# Commercialization of the South African Bush Tea, *Athrixia phylicoides*©

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## INTRODUCTION

South Africa has a rich diversity of indigenous plants and is ranked as one of the most biologically diverse countries in the world. *Athrixia phylicoides* is one of many plants, which is being harvested from the wild and due to its demand it has become threatened.

*Athrixia phylicoides* belongs to the family Asteraceae and its common name is bush tea, zulu tea, or bushman tea. It grows in the eastern mountain ranges of SA (Kwazulu-Natal), Eastern Cape, and in Zimbabwe (Van Wyk and Gericke, 2000). This small shrub (±1 m in height) is branched with thin woolly stems and has small, dark green pointed leaves with white hairy backs. It bears small mauve pink daisy flowers with a bright yellow centre (Roberts, 1990) from autumn to winter.

The local people use it for the following purposes (Roberts, 1990; Van Wyk and Gericke, 2000):

- Headaches, stomach aches, influenza, infested wounds, asthma, tuberculosis, and ulcers
- Blood purifier: to cleanse the veins, kidneys, womb, and the bladder
- The infusion of the roots is used as a face wash
- It has aphrodisiac properties
- Broom making

Results to date on pharmacological and chemical activities prove that this plant shows anti-bacterial activity, with the highest against *Staphylococcus aureus* and *Enterococcus faecalis* and slightly less against *Pseudomonas aeruginosa*. Its activity is the least against *E. coli*. Eight different antioxidant compounds have been identified. So far no harmful toxins have been detected and lastly the plant consists of essential oils (Olivier, 2004).

The bush tea is used either fresh or dried for both a medicine and a health tea, which is set as follows (Nkhumeleni, 2004):

**Medicine.**

- Infusion of the plant is used

**Tea Preparation.**

- Fresh or dried leaves and twigs are boiled and the extract is used as a tea. The time estimated for boiling the tea is 3 to 10 min. A research survey indicated that 97% of the respondents agreed that sugar and milk are added to the tea. Honey, lemon, and other teas could also be added to the tea.

When harvested for medicine, the plant is uprooted or the leaves and branches are harvested. For tea the leaves and branches are harvested and for broom making the mature plant is harvested except the roots. Thus, commercialization has become vital (Nkhumeleni, 2004).

## RESEARCH PLAN

Prof . J. Olivier from the University of South Africa received funding from the National Research Foundation for this project. She then co-opted co-investigators and team members from universities, governmental institutions, etc., to do research on the following projects:

1) Commercial potential of the plant via surveys
2) Chemical and pharmacological activities of the plant
3) Propagation and cultivation of the plant
    a) Vegetative propagation
    b) In vitro propagation
    c) Seed germination
    d) Nutritional requirements
    e) Fertigation under controlled environmental conditions
4) Product development
5) Community participating and training

For this paper, only certain recent results on vegetative propagation and propagation via tissue culture will be presented.

## VEGETATIVE PROPAGATION VIA MIST-BED

The objective of this study was to compile a procedure for vegetative propagation. Therefore the rooting media, rooting hormone, rooting of apical and basal cuttings, and the seasonal effects on cuttings were investigated.

**Materials and Methods.** Stock material from Venda-Muhuya village, Thohoyandou district, was collected from the field, planted into plastic bags and transferred to a glasshouse where the plants were maintained (Araya, 2004).

The propagation unit was supplemented with a fogging system, which worked automatically, based on the humidity of the greenhouse. The mist bed was supplied with an automatic misting system, operating through misting nozzles. Throughout the experimental period, the temperatures of the greenhouse and misting bed were measured using a thermohygrograph. For the experiment a complete randomised block design with four blocks and ten replications was planned to evaluate the effect of the position of the cutting (apical or basal), with or without hormone application, using two different rooting media (fine silica sand and decomposed fine pine bark) for four seasons (summer, autumn, winter, and spring) (Araya, 2004).

Shoots of 16 to 32 cm long were cut from the stock plants early in the morning (between 06:30 and 07:30). Working on the humid misting bed, shoots were divided into a total of 320 semihardwood cuttings, which were 8 cm in length and about 4 mm in circumference. Bottom leaves were stripped off, leaving only the top three. The bases of the cuttings were dipped in water and depending on the treatment type (with or without hormone), they were then dipped into a rooting hormone powder, Seradix No. 2, consisting of 0.3% IBA (4-indole-3-butyric acid) in a talc, to a depth of approximately 1 cm. Excess rooting powder was tapped off before planting. According to Hartmann and Kester (1983), in order to avoid brushing of the powder during planting, a trench was made in the rooting medium with a stick. The cuttings were then planted into the pre-wetted rooting medium (fine bark and 8 mm silica sand) to a depth of 2 cm. Throughout the experimental period, cuttings were assessed for percentage rooting and root number after 5, 10, 15, and 20 days

in summer and 15, 20, 25, and 30 days in autumn, winter, and spring from planting time. Data collection was done by carefully separating the rooted cuttings from the seedling trays, followed by washing the root zone with water (Araya, 2004).

## RESULTS AND DISCUSSION

The results of the experiment indicated that the propagation of bush tea (*Athrixia phylicoides*) could successfully be done with stem cuttings in a mistbed. Optimum rooting percentage of cuttings was recorded, however, the vegetative propagation of *Athrixia phylicoides* was affected by factors such as the cutting position, media, rooting hormone, and season (Araya, 2004).

Cuttings started to develop roots after 15 days from planting and afterwards the roots continued to develop. The major influencing factor for rooting success of bush tea cuttings was the cutting position. Apical cuttings performed better due to the higher rooting percentage of these cuttings and also due to the higher root number per cutting compared to those of the basal cuttings. This shows that apical cuttings are the best plant material for successful vegetative propagation of bush tea (Araya, 2004).

