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A REVIEW OF THE PHYTOCHEMISTRY, BOTANY, PHARMACOLOGY AND TOXICOLOGY OF *ARCTOTIS ARCTOTOIDES* (L.F.) O. HOFFM. (ASTERACEAE)

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Abstract

Background: *Arctotis arctotoides* (Asteraceae) is commonly used by the rural people of Eastern Cape for the treatment of epilepsy, indigestion, catarrh and stomach ache. The leaf paste or juice is applied topically in the treatment of wounds and skin disorders. Unfortunately, no previous reviews are available for this important medicinal plant. Hence, the aim of this review is to provide a comprehensive overview of the botany, phyto-chemistry, pharmacology and toxicology of *Arctotis arctotoides.*
Methodology: This review was carried out using a comprehensive and systematic literature search on the following databases: Google Scholar, PubMed, Science Direct and Scopus. Searches were undertaken using the key word "*Arctotis arctotoides"* and the six synonyms of *Arctotis arctotoides* identified in the Plant List.
Results: In the first phyto-chemical study of *Arctotis arctotoides*, the authors reported that sesquiterpenoids presence was predominant in the root oil whereas, the essential oils of the leaves, flowers and stems had both monoterpenoids and sesquiterpenoids. The literature survey revealed that *Arctotis arctotoides* has been investigated in four pharmacological areas, including anti-bacterial, anti-fungal, anti-cancer and anti-oxidant activities. Three toxicity screens for the crude extracts of *A. arctotoides* on cell lines, rats and brine shrimp were identified in the literature.
Conclusion: Detailed studies on the bioactivity of the crude extracts and the isolated phyto-chemicals have provided partial evidence as regards the traditional use of *A. arctotoides* in the treatment of wounds in Eastern Cape of South Africa. However, in order to fully exploit the medicinal potential of *A. arctotoides*, the expansion of existing traditional knowledge into neighboring communities where the plant is not currently in use for the stated indications will support a greater use of the plant in primary healthcare.

Key words: *Arctotis arctotoides*, botany, phyto-chemistry, pharmacology and toxicology

List of abbreviations: ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid; BHT: Butylated hydroxytoluene; DPPH: 1,1-diphenyl-2-picrylhydrazyl ; FRAP: Ferric reducing ability of plasma**;** MIC: Minimum inhibitory concentration**;** MTT: 3-(4,5-dimethythiazol-. 2-yl)-2,5-diphenyl tetrazolium bromide**;** RPMI 1640 medium: Roswell Park Memorial Institute medium; SEM: Scanning electron microscope**;** TLC: Thin layer chromatography.

Introduction

Arctotis arctotoides is a fast-growing, soft, herbaceous plant that is widespread in Eastern Cape Province of South Africa and Lesotho, usually in disturbed areas like road verges. The plant is used by the rural people of Eastern Cape for the treatment of epilepsy, indigestion, catarrh and stomach ache, with the leaf juice or a paste of the leaf applied topically to treat wounds (van der Walt, 2002). To the best of our knowledge, no previous reviews are available regarding the botany, phytochemistry and pharmacological studies of *Arctotis arctotoides*. Hence, the aim of this review is to provide a comprehensive overview of the botany, phytochemistry, pharmacology and toxicology of *Arctotis arctotoides.* Additionally, evidence of traditional uses of this important medicinal plant has been examined and recommendations pertaining to future research areas for the plant highlighted.

Methodology

This review was carried out using a comprehensive and systematic literature search on the medicinal plant *Arctotis arctotoides* (L.f.) O.Hoffm. The following databases were used: Google-Scholar (http://scholar.google.com), PubMed (http:www.ncbi.nlm.nih.gov/pubmed), Science-Direct (http://www.sciencedirect.com and Scopus (http://www.scopus.com). Additional search were also undertaken using the six synonyms identified by the plant list (www. the plantlist.org/): *Arctotis decurrens; Arctotis micrantha; Arctotis spathuligera; Venidium arctotoides; Venidium decurrens; Venidium spathuligerum*

