SELECTION OF HIGH YIELDING ACCESSIONS OF *CHROMOLAENA ODORATA L.* FOR FUTURE BREEDING ACTIVITIES

Farah Fazwa Md Ariff
Forestry Biotechnology Division,
Forest Research Institute Malaysia,
52109 Kepong, Selangor, Malaysia
Email: farah@frim.gov.my

Norhayati Saffie
Forestry Biotechnology Division,
Forest Research Institute Malaysia,
52109 Kepong, Selangor, Malaysia
Email: norhayatisaffie@frim.gov.my

Syafiqah Nabilah Samsul Bahari,
Forestry Biotechnology Division,
Forest Research Institute Malaysia,
52109 Kepong, Selangor, Malaysia
Email: syafiqah@frim.gov.my

Zunoliza Abdullah
Natural Product Division,
Forest Research Institute Malaysia,
52109 Kepong, Selangor, Malaysia
Email: zunoliza@frim.gov.my

Ling Sui Kiong,
Natural Product Division,
Forest Research Institute Malaysia,
52109 Kepong, Selangor, Malaysia
Email: lingsk@frim.gov.my

Mohd Zaki Abdullah
Forestry Biotechnology Division,
Forest Research Institute Malaysia,
52109 Kepong, Selangor, Malaysia
Email: farah@frim.gov.my

Sharmizi Ismail
Natural Product Division,
Forest Research Institute Malaysia,
52109 Kepong, Selangor, Malaysia
Email: sharmizi@frim.gov.my

ABSTRACT

Chromolaena odorata L. commonly known as Siam weeds or in Malaysia is known as 'pokok kapal terbang', 'pokok Jerman', 'rumput Jepun' or 'rumput Siam', belongs to family Asteraceaea. The plant is native in North America and has been introduced to South America, tropical Asia, West Africa and parts of Australia. It is a semi-woody shrub and able to reach 3.0-7.0 meter in height. It is very common on the road side, open areas, forest clearings, and abandoned gardens and near the beach. It is reported that C. odorata is an aggressive competitor that suppresses young plantation, agriculture crops and grows on other vegetation. However, the leaf of this species provides a lot of benefits especially in treating wound such as to stop bleeding and promote healing. The wound healing activity of C. odorata has been proven scientifically and is widely reported. In Forest Research Institute Malaysia (FRIM), isolated flavonoids from C. odorata leaf extract were found to have relatively strong inhibition on platelet-activating factor (PAF) receptor binding in vitro, indicating an anti-inflammatory activity, which is favourable in wound healing process. Among of the chemical constituents in the leaf parts that contributed to this activity are naringenin 4'-methyl ether and aromadendrin 4'-methyl ether. Therefore, screening of these two chemical constituents on 35 accessions of C. odorata from three provenances was conducted. The main attempt is to select high yielding accessions of the species for future production of high quality planting materials. This paper also highlights the process of collection, propagation, establishment of germplasm as part of the process in producing high quality planting materials of the species.

Keywords: collection, chemical screening, selection, propagation, new variety, quality herbal products

INTRODUCTION

Chromolaena odorata L. commonly known as Siam weeds or in Malaysia is known as 'pokok kapal terbang', 'pokok Jerman', 'rumput Jepun' or 'rumput Siam', belongs to family Asteraceaea. The plant is native in North America and has been introduced to South America, tropical Asia, West Africa and parts of Australia (McFadyen and Skaratt, 1996). It is a semi-woody shrub and able to reach 3.0-7.0 meter in height. It is very common on the road side, open areas, forest clearings, and abandoned gardens and near the beach (Syed, 1979). It is reported that *C. odorata* is an aggressive competitor that suppresses young plantation, agriculture crops and grows on other vegetations (Azmi, 2002). However, there was research showing that the species has positive contribution to the agricultural sector. The leaves of *C. odorata* are used as an ingredient for formulating animal feeds especially in rabbit's diet where the nutrient profile is similar to a concentrated feed (Bamikole et al., 2004). The leaves are also claimed to have high nutritive values and have potential to be used as protein supplements to ruminants (Apori et al., 2001).

