BREEDING PROGRAMME OF THREE IMPORTANT HERBAL SPECIES IN MALAYSIA

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ABSTRACT

Plant breeding has played important roles in improving the quality of crops in many aspects such as increasing the yield and nutritional quality of the crops. This paper aim to outline the breeding programme for three important herbal species in Malaysia namely Andrographis paniculata, Orthosiphon stamineus and Clinacantus nutans. These three herbal species have been used traditionally by Malaysian to treat various ailments. Previous studies found that these herbal species are rich in antioxidant which benefit human health. The collection of mother plants of these herbal species have been conducted in selected region in Peninsular Malaysia. A planting plot of 0.072 hectare has been established at FELCRA Nasaruddin, Seri Iskandar, Perak to evaluate the performance of these herbal plants based on several desirable characteristics. The way forward of this study is to identify elite clone materials which have good growth performances, high in yield and targeted phytochemical compounds. Findings of this study will be used as a guideline for plant breeders, herbal cultivators and herbal industry in selecting the best herbal planting materials for their plantation as well as for the development of high quality end products.

Key words: Cultivation, cloning material, medicinal plant, plant breeding, selection method

INTRODUCTION

Plant breeding is the application of genetic principles to produce plants that are more useful to humans. One major technique of plant breeding is selection, the process of selectively propagating plants with desirable characteristics and eliminating or "culling" those with less desirable characteristics. In Malaysia, many crop improvement was made for vegetables, rice and fruits through selecting superior plants from local and foreign genetic resources (Nordin et al., 2007). The goal of every breeding project is to identify variety that increase production yield, disease and drought tolerance and the one that improved nutrition.

In National Agrofood Policy (2011-2020), herbs have been classified as one of the high value agriculture activity. Several strategies were outlined by the government to strengthen the herbal industry in Malaysia. One of the highlighted strategy was to ensure sustainable and consistent supply of raw materials to the downstream sector. *Andrographis paniculata* (creat or green chiretta), *Orthosiphon stamineus* (Java tea) and *Clinacantus nutans* (Sabah snake grass) were among the preference species listed in Entry Project Point-1 of the National Key Economic Area (NKEA). Each of the species has its own medicinal value which associated with plant secondary metabolites. Secondary metabolites are important compounds for the human beings as they are sources for food additives, flavours and pharmaceuticals (Ravishankar and Rao, 2000).

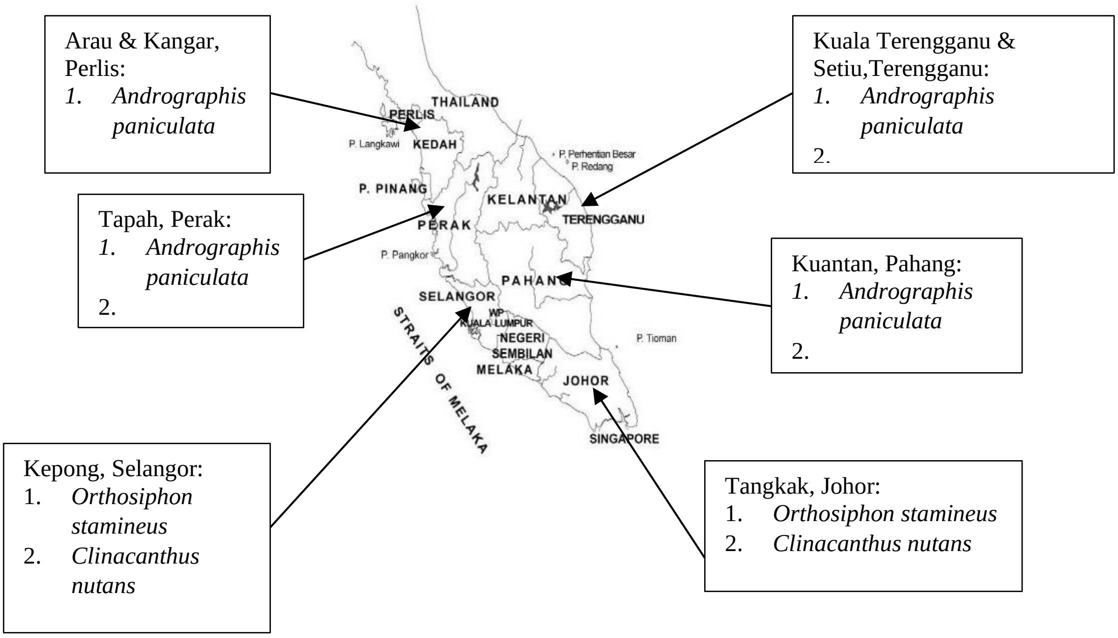
The breeding work of herbal species in Malaysia is still very limited. Most of the raw materials supply to the industry are being wildly collected from the forest or imported from other countries with uncertain quality (Zurinawati, 2004). It is therefore difficult for the country's herbal industry to penetrate the foreign market due to the uncertain quality and authentication of the raw materials. This study was initiated to outline the breeding program for the production of high-quality herbal plants of the selected herbal species.