Taxonomy, vernacular names and distribution

Asteraceae or Compositae (commonly referred to as the aster, daisy, or sunflower family) is an exceedingly large and widespread family of Angiosperms (Jeffrey, 2007). This is the largest family of the flowering plants with more than 24000 - 30000 species and 1600 - 1700 genera (Funk et. al, 2005) worldwide and inhabit almost every environment and continent except Antarctica. The main feature of the family is the composite flower type surrounded by involucral bracts (Bremer, 1994). The genus *Arctotis* comprises an estimated 50–60 species and belongs to the tribe Arctotideae. The representatives of this genus are indigenous to the *Flora of Southern Africa* region, with the highest concentration of taxa in Western Cape (Mckenzie, 2006). *Arctotis venusta* Norl., has the widest distribution, extending to Angola and Southern Zimbabwe, *Arctotis stoechadifolia* P.J. Bergius is in cultivation and has been introduced into other countries (Pope 1992), while *Arctotis arctotoides* is widespread throughout the summer /

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rainfall areas of South Africa and Lesotho, usually in disturbed areas like road verges. *A. arctotoides* is commonly called "botterblom" in Afrikaans, "putswa-pududu" in South Sotho, and "ubushwa" in Xhosa (van der Walt, 2002). The following names are considered as synonyms to *A. arctotoides* by the plant list (www.plantlist.org): *Arctotis decurrens* (Less.) O. Hoffm., *Arctotis micrantha* Thunb. ex DC., *Arctotis spathuligera* (DC.) O. Hoffm., *Osteospermum arctotoides* L.f., *Venidium arctotoides* (L.f.) Less., *Venidium decurrens* Less., and *Venidium spathuligerum* DC.

Botany and Traditional Uses

Arctotis arctotoides is a fast-growing, soft, herbaceous plant with green foliage and butter-yellow daisy flowers almost throughout the year. Each leaf is about 10-15 cm long with a wavy edge and prominent midrib. The stems are about 20 cm long and both the leaves and stems are covered with small white hairs. The single daisy flowers which are borne on the stems are about 4 cm in diameter and contain bright yellow ray florets at the centre. The undersides of the petals are purplish brown and are clearly visible when the flowers are in bud or closed during a cloudy day.
The rural people of Eastern Cape Province of South Africa are known to use *Arctotis arctotoides* for the treatment of epilepsy, indigestion and catarrh of the stomach and the leaf juice or paste of the leaf is applied topically to treat wounds and fungal infections of the skin (Afolayan, 2003; Otang et al., 2012a).

Figure 1: *Arctotis arctotoides* (Asteraceae)
(Source: http://www.biodiversityexplorer.org/plants/asteraceae/arctotis_arctotoides.htm)

Chemical constituents

Some of the key phytochemicals that have been isolated and characterized from *Arctotis arctotoides* and published in academic journals are presented in Table 1. The phytochemicals are labeled 1-33 and include isolated compounds: 27 terpenes, 1 sterol and 2 flavones and families of compounds (flavonoids, proanthocyanidins and polyphenols) as well. The first phytochemical study on the essential oil of *Arctotis arctotoides* was carried out by hydro-distillation of the leaves, flowers, stems and roots (Oyedeji et al., 2005). Sesquiterpenoids predominated in the root oil (97.9%) while the essential oils of the leaves, flowers and stems had both monoterpenoids (34.1–54.5%) and sesquiterpenoids (38.7–51.9%). Terpinen-4-ol (5.6–12.6%), β-caryophyllene (6.1–6.9%) and γ-curcumene (6.9–8.49%) were the major compounds (numbered 1-30) common to the three oils (Oyedeji et al., 2005).

Sultana and Afolayan (2007) reported the isolation and identification of a new daucosterol derivative 3-O-[β-D-(6'-nadeanoate) glucopyranosyl]- β-sitosterol and seven known compounds namely: serratagenic acid, stigmasterol, daucosterol, zaluzanin D, dehydrocostuslactone, nepetin, and pedalitin (Figure 1). Subsequently, bioassay guided fractionation of different extracts of *Arctotis arctotoides*, combined with NMR spectral analysis led to the isolation of glycerol-1-docosanoate, zaluzanin C and perydiscolic acid (Sultana et al., 2008).

Pharmacological Reports

The literature survey revealed that *Arctotis arctotoides* has been investigated in four pharmacological areas, including antibacterial, antifungal, anticancer and anti-oxidant activities. The available pharmacological studies with detailed conditions are listed in Table 2.

