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Genotype Screening and Propagation Techniques of Three Selected Medicinal Plant Species for Production of High-Quality Planting Material

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Abstract: Medicinal plants are a vital source of new bioactive compounds due to their ecological biodiversity and varied chemical properties of each species. Phenotypic selection coupled with the evaluation of genotypes based on the chemical profile can be used for the development of a high-yielding variety. However, most of the raw material that has been used for commercial production of herbal products is mainly derived from wild sources, with little knowledge of the quality of genetic materials. Thus, three medicinal species, *Chromolaena odorata* (Siam weed), *Andrographis paniculata* (Creat), and *Baeckea frutescens* (False ru), were chosen based on their significant benefits to human health. These medicinal species have been traditionally used to treat various illnesses, and have been shown to possess anti-inflammatory, antimicrobial, anticancer, and antioxidant properties. This paper highlights the harvesting method and collection of accessions from natural habitats for the selection of superior genotypes. Individual plants having superior phenotypic characteristics and bioactive compounds were identified. We successfully developed appropriate propagation techniques for each species for the mass production of high-yielding planting material. The establishment of breeding activities for these medicinal plants will provide quality raw materials to support the herbal industry in increasing the value of pharmaceutical products. These efforts will also ensure the sustainable production of high-quality planting materials for the establishment of herbal plantations.

Keywords: bioactive compound; breeding strategy; medicinal plant; plant propagation; superior genotypes

1. Introduction

The success of breeding programs for the improvement of certain characteristics of plant species depends on the existence of genetic variability for the selection of individuals with favorable attributes [1]. The primary aim of certain breeding programs is to produce plant varieties with unique or superior traits for wide application in the agricultural sector. The most frequently addressed characteristics are related to biotic and abiotic stress tolerance, the yield of the raw material, quality characteristics of the end-use product such as taste or concentration, and others [2]. This can be achieved through selection techniques practiced in conventional breeding programs. The selection and preservation of certain individual plants of desirable characters have been the basis of all crop improvement [3]. Most of the plant improvement programs in Malaysia are designed for vegetables, rice, and fruits through selecting superior plants from local and foreign genetic resources [4]. In the National Agrofood Policy (NAP 2011–2020), herbal plants were classified as one of the high-value agriculture activities in Malaysia [5]. One of the highlighted strategies

to strengthen the herbal industry is to ensure a sustainable and consistent supply of raw materials to the downstream sector. However, a very limited effort was made for the improvement of herbal plants to ensure sustainable production of high-yielding varieties. Medicinal plants serve a vital role in the maintenance of humans as a viable source of drugs, herbal products, and food supplements. In the search for novel therapeutic options, certain plant species and their secondary metabolites represent an important potential source of biomolecules [6]. They have been used not only for therapeutic purposes, but also as precursors for the development of various drugs. The chemical constituent of medicinal plants needs to be determined scientifically to evaluate their bioactivity before they can be used in the preparation of medicines and drugs. Thus, phytochemical screenings are often considered the first step towards the discovery and manufacturing of useful drugs [7]. Successive solvent extraction, chromatographic separations, and spectroscopic analysis are some of the most commonly applied techniques to isolate secondary metabolites. Numerous studies on medicinal plant extracts carried out globally verified their efficacy, and some of these findings have led to the production of plant-based medicines [8]. Herbal remedies have become popular in the treatment of minor illnesses and injuries due to the increasing awareness and costs of personal health maintenance [9]. Indeed, the market and public demand have been so great that there is also a great risk of losing most of these valuable medicinal plants due to overharvesting or loss of genetic diversity. The herbal industries are facing a major problem in the production of quality herbal products due to an insufficient supply of raw material for specific end use.

Most of the cultivated planting materials are from unimproved wild plants and are highly variable. Currently, herbal markets in Malaysia are claimed to be filled with some low-quality herbal teas and supplements made out of a limited number of plant species [10]. It is generally difficult to establish quality control parameters and maintain consistent batch-to-batch quality due to the inherent variability of the constituents of herbal products [11]. Sources from the wild are normally preferred due to the presence of secondary metabolites that respond to stimuli in the natural environment that may not be expressed under conditions outside of their natural distribution [12]. However, new techniques in the cultivation of herbal products can solve the problems related to the production of medicinal plants, such as the low content of active ingredients. The yield of active compounds and invariability in secondary metabolites can be improved through the selection of superior genotypes to ensure production stability. When examining this issue, therefore, initiatives have been taken to collect plant sources from various populations to screen individual plants for the production of high-quality planting material for the sustainable use of raw materials for industrial uses.

