Investigation towards propagation and cosmeceutical application of *Athrixia phylicoides* DC

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Abstract

The multitude of ailments related to human health has become a worldwide open challenge for scholars waged in the field of drug development. Plants have always been a gifted source of alternative medicine for countless disorders. The bush tea (*Athrixia phylicoides* DC.) is a South African indigenous plant with a diverse range of medicinal properties including cosmetic and antimicrobial properties. This plant has been used in different local communities to treat various ailments. Further, in rural areas it is popularly used as tea by the native people of South Africa. The present study was carried out to evaluate the effects of altered exogenous growth regulators in cutting production of bush tea in two dissimilar seasons. Based on the plant's traditional usage in cosmetics, the tyrosinase inhibition and antimicrobial potential against the strain *Propionibacterium acnes* were also investigated. In the propagation study, Indole-3-butyric acid (IBA) was found to have the highest rooting and sprouting percentage during spring, while 1-Naphthalene acetic acid (NAA) was found to promote the highest sprouting and rooting percentage during autumn. Both NAA and IBA improved the number of roots produced compared to that of the control, although not significantly. The addition of exogenous growth regulators is beneficial to obtain significantly more roots per cutting and increase the cutting production of *A. phylicoides* but did not affect the number of leaves. Although the tyrosinase inhibitory activity was found to be higher in plants from spring as compared to the other seasons it did not compare with the positive control.

Keywords: *Athrixia phylicoides*, Cuttings, Indole-3-butyric acid, 1- Naphthaleneacetic acid, tyrosinase, *Propionibacterium acnes*.

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1. Introduction

Bush tea (*Athrixia phylicoides* DC.) is a South African indigenous plant with a diverse range of medicinal properties. It is a popular plant, frequently known as Boesmans tee (Afrikaans), Mutshatshaila (Venda), Icholocholo (Zulu) and Mohlahlaishi (Pedi) in different localities (Lehlohonolo et al., 2013). This shrub is found in grasslands, forests, bushveld, rocky and sloping habitats of the eastern parts of South Africa (Lerotholi et al., 2017). This attractive bush plant can be grown in open spaces as a filler and works well as a specimen plant in the garden (Araya et al., 2007). Bush tea can flower throughout the year, with the best flowering time being from March to May, and is also reliant on climatic and edaphic factors. The plant requires well-drained soil and full sunlight with enough space for branching.

Bush tea has been used for many years by local populations, including Zulu, Venda, Sotho and Xhosa people, as a health beverage as well as to treat various ailments such as hypertension, heart disease, diabetes, boils, infected wounds, acne, headaches, sexually transmitted infections, erectile dysfunction, male libido, sore thought, coughs colds and loss of voice (Hutchings et al., 1996; Mbambezeli, 2005; Mashimbye et al., 2006; Joubert et al., 2008; Tshivhandekano et al., 2014). The wide use has led to extensive research on bush tea because of its benefits and medicinal potential in the folklore system. The scientific exploration and validation of this plant however, is not complete although phytochemical investigations have led to the isolation of many bioactive metabolites (Mashimbye et al., 2006; Mavundza et al., 2010; De Beer et al., 2011; Lerotholi et al., 2017). *A. phylicoides* was also found to exhibit significant antioxidant potential (Iswaldi et al., 2011) and an infusion produced from *A. phylicoides* was found to have a higher mineral content than other local teas (rooibos, honeybush, green) and non-local black and green teas (Olivier et al., 2012).

The potential for commercialization of *A. phylicoides* have been recognised by several authors (Rampedi and Olivier, 2005; Mudau et al., 2007; De Beer and Joubert, 2009; Lerotholi et al., 2017) however, more comprehensive research is needed on chemical characteristics, chemical standardization, quality control (Joubert et al., 2008) and chemical variation within the species (Lerotholi et al., 2017) to facilitate commercialization. Besides bush tea as beverage commercial products could also include cosmeceutical products/formulations especially since bush tea is also traditionally used against various skin disorders (Lall and Kishore, 2014). Extracts prepared from leaves were thus evaluated for its anti-tyrosinase activity and antibacterial activity against *Propionibacterium acnes* to identify possible cosmeceutical application for commercialization.