METHODOLOGY

Planting materials

Three important herbal species listed in NKEA EPP-1 viz. *Andrographis paniculata* (hempedu bumi), *Orthosiphon stamineus* (misai kucing) and *Clinacanthus nutans* (belalai gajah) were collected from several provenances in Peninsular Malaysia (Figure 1) and brought back to Forest Research Institute Malaysia (FRIM) for further cultivation. *Andrographis paniculata* were collected from four provenances such as Perlis (North), Perak (North), Terengganu (East coast) and Kuantan (East coast). Whereas, *Orthosiphon stamineus* and *Clinacanthus nutans* were collected at two provenances namely Selangor (Center) and Johor (South). Each accession was coded with different number and locality. These accessions were then propagated using stem cutting technique. Commercial rooting hormone (Seradix 1) were applied at the base of each cutting to enhance its rooting production. After four weeks, a total of 450 rooted stem cuttings of the three herbal species were selected based on high cutting survival rate and rooting percentage. The cuttings were transferred to polybags and hardened at the nursery for three months.

Figure 1: Collection of mother trees from selected provenance in Peninsular Malaysia



Field plantation and plant maintenance

The hardened plantlets were transported to R&D Centre, Felcra Nasarudin Belia, Seri Iskandar, Perak. The herbal plants were planted in 0.072 ha area. About 150 individual accessions from each herbal species were planted in randomized complete block design (RCBD). The field plantation was established on February 2020. The plants were watered manually once per day and fertilized once a month with 10g of NPK fertilizer (15:15:15). Weeding activity were conducted when needed.

Data collection

Among the data collected for quantitative and qualitative characters were shown in Figure 2. Data collection were conducted once per month until the plant reached the maturity age (16 weeks). The herbal plants were harvested randomly in each field at maturity age for biomass data collection. Fresh sample were weighted prior to drying process. The plants sample were oven dried at 60 °C until it reach constant weight (approximately 72 hours). All the data were analyse for analysis of variance (ANOVA) by using IBM SPSS Statistics version 22.

Figure 2: Quantitative and qualitative characters observed in the selection process

Quantitative characters	Qualitative characters
• Survival rate (%) • Plant height (cm) • Biomass (kg)	• Leaf density: (high, medium, low) • Chemical compounds

Analysis of chemical compounds

A total of 20g fresh leaves from each herbal species were harvested from selected individual plants. The leaves samples were washed with running tap water and proceed to oven dried at 60 °C for 48 hours. The dried leaves samples were grinded into powder form. A total of 0.5 g of sieved powder material (500 µm) was added in 5 mL of methanol and the mixture was ultra-sonicated for 15 minutes. The resulting solution was filtered using 0.45 µm syringe filter prior to analysis. Samples were analysed using HPLC system (WATERS 2535 quaternary gradient pump, WATERS 2707 auto sampler and WATERS 2998 PDA). Two gradient system consist of two different solvent were used; A (0.1% Acid formic in water) and B (acetonitrile). The flow rate used was 1.0 mL/min and the injection volume was 10 µL. The retention times and UV spectra of the targeted compounds were analysed at wavelength of 220 nm. Each herbal plant used different marker for quantitative analysis as shown in Table 1.

Table 1: Reference compound in each herbal plant for quantitative analysis

Herbal species	Reference compound
Clinacanthus nutans	Shaftoside
Andrographis paniculata	Andrographolide
Orthosiphon stamineus	Sinensetin and Rosmarinic acid

RESULTS AND DISCUSSION

Selection of accession based on survival rate and phenotypic characteristics

The percentage of plants survival were recorded after 16 weeks of planting. The total survival rate of the herbal species were above 85% (Table 2). The survived plants were further evaluated for desired height characteristics (Table 2). Each species has different plant height criteria. For *A. paniculata,* plants with height above 25 cm were selected, whereas for *C. nutans* and *O. stamineus* were selected above 50 cm.