Three important herbal species were selected through bioactive compound screening and mass production of high-yielding genotypes through the development of suitable propagation techniques. The selected species were *Chromolaena odorata* (L.) (Family: *Asteraceae*) or Siam weed, *Andrographis paniculata* Wall (Family: *Acanthaceae*) or Creat, and *Baeckea frutescens* (L.) (Family: *Myrtaceae*) or False ru. These species have been used traditionally for decades to treat various illnesses and discomfort, apart from being used as dietary supplements. There has been numerous research on the medicinal usage, pharmacological properties, therapeutic usage, phytochemistry, and cytotoxicity activities of these species [13–16]. Leaf extract of C. *odorata* has an inhibition effect on platelet-activating factor (PAF) receptor binding, with excellent anti-inflammatory activity to assist in the wound-healing process, cessation of bleeding, and treating various skin infections [16,17]. It has been reported to exhibit antimicrobial, antispasmodic, antiprotozoal, antihypertensive, astringent, diuretic, and anticancer effects [6]. Andrographolide, a major compound in terms of bioactive properties and abundance in *A. paniculata*, has shown great pharmacological properties such as immunostimulatory, anti-infective, antiatherosclerotic, anti-inflammatory, antihepatotoxic, and anticancer potential in various research [18]; whereas *B. frutescens* is used in traditional medicine in Malaysia during confinement and in massaging postpartum women to relieve body aches and numbness, as well as in the treatment of rheumatism [15].

It has the potential to be used as a natural xanthine oxidase (XO) inhibitor for the long-term management and treatment of gout and related disorders. The active compound in *B. frutescens*, namely 6-methyl quercetin, is suitable for use in the treatment of gout by inhibiting XO activity [19]. Research indicates that some active compounds extracted from plant parts of *B. frutescens* are useful in promoting uric acid secretion and inhibiting its formation [20]. These medicinal plant species are commonly propagated by utilizing seeds, and several studies also highlight the vegetative propagation for the production of true-to-type plants [21–23]. However, we have very limited information on the vegetative propagation of *B. frutescens*. The present study was designed to evaluate the chemical profile and biological properties of selected genotypes of these medicinal species, and to develop an efficient regeneration method for the mass propagation of high-yielding superior plant material. This research was also conducted to obtain new genetic materials to add value to the products and to increase the yield to meet the demand for sustainable development of the herbal industry.

2. Materials and Methods

2.1. Collection of Accessions from Natural Populations in Peninsular Malaysia

2.1.1. Collection of *C. odorata*

A total of 35 accessions of *C. odorata* were collected from three populations: Kota Tinggi, Johor (JKT); Jasin, Melaka (MKT); and Maran, Pahang (CKT) were selected for screening of their chemical constituents. The accessions were coded as accessions from Johor, accessions from Melaka, and accessions from Pahang. Leaf samples were collected from each of the populations for the chemical screening process. Some of the major superior traits, such as height, diameter, number of clumps, leaf size, and crown diameter, were measured for mother tree identification (data not shown). These stumps were then planted and grown at FRIM's nursery under 50% shade.

2.1.2. Collection of *A. paniculata*

A total of 30 accessions of *A. paniculata* were collected from four different populations and were coded as AHB (Tapah, Perak), JHB (Kota Tinggi, Johor), THB (Kuala Terengganu, Terengganu), and CHB (Kuantan, Pahang). Morphological data such as height, diameter, length, and width of leaf were recorded for the superior tree identification (data not shown). Leaf samples from each accession were collected for chemical screening in the laboratory. The collected plants were placed under nursery conditions for acclimatization and irrigated whenever necessary.

2.1.3. Collection of *B. frutescens*

A total of 84 accessions of *B. frutescens* were identified from three populations: (i) Gunung Pulut, Perak; (ii) Setiu, Terengganu; and (iii) Sungai Baging, Pahang. Superior trees with good vegetative growth in terms of tree height and bole diameter with dense branching patterns were selected for the study. Leaves and secondary branches were collected from the mother tree and packed into plastic bags for chemical screening.

2.2. Screening of Chemical Constituents

Chemical and general methods: all reagents and solvents used were of analytical or gradient grade for liquid chromatography and were obtained from Merck (Darmstadt, Germany). High-performance liquid chromatography (HPLC) analysis was carried out on a Waters HPLC system equipped with a quaternary gradient module, autosampler, and photodiode array detector. Andrographolide (98% purity) was purchased from Sigma Aldrich, Steinheim, Germany All other standards: naringenin 4′-methyl ether and aromadendrin 4′-methyl were isolated from the previous study (Figure 1). All samples and standards were filtered through a 0.45 μm PTFE membrane filter obtained from Bioflow Lifescience Sdn Bhd.

