Journal of Natural Pesticide Research 11 (2025) 100105

Contents lists available at ScienceDirect

Journal of Natural Pesticide Research

journal homepage: www.journals.elsevier.com/journal-of-natural-pesticide-research

Phytochemical characterization and biological properties of *Arctotis arctotoides* (L.f.) O. Hoffm related to the management of *Pythium* root rot disease

James Lwambi Mwinga [a], Peter Tshepiso Ndhlovu [b], Bongisiwe Gladys Shelembe [b], Olaniyi Amos Fawole [c], Adeyemi Oladapo Aremu [a,d,*], Wilfred Otang-Mbeng [b]

[a] Indigenous Knowledge Systems Centre, Faculty of Natural and Agricultural Sciences, North-West University, Private Bag X2046, Mmabatho 2790, South Africa
[b] School of Biology and Environmental Sciences, Faculty of Agriculture and Natural Sciences, University of Mpumalanga, Private Bag X11283, Mbombela 1200, South Africa
[c] South African Research Chairs Initiative in Sustainable Preservation and Agroprocessing Research, Postharvest and Agroprocessing Research Centre, Department of Botany and Plant Biotechnology, University of Johannesburg, P.O. Box 524, Auckland Park, Johannesburg 2006, South Africa
[d] School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg 3209, South Africa

ARTICLE INFO

Keywords:
Antioxidant activity
Crop protection
LC-MS
Plant diseases
Soil-borne diseases

ABSTRACT

Fungal phytopathogens are the primary causative agents of plant diseases and can significantly limit crop production. As an alternative to synthetic fungicides, often associated with detrimental environmental effects, the use of botanicals is a promising tool for managing fungal infections. This study analysed the phytochemical profiles, as well as the antioxidant and antifungal effect of *Arctotis arctotoides* (L.F.) Hoffm. The phytochemical profiles were determined using spectrophotometry and Liquid Chromatography-Mass Spectrometry (LC-MS), while two chemical test models were used to assess the antioxidant effect of the plant extract. The antifungal activity was evaluated against *Pythium ultimum* using the agar well diffusion method. Varying concentrations of total phenolics (131.70 mg gallic acid equivalents per gram (GAE/g) of dry weight (DW) for acetone and 231.56 mg GAE/g DW for methanol extracts) and flavonoids (11.36 mg quercetin equivalents per gram (QE/g) of dry weight (DW) for acetone and 9.86 mg QE/g DW for methanol extracts) were recorded. The LC-MS analysis of the plant extract revealed 14 tentatively identified compounds and 7 unknown bioactive compounds. Two of the compounds, dehydrocostus lactone and methyl pheophorbide A, have been associated with antioxidant effects. Acetone extract (16.67 mm) and methanol extract (18.33 mm) of *Arctotis arctotoides* exhibited considerable inhibitory effects against *P. ultimum*. Further antifungal assessment of the identified bioactive compounds remains essential to establish their activity against phytopathogens relevant in the agricultural sector, especially under field conditions.

1. Introduction

Food security is crucial for achieving the United Nations Sustainable Development Goal (UN SDGs) #2, geared at zero hunger across all nations. However, it is negatively impacted by climate variability and extremes, which limits the agricultural production of food. Climate variability and extremes can lead to plant diseases caused predominantly by fungal phytopathogens, which infect crops and intensify yield loss (Agrios, 2009; De Lucca, 2007; FAO et al., 2018; Lahlali et al., 2024). Fungicides are used widely to manage plant diseases (Panth et al., 2020); however, some are detrimental to humans (Harris et al., 2001) and the environment (Aktar et al., 2009). Furthermore, fungicides have been linked to the development of fungicide resistance (Daferera et al., 2003; Ramaiah & Garampalli, 2015; Yin et al., 2023). These challenges have prompted the use of plant extracts, which are readily available, biodegradable, and eco-friendly (Cherry et al., 2005; Sukanya et al., 2011; Ugwu & Nwaokolo, 2020) in managing diseases in crops (Mwinga et al., 2022). Plants can play a significant role in managing these diseases due to their antimicrobial properties against phytopathogens (Mahlo et al., 2010; Mdee et al., 2009).

* Corresponding author at: Indigenous Knowledge Systems Centre, Faculty of Natural and Agricultural Sciences, North-West University, Private Bag X2046, Mmabatho 2790, South Africa.
E-mail address: Oladapo.Aremu@nwu.ac.za (A.O. Aremu).

https://doi.org/10.1016/j.napere.2024.100105
Received 25 March 2024; Received in revised form 6 November 2024; Accepted 22 November 2024
Available online 26 November 2024