Besides that, the leaves and other parts of *C. odorata* also provide a lot benefits especially in traditional medicine. A decoction of the leaves is used as a cough remedy while the flower is used as tonic. The leaves are also traditionally applied onto cuts or wounds to stop bleeding and promote healing (Muhamad and Mustafa, 1994). Scientific findings on *C. odorata* leaf extract towards wound healing show positive results especially in helping to form new tissue cells (Chee, 2012). In addition, fresh leaves or decoction are used to treat leech bite, soften tissue wounds, burn wounds and skin infection (Phan et al., 2001). In Forest Research Institute Malaysia (FRIM), isolated flavonoids from *C. odorata* leaf extract were found to have relatively strong inhibition on platelet-activating factor (PAF) receptor binding *in vitro*, indicating an anti-inflammatory activity, which is favourable in wound healing process. Among of the chemical constituents found in the leaf parts that contributed to this activity is naringenin 4'-methyl ether and aromadendrin 4'-methyl ether (Ling et al. 2007).

Looking at the benefits in pharmaceutical aspects of the species, therefore an initiative has been taken to screen these two chemical constituents on 35 accessions of *C. odorata* collected from three provenances in Peninsular Malaysia. The main attempt is to select high yielding accessions of the species which contain high chemical constituents for future production of high quality planting materials. The output of this study will support the herbal industries in getting quality raw materials for production of herbal products in the market. This is because nowadays most of plant raw materials used in producing herbal products and its development were being sourced from the wild, with little knowledge on the quality of the raw materials were known. This paper also highlights the process of collection, propagation, establishment of germplasm as part of the process in screening of high quality accessions of the species.

MATERIALS AND METHODS

Collection of Chromolaena odorata accessions

A total of 35 accessions of *C. odorata* collected from three provenances such as Kota Tinggi, Johor (accessions 1, 2, 4, 5, 12, 13, 15, 16, 18, 19, 20, 21, 22, 25, 28), Jasin, Melaka (accessions 4, 9, 12, 23, 26, 28, 29, 30) and Maran, Pahang (accessions 1, 2, 3, 4, 5, 7, 10, 11, 18, 19, 21) were selected for screening their chemical constituents. The accessions were coded as JKT for accessions from Johor, MKT for accessions from Melaka and CKT for accessions from Pahang. The topographic information such as coordinates, altitudes and dates of assessment were also recorded. Samples of seeds, stumps and stems of each mother plants were collected from each of the populations. The morphology characteristics of all mother plants such as number of clumps, height, diameter, leaf length, leaf width, crown diameter and number of nodes were measured (Table 1). These plants (stumps) were then planted and grown at FRIM's nursery under 50% shades before transferred for field planting.

Table 1: Morphological characteristics of mother plants for *Chromolaena odorata* from three provenances

Population	No. of Clumps	Height (m)	Diameter (mm)	Leaf Length (cm)	Leaf Width (cm)	Silara X (cm)	Silara Y cm)	No of Nodes
JKT	2-5	1.38-2.35	5.2-12.3	9.4-12.5	4.5-7.2	15-48	20-57	9-23
MKT	2-5	1.32-2.50	5.6-12.6	8.0-19.8	3.5-9.0	10-55	9-45	8-24
CKT	1-7	1.35-2.74	3.2-12.3	8.7-13.4	4.7-7.9	21-55	24-72	9-27

Figure 1. Stumps of *Chromolaena odorata* were collected from three provenances and replanted at FRIM's nursery for screening process

Establishment of Chromolaena odorata germplasm

FRIM's Substation in Maran, Pahang was selected as a location for the establishment of germplasm plot. The site was selected due to its suitable soil condition which is categorized as clay loam. At the initial phase of establishment, the selected area was cleared and ploughed to further improve the soil condition. Planting distance used was 1.0 m x 1.0 m. A total of 35 stumps of *C. odorata* from three provenances were planted. The plot was covered with 50% shade netting and irrigated with sprinkler system. The planting plots were also covered with silver shine plastics for weed control. The accessions were arranged and planted accordingly.