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Antibacterial Activities

A number of *in vitro* studies have been reported regarding the antibacterial activity of the aqueous, ethanol, methanol and acetone extracts of *Arctotis arctotoides* (Afolayan, 2003; Afolayan et al., 2007; Sultana and Afolayan, 2007 and Sultana et al., 2008). Afolayan (2003) reported strong antibacterial activity of the acetone, methanol and water whole shoot extracts of *Arctotis arctotoides* against gram positive bacteria: *Bacillus cereus, Bacillus pumilus, Bacillus subtilis, Micrococcus kristinae* and *Staphylococcus aureus.* None of the extracts inhibited *Klebsiella pneumoniae* and *Pseudomonous aeruginosa*, both Gram negative bacteria. Afolayan et al. (2007) reported strong antibacterial activity by the acetone extract of *Arctotis arctotoides* root against *Bacillus cereus, Staphylococcus aureus, Micrococcus kristinae*, and *Streptococcus pyrogens*. No MICs were determined and owing to a lack of positive control, the antibacterial activity of *Arctotis arctotoides* extracts cannot be compared objectively with modern antibiotics, making it difficult to draw conclusion from this study as regards the utilization of the plant for the management of bacterial infections in traditional medicine.

Table 1: Key phytochemicals isolated from *Arctotis arctotoides*

No	Phytochemical	Plant part	Reference
	Terpenes		
1	α-Phellandrene	Leaf, stem	
2	δ-3-Carene	Leaf	
3	α-Terpinene	Flower, stem	
4	*p*-cymene	Leaf, stem	
5	Limonene	Leaf, flower, stem	
6	1,8-Cineole	Leaf, flower	
7	γ-Terpinene	Leaf, flower, stem	
8	*trans*-Sabinene hydrate	Leaf, flower, stem	
9	Terpinolene	Leaf, flower, stem	
10	Linalool	Leaf, flower	
11	*cis*-Sabinene hydrate	Stem	
12	*cis-p*-Menth-2-en-1-ol	Leaf, flower, stem	
13	*trans-p*-Menth-2-en-1-ol	Leaf	1-23: Oyedeji et al., 2005
14	Verbenol	Flower	
15	*p*-Mentha-1,5-dien-8-ol	Flower, stem	
16	Terpinen-1-ol	Flower	
17	Terpinen-4-ol	Leaf, flower	
18	Cymen-8-ol	Flower	
19	α-Terpineol	Leaf, flower, stem	
20	Myrtenol	Leaf, flower, stem	
21	*trans*-Pipertiol	Leaf, flower, stem	
22	Piperitone	Leaf, flower, stem	
23	Perlialdehyde	Leaf	
24	Zaluzanin	Shoot	Sultana and Afolayan, 2007; Sultana et al., 2008
25	Perydiscolic acid	Shoot	Sultana et al., 2008
26	Serratagenic acid	Shoot	Sultana and Afolayan, 2007
27	Dehydrocostuslactone	Shoot	Sultana and Afolayan, 2007
	Sterol		
28	3-O-[_β-D-(60-nonadeanoate)glucopyranosyl]-β-sitosterol	Shoot	Sultana and Afolayan, 2007
	Flavone		
29	Nepetin	Shoot	Sultana and Afolayan, 2007
30	Pedalitin	Shoot	Sultana and Afolayan, 2007
31	***Flavonoids***	Roots	Afolayan et al., 2007
32	***Polyphenol***	Roots	Afolayan et al., 2007
33	***Proanthocyanidins***	Roots	Afolayan et al., 2007