The Asteraceae family is well known for its medicinal applications in African pharmacopoeia (Van Wyk, 2020). Some members of the Asteraceae, for instance, *Acmella oleracea* and *Sphagneticola trilobata*, are characteristic for protecting plants against phytopathogens due to their allelopathic properties (Araújo et al., 2021). As a member of Asteraceae, *Arctotis arctotoides* (L.F.) Hoffm. is a perennial weed found in all provinces of South Africa except Limpopo (Saleh-e-In & Van Staden, 2018). The local names for *Arctotis arctotoides* include "putswa-pududu" (South Sotho), "ubushwa" (isiXhosa), and "botterblom" (Afrikaans) (https://pza.sanbi.org/arctotis-arctotoides). Traditionally, the plant is used for medicinal purposes. For instance, Otang et al. (2012) reported that its leaves and roots are used to manage fungal infections in patients with HIV/AIDS. Among the Xhosa of South Africa, the leaves are used to treat skin-related disorders and diseases such as boils, pimples, and ringworms (Afolayan et al., 2014) Additionally, the plant is used to treat indigestion and epilepsy (Van der Walt, 2002). *Arctotis arctotoides* has demonstrated antifungal activity against various plant pathogenic fungi such as *Aspergillus flavus, A. niger, A. tamarii, Cladosporium herbarum, C. sphaerospermum, Fusarium oxysporium, Mucor canis, M. heamalis, Penicillium digitatum, P. italicum* and *P. notatum* (Afolayan, 2003; Afolayan et al., 2007). For instance, aqueous extracts of the shoots demonstrated strong antifungal effects against *Aspergillus tamarii* and *Penicillium digitatum* at 0.1 mg/mL, with inhibition effects ranging from 50.7 % to 95.2 % (Afolayan, 2003). The study by Otang et al. (2011) revealed the methanol extracts of the leaves exhibit strong antifungal activity against *A. fumigatus*, *Candida albicans*, *C. glabrata*, and *Microsporum canis*. The plant has also demonstrated antibacterial potential. For instance, hexane extract of the shoot was able to inhibit *Bacillus subtilis* (Sultana et al., 2003). Plants contain therapeutic phytochemicals which are relevance in managing crop diseases (Gurjar et al., 2012). The roots of *A. arctotoides* contain flavonoids, polyphenols, and proanthocyanidins (Afolayan et al., 2007). Furthermore, terpenes have been quantified in the leaves and stem (Oyedeji et al., 2005), and as sterols and flavones in the shoot (Sultana & Afolayan, 2007).

This evidence partially points to the relevance of *A. arctotoides* in managing fungal diseases. *Pythium* spp. is responsible for soil-borne diseases that can cause considerable crop losses (Hausbeck, 1985; Mihajlović et al., 2017). Less severe and early stages of root rot may delay flowering and cause plant stunting (Hausbeck, 1985). *Pythium* spp. is associated with damping-off and root rot, which can cause up to 70 % crop losses (Baysal-Gurel & Kabir, 2018; Drizou et al., 2017; Mihajlović et al., 2017). Plant diseases associated with *Pythium* spp. are economically significant, causing substantial crop losses, during wet and rainy seasons (Matthiesen et al., 2016). Thus, this study evaluated the biological activities of *A. arctotoides* relevant for managing *Pythium* root rot. Specifically, we characterised the phytochemical profiles, antioxidant and antifungal activities of *Arctotis arctotoides* against *P. ultimum*.

2. Material and methods

2.1. Collection of Arctotis arctotoides

Whole plants of *Arctotis arctotoides* were collected in April 2023 during autumn from their natural habitat (32°476.0003′ S, 26°5034.42632′ E) at Alice district in Eastern Cape Province, South Africa. The plant was identified by Xhosa traditional healers, locally known as *amaxhwele*. We followed the procedure as outlined by Fish (1999), and recorded the date and location of the collection. The plant was dried by pressing in a plant presser and a voucher specimen, designated as W28, was prepared and deposited at the Griffin Herbarium at the Department of Botany of the University of Fort Hare, South Africa.

2.2. Plant extracts preparation

The whole plants were shade-dried at room temperature (25 ± 2 °C) and ground into fine powder, which was non-sequential extracted with acetone and methanol at a ratio of 1:10 for plant material and solvent. Following extraction in an ultrasonic bath for 30 min, we filtered the mixture using Whatman No. 1 filter paper. The resultant solution was concentrated under reduced pressure using a rotary evaporator and air-drying in a fume hood.

2.3. Phytochemical analysis

2.3.1. Quantification of total phenolic and flavonoid contents

The Folin-Ciocalteu colourimetric method was used to quantify the total phenolic content in the acetone and methanol extracts (Makkar, 2003), with few modifications. An aliquot (1 mL of 100 μg/mL) of plant extracts was used for the assay, and the absorbance of the resultant mixture was measured at 760 nm using a UV-Vis spectrophotometer. Total phenolic content of the acetone and methanol extracts was expressed as milligrams of gallic acid equivalents (GAE) per gram of dry extract (mg GAE/g DW).

Flavonoid content in the plant extracts was quantified using the aluminium chloride colorimetric method (Yang et al., 2004), with modifications. An aliquot (1 mL of 2 mg/mL) of plant extracts was used to prepare the resultant mixture, and its absorbance was measured at 510 nm using a UV-Vis spectrophotometer. The flavonoid content in the extracts was expressed as milligrams of quercetin equivalents per gram of dry extract (mg QE/g DW).

2.3.2. Liquid Chromatography-Mass Spectrometry (LC-MS) analysis

A previously established extraction protocol as described by Khoza et al. (2016) was applied. Based on the procedure outlined by Magangana et al. (2021), the compounds in the methanol extract were identified using high-resolution ultra-performance liquid chromatography-mass spectrometry. Leucine enkephalin was used as reference mass for accurate mass determination, and sodium formate was used to calibrate the instrument. Data were acquired by scanning from 150 to 1500 m/z in MSE and resolution modes. Additional details on the acquisition of data and separation followed the previous study (Magangana et al. 2021).

2.4. Antioxidant tests

As outlined by Arnao et al. (2001), we applied 2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid, ABTS) to evaluate the antioxidant effect of the acetone and methanol extracts. In addition, 2, 2-diphenyl-1-picryhydrazyl (DPPH) free radical scavenging activity of the acetone and methanol extracts was determined following the procedure of Karioti et al. (2004). For both tests, 10 μL of the different concentrations (0.009766–1.25 mg/mL) of each plant extract were used for the assay. The absorbances were read spectrophotometrically at 700 nm (ABTS) and 520 nm (DPPH). The antioxidant activities of *A. arctotoides* were expressed as IC_{50} values in mg/mL.