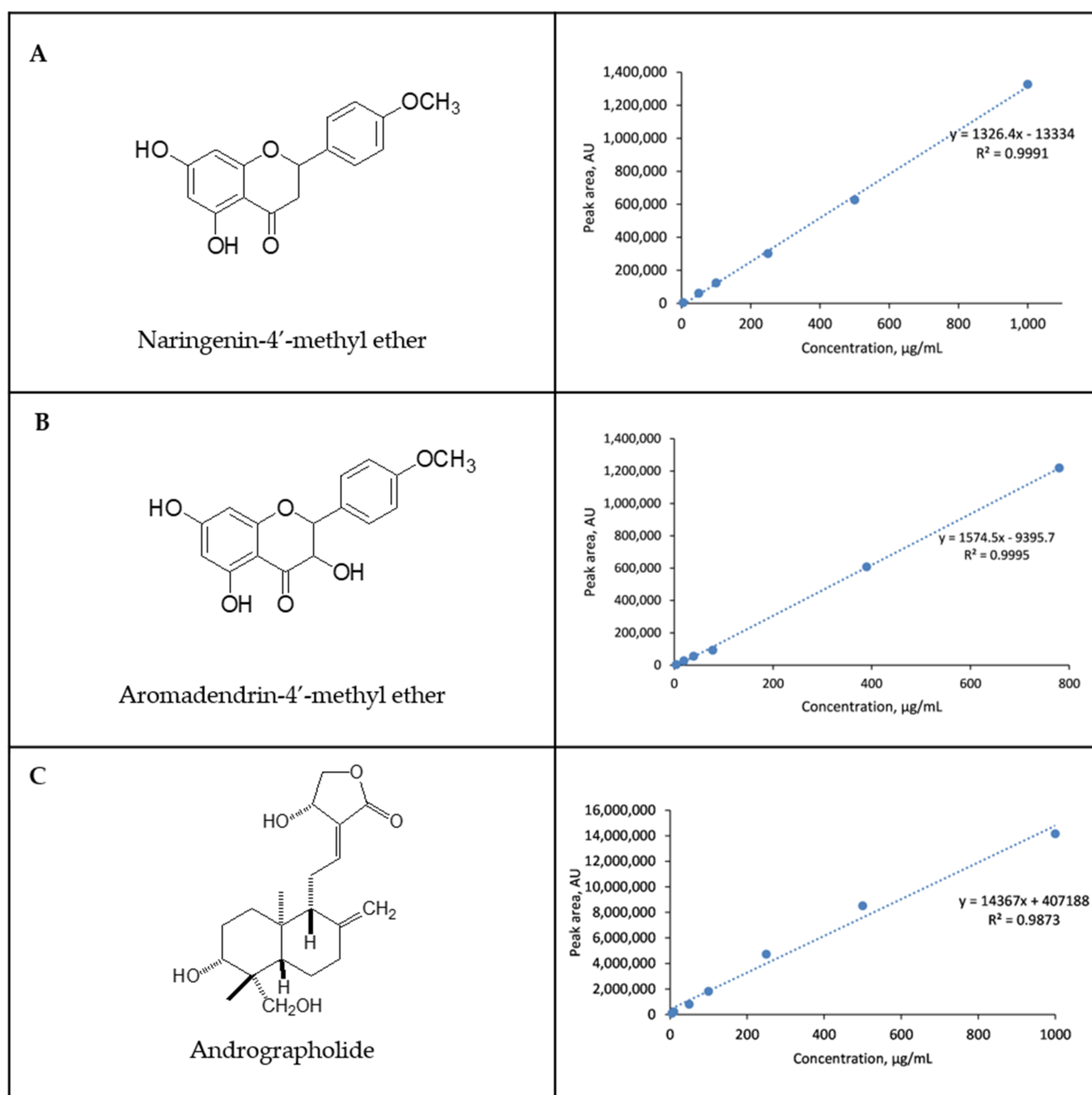

Figure 1. Chemical structures of isolated compounds and plot of calibration curves of standards: (**A**) naringenin-4′-methyl ether; (**B**) aromadendrin-4′-methyl ether; (**C**) andrographolide.

The collected leaf samples from all accessions for the four species were covered with clean tissue paper and carefully placed into a polythene bag to be transported to the laboratory for chemical analysis at Forest Research Institute of Malaysia (FRIM) in Kepong Selangor.

2.2.1. Screening of Naringenin 4′-Methyl Ether and Aromadendrin 4′-Methyl of *C. odorata* Leaves

A total of 0.5 g of leaf from *C. odorata* was ultrasonicated in 5 mL of methanol (MeOH) for 15 min. The solution was filtered through a 0.45 µm membrane filter. All samples were diluted 5 times for quantification of naringenin 4′-methyl ether and aromadendrin 4′-methyl ether before analysis. A series of working solutions with a concentration range of 5–1000 µg/mL of naringenin 4′-methyl ether and aromadendrin 4′-methyl ether was prepared in MeOH, and the calibration curve was plotted. The samples were analyzed using an HPLC system (Waters Delta 600 with 600 controllers, Waters Corporation,

Milford, MA, USA) with a photodiode array detector (Water 2996, Waters Corporation, Milford, MA, USA). The chromatographic analysis was performed on a WATERS X- bridge C-18 HPLC column (Waters Corporation, Milford, MA, USA). The mobile phase consisted of two solvents denoted A (0.1% formic acid in water) and B (acetonitrile). The gradient elution system was set as follows: 0–3 min, 80–65% A; 5–8 min, 65% A; 8–18 min, 65% A–55% A; 18–28 min, 55–40% A; 28–38 min, 40–55% A; 38–41 min, 55–65% A; and 41–45 min, 80–20% A, and followed by a 10 min equilibrium period prior to the next injection. The flow rate used was 1.0 mL/min, and the injection volume was 10 µL. The chromatogram of the targeted compounds was monitored at the wavelength of 356 nm.