Figure 2. Planting of Chromolaena odorata accessions in germplasm plot located at FRIM's Substation in Maran, Pahang

Sample collection and screening of chemical constituents

After 6 months, leaf samples with the uniform age were collected from each accession. The leaf samples were carefully covered with tissue paper and packed in plastic bags before they were transported back to FRIM in Kepong, Selangor. Upon arrival, the samples were deposited to Chemistry Lab for chemical analysis.

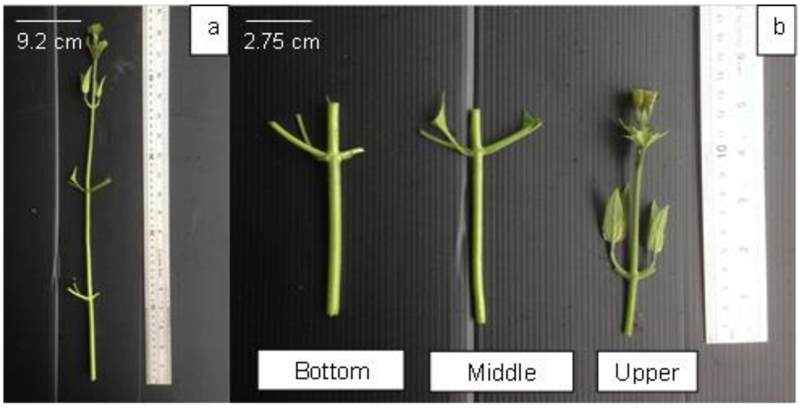
Quantification of chemical constituents using High Performance Liquid Chromatography (HPLC) of Chromolaena odorata extract

A total of 0.5 g *C. odorata* dry samples was dissolved in 5 ml of MeOH and sonicated for 15 minutes. Each of resulting solution were filtered through membrane filter (pore size 0.45 µm) prior to analysis. All samples were diluted 5 times for quantification of naringenin 4'-methyl ether and aromadendrin 4'-methyl ether prior to analysis. A series of working solution with concentration range from 5-1000 µg/ml of naringenin 4'-methyl ether and aromadendrin 4'-methyl ether were prepared in MeOH. The samples were analyzed by means of HPLC system (Waters Delta 600 with 600 controllers) with Photodiode array detector (Water 2996). A WATER x- bridge C-18 was used and for elution of constituents, two solvent denoted as A and B was employed. A was 0.1% aqueous formic acid and B was acetonitrile. Initial conditions were 80% A and 20% B. Analysis was performed using gradient solvent system in 45 minutes of running time. The flow rate used was 1.0 ml/min and the injection volume was 10 µl.

Propagation of selected high yielding accessions of Choromolaena odorata using stem cuttings

An experiment using stem cuttings of *C. odorata* collected from selected high yielding accessions was carried out in FRIM's nursery. Each stem was cut into three portion, i.e. top, middle and bottom (Figure 3). The length of each cutting was 11 cm. The base of the cutting was treated with commercial powdered hormone, Seradix 1 (0.1% IBA). A total of 90 cuttings were used and they were arranged randomly in 3 blocks with 30 cuttings per block. These cuttings were planted in cleaned river sand in a propagation tray. Observation on cuttings was made weekly and the experiment was terminated at week 7 since most of them had rooted. Variables collected were the number of cuttings rooted, dead and root length. Data were subjected to analysis of variance using a statistical package for social study (SPSS) version 22.0 International Business Machines Corporation, New York, USA). This was followed by Duncan Multiple Range Test (DMRT) to see the effect of different cutting part on rooting. The results of the analysis were considered significant when $P < 0.05$.