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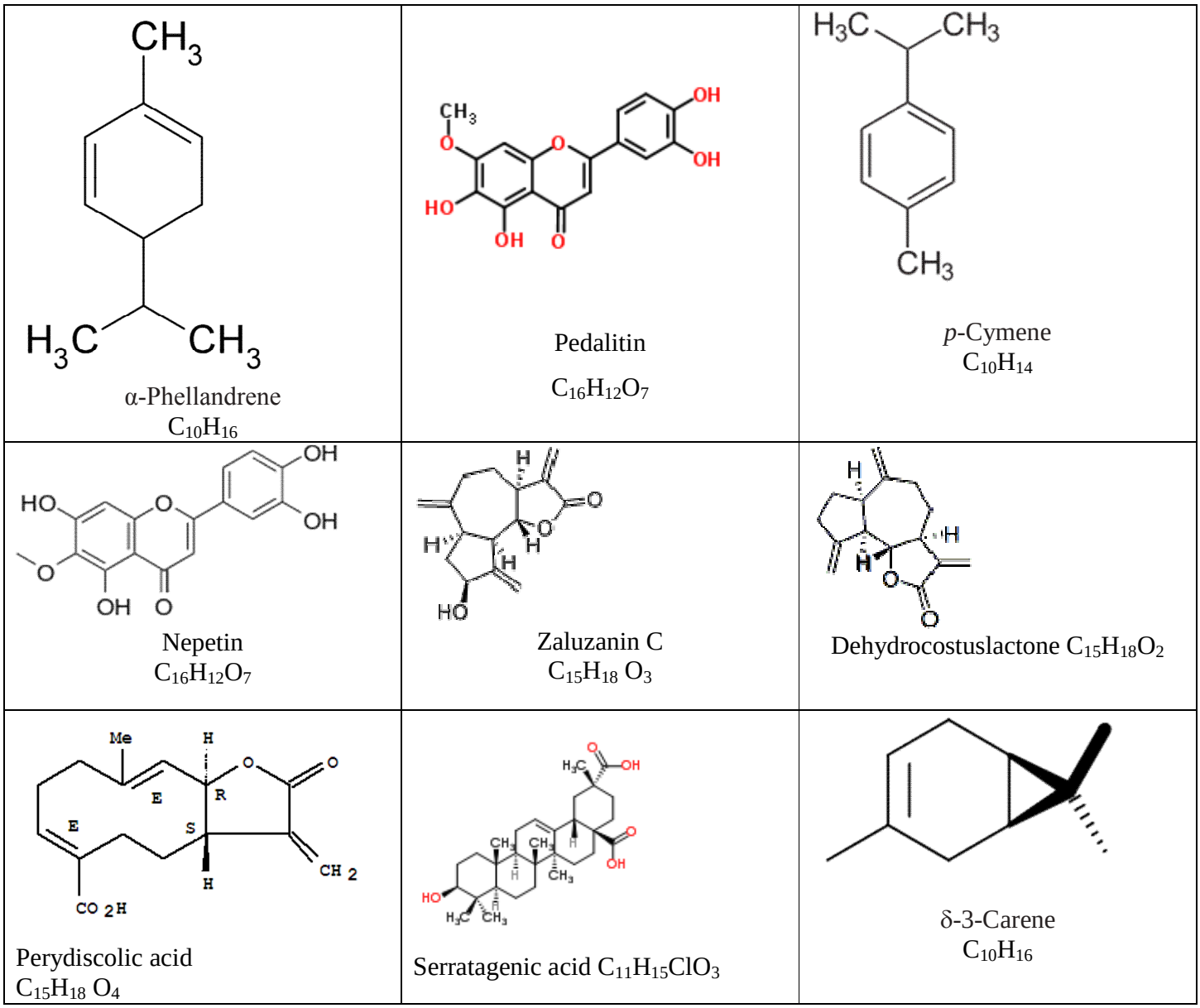

Figure 1: Chemical structures and molecular formulae of some compounds isolated from *A. arctotoides* (Sultana and Afolayan, 2007; Sultana et al., 2008).

Bio-autographic assay was performed on TLC plates using two Gram positive bacteria: *Bacillus subtilis* and *Staphylococcus aureus* and two Gram negative: *Escherichia coli* and *Shigella sonnei* and streptomycin as the positive control. Among the compounds (nepetin, stigmasterol, daucosterol, dehydrocostuslactone, serratagenic acid, zaluzanin and pedalitin) isolated from the shoot of *A. arctotoides*, weak antibacterial activity was observed for nepetin against *Bacillus subtilis* and *Staphylococcus aureus* with MICs of 4mg/ml and 31mg/ml respectively. No antibacterial activity was reported for stigmasterol, daucosterol and dehydrocostuslactone while moderate antibacterial activity by serratagenic acid and pedalitin was observed (Sultana et al., 2008).

Antifungal activity

A series of studies on the antifungal activity of *A. arctotoides* have been reported (Afolayan, 2003; Afolayan et al., 2007; Otang et al., 2011; Otang et al., 2012b). The water extract of the shoot of *A. arctotoides* was particularly inhibitory to the growth of *Aspergillus tamari* and *Penicillum digitatum* with inhibitory activity ranging from 50.7 to 95.2% respectively (Afolayan, 2003). Significant antifungal activity by the acetone extract of the root of *A. arctotoides* was reported (Afolayan et al., 2007) with growth inhibition ranging from 56.23% on *Aspergillus flavus* to 100% on *Penicillium notatum* and *Mucor heamalis* at 5.0 mg/ml. The water extract showed the least inhibitory activity. However, the lack of positive controls in the above studies makes it difficult to conclude about the antifungal activity of *A. arctotoides*.