Table 2: Percentage of plants survival and screening of accessions based on plant height

Herbal species	Percentage of plant survival at week 16 (%)		
Andrographis paniculata	88	No. of accessions with height > 25 cm	103
		No. of accessions with height < 25 cm	29
Orthosiphon stamineus	85	No. of accessions with height > 50 cm	71
		No. of accessions with height < 50 cm	56
Clinacanthus nutans	95	No. of accessions with height > 50 cm	122
		No. of accessions with height < 50 cm	20

From the survived plants, 78% of *A. paniculata* accessions has plant height more than 25 cm, while 56% and 86% of *O. stamineus* and *C. nutans* has plant height more than 50 cm respectively. Accessions below the par were eliminated though the selected were further evaluated for leaf density by qualitative observation. The leaf density parameter were categorized as dense, medium and sparse (Table 3).

Table 3: Screening of plants based on leaf density

Herbal species	Leaf density classification	No. of accessions	Percentage (%)
Andrographis paniculata	Dense	40	38.8
	Medium	41	39.8
	Sparse	22	21.4
Orthosiphon stamineus	Dense	22	31
	Medium	34	47.8
	Sparse	15	21.2
Clinacanthus nutans	Dense	43	35.2
	Medium	69	56.6
	Sparse	10	8.2

Based on the screening, accessions with higher leaf density were below 40% for all species. Most of the accessions in each species recorded medium leaf density (<40%). *Clinacanthus nutans* recorded the lowest number of accession with sparse leaf density (8.2%). Leaf density is one of the important characteristics for selection of high quality planting materials. High leaf density may results in high yield per hectare in commercial plantation. About 10 accessions (from dense group) were randomly selected and the leaves were harvested for biomass analysis. The data were presented in Table 4.

The high yielding selection was made based on the dried weight results. In herbal industry, most of the raw materials used were in the dried form as it has longer shelf life and low risk of contamination compared to fresh samples. In *A. paniculata* the top five high yielding accessions were identified in HB 88, HB 42, HB 79, HB 69 and HB 63. The highest dried yield in *A. paniculata* was 0.039 kg (HB 88) while the lowest were 0.007 kg (HB 9). In *O. stamineus* the sequence of selected high yielding accessions were MK 45, MK 72, MK 97, MK 126 and MK 22 with the dried yield range from 0.013 kg to 0.018 kg. In *C. nutans*, the selected dried yield were in the range of 0.012 kg to 0.016 kg and the top five high yielding accessions were BG 26, BG97, BG 24, BG 23, BG27. The top five accessions in each herbal species were proceed for quantification of chemical constituent by HPLC as tabulated in Figure 3, Figure 4 a) and b) and Figure 5.

Table 4: Fresh and dry weight of 10 selected accessions in *Andrographis panicualata, Orthosiphon stamineus* and *Clinacanthus nutans*

Herbal species	Accession code	Leaf fresh weight (kg)	Leaf dry weight (kg)
Andrographis panicualata	HB 9	0.035	0.007
	HB 42	0.080	0.024
	HB 43	0.070	0.014
	HB 56	0.070	0.015
	HB 57	0.058	0.010
	HB 63	0.082	0.019
	HB 69	0.099	0.022
	HB 71	0.081	0.016
	HB 79	0.100	0.024
	HB 88	0.170	0.039
Orthosiphon stamineus	MK 12	0.051	0.006
	MK 16	0.051	0.009
	MK 22	0.082	0.013
	MK 45	0.080	0.018
	MK 68	0.050	0.008
	MK 72	0.099	0.018
	MK 97	0.071	0.016
	MK 100	0.048	0.006
	MK 124	0.061	0.012
	MK 126	0.060	0.013
Clinacanthus nutans	BG 9	0.050	0.007
	BG 10	0.050	0.007
	BG 23	0.065	0.012
	BG 24	0.070	0.013
	BG 26	0.085	0.016
	BG 27	0.075	0.012
	BG 34	0.055	0.009
	BG 55	0.050	0.007
	BG 78	0.065	0.012
	BG 97	0.080	0.015

Figure 3: Mean percentage of andrographolide in Andrographis panicualata sample ± RSD (w/w)