A consistent supply of quality material is however needed for successful commercialization of any product. The propagation of *A. phylicoides* is generally carried out through the use of mature seeds, collected at the expiration of summer. Vegetative propagation of *A. phylicoides* was found to be successful using apical cuttings and dry exogenous growth regulator supplementation (IBA) (Araya, 2005). Araya (2005) also found that apical cuttings gave rise to a higher root length, root number and rooting percentage than basal cuttings. Due to the diverse range of uses of *A. phylicoides*, there is a need to produce rapid vegetation by using new techniques. Plant growth regulators (PGR's) can play a paramount role in the propagation of apical cutting vegetation as reported by many researchers (Saifuddin et al., 2013; Yan et al., 2014; Elhaak et al., 2015). To further evaluate the propagation of *A. phylicoides*, a trial was carried out in the present study in two different seasons using application of different PGR's in liquid instead of powder formulation.

2. Materials and methods

2.1. Apical cutting propagation

Apical cuttings were prepared in spring (October 2012) and autumn (April 2013) by cutting the stem 5-6 cm away from the tip. Approximately 4-6 leaves were left on the cutting which was then planted in trays with movable seedling wells in a composted pine bark medium. Nine aqueous treatments; 1-Naphthalene acetic acid (NAA 0.1 mg/ml, 0.3 mg/ml), Indole-3-butyric acid (IBA 0.1 mg/ml, 0.3 mg/ml), Indole-3-acetic acid (IAA 0.1 mg/ml, 0.3 mg/ml), Gibberellic acid (GA 0.1 mg/ml, 0.3 mg/ml) and a control (distilled H_2O) were included in this study. Twenty cuttings per treatment were prepared for each of three replicates in spring and 30 cuttings were prepared per treatment for each of four replicates during autumn. Due to large variances obtained in the spring trial that resulted in no significant differences an additional replicate was added in the autumn trial to increase the degrees of freedom for error. Additional cuttings were also added per experimental unit to obtain higher precision of measurement. Planted cuttings were then placed in a glasshouse (25-30°C) and watered 3 times a day for a period of 10 minutes. After two months, cuttings were transplanted to 20 litre (*l)* plastic bags containing pine bark: sand (2:1).

The experiment was designed as a randomized complete block design with 3 replications during spring and four replications during autumn. The treatment design was a 4 x 2 factorial plus a control with factors four plant growth regulators (NAA, IBA, IAA and GA) and 2 concentrations (0.1 mg/ml and 0.3 mg/ml). The variables measured were sprouting, rooting, number of roots produced and number of old and new leaves. These variables were subjected to a factorial analysis of variance (ANOVA). Means of significant source effects were separated using Fisher's protected t-Least Significant Difference (LSD) at a 5% significance level (Snedecor and Cochran, 1980). The Shapiro-Wilk test was performed on residuals to test for non-normality and outliers were identified and removed (Shapiro and Wilk, 1965). All analyses were performed using SAS statistical software (SAS Institute Inc 2008).

2.2. Plant collection and extract preparation

Leaves and stems of *A. phylicoides* were collected from the Agricultural Research Council's Vegetable and Ornamental Plants (ARC-VOP) in January, March, June and September and voucher specimens numbers were deposited at the H.G.W.J Schwelckerdt Herbarium (Collection 1, PRU 119652). The material to be tested was washed in dH_2O and allowed to dry for 10 days away from sunlight. Dry material (6.0 g) was then ground and extracted with 50 ml 99.9% ethanol (EtOH). This mixture was placed on a shaker for 48 hours. The resulting solution was filtered and dried in a fume hood to obtain a dry ethanol extract.