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Table 2: Summary of pharmacological and toxicological studies carried out on *Arctotis arctotoides*

Activity tested	Model used	Plant part used/Tested material	Extract type	Dosage	Control	Results	Reference
Antibacterial	Nutrient agar medium was mixed with extract and bacterial suspension. Species: *Bacillus cereus, B. pumilus, B. subtilis, Micrococcus kristinae, Staphylococcus aureus, Enterobacter cloacae, Escherichia coli, Klebsiella pneumonia, Pseudomomas aeruginosa, Serratia marcescens*	Whole shoot	Acetone, methanol and water	0.1-5 mg/ml	Negative: nutrient agar + 2% solvent. No positive control	Strong antibacterial activity against gram positive bacteria: *Bacillus cereus, B. pumilus, B. subtilis, Micrococcus kristinae* and *Staphylococcus aureus.* No MICs determined. None of the extracts inhibited *Klebsiella pneumoniae* and *Pseudomonous aeruginosa*, both Gram negative bacteria	Afolayan, 2003
Antibacterial	Nutrient agar medium was mixed with extract and bacterial suspension. Species: *Bacillus cereus, Staphylococcus epidermidis, Staphylococcus aureus, Micrococcus kristinae, Streptococcus pyrogens, Escherichia coli, Salmonella pooni, Serratia marcescens, Pseudomonas aeruginosa, Klebsiella pneumonia*,	Root	Acetone, methanol and water	0.1-5 mg/ml	Negative: 5ml acetone or methanol Positive: Streptomycin	Strong antibacterial activity by the acetone extract against *Bacillus cereus, Staphylococcus aureus, Micrococcus kristinae*, and *Streptococcus pyrogens*.	Afolayan et al., 2007
Antibacterial	Bioautographic assay was performed on TLC plates using two Gram positive bacteria: *Bacillus subtilis and Staphylococcus aureus and two Gram negative: Escherichia coli and Shigella sonnei*	Compounds isolated from shoot of *A. arctotoides:* nepetin stigmasterol, daucosterol, dehydrocostuslactone. serratagenic acid, zaluzanin and pedalitin		2-250 µg/ml	Negative: Dimethyl sulphoxide Positive: Streptomycin	Significant antibacterial activity of nepetin against *Bacillus subtilis* and *Staphylococcus aureus* with MICs of 4mg/ml and 31mg/ml respectively. No antibacterial activity by stigmasterol, daucosterol and dehydrocostuslactone. Moderate antibacterial activity by serratagenic acid and pedalitin	Sultana and Afolayan, 2007
Antibacterial	Bioautographic assay was performed on TLC plates using *Bacillus cereus, B. subtilis, Staphylococcus aureus, Staphylococcus epidermidus, Escherichia coli,* and *Shigella sonnei*	Compounds isolated from shoot of *A. arctotoides:* glycerol-1-docosanoate, zaluzanin C and perydiscolic acid	Ethanol	Not mentioned	Negative: DMSO Positive: Streptomycin	Moderate antibacterial activity of compounds isolated from *A. arctotoides* against Gram positive and Gram negative bacteria	Sultana et al., 2008
Antifungal	Nutrient agar medium was mixed with extract and fungal suspension. Species: *Aspergillus flavus, Aspergillus tamarii, Cladosporium herbarum, Cladosporium sphaerospermum, Penicillium digitatum, Penicillium italicum*	Shoot	Acetone, water and methanol	0.1-5 mg/ml	Negative: nutrient agar + 2% solvent. No positive control	Extracts showed significant growth inhibition against all the fungi tested. The water extract was particularly inhibitory to the growth of *Aspergillus tamari* and *Penicillum digitatum* with inhibitory activity ranging from 50.7 to 95.2% respectively	Afolayan, 2003
Antifungal	Prepared potato dextrose agar plates containing *A. arctotoides* extract at concentrations of 5.0, 1.0, 0.5 and 0.1 mg/ml, were inoculated with plugs obtained from the actively growing margin of the	Root	Acetone, water and methanol	0.1-5 mg/ml	Negative: 5ml acetone or methanol Positive: Streptomycin	Significant antifungal activity by the acetone and methanol extracts with growth inhibition ranging from 56.23% on *Aspergillus flavus* to 100% on *Penicillium*	Afolayan et al., 2007