2.5. Antifungal activity assay

Pythium ultimum was obtained from the fungal culture collection at the Discipline of Plant Pathology, University of KwaZulu-Natal, Pietermaritzburg, South Africa. The pathogen was isolated from carrots (*Daucus carota* subsp. *sativus*) using the baiting method described by Ferguson and Jeffers (1999) and later characterised as *P. ultimum*. *Pythium ultimum* pathogen was maintained on potato dextrose agar (PDA). Afterward, the fungal suspension was withdrawn and suspended aseptically into potato dextrose broth (PDB), which was further incubated for 3 days. A microscope and haemocytometer were used to determine the number of fungal spores and adjusted to a final concentration of 1.0×10^6 spores/mL (Aberkane et al., 2002; Mahlo et al., 2010).

The agar well diffusion method was used to assess the antifungal effect of *A. arctotoides* extracts, as described by Mishra and Arora (2012).

The acetone and methanol extracts were diluted up to 100 mg/mL. A sterile spreader was used to spread the fungal suspension on the PDA evenly. Afterwards, four wells of 9 mm diameter were punched into the PDA using a sterile cork borer. Approximately 50 µL of each plant extract was added to the wells. Blank plates containing 2 % acetone or PDA only served as controls. After 48 h of incubation at 30 °C, the zone of inhibition (mm) was measured.

2.6. Data analysis

A non-linear regression model facilitated with the use of GraphPad Prism 10.1.2 generated the IC_{50} values, which implied the concentration whereby 50 % antioxidant effect by *A. arctotoides* extract was achieved. Significance differences within the means of the treatments were calculated using student's t-test.

3. Results and discussion

3.1. Concentrations of total phenolic and flavonoid

The current study revealed varying concentrations of total phenolics and flavonoids in the two extracts (Table 1). Total phenolic content was higher in methanol than in acetone extracts, while the flavonoid content was higher in acetone than in methanol extracts. Phenols have been attributed to antifungal activity. For instance, salicylic acid (a phenolic compound) exhibited antifungal properties against fungal pathogens such as *Botrytis cinerea*, *Penicillium expansum* and *Rhizopus stolonifera*. Flavonoids are known for their antifungal activity against phytopathogens, and they are also considered signalling molecules in defence against plant pathogens (Cesco et al., 2012). For instance, hesperidin, a major flavonoid found in citrus species, displayed a good antifungal effect against *Penicillium digitatum* (Ortuño et al., 2006).

3.2. Identified compounds in Arctotis arctotoides

Based on the LC-MS analysis, *A. arctotoides* methanol extracts yielded 21 compounds, of which 14 were tentatively identified and 7 were unknown bioactive compounds (Table 2, Supplementary Fig. S1). The categories of the compounds were alkaloids (2), benzene and substituted derivatives (3), fatty acyl (2), flavonoids (1), indoles and derivatives (1), prenol lipids (2), and 10 unclassified polyphenols. Previous studies have profiled the bioactive compounds of *A. arctotoides* using Gas Chromatography-Mass Spectrometry (Oyedeji et al., 2005), gel filtration chromatography and silica gel column chromatography (Sultana et al., 2008), silica gel column chromatography (Sultana & Afolayan, 2007), High-Field NMR (Dahmy et al., 1986; Tsichritzis et al., 1990). Various phytochemicals have been identified from these studies, including terpenes such as α-Phellandrene, 1,8-Cineole, terpinen-4-ol, and *rans*-Pipertiol (Oyedeji et al., 2005), and flavones such as nepetin, and pedalitin (Sultana & Afolayan, 2007). Some of the phytochemicals exhibit antimicrobial activity; for instance, dehydrobrachylaenolide and 4β, 15-dihydro-3-dehydro-zaluzanin C have demonstrated antibacterial activity against *Bacillus subtilis* (Sultana et al., 2003). However, the use of LC-MS revealed one common compound (dehydrocostus lactone), which was also reported by other authors (Oyedeji et al., 2005; Sultana & Afolayan, 2007; Sultana et al., 2008). The observation could also be influenced by the extract preparation methods and the exposure time between extract preparation and analysis (Clemente et al., 2011).

Table 1
Total phenolic and flavonoid contents from different extract solvents of *Arctotis arctotoides*.

Solvent	Total phenolic content (mg GAE/g DW)	Total flavonoid content (mg QE/g DW)
Acetone	131.7 ± 0.01^{b}	11.36 ± 0.373^{a}
Methanol	231.56 ± 0.001^{a}	9.86 ± 0.306^{b}

mg GAE/g DW = milligrams of gallic acid equivalents per gram of dry weight; mg QE/g DW = milligram quercetin equivalents per gram of dry weight. Values are means of total phenolics and flavonoids of three replicates; values within a column followed by the same superscript are not significantly different at $p < 0.05$ according to student's t-test

The identified compounds in this study (individually or in synergetic interactions) may have played a role in the antifungal and antioxidant effects of *A. arctotoides*. As reported by Zheng and Wang (2001), polyphenols have a characteristic antioxidant activity as a result of their redox properties. For example, methyl pheophorbide A has been demonstrated to exert antioxidant effects (Yoon et al., 2011). Dehydrocostus lactone has also been reported to produce an antioxidant effect, demonstrating anticancer activities by producing reactive oxygen species that eliminate cancer cells (O'Neill & Posner, 2004).