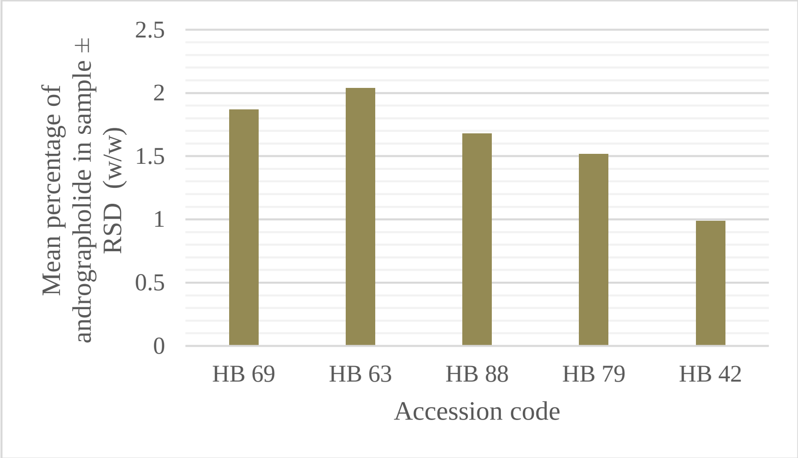


Figure 4: Mean percentage of a) rosmarinic acid and b) sinensetin in Orthosiphon stamineus sample ± RSD (w/w)

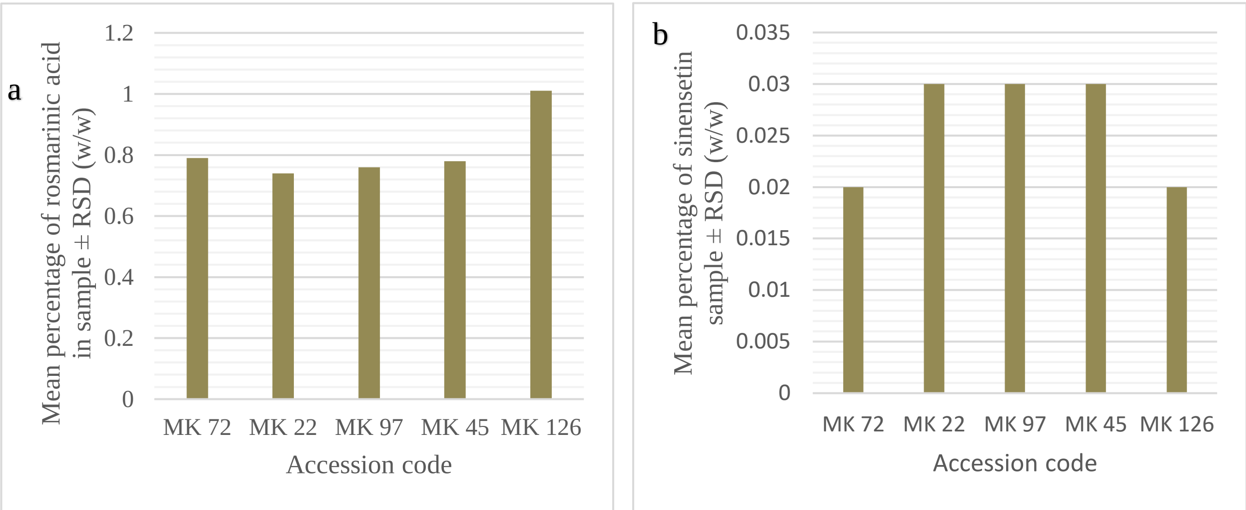
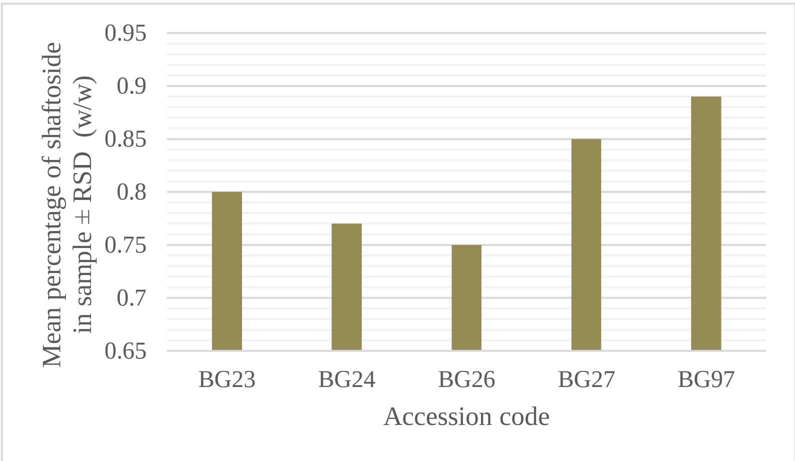


Figure 5: Mean percentage of shaftoside in Clinacantus nutans sample ± RSD (w/w)



The andrographolide in *A. paniculata* sample was higher in HB 63 (2.04±0.31 w/w) followed by HB 69 (1.87±0.15 w/w) and HB 88 (1.68±0.6 w/w). Andrographolide is a major bioactive constituent which possess pharmaceutical activities such as immunostimulatory, antidiabetic, hypolidemic, antiviral, antibacterial, antitumor, antidiabetic, antimalaria, hepatoprotective effects and antiscorpion venom activity (Okoye et al., 2014). The production of high yielding accession of this species in Malaysia is important as it is widely used in different medicinal formulations in national and international market.