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	fungi plates and incubated at 25°C. The diameter of the fungal growth was measured					*notatum* and *Mucor heamalis* at 5.0 mg/ml. The water extract showed the least inhibitory activity	
Antifungal	Diffusion method on sabouraud dextrose agar plate. Wells were made on the plate to put the extract, negative and positive control. Species: *Candida albicans*, *Candida krusei*, *Candida glabrata*, *Cryptococcus neoformans*, *Aspergillus fumigatus*, *Aspergillus niger*, *Trychophyton tonsurans*, *Trychophyton mucoides*, *Microsporum gypseum* and *Microsporum canis*	Leaves	Hexane and acetone	0.005-5mg/ml	Negative: corresponding solvent Positive: Nystatin, Amphotericin B and Grieseofulvin	The hexane extract was active against 6 out of the 10 fungi, the acetone extract was active against 7 out of the 10 tested fungi with zones of inhibition varying from 8 to 32 mm, while none of the aqueous extracts was active against any of the fungi. Highest antifungal activity was obtained with the hexane extract of *A. arctotoides* with MICs of 0.005 mg/ml against *T. mucoides* and 0.04 mg/ml against *A. niger*	Otang et al., 2012
Antifungal	Spore suspensions of *Candida albicans*, *Candida glabrata*, *Aspergillus fumigatus*, and *Microsporum canis* were aseptically transferred into the test tubes containing various concentrations of the acetone extract of *A. arctotoides* dissolved in sterilised sabouraud dextrose. The tubes were incubated at 37 °C. Morphological alterations of fungal mycelia were assessed by scanning electron microscope	Leaves	Acetone	0.32-5mg/ml	Negative: sabouraud dextrose broth. No positive control	Remarkable morphological alterations in the fungal mycelia, loss of turgidity and uniformity, collapse of entire hyphae to evident destruction of the hyphae	Otang et al., 2011
Anticancer	Extracts of *A. arctotoides* were assayed in 3 cell lines (renal TK10, breast MCF7, melanoma UACC62) in 96 well microtitre plates containing RPMI 1640 medium and incubated at 37°C, 5% CO_2. The assay results for the extracts screened were reported as total growth inhibition values	Whole plant	Dichloromethane and methanol	6.25-100ug/ml	Negative: DMSO + RPMI Positive: etoposide	Moderate anticancer activity with total growth inhibition of 12.70, 16.81 and 13.74µg/ml in renal TK10, breast MCF7 and Melanoma UACC62 cell lines respectively	Fouche et al., 2008
Toxicity	Male Wistar rats which had fasted for 16 hours were administered with graded doses of *A. arctotoides* extract, allowed assess to food and water for 48 hours. Serum and tissue homogenate (liver, kidney) were used to estimate haematological parameters	Shoot	Water	400- 3,200 mg/kg body weight	Negative: Group of rats receiving distilled water	No significant change in the red blood cell count and haemoglobin concentration, no significance increase in white blood cell count and its differentials. Significant decrease in the levels of some liver enzymes and blood urea nitrogen. No significant lesions were observed in the organs examined	Jimoh et al., 2008
Toxicity	Toxicity assessment was	Leaf	Hexane and	0.125-2	Negative: Sea	Hatching success was	Otang et

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	based on both the percentage of hatching of cysts and lethality of hatched nauplii in different concentrations of plant extracts and controls		acetone	mg/ml	water Positive: amphotericin B	significantly lower in cysts incubated in the hexane (5.10%) and acetone (5.20%) extracts as compared to the control. Both the acetone and the hexane extract exhibited significant brine shrimp lethality with LC_{50} values of 0.87 and 0.89mg/ml respectively	al., 2013a
Toxicity	*In vitro* cytotoxicity evaluation of *A. arctotoides* extracts against the Chang liver cell line. Different concentrations of the extracts were added into 24-hour cultured cells and incubated for 72 hours, 37 °C, 5% CO_2. Cell survival was evaluated using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay	Leaf	Hexane and acetone	0-50ug/ml	Positive: Griseofulvin	The LC_{50} values of the acetone and hexane extracts of *A. arctotoides* were 17.4 and 12.4µg/ml respectively and were not significantly lower than that of the positive griseofulvin (9.02 µg/ml)	Otang et al., 2013b
Antioxidant	*In vitro* DPPH free-radical scavenging activity	Root	Acetone, methanol and water	0.02-0.1mg/ml	Negative: corresponding solvent. Positive: Ascorbic acid and BHT	The methanol, water and acetone extracts showed a lower potency than ascorbic acid and BHT at all tested concentrations. % inhibition at 0.1mg/ml - methanol: 68.2%, water: 7.5%, acetone: 61.8%	Afolayan et al., 2007
Antioxidant	*In vitro* ABTS free-radical scavenging activity	Root	Acetone, methanol and water	0.02-0.1mg/ml	Negative: corresponding solvent. Positive: BHT	The acetone root extract showed a higher potency than BHT at 0.1mg/ml. % inhibition. Acetone: 96.7%, methanol: 95.5%, water: 91.1% and BHT: 96.3%	Afolayan et al., 2007
Antioxidant	*In vitro* ABTS free-radical scavenging activity	Root	Acetone, methanol and water	0.02-0.1mg/ml	Negative: corresponding solvent Positive: BHT	The acetone root extract showed a higher potency than BHT at 0.1mg/ml. % inhibition. Acetone: 96.7%, methanol: 95.5%, water: 91.1% and BHT: 96.3%	Afolayan et al., 2007
Antioxidant	*In vitro* FRAP assay: ability of the extracts to reduce TPRZ-Fe (III) complex to TPTZ-Fe (II) was assessed	Root	Acetone, methanol and water	0.02-0.1mg/ml	Negative: corresponding solvent. Positive: BHT, ascorbic acid, catechin	The FRAP values for the acetone (80.43) and methanol (81.86) extracts were significantly lower than those of ascorbic Acid (1626.5) and catechin (971.6) but higher than that of BHT (62.3)	Afolayan et al., 2007