3.3. Antioxidant activity of Arctotis arctotoides

A higher antioxidant response was observed in DPPH relative to the ABTS assay, which suggests the extracts were more sensitive in DPPH than in the ABTS assay (Table 3). The acetone and methanol extracts revealed no significant difference in both antioxidant models, indicating that acetone and methanol extracts of *A. arctotoides* exerted a similar potency in the ABTS and DPPH assays.

The resultant antioxidant effect of *A. arctotoides* could be due to the presence of phenolics in varying concentrations in the different solvent extracts (Kähkönen et al., 1999). The antioxidant effect of *A. arctotoides* can be significant in protecting against the toxicity of fungal phytopathogens. Antioxidants can protect cell membranes from fungal phytopathogen-induced damage by acting as superoxide anion scavengers (Atroshi et al., 1997; Atroshi et al., 1998; Wu et al., 2017). Proton radical scavenging (Matthew & Abraham, 2006) and hydrogen-donating ability are important attributes of antioxidants in neutralizing free radicals (Contreras-Guzman & Strong, 1982). The antioxidant activity of *A. arctotoides* was compared to the standard (Trolox). In ABTS, the order of scavenging activity was found as Trolox > *A. arctotoides* extract; in DPPH, the extracts demonstrated a higher antioxidant activity than the standard.

3.4. Antifungal activity of A. arctotoides against P. ultimum

The current findings revealed the potential of *A. arctotoides* extract in managing diseases associated with *P. ultimum,* indicated by varying degrees of zones of inhibition. The methanol extracts had slightly stronger antifungal activity (18.33 ± 1.33 mm) than acetone extracts (16.67 ± 0.67 mm) against the tested fungal strain. The presence of flavonoids in various concentrations could have played a role in the antifungal activity of *A. arctotoides* extract against *P. ultimum* (Cesco et al., 2012). The moderate antifungal activity of the acetone extracts could be as a results of lower concentrations of relevant phytochemicals in the extract. Eloff et al. (2017) tested the antifungal effect of *Melianthus comosus* extract against *P. ultimum*. However, acetone displayed moderate antifungal activity (minimum inhibitory concentration (MIC) = 0.16 mg/mL), while methanol extracts did not exhibit significant antifungal activity (MIC = 1.25 mg/mL). Following the evaluation of seven South African plants against *P. ultimum*, Mdee et al. (2009) reported MIC ranging from 0.63 to 2.5 mg/mL, indicating a limited antifungal effect.

Different studies have revealed promising antifungal effects of *A. arctotoides* against various phytopathogens. The studies by Afolayan et al. (2002) reported 100 % growth inhibition of *A. alternaria* and *A. niger* using *A. arctotoides* acetone shoot extracts. The shoot water extracts of this plant also demonstrated promising antifungal activity against *A. flavus*, *A. tamarii*, *Cladosporium herbarum*, *C. sphaerospermum*, *Penicillium digitatum* and *P. italicum* with growth inhibitions ranging from 70.3 % to 95.2 % at 5 mg/mL (Afolayan, 2003). The methanol and acetone root extracts also displayed strong antifungal effects against *A. flavus, A.*

Table 2
Compounds tentatively identified in *Arctotis arctotoides* methanol extract showing retention times, detected $[M-H]^-$ and $M+H]^+$ ion, elemental composition, MS^E fragments and class.