2.3. Anti-tyrosinase and antibacterial assays

The colorimetric tyrosinase assay was carried out in accordance with the method described by Nerya et al. (2003) and Curto et al. (1999). Plant extracts and kojic acid (positive control) were dissolved in dimethyl sulfoxide (DMSO) to a stock solution of 20 mg ml^{-1} (Lee et al., 1997). The solutions were diluted in 50 mM potassium phosphate buffer (pH 6.5). In a 96 well microtitre plate, 70 µl of each solution (extract, positive and negative control) of different concentrations were added in triplicate. Thereafter, 30 µl of tyrosinase (333 Units ml^{-1} in phosphate buffer, pH 6.5) was added. After an incubation period of 5 minutes at room temperature, 110 µl of substrate (2 mM L-tyrosine) was added. Extracts from autumn, spring, summer and winter seasons and kojic acid (control) were tested from 1.56 µg ml^{-1}-200 µg ml^{-1}. The optical density (OD) was determined at 492 nm with the BIO-TEK power Wave XS multi-well plate reader (A.D.P., Weltevreden Park, South Africa) at room temperature for 30 min.

The anti-microbial activity of the seasonal ethanolic extracts (400-3.125 µg ml^{-1}) of *A. phylicoides* was determined using the microdilution assay. This assay was carried out in accordance with the method outlined by Eloff (1998). The *Propionibacterium acnes* bacteria were cultured on nutrient agar plates under anaerobic conditions at 37 °C for 72 hours. The

bacterial culture was added into solution and adjusted by trial and error to 0.5 McFarland standards. Tetracycline was used as a positive control (0.2 mg ml^{-1}). The plates were incubated under anaerobic conditions at 37 °C for 72 hours in an incubator. The colour changes in the plate were observed after the addition of presto blue as an indicator. The minimum inhibitory concentration (MIC) was determined visually at the point where no pink colouration occurred, which is indicative of no reaction.

3. Results and discussion

3.1. Propagation trial

Apical cuttings of *A. phylicoides* were subjected to various exogenous plant growth regulators (PGR) for propagation trials over two seasons to promote rooting. The application of auxin significantly affected the number of roots produced in spring (Table 1), but did not expressively improve the rooting percentage of *A. phyllicoides* apical stem cuttings when compared to the control. These findings were in accordance with the findings of Araya (2005) who reported that the application of Seradix no. 2 (0.3% IBA) improved root quality, but not rooting percentage. The PGR concentration used in the current study did not affect any of the parameters measured, which was contrary to what Araya et al. (2007) found. In the author's study the rooting percentage of basal cuttings could be improved by applying the lower IBA concentration. Araya et al. (2007) however did not test different concentrations on apical cuttings. .

Rooting and sprouting percentages in general were better in autumn as compared to that of spring (Table 1). In contrast spring was found to have better results than autumn on overall rooting percentage in the study of Araya (2005), although differences were not significant. If only apical cuttings from the study of Araya (2005) are taken into account then autumn also produced a better rooting percentage. Both the current study and that of Araya (2005) thus indicated better rooting in autumn, where apical cuttings were concerned. Season

Table 1: The effect of exogenous growth regulator application on the sprouting, rooting, number of roots and number of leaves formed and retained in apical stem cuttings of *Athrixia phylicoides* during spring and autumn

Hormone	Sprouting (%)		Rooting (%)		No. roots		New leaves (no.)	
	Spring	**Autumn**	**Spring**	**Autumn**	**Spring**	**Autumn**	**Spring**	**Autumn**
NAA	30.00 [a]	77.50 [a]	38.34 [a]	78.75 [a]	26.29 [a]	6.68 [a]	9.38 [a]	4.18 [a]
IBA	51.67 [a]	63.33 [a]	51.67 [a]	67.50 [a]	31.67 [a]	6.11 [a]	13.03 [a]	3.57 [a]
IAA	25.84 [a]	68.33 [a]	20.84 [a]	67.08 [a]	21.22 [a]	6.07 [a]	9.45 [a]	4.50 [a]
GA	23.33 [a]	30.00 [b]	19.17 [a]	35.42 [b]	7.99 [b]	2.41 [b]	4.5 [a]	1.71 [b]
Control	35.00 [a]	76.67 [a]	31.61 [a]	69.17 [a]	7.89 [b]	4.88 [a]	5.29 [a]	4.12 [a]
*LSD p=0.05	-	19.63	-	19.46	12.60	3.32	-	1.41
**Shapiro-Wilk (P>W)	0.83	0.81	1.00	0.77	0.51	0.40	0.91	0.50

*$LSD_{p=0.05}$ = Fisher's Least significant difference at a 5% significance level. Means within columns with the same letter or letters do not differ significantly at the 5% level.