Otang et al. (2011) investigated the effect of the acetone extract of *A. arctotoides* on the growth and ultra-structure of some opportunistic fungi (Candida *albicans*, *Candida glabrata*, *Aspergillus fumigatus*, and *Microsporum canis*) associated with HIV/AIDS by means of scanning electron microscope (SEM). Remarkable morphological alterations in the fungal mycelia which were attributed to the loss of cell wall strength ranged from loss of turgidity and uniformity, collapse of entire hyphae to evident destruction of the hyphae. The inhibition of mycelia growth ranged from 0% in *A. fumigatus* at a concentration of 0.32 mg/ml of plant extract to 65.3% in *C. albicans* at the highest concentration of 5 mg/ml. The plant extract inhibited mycelia growth in *C. albicans* and *M. canis* in a dose-dependent manner. The observed alterations in the fungal ultra-structure were attributed to the impact of the plant extract on the cell wall with the resultant changes in the fungal hyphae. The hexane and the acetone extracts of the leaves of *A. artotoides* were reported to be active against 6 out of 10 fungi, against 7 out of the 10 tested fungi respectively, with zones of inhibition varying from 8 to 32 mm (Otang et al., 2012). Highest antifungal activity was obtained with the hexane extract of *A. arctotoides* with MICs of 0.005 mg/ml against *T. mucoides* and 0.04 mg/ml against *A. niger*, and considering the fact that the mean inhibition zone diameters of *A. arctotoides* (hexane) and the positive control (Nystatin) were not significantly different ($P > 0.05$) suggests that *A. arctotoides* appear to have high efficacy with a broad spectrum of antifungal activity

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Anticancer activity

Dichloromethane and methanol extracts of *A. arctotoides* were assayed in 3 cell lines (renal TK10, breast MCF7, melanoma UACC62) in 96 well micro-titre plates containing RPMI 1640 medium and incubated at 37^{o}C, 5% CO_2 (Fouche et al., 2008). The assay results for the extracts screened were reported as total growth inhibition values. Moderate anticancer activity with total growth inhibition at 12.70, 16.81 and 13.74µg/ml in renal TK10, breast MCF7 and Melanoma UACC62 cell lines respectively

Antioxidant activity

The *in vitro* antioxidant properties of the acetone, methanol and water extracts of *A. arctotoides* root extracts were screened through ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid), FRAP (ferric reducing ability of plasma) and DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging effects (Afolayan et al., 2007). For the DPPH assay, the methanol, water and acetone extracts showed a lower potency than ascorbic acid and BHT at all tested concentrations. In the ABTS assay, the scavenging activity of the acetone root extract (96.7%) was not significantly higher than that of BHT (96.3%) at 0.1 mg/ml and similarly, the FRAP values for the acetone (80.43) and methanol (81.86) extracts were comparable to that of BHT (62.3).