No.	Rt	Experimental *m/z* $[M-H]^-$/$[M+H]^+$		MS^E Fragmentation Ions	Elemental Formula	Class	Tentative identity
1	0.87	274.8738	$[M+H]^+$	**275.1215,** 259.2366, 479.3282, 495.2822, 463.1211, 411.2222, 395.2332	$C_8H_4O_{15}$	-	Unknown 1
2	1.18	341.1087	$[M-H]^-$	**341.2322,** 191.2234, 377.2332, 533.5933, 683.2322	$C_7H_5O_4$	-	Unknown 2
3	2.65	243.0622	$[M-H]^-$	**499.2322,** 827.2355, 989.3444	$C_5H_7O_{11}$	-	Unknown 3
4	3.49	487.1814	$[M-H]^-$	**487.2355**, 477.2622, 390.3733, 361.2323, 353.2323	$C_{22}H_{31}O_{12}$	-	Unknown 4
5	4.18	471.18	$[M-H]^-$	**471.2733**, 461.2844, 515.1115		-	Unknown 5
6	4.24	362.1970	$[M+H]^+$	**362.1211**, 316.1211, 384.1010, 745.2322	$C_{20}H_{27}NO_5$	Alkaloids	Phalaenopsine T
7	4.72	362.1970	$[M+H]^+$	**362.3222,** 316.2322, 384.2220, 745.2333	$C_{20}H_{27}NO_5$	Alkaloids	Epihernandolinol
8	5.34	412.2129	$[M+H]^+$	**412.1010,** 366.2333, 301.3222, 477.2322	$C_{24}H_{29}NO_5$	Benzene and substituted derivatives	2-({2-[4-(2-methoxyphenyl)−2,2-dimethyloxan−4-yl]ethyl}carbamoyl) benzoic acid
9	5.72	404.2078	$[M+H]^+$	**317.3322**, 354.2333, 302.3222, 685.2322, 231.2112, 157.2322	$C_{22}H_{29}NO_6$	-	6-{2-[(8-methyl−2-oxo−4-propyl-2H-chromen−7-yl)oxy]propanamido} hexanoic acid
10	5.88	426.2288	$[M+H]^+$	**433.2222,** 231.1211, 843.2322, 157.2322	$C_{25}H_{31}NO_5$	Fatty acyls	7-(2-hydroxyethyl)-monascorubramine
11	6.20	315.0508	$[M-H]^-$	**315.3254**, 300.2333, 285.1111	$C_{16}H_{12}O_7$	Flavonoids	Isorhamnetin
12	6.68	338.1239	$[M+H]^+$	**787.2322**, 641.3232, 809,3222, 189.3222, 320.1111	$C_{16}H_{19}NO_7$	Indoles and derivatives	1H-Indol−3-ylacetyl-myo-inositol
13	6.92	229.1228	$[M+H]^+$	**229.1211**, 269.4733, 331.3322, 183.4433, 459.2322,475.3232	$C_{15}H_{16}O_2$	Benzene and substituted derivatives	Bisphenol A
14	7.03	231.1382	$[M+H]^+$	**271.2232**, 353.2322, 157.2322	$C_{15}H_{18}O_2$	Prenol lipids	Dehydrocostus lactone
15	8.60	229.1227	$[M+H]^+$	**229.1224**, 269.2333, 331.3222, 191.2111, 183.1233, 459.3222, 475.2333	$C_{15}H_{16}O_2$	Benzene and substituted derivatives	Bisphenol A
16	9.60	291.1962	$[M-H]^-$	**291.2522**, 480.1722, 540.1222, 597.1555	$C_{18}H_{28}O_3$	Fatty acyls	(2′*E*,4′*Z*,7′*Z*,8*E*)-Colnelenic acid
17	9.84	353.2693	$[M+H]^+$	**376.5443**, 565.3344, 398.3444, 229.1255	$C_{21}H_{36}O_4$	-	(-)-Ebelactone B; Ebelactone B
18	11.83	555.2844	$[M-H]^-$	**555.1521,** 325.1633, 719.1333, 397.1532	$C_{23}H_{45}O_{16}$	-	Unknown 6
19	12.65	457.3483	$[M+H]^+$	**457.2322**, 746.2333, 501.3223, 762.2322, 818.1222, 413.4433, 369.2321,313.2444, 607.3433, 547.4555	$C_{33}H_{44}O$	Prenol lipids	Citranaxanthin
20	13.33	607.2925	$[M+H]^+$	**227.5655,** 858.3433, 431.3222, 247.3444, 365.3444, 467.3433, 552.4333,746.2322, 159.3433	$C_{36}H_{38}N_4O_5$	-	Methyl pheophorbide a
21	14.33	238.8921	$[M-H]^-$	**390.1633,** 293.1444, 310.2672, 361.4103	$C_9H_4NO_7$	-	Unknown 7

MS^E fragments in bold refers to the base peak (the highest peak) (Alberts et al., 2012).

Table 3
Antioxidant activity of *Arctotis arctotoides* extracts based on IC_{50} values (mg/mL) using 2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picryhydrazyl (DPPH) models.

	ABTS	DPPH
Acetone extract	0.28 ± 0.077^a	0.23 ± 0.105^b
Methanol extract	0.37 ± 0.013^a	0.12 ± 0.024^b

Standard positive control; Trolox = 0.04 ± 0.027 (ABTS assay), 0.35 ± 0.006 (DPPH assay). Values are means of ABTS and DPPH IC_{50} values of three replicates; values within a column followed by the same superscript are not significantly different at $p < 0.05$ according to student's t-test.

niger, Fusarium oxysporium, Mucor heamalis, and *P. notatum*. The highest antifungal activities were observed against *M. heamalis* and *P. notatum* with 100 % growth inhibitions at 5 mg/mL (Afolayan et al., 2007).

4. Conclusion

The current study partially provides evidence on the efficacy of *A. arctotoides* in the management of plant diseases related to *P. ultimum*. Various concentrations of phenolics and flavonoids revealed in the extracts, as well as the identified bioactive phytochemicals in the methanolic extracts (especially dehydrocostus lactone and methyl pheophorbide A), could partially be responsible for the antioxidant and antifungal activities of *A. arctotoides*. Overall, *A. arctotoides* extracts displayed moderate antioxidant and antifungal effects against *P. ultimum*. Antioxidant effect was more potent in methanol extracts (IC_{50} = 0.12 mg/mL) with DPPH. Antifungal activity against *P. ultimum* was more potent with methanol extracts (inhibition zone of 18.33 mm). Future research should focus on the isolation, characterization, and detailed antifungal activities of the tentatively identified polyphenols. Additionally, studies on the mode of action of these phytochemicals are crucial to understanding how they exert their antifungal effects. Further studies are also recommended to evaluate the field efficacy of *Arctotis arctotoides* extracts under various environmental conditions and to explore the potential for large-scale production and commercialization. This will help understand the practical viability and economic benefits of using these extracts in real-world agricultural settings.

Authors contributions

JLM, with guidance from WOM, OAF and AOA, conceptualized the study. JLM, BGS and PTN performed the experiments. JLM analysed the data and prepared the draft manuscript. WOM, OAF and AOA supervised the whole project. Funding acquisition was done by WOM, OAF and AOA.

CRediT authorship contribution statement

Dr Peter Tshepiso Ndhlovu: Writing – review & editing, Investigation, Formal analysis. **Dr Bongisiwe Gladys Shelembe:** Writing – review & editing, Investigation, Formal analysis. **Prof Olaniyi Amos Fawole:** Writing – review & editing, Supervision, Project

administration, Funding acquisition. **Adeyemi Oladapo Aremu:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Prof Wilfred Otang-Mbeng:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **James Lwambi Mwinga:** Writing – original draft, Methodology, Investigation, Conceptualization.