2.2.2. Screening of Andrographolide of *A. paniculata* Leaves

A total of 0.5 g of leaf of *A. paniculata* was ultrasonicated in 5 mL of MeOH for 15 min. A series of working solutions with a concentration range of 5–1000 µm/mL of andrographolide was prepared in MeOH, and the calibration curve was plotted. The sample solution was filtered through a 0.45 µm microfilter and analyzed using an HPLC system (Waters 2535 quaternary gradient module, Waters 2707 Autosampler, and Waters 2998 photodiode array detector). The chromatographic analysis was performed on a Phenomenex Luna C18 HPLC column (250 mm × 4.6 mm, 5 µm). The analysis was carried on a mobile phase consisting of two solvents denoted as A 0.1% formic acid in the water, and B (acetonitrile). The gradient elution system was set as follows: 0–7 min, 75% A; 7–15 min, 60–25% A; 15-20 min, 25–0% A; 20–25 min, and 5–75% A, and followed by 10 min equilibrium period prior to the next injection. The flow rate used was 1.0 mL/min, and the injection volume was 10 µL. The chromatogram of the targeted compound was monitored at a wavelength of 220 nm.

2.2.3. Screening of In Vitro Xanthine Oxidase (XO) Inhibitory Activity of *B. frutescens* Extract

Extracts from *B. frutescens* leaves were obtained using the solvent extraction method, whereby fresh leave samples were dissected into smaller pieces and dried in a ventilated oven at 45 °C for three days before being ground into fine powder form. About 30 g of ground powder was soaked in a mixture of ethanol and water at a 1:10 ratio for a few hours. The extract was filtered using a 0.45 µm PTFE membrane filter to remove unwanted fragments in the extracts. Excess solvent was removed using a rotary evaporator. The dark green crude extract was stored in a freezer at 0 °C for further analysis. The average yield of *B. frutescens* crude extract was about 4%. The method of in vitro XO inhibitory analysis was adapted from Noro et al. [24]. with a few modifications. The *B. frutescens* extract was dissolved with dimethyl sulfoxide (DMSO) at 20 mg/mL as an extract solution. The potassium phosphate monobasic (KH_2PO_4) buffer solution, pH 7.5 was used as the main buffer in the system. The XO enzyme solution was freshly prepared. The 96-well microplate was pipetted in with main buffer solution, extract solution, and XO enzyme before being incubated for 15 min at 25 °C. After the incubation period, the reaction of the enzyme was induced with the addition of substrate solution. The microplate was then incubated again for 10 min before analysis. The production of uric acid was measured using a spectrophotometer at 295 nm. Allopurinol is a common drug used to treat gout patients. Therefore, allopurinol was selected as a positive control in this assay system.

2.3. Development of Propagation Techniques for Mass Production of Quality Planting Material

2.3.1. Stem Cuttings of *C. odorata*

To mass-propagate the selected high-yielding accessions, another experiment similar to the one described above was designed with some modifications of the cutting sources based on our preliminary findings. The stem segments were divided into top, middle, and bottom sections, with each of the cuttings measuring 11 cm in length. The basal part of the cuttings was treated with commercial rooting hormone in the form of powder, Seradix 1 (0.1% indole butyric acid), before being transplanted in propagation trays containing moist river sand. A total of 90 stem cuttings were arranged randomly on a propagation tray,

with 30 cuttings for each stem section. The propagation tray was placed under the shaded condition with a relative humidity of 70%. These cuttings were irrigated with an automatic mist sprinkler system that operated at an hourly interval with a one-minute duration of each spray. The observation was made weekly for 7 consecutive weeks, and the number of rooted cuttings and root length were recorded. Data were subjected to analysis of variance using the Statistical Package for the Social Sciences (SPSS) version 22, followed by Duncan's multiple range test (DMRT) to detect the mean differences.

2.3.2. Stem Cuttings of *Andrographis paniculata*

Mature stems from selected high-yielding mother plants were chosen to be used as planting material for the propagation of *A. paniculata* through the cutting method. The stem segments were cut slanted individually within 5–7 cm and dipped into commercial rooting hormone in the form of powder, Seradix 3 (0.8% indole butyric acid). The treated stem segments were planted vertically at a depth of 1/3 of their length, and remained immersed in a moist sand bed under nursery condition with 50% shading and relative humidity of 70%. These cuttings were irrigated with an automatic mist sprinkler system that operated at an hourly interval with a one-minute duration of each spray. After 30 days, the cuttings were removed from the sand bed to evaluate the percentage of sprouting, and the average number and length of the roots per cuttings. Rooted stem cuttings were transplanted into new growth substrates containing two parts topsoil and one part sand mixed with either one part sawdust, cocopeat, or CompAcc. A growth substrate containing two parts topsoil mixed with one part sand was used to serve as a control. A total of 240 stem cuttings were used in this experiment, with 30 stem cuttings for each substrate. The potted rooted cuttings were placed under 50% shade and in an open area receiving full sunlight (59 $\mu mol^{-2}\ s^{-1}$). After a month, morphological data such as leaf number, leaf width, leaf length, and collar diameter were recorded. Data were subjected to analysis of variance using SPSS (version 22), followed by Duncan's multiple range test (DMRT) to detect the mean differences.