Toxicity

The toxicity tests undertaken on *A. arctotoides* reported in the literature are summarized in Table 2. Three toxicity screens for the crude extracts of *A. arctotoides* on cell lines, rats and brine shrimp were identified in the literature. Graded doses of the aqueous extract from the shoot of *A. arctotoides* (400, 800, 1,600 and 3,200 mg/kg body weight) were separately administered to mice in a test group while the control group was treated with orally administered distilled water (3ml/kg) (Jimoh et al., 2008). Clinical chemistry and histopathological examination of major organs were performed on all the animals. The extract did not cause any significant change in the red blood cell count, packed cell volume, haemoglobin concentration, mean corpuscular volume, mean corpuscular haemoglobin concentration, and mean corpuscular haemoglobin even up to the dose of 2000 mg/kg body weight. No significant lesions were observed in the organs examined and no behavioral changes or deaths were recorded in the mice after 48 hr of administration of the extract. The extract also caused significant decrease in the level of platelets, total conjugated and un-conjugated bilirubin, blood urea nitrogen (BUN) in all the experimental groups when compared to the control group (Jimoh et al., 2008). However, all doses caused a significant increase in the levels of white blood cell count and its differentials. Based on the findings of Jimoh et al. (2008), it may be inferred that the aqueous extract of *A. arctotoides* has no toxic effect on the red blood cell parameters. The decrease in the level of platelets induced by the extracts indicates that the continued administration of the extract may lead to haemorrhage, however, considering the absence of neither mortality nor behavioral changes in the treated animals (Jimoh et al., 2008), the plant may be safe for medicinal uses.

Otang et al., (2013a) investigated the cytotoxicity of the hexane and acetone extracts of *Arctotis arctotoides* against the Chang Liver cell line using the *in vitro* MTT assay. The IC_{50} values for the acetone and hexane extracts were 17.4 and 12.4 µg/ml respectively, lower than that of grieseofulvin (9.02 µg/ml), which was used as positive control. In another study (Otang et al., 2013b), the hexane and acetone extracts of *A. arctotoides* were assayed for toxicity to hatching and larval mortality of *Artemia salina.* Based on Meyer's toxicity index, the acetone extract of *A. arctotoides* with LC_{50} values > 1 mg/ml was considered as non-toxic and may be further explored for the development of plant-based pharmaceuticals (Otang et al., 2013b).

Conclusion

A. arctotoides is an important medicinal plant in South Africa and continues to be frequently prescribed in traditional medicine especially by the rural people of Eastern Cape Province for the treatment of epilepsy, indigestion and catarrh of the stomach, skin infections and wounds (van der Walt, 2002). Justification of its traditional use has been supported by a number of studies detailing the bioactivity of the crude extracts and the isolated phytochemicals (Tables 1 & 2). Crude extracts and phytochemicals from the root and shoot of *A. arctotoides* were reported to have demonstrated potent anti-microbial properties against a number of bacteria and fungi. Three of the compounds isolated from the shoot of *A. arctotoides* (Sultana et al., 2008) showed moderate antibacterial activity. In addition, the hexane extract of the plant showed high antifungal activity with a broad spectrum of action. These results provide partial evidence regarding the traditional use of *A. arctotoides* in the treatment of wounds in Eastern Cape of South Africa.

One of the traditional uses of *A. arctotoides* is the management of skin diseases (Otang et al., 2014). However, no studies have been carried out on the anti-inflammatory properties of *A. arctotoides* extract although inflammation is common to many skin problems. Skin diseases have been of major concern recently due to the association of skin opportunistic infections and HIV/AIDS. With an HIV prevalence rate of 16.6% amongst South Africans aged between 15 – 49 years, South Africa has the largest HIV epidemic in the world (Statistics South Africa, 2011). Skin diseases such as dermatitis, prurigo, scabies, and papular urticaria are either untreated or over-treated with strong topical steroids or antibiotics which have been found to cause considerable disability (Njoroge and Bussmann, 2007). These short-comings, including the appearance of drug-resistant microbial strains have resulted in increased efforts for the search of better antimicrobial agents and much attention is now being directed towards natural products (Abbasi et al., 2010). Hence, further research on the anti-inflammatory properties of *A. arctotoides* seems warranted.

Given the widespread search for alternative medicines with novel mechanisms of actions from plants, it is surprising that there are currently no reports on the traditional uses of *A. arctotoides* outside the borders of South Africa. This endemism of *A. arctotoides* to South Africa may partially account for the limited amount of scientific research that has been carried out on the plant. Hence, in order to fully exploit the medicinal potential of *A. arctotoides*, there are perhaps two main areas for potential development. The first is to ensure the expansion of existing traditional knowledge from the areas where *A. arctotoides* is currently used for traditional medicine into neighboring communities where the plant is not used for the stated indications. Ultimately such knowledge expansion and sharing will support the greater use of the plant in primary healthcare (TRAMIL, 2011). Secondly, the possible mechanisms explaining the antibacterial and antifungal activity deserve to be investigated.

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