stem extract (6.375 mg/mL); the cold-water stem extract (11.002 mg/mL) was the only extract above 10.

Antibacterial activity

The antibacterial properties of *P. afra* were measured against two different bacterial strains, *E. coli* and *S. aureus*. The zones of inhibition were measured with a ruler and compared to the standard zone of inhibition ranges.

The methanolic stem and hot water stem extracts displayed the largest zone of inhibition of 20 mm against *E. coli*. The cold-water and room-temperature water extracts of all three plant parts showed no zone of inhibition against either bacterial strain (Table 2).

In all three plant parts, the methanolic and hot water extract zones of inhibition were identical against *E. coli*. The largest zones of inhibition were against *E. coli*, with the exception of the hexane extracts, which exhibited a greater or equal activity against *S. aureus*.

Discussion

The phytochemical constituents of a plant represent a good indication of the medicinal value of the plant (14). Of the 10 phytochemicals tested in this study, eight of them were present in all plant parts. Methanol is a commonly suggested solvent in literature for phytochemical

Table 2. The zone of inhibition (mm) of the different plant extracts of *Portulacaria afra* against *Escherichia coli* and *Staphylococcus aureus*

Plant part	Extract	Zone of inhibition (mm)	
		E. coli	*S. aureus*
Stem	Methanol	20	16
	Cold water	-	-
	Room temperature water	-	-
	Hot water	20	15
	Ethyl acetate	-	-
	Hexane	13	13
Leaves	Methanol	19	16
	Cold water	-	-
	Room temperature water	-	-
	Hot water	19	15
	Ethyl acetate	-	14
	Hexane	15	18
Roots	Methanol	12	15
	Cold water	-	-
	Room temperature water	-	-
	Hot water	12	16
	Ethyl acetate	-	-
	Hexane	15	17

extraction due to its high polarity of 0.763 (15). This study conforms with the current literature review, as methanolic solvents detected the highest number of phytochemicals in all three plant parts. The hot water extracts showed the presence of five phytochemical groups in all three plant parts and indicated that hot water was able to extract a larger number of phytochemicals than both cold water and room-temperature water. This validates the use of hot water extractions performed traditionally. Hexane has the lowest polarity among all solvents used in this study (15) and the hexane extracts had the lowest presence of phytochemical groups in the leaves, stems, and roots.

Coumarins are a phytochemical group, which displayed a high presence in the stems and leaves of the plant. This suggests a large biological potential of these plant parts because coumarins have shown to possess high antioxidant and antibacterial activities and have recently been associated with anti-cancer, anti-inflammatory, and analgesic properties (16). In addition, saponins, phenolics, and glycosides are phytochemical groups which appear frequently in the phytochemical screening. The high frequency of phytochemicals detected in *P. afra* coincides with the study performed by Tabassum et al (17). The leaves and stems showed a higher overall presence of photochemical groups. Phytochemicals are the bioactive compounds within plants, which produce medicinal properties (18). As such, the leaves may exhibit a larger range of health benefits than the stems and roots.

Antioxidants are important in overall health because they stop the effects of free radicals, which attack the human body, and have been associated with the risk of chronic human diseases (19). The IC_{50} value represents the extracts' ability to reduce oxidation activity by half. The lower the IC_{50} value, the greater the scavenging activity of the extracts and thus the greater the antioxidant activity (20).

The DPPH scavenging assay is the commonly used antioxidant test because it is rapid and inexpensive; it also provides a general idea of the extracts' ability to donate an electron, neutralising oxidative molecules (11). All IC_{50} values were below 10, with the exception of the hexane leaf extract. The high IC_{50} coincides with the low phytochemical presence in the hexane extracts.

Hydrogen peroxide is not a very reactive molecule; however, it gives rise to a hydroxyl radical, which is highly reactive (12). Hydroxyl radicals have a highly oxidising nature, as such, are toxic to cells. Thus, a strong H_2O_2 scavenging activity is important in reducing oxidative substrates in the body. All IC_{50} values for the leaves, stem, and roots were below 5 mg/mL, except for the hexane stem extract (6.375 mg/mL) and cold-water stem extract which was above 10 (11.002 mg/mL). The strongest activity against H_2O_2 was displayed by the hot water leaves extracts (0.217 mg/mL). In the study performed by Tabassum et al (17), it was also determined that the antioxidant activity against DPPH was considered moderate whilst the plant showed strong antioxidant activity against other methods of antioxidant testing. The overall low IC_{50} values obtained in this study suggest the use of the plant as an antioxidant to improve overall health.

The antibacterial activity was tested against *E. coli,* a prominent bacterial strain in South Africa (21) and *S. aureus,* a bacterial strain which is a common cause of skin diseases (22).

The methanolic stem and hot water stem extracts displayed the largest zone of inhibition of 20 mm against *E. coli.* Thus, *E. coli* is susceptible to these extracts. The rest of the extracts, which displayed inhibition zones, varied between 12 and 19 mm and thus fall within the intermediate category. The cold water and room-temperature water extracts showed no zones of inhibition against either bacterial strain, indicating no bacterial activity.

The antibacterial activity against *S. aureus* was considered intermediate with the zones of inhibition ranging from 13 to 18 mm. Due to *S. aureus* being one of the most common skin pathogens, the intermediate bacterial activity of *P. afra* against *S. aureus* may provide relief against diseases caused by *S. aureus*, such as skin and soft skin diseases (22).

This study provides a good general assessment of the potential medicinal properties of the different plant parts of *P. afra*. The use of different solvents of varying polarities allowed for a more comprehensive analysis of the phytochemicals present. Future studies on *P. afra* should include antibacterial activity analyses against other common skin pathogens in order to further validate the use of *P. afra* to treat skin conditions.

Conclusion

In conclusion, *P. afra* possesses phytochemical groups, which contribute to the medicinal properties exhibited by the plant. The plant is rich in coumarins, making the plant a potential species of interest in anticancer studies. The plant extracts displayed high antioxidant properties against DPPH and H_2O_2, thus confirming the nutritional properties of the plant. The antibacterial properties indicated that the stem and leaf extracts might be used as antibacterial agents against *E. coli* and might provide relief against *S. aureus* infections. *P. afra* is a medicinal plant species with a high phytochemical presence and strong biological activities.

Authors' contributions

DCB: Data collection, contribution to methodology, data analysis and interpretation, manuscript writing. TKT: Contribution to methodology, study supervision, manuscript edit and review. IMR: Conceptualisation, contribution to methodology, study supervision, manuscript edit and review.

Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical considerations

Ethical issues regarding authorship, data acquisition, review, and analysis have been carefully observed by authors.

Funding/Support

National Research Foundation (Reference number: TTK190401426371).

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