2.3.3. Air Layering of *B. frutescens*

An experiment was designed to investigate the possibility of some selected *B. frutescens* genotypes being propagated through the air-layering method. The rooting ability of *B. frutescens* air-layered branches were evaluated through the application of different rooting substrates. Air layering was done on 10 superior accessions with the highest inhibitory activity on xanthine oxidase based on their bioactive compound screening. Healthy branches were girdled by removing about a 2.5 cm wide section of the outer layer, and the cambium layer was removed completely by scraping the woody part of the exposed section. A thin layer of commercial rooting hormone in the form of powder, Seradix 3 (0.8% indole butyric acid), was applied to the exposed wound and covered with six different types of damp rooting substrates. Polythene film was wrapped around the rooting medium, and both ends of the film were secured with twist ties to retain the moisture of the rooting medium. A few holes were created in the polythene film for better aeration, and the rooting medium was sprayed once a week to maintain the moisture content. The rooting substrates prepared for the air-layering treatment were: topsoil (M1), sphagnum peat moss (M2), topsoil and sand (1:1) (M3), topsoil and sawdust (1:1) (M4), topsoil and sphagnum peat moss (1:1) (M5), and topsoil and coconut husk (1:1) (M6). The experiment was repeated twice with a total number of five samples per treatment. Air-layered branches were removed from the parent plants once the roots were visible throughout the rooting substrates. The rooted layers were transplanted into a new rooting substrate containing a mixture of topsoil and river sand at a ratio of 1:2 and placed in the nursery under shade conditions for acclimatization purposes. Data on vegetative growth, such as the number and length of roots per plant and the rooting percentage, were recorded. Data were subjected to analysis of variance in SPSS (version 22), followed by Duncan's multiple range test (DMRT) to detect the mean differences.

3. Results and Discussion

3.1. Chemical Screening and Propagation of *C. odorata*

Screening of chemical constituents from 35 accessions of *C. odorata* found that the concentration of aromadendrin 4′-methyl ether was higher than the concentrations of naringenin 4′-methyl ether. The concentration of chemicals ranged from 0.22–1.11% for naringenin 4′-methyl ether and 0.50–5.77% for aromadendrin 4′-methyl ether. Profiles of HPLC chromatogram standards of both compounds are presented in Figure 2A. To screen for high-yielding accession, the six best accessions with a high concentration of each chemical were selected for mass propagation (Figure 3). Accession JKT22, which was from Kota Tinggi, Johor recorded the highest concentrations for both chemical constituents. The findings of the study were in line with Hung et al. [25], Johari et al. [26], and Omokhua [27], who reported the same chemical constituents in *C. odorata*.

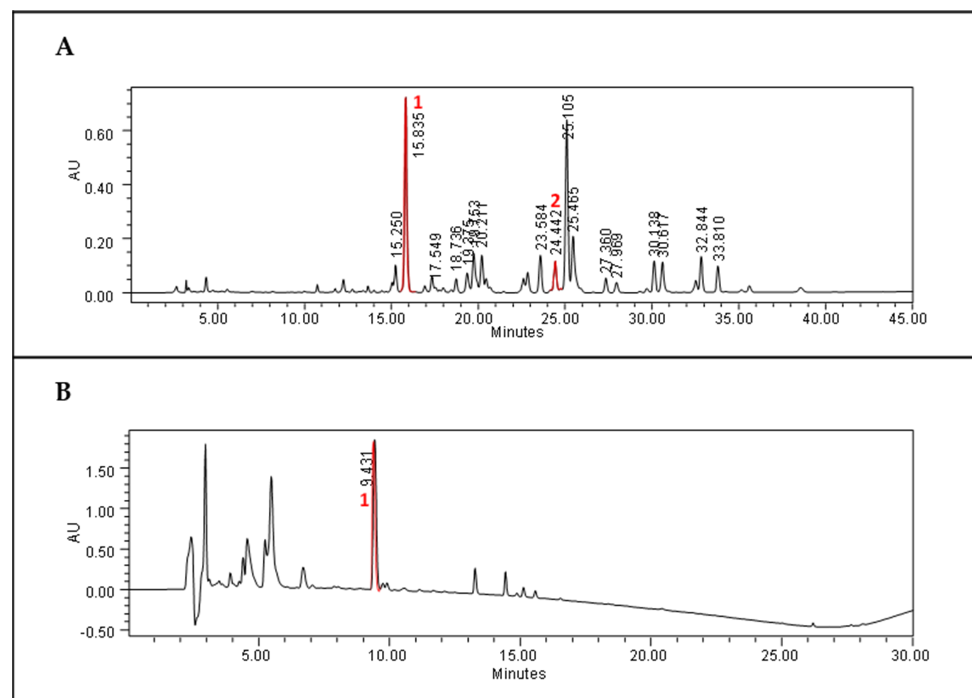

Figure 2. (**A**) HPLC chromatogram of representative samples of *C. odorata*: (**1**) naringenin-4′-methyl ether and (**2**) aromadendrin-4′-methyl ether; (**B**) HPLC chromatogram of representative samples of *A. paniculata* and (**1**) andrographolide.