**The standardised residuals were considered as normal distributed if the Shapiro-Wilk probability is greater than 0.01.

or time when cuttings are made has also played a significant role in the root cutting success of honey bush (*Cyclopia subternata)* tea (Mabizela et al., 2017). Seasonal changes in rooting success were also observed in mandarin (Sarmiento et al., 2016) and *Boswellia* (Haile et al., 2011). These changes in rooting success can be related to seasonal temperature changes (Sarmiento et al., 2016; Mabizela et al., 2017), as well as carbohydrate content differences in the cuttings (Sarmiento et al., 2016).

In spring, the highest rooting and sprouting percentage and root number were observed with the application of IBA, but in autumn NAA gave the best results for all three parameters. In previous reports it has been noticed that IAA was found to be more effective in cutting treatments than IBA and NAA (Hassanain, 2013), but this was not the case in the current study. In contrast the application of GA_3 significantly reduced all measured parameters in autumn and also had a negative effect on the number of roots produced in spring (Table 1). Maudu et al. (2011) reported that the application of gibberellic acid had a positive effect on growth parameters of already rooted cuttings of bush tea. Gibberellins could thus be applied after rooting to promote growth, but effects rooting negatively.

3.2. Anti-tyrosinase and antibacterial assays

The ethanolic leaf extract of *A. phylicoides* was tested for its tyrosinase inhibitory activity using the substrate $_L$-tyrosine (monophenolase inhibition). The crude spring and autumn extracts displayed moderate tyrosinase inhibition (223.65-301.40 µg ml^{-1}) activity for monophenolase activity (Table 2). It is possible if extracts are prepared using non-polar solvent, one can expect better anti-tyrosinase activity than that of the ethanolic extract. Although seasonal variation was observed in the leaf extracts, the IC_{50} values were not significant as compared to the positive control (IC_{50} 7.14 µg ml^{-1}). All the plant samples did not show bacterial inhibition at the highest concentration (400 µg ml^{-1}) tested during the antimicrobial bioassay. As the traditional usage of the plant is for sores and wounds, it is

Table 2: Tyrosinase inhibition activity of seasonal leaf extracts of *Athrixia phylicoides* mother plant material

Extract	[a]**IC_{50}** (µg ml^{-1})
Spring	223.65 ± 3.2
Summer	767.40 ± 2.2
Autumn	301.40 ± 1.2
Winter	402.20 ± 3.5
Positive control (kojic acid)	7.144 ± 2.2
Extracts from spring, summer, autumn and winter for antibacterial activity	na[b]

[a]Fifty percent inhibitory concentration for tyrosinase activity using L-tyrosine as a substrate.

[b]No activity at the highest concentration tested i.e., 400 µg ml^{-1}

important to note that at a low concentration the plant extract did not show antibacterial activity. However, the methanolic extract of the plant has been reported earlier to have antibacterial activity against other bacteria i.e., *Staphylococcus aureus, Bacillus cereus* and *Bacillus subtilis* at a concentration of 1 mg ml^{-1} (Padayachee, 2011).

4. Conclusion

In addition to IBA, NAA can be used as a PGR for the propagation of *A. phylicoides* apical stem cuttings, to obtain more roots and a higher rooting percentage especially during the autumn season. More roots can assist with better cutting transplantation success and higher rooting percentages are essential for commercial viability. In general, a higher number of roots were formed during the spring trial but a higher rooting percentage was observed during autumn. Some commercial PGR's contain both IBA and NAA and should be investigated as alternative to PGR's with only IBA for *Athrixia* cutting production. The autumn and spring ethanol extract of *A. phylicoides* displayed better tyrosinase inhibition activity than the summer and winter extracts. No noticeable MIC was detected for the seasonal ethanol extracts of *A. phylicoides* against the bacterium *P. acnes*. As a way forward, one can consider testing the extracts made from spring sample using non-polar solvents, which might extract non-polar compounds, and which might show better activity than the one showed in the present study when ethanolic extract was used. For antibacterial evaluation one can consider testing extract at a higher concentration as *P. acnes* is considered to be one of the resistant bacteria.

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