Rosmarinic acid and sinensetin were among important flavonoid compound in *O. stamineus* leaf extract (Abdullah et al., 2014). The medical properties of rosmarinic acid are described as antioxidant, antimicrobial, antiviral, and anti-inflammatory while the biological activity of rosmarinic acid was reported to have antibacterial, antiviral, and ant oxidative (Almatar et al., 2014). In this study, MK 126 recorded highest value of rosmarinic acid 1.01±1.37 w/w. On the other hand, sinensetin in Orthosiphon species was reported to exhibit potential anti-cancer characteristic (Dong et al., 2011). Three accessions were found high in sinensetin compound namely MK 97 (0.03±0.68 w/w), MK 22 (0.03±0.58 w/w) and MK 45 (0.03±0.28 w/w).

Shaftoside has been found as one of the major flavone in *C. nutans.* In study conducted by Abd Samat et al. (2020), shaftoside was found abundant at all level of harvesting age (week 8, week 12 and week 16) and harvesting frequency of *C. nutans* thus suggested that it would be a possible chemical marker for *C. nutans* raw materials. From the analysis, shaftoside was found higher in BG 97 (0.89±2.79 w/w) followed by BG 27 (0.85±1.95 w/w) and BG 23 (0.80±1.3w/w).

Future work in the breeding programme

Of all the selection process, accessions with desired phenotype and high yielding chemical compounds will be further mass produce through vegetative propagation technique. The superior accessions should be reliable over a wide range of environmental conditions. Therefore, the stability analysis should be conducted to study the genotype-environment interaction (Becker & Leon, 1988). Superior accessions will be planted at different geographical location within the Peninsular Malaysia. The growth performance, biomass and chemical compounds will be evaluated for stability analysis. Clones or accessions with high stability index will be promoted to the industry for commercialization.

CONCLUSION

The practiced of plant breeding in herbal trade in Malaysia could open more economic opportunity for local and international market. High quality raw materials would be the added value of the raw materials produced. The breeding programme outlined in this study might perhaps useful for the country's herbal industry.

ACKNOWLEDGEMENTS

This study was supported by FRIM-FELCRA Memorandum of Understanding (MoU).

REFERENCES

Abd Samat, N. M. A., Ahmad, S., Awang, Y., Bakar, R. A. H., & Hakiman, M. (2020). Alterations in Herbage Yield, Antioxidant Activities, Phytochemical Contents, and Bioactive Compounds of Sabah Snake Grass (*Clinacanthus Nutans* L.) with Regards to Harvesting Age and Harvesting Frequency. *Molecules*, *25*(12), 2833.

Abdullah, S., Shaari, A. R., & Azimi, A. (2012). Effect of drying methods on metabolites composition of misai kucing (*Orthosiphon stamineus*) leaves. *Apcbee Procedia*, *2*, 178-182.

Almatar, M., Ekal, H., & Rahmat, Z. (2014). A glance on medical applications of *Orthosiphon stamineus* and some of its oxidative compounds. *Int. J. Pharm. Sci. Rev. Res*, *24*(2), 83- 88.

Becker, H. C., & Leon, J. (1988). Stability analysis in plant breeding. *Plant breeding*, *101*(1), 1-23.

Dong Y, J.G., Cao A, Shi J, Shi H, Xie J, Wu D., Effects of sinensetin on proliferation and apoptosis of human gastric cancer AGS cells. Zhongguo Zhong Yao Za Zhi, 2011. 36(6): p. 790-4.

Nordin, S. M., Ariffin, Z., Jajuli, R., Abdullah, W. D. W., & Denis, M. G. (2007). Country Report on the State of Plant Genetic Resources for Food and Agriculture in Malaysia (1997-2007). *FAO, Rome, Italy*.

Ravishankar, G.A. & Rao, S.R. (2000). Biotechnological production of phytopharmaceuticals. *J Biochem Mol Biol Biophys* 4: 73-102.

Okoye, T. C., Uzor, P. F., Onyeto, C. A., & Okereke, E. K. (2014). Safe African medicinal plants for clinical studies. In *Toxicological Survey of African Medicinal Plants* (pp. 535-555). Elsevier.

Zurinawati, Z. A. (2004). Medicinal Plants and Products Malaysian Industry Status Report. In *Proceedings of Medicinal Herbs & Plants: Scope for Diversified and Sustainable Extraction*, 22-26 July 2004, Bangalore, India.