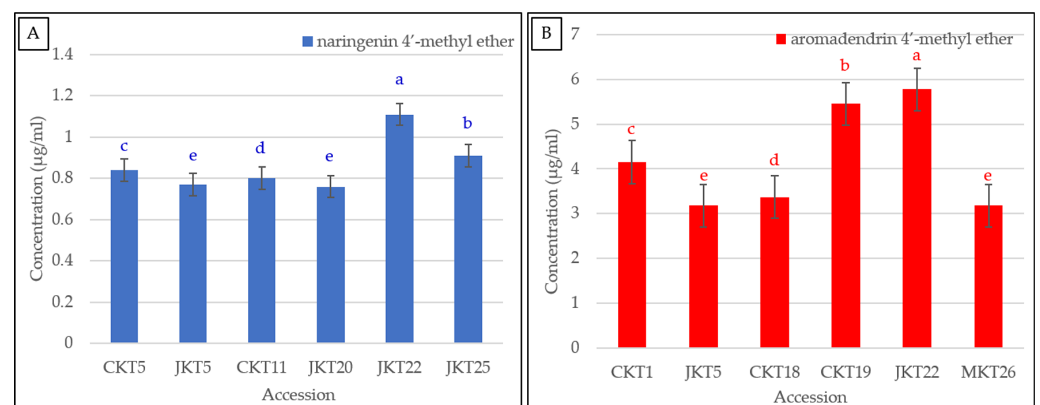

Figure 3. Selection of accessions of *C. odorata* with high concentrations of (**A**) naringenin 4′-methyl ether and (**B**) aromadendrin 4′-methyl ether after screening. Note: bar = ± standard error of the mean. Means of each variable with the same letters are not significantly different at $t < 0.05$.

Analysis of variance conducted on parameters taken on the rooting ability of the cuttings after seven weeks of planting showed there was a significant difference among the cutting positions. Stem cuttings taken from the top part of the branch showed better rooting performance in terms of survival rate and root length of the cuttings than the cuttings collected from other parts of the stem, as presented in Table 1.

Table 1. Effect of cutting position on rooting percentage and root length of *C. odorata* after seven weeks under nursery conditions.

Position of Stem Cutting	Rooting Percentage (%)	Mean Root Length ± SE (cm)
Top	90.0 [a]	11.47 ± 1.37 [a]
Middle	63.3 [b]	6.61 ± 1.30 [b]
Bottom	60.0 [b]	4.02 ± 0.91 [b]

Means followed by the same letters were not significantly different at $p < 0.05$. SE: standard error.

The difference in rooting percentages of cuttings taken from different parts of the stem could have been due to the different degrees of juvenility along the stem. Different responses of the rooting percentage of the cuttings might have been related to the size of the cuttings where middle and bottom segments had significantly bigger diameters compared with the top segments. Thus, it could be assumed that the juvenile stock plants rooted significantly better (90%) than other parts of the plants, and they also developed the longest roots (11.47 cm). These findings also supported the theory of Otiende et al. [28] on *Rosa hybrida* juvenile rootstocks. To reduce the transpiration rate from the exposed leaves of the cuttings, larger leaves were removed and smaller leaves were maintained on the cuttings from each segment. Thus, the number of leaves attached to the stem cuttings might have been another factor influencing the rooting ability of the cutting. More leaves on the top part might have enhanced the rooting ability of the cuttings compared to the less leafy part of the middle and bottom parts of the cuttings. Translocation of auxin from leaves to the base of the cuttings for the production of carbohydrates during photosynthesis might have enhanced the rooting ability of the cuttings [29,30]. It also acted as a trigger factor in the developmental process of rhizogenesis [31]. It was also recorded that 2/3 of leaf retention on *Lavandula dentata* L. cuttings resulted in an increase in the root length and fresh weight compared to the leafless cuttings [32]. However, excessive leaf retained on cuttings can lead to the dehydration of cuttings, which will affect the root formation [33].

3.2. Chemical Screening and Propagation of A. paniculata

Table 2 indicates that all samples from four populations exhibited andrographolide constituents. Profile of HPLC chromatogram standard of andrographolide is presented in Figure 2B. Accessions from Terengganu (THB) and Perak (AHB) contained andrographolide with more than the average (2.89%). The lowest value of andrographolide was recorded by accessions from Johor (JHB), which was 2.59% ± 2.61. The concentration of andrographolide for four populations was presented during the injection time of 9 to 10 min between 220 and 230 nm. The concentration of andrographolide obtained in this study was relatively higher compared to the content reported in several studies due to the origin of the sample collection, different genetic materials, and different types of extraction methods used in each study [34]. Rafi et al. [34] found significant variation in extraction methods and their conditions in obtaining extract with the maximum amount of andrographolide. The highest amount of andrographolide was obtained with the maceration method.

Table 2. Screenings for high andrographolide chemical constituent in four populations of *A. paniculata*.

Accessions Code No.	Average Concentration (%) ± RSD (*W/W*)
THB	3.21 ± 0.75
AHB	2.94 ± 1.18
CHB	2.83 ± 1.24
JHB	2.59 ± 2.61

A survival rate of more than 87% was recorded for *A. paniculata* cuttings selected from the high-yielding accessions. All stem cuttings of *A. paniculata* rooted through the application of the Seradix 3 rooting hormone. Successfully rooted cuttings were selected to be tested for suitable growing medium under two different environmental conditions; i.e., open and shade areas. Growth parameters collected from cuttings grown in different growth substrates are presented in Table 3.