Figure 3. Chromolaena odorata L. a) Stem b) top, middle and bottom cuttings

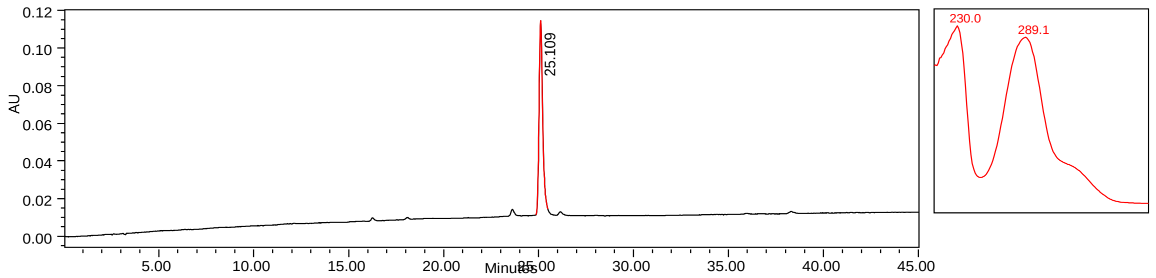


RESULTS AND DISCUSSION

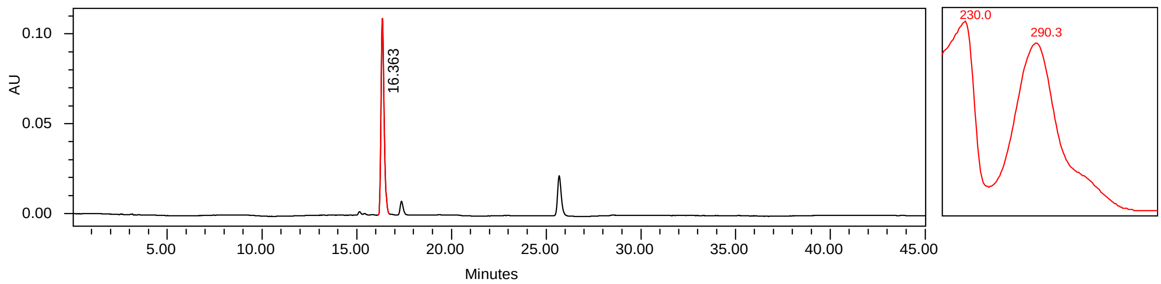
HPLC profiles of Chromolaena odorata

In this study determination of the presence compounds were carried out by comparing the retention time and the UV spectra pattern of standards and extracts. HPLC results of *C. odorata* extracts showed that all provenances consisted of naringenin 4'-methyl ether and aromadendrin 4'-methyl at retention time of 25 and 16 minutes. Profiles of HPLC chromatogram standards of naringenin 4'-methyl ether and aromadendrin 4'-methyl were presented in Figure 3. Both compounds detected were found to have relatively strong inhibition on platelet-activating factor (PAF) receptor binding *in vitro*, indicating an anti-inflammatory activity, which is favourable in wound healing process as reported by Ling et al. 2007.

Figure 3: HPLC chromatogram of standards, naringenin 4'-methyl ether and aromadendrin 4'-methyl



Naringenin 4'-methyl ether



Aromadendrin 4'-methyl

Screening and selection of high yielding accessions

Ranges of concentration for both chemical constituents in 35 accessions are presented in Table 2. It showed that concentrations of aromadendrin 4'-methyl ether are ranging from 0.50-5.77% while naringenin 4'-methyl ether is ranging from 0.22-1.11% in the leaf samples of *C. odorata*. The results also found that chemical constituent of aromadendrin 4'-methyl ether are having higher concentrations compared to naringenin 4'-methyl ether. Findings of the study are in line with Hung et al. (2011), Johari et al. (2012) and Omukhua (2016) where they reported the same chemical constituents were found in the species of *C. odorata* (L.) R.M. King & H. Rob.