The rooting percentage of bush tea was not significantly affected by the type of rooting media used or by the treatment of Seradix No. 2 hormone. This shows that the plant can be propagated either in decomposed fine pine bark or fine silica sand with or without a hormone treatment. Differences in the rooting system were nevertheless observed. Cuttings propagated in decomposed fine pine bark produced a highly branched and fine root system, which is good for transplanting. Elongated coarse roots developed from cuttings propagated in fine silica sand. The type of rooting media also affected the number of roots produced. Cuttings propagated in decomposed fine pine bark produced a higher number of roots per cutting than those from the fine silica sand. Seradix No. 2 hormone also increased the number of roots on the cuttings, which rooted in both the rooting media (Araya, 2004).

Since root number is the most important quality in the establishment of a cutting, it is recommended to propagate bush tea cuttings in decomposed pine bark with the application of Seradix No. 2 hormone. The other major factor in rooting of bush tea was time of the year or season when the cuttings were taken. A high rooting percentage of cuttings and also the root number per cutting can be obtained by taking cuttings during autumn and spring.

## PROPAGATION VIA TISSUE CULTURE

The objective of the study was to develop a protocol for successful in vitro propagation. To accomplish the above mentioned, the composition of a growth medium most suited for the establishment, multiplication, and rooting of *A. phylicoides* was determined. The leaf ultrastructure of plantlets growing in tissue culture was also studied to find out if any morphological changes occurred in plantlets and if so, will it affect the plant characteristics.

**Explant Sterilisation.** Nonglandular trichomes cover the leaf surface of *A. phylicoides* almost entirely, giving the leaves a woolly or hairy appearance. These hairs are likely to be involved in protecting leaf surfaces against insects and other pests during early development. Therefore, the sterilization of this type of material was a barrier that had to be overcome due to the high percentage of contamination that occurred with previous pilot trials.

After five sterilisation trials a successful method for sterilisation was established. Fungi were best controlled by an application of Benomyl™ 2–4 weeks prior to initiation. Bacteria was successfully controlled by a 90 sec immersion in a 60% (v/v) ethanol solution followed by a 10-min emersion in 100 ml·L$^{-1}$household bleach (3.5% NaOCl) (Möller, 2004).

**Figure 1.** In vitro rooting on *Athrixia phylicoides* after 9 weeks on ½ Murashige and Skoog medium (left) and ½ Murashige and Skoog medium supplemented with 30% sucrose and 1.5 mg·L$^{-1}$ IBA (right).

**Establishment, Multiplication, and Rooting.** Plants were initiated on four different growth media and kept at 26 °C with 16h illumination. The growth media used were ½ MS (Murashige and Skoog) medium supplemented with 30% sucrose as the basic medium and IBA, BAP, or both IBA and BAP were added to the basic medium at a concentration of 1.5 mg·L$^{-1}$ (Möller, 2004).

Explants rooted between 9 and 11 weeks and the best growth media was on ½ MS medium which was supplemented with 30% sucrose and 1.5 mg·L$^{-1}$ IBA (Fig. 1). A few plants were not affected by hyperhydricity and were successfully multiplied and maintained in culture. Shoots showing symptoms of hyperhydricity do not normally produce adventitious roots and root poorly. Each pant was multiplied and transplanted onto the same growth medium it has been initiated on. Transplanting or multiplication took place as soon as plants showed sufficient vegetative growth (Möller, 2004).

**Leaves Versus No Leaves.** Due to difficulty in surface sterilizing hairy leaves on explants, explants without leaves were initiated. Results illustrated that no significant differences were found in the new vegetative bud growth of explants with or without leaves. However, the percentage rooting was higher in explants with leaves than in those without leaves. Leaves present on explants may stimulate vegetative growth either by altering the endogenous hormone levels in explants or by contributing to photosynthesis (Möller, 2004).

**Leaf Ultrastructure.** Greenhouse leaves are between 50%–80% larger than in vitro propagated leaves, darker in colour, and more rigid. With microscopic comparisons made between greenhouse and in vitro grown leaves, no noticeable difference in the morphology of glandular and nonglandular trichomes were visible. However, greenhouse plants had more glandular and nonglandular trichomes present on their leaf surfaces (white woolly appearance) than in vitro grown leaves (Möller, 2004).

Glandular trichomes are present on the lower surfaces of leaves. They are distributed randomly across the leaf surface with a slightly higher density near the central vein of the leaf. These trichomes are probably responsible for producing the essential oils stored inside them. Thus, a decrease in the number of glandular trichomes on in vitro-grown leaves will result in a decrease in quantitative production of essential oils, if harvested in this stage.

Scanning electron micrographic images of in vitro-grown leaves showed almost 100% of stomata open compared to less than 5% of stomata open on images of

greenhouse-grown leaves. Stomata on in vitro grown leaves seem unable to close in response to a change in atmospheric conditions. The inability of stomata to regulate water loss through leaves is often the case with in vitro-grown plants. This results in poor adaptation of these plants to natural conditions and great losses after transplanting (Möller, 2004).

## FUTURE RESEARCH

Above mentioned research results are part of the basic research that needs to be completed before more sophisticated research will follow, such as:

- To increase production rate
- To improve quantity and quality of active ingredients
- Potential ornamental value:
- Cut flower leaves do not drop after harvest
- Ground cover / shrub (attractive purple flowers during winter)
- Manipulation techniques
- Growth regulators, pruning, etc.
- Pest and disease control
- Plant selection and breeding

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