Table 3. Vegetative growth traits of *A. paniculata* in different growth substrates under shaded and open areas at four weeks after planting.

Area	Treatment	No. of Leaves	Leaf Length (cm)	Leaf Width (cm)	Collar Diameter (mm)
Open	Control	$3.50^{b} \pm 1.00$	$3.14^{a} \pm 0.47$	$0.78^{ab} \pm 0.10$	$2.05^{a} \pm 0.15$
	Sawdust	-	-	-	-
	Cocopeat	$4.25^{b} \pm 0.75$	$3.75^{a} \pm 0.58$	$0.98^{a} \pm 0.13$	$2.43^{a} \pm 0.11$
	CompAcc	$14.53^{a} \pm 1.73$	$3.05^{a} \pm 0.19$	$0.61^{b} \pm 0.05$	$1.33^{b} \pm 0.11$
Shade	Control	$11.19^{a} \pm 2.12$	$3.86^{a} \pm 0.31$	$0.95^{a} \pm 0.08$	$1.89^{a} \pm 0.14$
	Sawdust	-	-	-	-
	Cocopeat	$4.43^{b} \pm 1.80$	$3.07^{a} \pm 0.65$	$0.64^{a} \pm 0.15$	$1.42^{a} \pm 0.18$
	CompAcc	$11.47^{a} \pm 1.34$	$3.45^{a} \pm 0.27$	$0.85^{a} \pm 0.09$	$1.90^{a} \pm 0.17$

Values are expressed as mean ± standard error; means followed by the same letters were not significantly different at $p < 0.05$.

Significant differences were found among treatments on the amount of leaf from both growth conditions and leaf width on cuttings placed in an open area. Stem cuttings grown in sawdust did not survive and were not suitable for rooted cuttings of *A. paniculate* due to poor drainage. Generally, cuttings grown in CompAcc, which is a compost, showed better growth performance in terms of the number of leaves. Different organic wastes in compost as an improver of soil characteristics and plant growth might have been the contributing factor for the good growth of cuttings [35]. Different growth substrates did not have any effects on the growth of cuttings grown under shade conditions in most of the growth parameters tested.

3.3. Chemical Screening and Propagation of B. frutescens

Based on the screening, it was recorded that 10 out of 84 genotypes of *B. frutescens* were selected as superior due to the value of more than 70% inhibitory activity on XO (Table 4). Accession TCA8 from Setiu, Terengganu showed the highest value (78.12% ± 1.68), whereas ACA 4 returned the lowest value (70.39% ± 3.57). It was observed that a total of seven accessions were originated from the population Setiu, Terengganu, and three accessions from Gunung Pulut, Perak. Only one accession from Sungai Baging, Kuantan was categorized as superior.

Table 4. Selection of 10 superior accessions of *B. frutescens* with high inhibitory activity on xanthine oxidase.

Accession Code No.	Xanthine Oxidase (%) ± SEM *
TCA 8	78.12 ± 1.68
ACA 10	75.79 ± 4.79
TCA 15	74.14 ± 5.11
TCA 12	73.39 ± 2.98
TCA 6	72.24 ± 3.24
TCA 14	72.00 ± 1.95
CCA 9	71.77 ± 2.22
ACA 6	71.28 ± 1.28
TCA 13	71.10 ± 0.93
ACA 4	70.39 ± 3.57

* Values are expressed as mean inhibition (%) ± SEM of triplicate measurements from three independent experiments.

Besides *B. frutescens*, several other herbs have proven to show antigout potential, mainly through an XO inhibition assay. This assay is regarded as an essential standard for discovering antigout potential among medicinal plants [36]. It was reported that bitter melon (*Momordica charantia*) showed the highest percentage of XO inhibitory activity (96.5%) at 100 µg/mL using 70% methanol extract [37]. The highest XO inhibitory activity at 100 µg/mL (90.6%) was also discovered using the extract of aromatic ginger (*Kaempferia galangal*) [38]. Superior genotypes usually referred to good growth characteristics and/or contained high-quality active ingredients [39]. A few investigations on the selection of superior plants were previously conducted by FRIM on selected species such as *Citrus hystrix* [40], *Citrus microcarpa* [41], *C. odorata* [42], and *Labisia pumila* [43].

The results indicated that there was a significant difference between the different types of rooting substrates in promoting the rooting ability of *B. frutescens*. Branches treated with topsoil and sphagnum peat moss (M5) as rooting substrates produced the highest rooting percentage of 60.0% compared to other treatments of rooting substrates. The lowest rooting percentage was recorded using topsoil and sawdust (M4), with only 10.0% of the branches producing roots (Figure 4). The high capacity of sawdust in holding moisture might be the causal factor that led to poor rooting among the treated branches. Low air porosity due to high moisture content might have led to insufficient oxygen during respiration and disrupted the rooting process [44].