Table 2. Range of concentration of two chemical constituents in 35 accessions of *Chromolaena odorata* leaf extract

Compound	Average Concentration in % Sampel ± RSD (W/W)
Naringenin 4'-methyl ether	0.22-1.11
Aromadendrin 4'-methyl ether	0.50-5.77

Based on the screening results, 14 accessions were identified for having more than average of 0.6% concentration of naringenin 4'-methyl ether chemical constituents, whereas for aromadendrin 4'-methyl ether, a total of 15 accessions which having more than average of 2.23% concentrations were selected as high yielding accessions (Table 3 & 4). Accession JKT22 which is from Kota Tinggi, Johor recorded the highest concentrations for both chemical constituents, naringenin 4'-methyl ether and aromadendrin 4'-methyl ether with 1.11 % and 5.77%, respectively (Figure 4). As of now all the selected accessions were propagated using cutting technique before undergo for the next process which is named as clonal test.

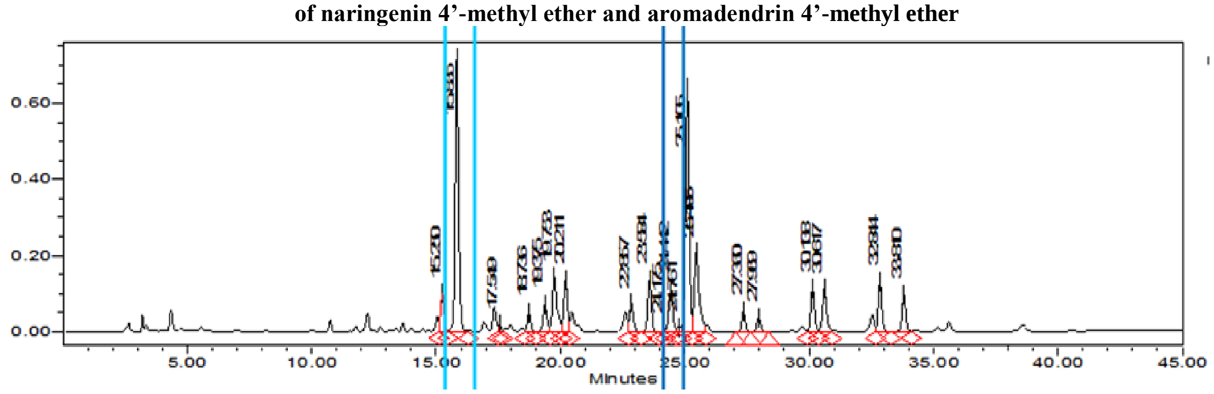
Table 3. Selection of 14 high yielding accessions of *Chromolaena odorata* with high concentrations of naringenin 4'-methyl ether chemical constituent

No.	Accessions Code No.	Average Concentration in % Sampel ± RSD (W/W)
1.	JKT22	1.11 ± 0.01
2.	JKT25	0.91 ± 0.59
3.	CKT5	0.84 ± 0.44
4.	CKT11	0.80 ± 0.80
5.	JKT5	0.77 ± 5.43
6.	JKT20	0.76 ± 4.44
7.	MKT12	0.75 ± 1.72
8.	MKT28	0.75 ± 0.34
9.	MKT29	0.73 ± 1.76
10.	CKT19	0.69 ± 0.61
11.	CKT4	0.67 ± 2.98
12.	MKT9	0.66 ± 5.08
13.	CKT21	0.65 ± 4.78
14.	JKT4	0.62 ± 0.30

Table 4. Selection of 15 high yielding accessions of *Chromolaena odorata* with high concentrations of aromadendrin 4'-methyl ether chemical constituent

No.	Accessions Code No.	Average Concentration in % Sampel ± RSD (W/W)
1.	JKT22	5.77 ± 0.15
2.	CKT19	5.45 ± 0.35
3.	CKT1	4.15 ± 0.04
4.	CKT18	3.37 ± 1.35
5.	MKT26	3.18 ± 0.93
6.	JKT5	3.18 ± 0.72
7.	CKT3	3.12 ± 1.19
8.	CKT10	3.07 ± 1.12
9.	CKT2	2.92 ± 1.34
10.	CKT4	2.66 ± 0.84
11.	JKT2	2.55 ± 0.32
12.	CKT5	2.52 ± 0.36
13.	JKT18	2.48 ± 0.69
14.	CKT21	2.34 ± 0.07
15.	CKT11	2.24 ± 0.73