Declaration of Competing Interest

We declare no conflict of interest. The design of the study and the findings, opinions, conclusions, and recommendations expressed in this study are solely those of the authors, and the NRF accepts no liability whatsoever in this regard.

Acknowledgements

The National Research Foundation (NRF) of South Africa, grant number 135452 (WOM), SPAR231013155231(OAF) and SRUG2204224395, 150734 (AOA) provided financial support for this study. The North-West University, University of Mpumalanga and University of Johannesburg provided the necessary facilities used for this work. We thank Dr Trust Pfukwa for assisting with the antioxidant activity and LC-MS analyses.

Conflict of interests

We declare no conflict of interest. The design of the study and the findings, opinions, conclusions, and recommendations expressed in this study are solely those of the authors, and the NRF accepts no liability whatsoever in this regard.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.napere.2024.100105.

Data availability

Data will be made available on request.

References

Aberkane, A., M, C.-E., Gomez-Lopez, A., Petrikkou, E., Mellado, E., Monzón, A., Rodriguez Tudela, J.L., 2002. Comparative evaluation of two different methods of inoculums preparation for antifungal susceptibility testing of filamentous fungi. J. Antimicrob. Chemother. 50, 719–722.

Afolayan, A., 2003. Extracts from the shoots of *Arctotis arctotoides* inhibit the growth of bacteria and fungi. Pharm. Biol. 41, 22–25. https://doi.org/10.1076/phbi.41.1.22.14692.

Afolayan, A.J., Grierson, D.S., Mbeng, W.O., 2014. Ethnobotanical survey of medicinal plants used in the management of skin disorders among the Xhosa communities of the Amathole District, Eastern Cape, South Africa. J. Ethnopharmacol. 153, 220–232. https://doi.org/10.1016/j.jep.2014.02.023.

Afolayan, A.J., Grierson, D.S., Kambizi, L., Madamombe, I., Masika, P.J., 2002. *In vitro* antifungal activity of some South African medicinal plants. South Afr. J. Bot. 68, 72–76.

Afolayan, A.J., Jimoh, F.O., Sofidiya, M.O., Koduru, S., Lewu, F.B., 2007. Medicinal potential of the root of *Arctotis arctotoides*. Pharm. Biol. 45, 486–493. https://doi.org/10.1080/13880200701389383.

Agrios, G.N., 2009. Plant pathogens and disease: General introduction. Encyclopedia of Microbiology, Third Edition. Elsevier Inc., University of Florida, pp. 613–646.

Aktar, M.W., Sengupta, D., Chowdhury, A., 2009. Impact of pesticides use in agriculture: their benefits and hazards. Interdiscip. Toxicol. 2, 1–12.

Alberts, P., Stander, M.A., De Villiers, A., 2012. Advanced ultra high-pressure liquid chromatography-tandem mass spectrometric methods for the screening of red wine anthocyanins and derived pigments. J. Chromatogr. A 1235, 92–102.

Araújo, C.A., Morgado, C.S., Gomes, A.K.C., Gomes, A.C.C., Simas, N.K., 2021. Asteraceae family: a review of its allelopathic potential and the case of *Acmella oleracea* and *Sphagneticola trilobata*. RodriguéSia 72, 1–25.

Arnao, M.B., Cano, A., Acosta, M., 2001. The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chem. 73, 239–244.

Atroshi, F., Rizo, A., Antila, E., Westermarck, T., 1997. Free radicals and antioxidants in trichothecens toxicity. In: Hiramatsu, In.M., Yoshikawa, T., Inoue, M. (Eds.), Food and Free Radicals. Springer US, pp. 157–160.

Atroshi, F., Rizzo, A., Westermarck, T., Ali-Vehmas, T., 1998. Effects of tamoxifen, melatonin, coenzyme Q10, andi-carnitine supplementation on bacterial growth in the presence of mycotoxins. Pharmacol. Res. 38, 289–295.

Baysal-Gurel, F., Kabir, N., 2018. Comparative performance of fungicides and biocontrol products in suppression of *Rhizoctonia* root rot in viburnum. J. Plant Patholol. Microbiol. 9, 451.

Cesco, S., Mimmo, T., Tonon, G., Tomasi, N., Pinton, R., Terzano, R., Neumann, G., Weisskopf, L., Renella, G., Landi, L., Nannipieri, P., 2012. Plant-borne flavonoids released into the rhizosphere: impact on soil bio-activities related to plant nutrition. A review. Biol. Fertil. Soils 48, 123–149.

Cherry, A.J., Bantino, A., Djegui, D., Lomers, C., 2005. Suppression of the stem borer *Sesamia calamistis* (*Lepidoptera: Noctuidae*) in maize following seed dressing, topical application and stem injection with African isolates of *Beauveria bassiana*. Int. J. Pest Manag. 50, 67–73.

Clemente, J.G., Williams, J.D., Cross, M., Chambers, C.C., 2011. Analysis of garlic cultivars using head space solid phase microextraction/gas chromatography/mass spectroscopy. Open Food Sci. J. 6, 1–4. https://doi.org/10.2174/1874256401206010001.

Contreras-Guzman, E.S., Strong, F.C., 1982. Determination of tocopherols (Vitamin E) by reduction of cupric ion. J. Assoc. Off. Anal. Chem. 65, 1215–1222.

Daferera, D.J., Ziogas, B.N., Polissiou, M.G., 2003. The effectiveness of plant essential oils on the growth of *Botrytis cinerea*, *Fusarium* sp. and *Clavibacter michiganensis* subsp. *michiganensis*. Crop Prot. 22, 39–44.