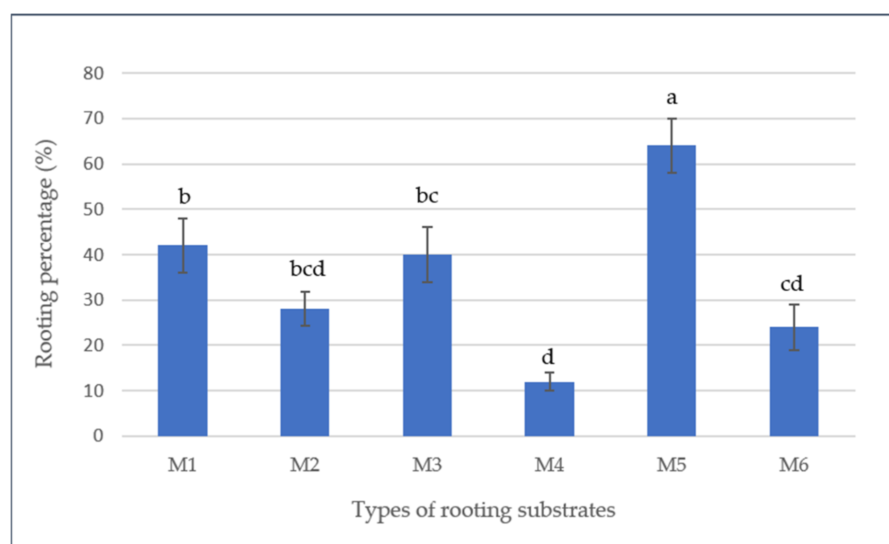

Figure 4. Rooting percentage in different types of substrates in air layering propagation of *B. frutescens*. Note: bar = ± standard error of the mean. Means of each variable with the same letters were not significantly different at $t < 0.05$.

Similarly, branches treated with topsoil and sphagnum peat moss (M5) also returned the highest root numbers, followed by branches treated with topsoil and coconut husk (M6) (Figure 5). A combination of topsoil and sphagnum peat moss was also found to be the best rooting substrate for air layering of Guava (*Psidium guajava*) from the same family Myrtaceae [45]. High porosity and its capacity to retain higher moisture retention might have been one of the reasons for better rooting compared to the other rooting substrates. In terms of root lengths, treatment M4 with the lowest rooting percentage produced the highest root lengths, whereas treatment M1 with only sphagnum peat moss resulted in the lowest root lengths. Sphagnum peat moss has large holes with thin-walled cells for better water absorption and transportation. However, it is important to maintain the moisture content and good aeration by keeping the substrate wet. Thus, the application of sphagnum peat moss as a rooting substrate is not suitable in the air-layering method in a dry area such as Setiu, Terengganu. Application of a much higher concentration of rooting hormone might stimulate better root formation and growth.

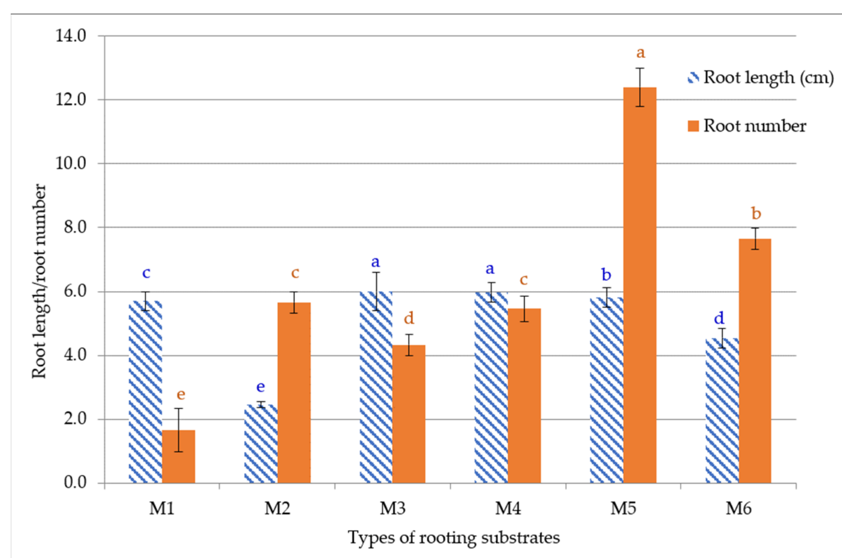

Figure 5. Number and length of roots produced from six types of rooting substrates in air-layering propagation of *B. frutescens*. Note: bar = ± standard error of the mean. Means of each variable with the same letters are not significantly different at $t < 0.05$.

4. Conclusions

The superior accessions of the three herbal plants were identified based on the value of bioactive compounds. The selected individual plants were mass-propagated by developing appropriate vegetative propagation techniques for each species. It was concluded that rooting substrates play an important role in the propagation of *A. paniculata* and *B. frutescens*. Plant propagation of *C. odorata* and *A. paniculata* was possible through stem cuttings; however, further investigation is needed to enhance the rooting ability of the cuttings through the application of different concentrations of rooting hormone and manipulation of other environmental conditions. Through screening and individual selection, plant genotypes with wide variation and genetic backgrounds can be improved for the development of new clones or varieties. Outputs from the study will be beneficial not only to plant breeders in terms of producing new varieties, but also to herbal industries in producing high-quality herbal products.

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