Figure 4. HPLC chromatogram of accession JKT22 which have the highest concentrations for both chemical constituents of naringenin 4'-methyl ether and aromadendrin 4'-methyl ether



Beside *C. odorata* species, selection of high yielding plants was done previously for other herbal plants in Malaysia such as *Citrus hystrix* (Farah Fazwa et al. 2005) and *Citrus microcarpa* (Farah Fazwa et al. 2007) where the selection was based on the leaf oil chemical contents such as citronellal and linalool. In 2009, Mohamad et al. conducted a study on selection of high yielding on roselle accessions (*Hibiscus sabdariffa*) based on the compound of hidroxycitric acid. Selection of 119 high yielding accessions of *Labisia pumila* based on the yield of total phenolic compound was also successfully conducted by Farah Fazwa et al. (2012). The efforts on producing high and good quality planting materials for the herbal species is important because it can support the herbal industries for using quality raw materials for the development of herbal products. Other than that, response on propagation of the selected species should also be carried out in order to determine the best method for future mass production.

Propagation of selected high yielding accessions through stem cuttings

Analysis of variance on variables taken 7 weeks after planting showed that there was significant difference among the positions of cuttings. The cuttings taken from upper showed higher percentage of rooted cutting and higher survivality than those of middle and bottom part as presented in Table 5. For root length, upper part showed the highest measurement (11.47±1.37cm) while bottom part showed poor performance of root length (4.02±0.91 cm).

Table 5: Effect of cutting position on rooting ability of *Chromolaena odorata* 7 weeks after cutting

Cutting position	Rooted cuttings (%)	Unrooted cuttings (%)	Dead cuttings (%)	Mean root length±SEM (cm)
Top	90.0a	NA	10.0b	11.47±1.37a
Middle	63.3b	NA	36.7a	6.61±1.30b
Bottom	60.0b	NA	40.0a	4.02±0.91b

Means followed by the same letters in the same column are not significantly different at P<0.05. SEM: Standard error of means.

The difference in rooting percentages with the position of cutting could be due to the different degree of juvenility along the stem. This assumption is based on the diameter of cuttings where the top cutting has significantly smaller diameter and more juvenile than the middle and bottom parts. The juvenility of stem reduces from top to bottom thus give different response on the rooting percentage. This finding is in line with Otiende et al. (2016) study on the effects of cutting position (top, middle and bottom) of *Rosa hybrida* rootstocks. Porlingis & Therois (2015) they also found that juvenile cuttings rooted faster and in higher percentages as compared to adult cuttings of leafy olive. Leaf retention could also be a factor that influences the rooting capacity of cuttings. As in Figure 3, the top part has leafy stem cuttings compared to middle and bottom with leafless cuttings. The leaves function as an auxin holder which translocated the auxin to the cutting base and allowing the production of carbohydrates by photosynthesis (Hartmann et al., 2002; Bordin et al., 2005), and acting as a trigger to the developmental process of rhizogenesis (Robert & Friml, 2009). Bona and Biasi (2010) also found the root length and fresh weight of *Lavandula dentata* L. were statistically greater with 2/3 of leaf retention as compared to the leafless cutting. However, excessive leaf area left on the cutting should be avoided as it can cause dehydration on cuttings and affect the rooting formation (Hartmann et al., 2002; Lima et al., 2006).

CONCLUSION

The selected high yielding accessions may provide the plant breeder to use these basic planting materials to initiate a breeding programme for the species. Through selection, plants from different origins can be improved to develop new clone or varieties. The established germplasm is also one of the conservation effort and a method to sustain the production of quality planting materials in the future. In addition, the best propagation method also important in order to mass produce the selected varieties in the future. As conclusion, outputs from the study are not only beneficial to the plant breeder in the aspect of producing new variety but also to the herbal industries in developing high quality products in the market.

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