Dahmy, S.E., Sarg, T., Salem, S.A., Jakupovic, J., Bohlmann, F., 1986. New guaianolides and farnesol derivatives from *Arctotis arctotoides*. Planta Med. 5, 365–367. https://doi.org/10.1055/s-2007-969186.

De Lucca, A.J., 2007. Harmful fungi in both agriculture and medicine. Rev Iberoam Micol 24, 3–13.

Drizou, F., Graham, N.S., Bruce, T.J.A., Ray, R.V., 2017. Development of high-throughput methods to screen disease caused by *Rhizoctonia solani* AG 2-1 in oilseed rape. Plant Methods 13.

Eloff, J.N., Angeh, I.E., McGaw, L.J., 2017. Solvent-solvent fractionation can increase the antifungal activity of a *Melianthus comosus* (Melianthaceae) acetone leaf extract to yield a potentially useful commercial antifungal product. Ind. Crops Prod. 110, 103–112.

FAO, IFAD, UNICEF, WFP and WHO. 2018. The State of Food Security and Nutrition in the World 2018. Building climate resilience for food security and nutrition. Rome, FAO. Licence: CC BY-NC-SA 3.0 IGO.

Ferguson, A.J., Jeffers, S.N., 1999. Detecting multiple species of *Phytophthora* in container mixes from ornamental crop nurseries. Plant Dis. 83, 1129–1136. https://doi.org/10.1094/PDIS.1999.83.12.1129.

Fish, L., 1999. Preparing herbarium specimens. Strelitza 7, 4–44.

Gurjar, M.S., Ali, S., Akhtar, M., Singh, K.S., 2012. Efficacy of plant extracts in plant disease management. Agric. Sci. 3, 425–433.

Harris, C.A., Renfrew, M.J., Woolridge, M.W., 2001. Assessing the risk of pesticide residues to consumers: recent and future developments. Food Addit. Contam. 18, 1124–1129.

Hausbeck, M.K. (1985). Influence of silver thiosulphate and fungicides on plant mortality caused by *Pyhium ultimum* in the seed-propagated geranium (*Pelargonium* X *hortorum*). MSc thesis, Department of Horticulture, Michigan State University, USA.

Kähkönen, M.P., Hopia, A.I., Vuorela, H.J., Rauha, J.P., Pihlaja, K., Kujala, T.S., Heinonen, M., 1999. Antioxidant activity of plant extracts containing phenolic compounds. J. Agric. Food Chem. 47, 3954–3962.

Karioti, A., Hadjipavlou-Litina, D., Mensah, M.L., Fleischer, T.C., Skaltsa, H., 2004. Composition and antioxidant activity of the essential oils of *Xylopia aethiopica* (Dun) A. Rich. (Annonaceae) leaves, stem bark, root bark, and fresh and dried fruits, growing in Ghana. J. Agric. Food Chem. 52, 8094–8098. https://doi.org/10.1021/jf040150j.

Khoza, B.S., Gbashi, S., Steenkamp, P.A., Njobeh, P.B., Madala, N.E., 2016. Identification of hydroxylcinnamoyl tartaric acid esters in *Bidens pilosa* by UPLC-tandem mass spectrometry. South Afr. J. Bot. 103, 95–100.

Lahlali, R., Taoussi, M., Laasli, S.-E., Gachara, G., Ezzouggari, R., Belabess, Z., Aberkani, K., Assouguem, A., Meddich, A., El Jarroudi, M., Barka, E.A., 2024. Effects of climate change on plant pathogens and host-pathogen interactions. Crop Environ. 3, 159–170. https://doi.org/10.1016/j.crope.2024.05.003.

Magangana, T.P., Makunga, N.P., Fawole, O.A., Opara, U.L., 2021. Effect of solvent extraction and blanching pre-treatment on peel extracts. Processes 9, 1012. https://doi.org/10.3390/pr9061012.

Mahlo, S.M., McGaw, L.J., Eloff, J.N., 2010. Antifungal activity of leaf extracts from South African trees against plant pathogens. Crop Prot. 29, 1529–1533.

Makkar, H., 2003. Quantification of Tannins in Tree and Shrub Foliage. A Laboratory Manual. Springer Science and Business Media, Doi. https://doi.org/10.1007/978-94-017-0273-7.

Matthew, S., Abraham, E.T., 2006. In vitro antioxidant activity and scavenging effects of *Cinnamomum verum* leaf extract assayed by different methodologies. Food Chem. Toxicol. 44, 198–206.

Matthiesen, R.I., Ahmad, A.A., Robertson, A.E., 2016. Temperature affects aggressiveness and fungicide sensitivity of four *Pythium* spp. that cause soybean and corn damping-off in Iowa. Plant Dis. 100, 583–591.

Mdee, L.K., Masoko, P., Eloff, J.N., 2009. The activity of extracts of seven common invasive plant species on fungal phytopathogens. South Afr. J. Bot. 75, 375–379.

Mihajlović, M., Rekanović, E., Hrustić, J., Tanović, B., 2017. Methods for management of soilborne plant pathogens. Pestic. Phytomed. 32, 9–24.

Mishra, S., Arora, N.K., 2012. Evaluation of rhizospheric Pseudomonas and Bacillus as biocontrol tool for *Xanthomonas campestris* pv. *campestris*. World J. Microbiol. Biotechnol. 28, 693–702.

Mwinga, J.L., Otang-Mbeng, W., Kubheka, B.P., Aremu, A.O., 2022. Ethnobotanical survey of plants used by subsistence farmers in mitigating cabbage and spinach diseases in OR Tambo Municipality, South Africa. Plants 11, 3215.

O'Neill, P.M., Posner, G.H., 2004. A medicinal chemistry perspective on artemisinin and related endoperoxides. J. Med. Chem. 47, 2945–2964. https://doi.org/10.1021/jm030571c.

Ortuño, A., Báidez, A., Gómez, P., Arcas, M.C., Porras, I., García-Lidón, A., Del Río, J.A., 2006. *Citrus paradisi* and *Citrus sinensis* flavonoids: their influence in the defence mechanism against *Penicillium digitatum*. Food Chem. 98, 351–358.

Otang, W.M., Grierson, D.S., Ndip, R.N., 2011. The effect of the acetone extract of *Arctotis arctotoides* (Asteraceae) on the growth and ultrastructure of some opportunistic fungi associated with HIV/AIDS. Int. J. Mol. Sci. 12, 9226–9235. https://doi.org/10.3390/ijms12129226.

Otang, W.M., Grierson, D.S., Ndip, R.N., 2012. Antifungal activity of *Arctotis arctotoides* (L.f.) O. Hoffm. and *Gasteria bicolor* Haw. against opportunistic fungi associated with human immunodeficiency virus/acquired immunodeficiency syndrome. Pharmacogn. Mag. 8, 135–140. https://doi.org/10.4103/0973-1296.96564.

Oyedeji, A., Yani, V., Afolayan, A., 2005. Chemical composition of the essential oil from *Arctotis arctotoides* (L.F.) O. Hoffm. (syn. *Vendium arctotoides* Less.). Flavour Fragr. J. 20, 232–234. https://doi.org/10.1002/ffj.1437.

Panth, M., Hassler, S.C., Baysal-Gurel, F., 2020. Methods for management of soilborne diseases in crop production. Agriculture 10, 16.

Ramaiah, K.A., Garampalli, R.K.H., 2015. *In vitro* antifungal activity of some plant extracts against *Fusarium oxysporum f.* sp. *lycopersici*. Asian J. Plant Sci. Res. 5, 22–27.

Saleh-e-In, M.M., Van Staden, J., 2018. Ethnobotany, phytochemistry and pharmacology of *Arctotis arctotoides* (L.f.) O. Hoffm.: a review. J. Ethnopharmacol. 220, 294–320. https://doi.org/10.1016/j.jep.2018.01.011.

Sukanya, S.L., Yamini, D., Fathima, S.K., 2011. Eco-friendly management of *Pyricularia oryzae*, the causal agent of blast of paddy. Curr. Bot. 2, 46–49.

Sultana, N., Afolayan, A., Bhuiyan, R., 2008. Antimicrobial compounds from the shoots of *Arctotis arctotoides*. Bangladesh J. Sci. Ind. Res. 43, 89–96. https://doi.org/10.3329/bjsir.v43i1.860.

Sultana, N., Afolayan, A., 2007. A novel daucosterol derivative and antibacterial activity of compounds from *Arctotis arctotoides*. Nat. Prod. Res. 21, 889–896. https://doi.org/10.1080/14786410601129606.

Sultana, N., Afolayan, A.J., Houghton, P.J., 2003. Bioactive sesquiterpene lactones isolated from the shoots of *Arctotis arctotoides*. South Afr. J. Bot. 69, 158–160. https://doi.org/10.1016/S0254-6299(15)30339-2.

Tsichritzis, F., Jakupovic, J., Bohlmann, F., 1990. Sesquiterpene lactones and farnesol derivatives from *Arctotis* and *Aarctotheca* species. Phytochemistry 29, 195–203. https://doi.org/10.1016/0031-9422(90)89036-9.

Ugwu, J.A., Nwaokolo, V.M., 2020. Biocidal activity of selected botanicals and *Beauveria bassiana* on oriental fruit fly, *Bactrocera dorsalis* (Diptera:Tephritidae). J. Res. For. Wildl. Environ. 12, 53–61.

Van der Walt, L., 2002. Arctotis arctotoides (L.f.) O.Hoffm. South African National Biodiversity Institute's Plant Information.

Van Wyk, B.E., 2020. A family-level floristic inventory and analysis of medicinal plants used in Traditional African Medicine. J. Ethnopharmacol. 249, 112351.

Wu, Q., Wang, X., Nepovimova, E., Wang, Y., Yang, H., Li, L., Zhang, X., Kuca, K., 2017. Antioxidant agents against trichothecenes: new hints for oxidative stress treatment. Oncotarget 8, 110708–110726.

Yang, J., Meyers, K., Heide, J., Liu, R., 2004. Varietal differences in phenolic content and antioxidant and antiproliferative activities of onions. J. Agric. Food Chem. 52, 6787–6793.

Yin, Y., Miao, J., Shao, W., Liu, X., Zhao, Y., Ma, Z., 2023. Fungicide resistance: progress in understanding mechanism, monitoring, and management. Phytopathology 113, 707–718. https://doi.org/10.1094/PHYTO-10-22-0370-KD.

Yoon, I., Park, H.-S., Cui, B., Li, J., Kim, J.-H., Byambajav, L., Shim, Y., 2011. Photodynamic and antioxidant activities of divalent transition metal complexes of methyl pheophorbide-A. Bull. Korean Chem. Soc. 32, 2981–2987. https://doi.org/10.5012/bkcs.2011.32.8.2981.

Zheng, W., Wang, S.Y., 2001. Antioxidant activity and phenolic compounds in selected herbs. J. Agric. Food Chem. 49, 5165–5170. https://doi.org/10.1021/jf010697n.