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INTRASPECIFIC VARIATION of *Flueggea leucopyrus* Willd. GROWN IN SRI LANKA AND ESTABLISHING A SUITABLE PROPAGATION SYSTEM

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ABSTRACT

Flueggea leucopyrus belongs to family Phyllanthaceae, commonly known as 'Katupila'or 'Heen katupila' and has become increasingly popular as an ingredient in several traditional therapeutic systems in Sri Lanka as well as in other Asian countries. A questionnaire survey was carried out using a hundred population sample to determine the awareness of the distribution and therapeutic uses of *F. leucopyrus* in Sri Lanka as a preliminary survey. The aims of the present study were to interpret the phenetic variation and determine the variation of phenolic composition in leaf extracts of *F. leucopyrus* in different climatic zones in Sri Lanka and to develop a suitable propagation system for *F. leucopyrus.* Eleven floral and vegetative characters of *F. leucopyrus* were observed and cluster analysis was carried out to infer phenetic relationships among populations collected from localities, Colombo, Kurunegala, Puttalam and Hambanthota, .representing different climatic zones. Medicinal value of most of plants lies in phytochemicals such as phenolics, flavonoids and anthocyanidins. Thus, leaf samples were extracted into ether, and ether extracts were used to isolate phenolic compounds using 2-Dimensional paper chromatography in solvent systems, butanol: acetic acid: water; 6:7:3 and sodium formate: formic acid: water; 10:1:200. Colors of the spots were observed under UV (366nm), and R_f values were calculated. Intra specific variations were observed in the distribution of phenolics in leaves and phenetic characters of *F. leucopyrus* with respect to their habitats

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in different climatic zones. Due to low seed production in *F. leucopyrus*, stem cuttings (Semi-hardwood, hardwood and softwood) in three different potting media were used to evaluate the successfulness in propagation. Semi hardwood and hardwood cuttings of *F. leucopyrus* were well established within a period of one week in sand potting medium while softwood cutting has not responded in any of the media during the period. Medium with only coir dust was also not successful in establishing the cuttings.

Keywords:*Flueggea leucopyrus,* Phenetic variation, Leaf phenolics, Propagation system

INTRODUCTION

Flueggea (Vern: Katupila) is a genus belongs to family Phyllanthaceae and represented by a single species, *F. leucopyrus,* in Sri Lanka. *F. leucopyrus* is distributed in throughout tropical and subtropical areas such as Saudi Arabia, India, Sri Lanka and China and has recorded in different climatic zones of Sri Lanka. *F. leucopyrus* is a thorny shrub with spiny branch ends; leaves simple, alternate; flowers small in clusters, male greenish yellow, female reddish and fruits globose, fleshy berries (Dassanayake *et al.,* 1997).

F. leucopyrus is commonly used as an ingredient in several traditional therapeutic systems in Sri Lanka as well as in other countries. *F. leucopyrus* is mostly used as a remedy for burning sensation, leaves act as a disinfectant and it has anticancer properties (Ramanayake *et al.,* 2002). The whole plant of *F. leucopyrus* is used in medicine for seminal weakness and as a disinfectant due to high antioxidant activity. *F. leucopyrus* has less anthocyanin compounds (0.34/100g), high phenolic compounds (31.7/100g) and flavonoid compounds (42.7/100g), and high antioxidant activity with the rate of 76.8 µg/ml (Karuppusamy *et al.,* 2011).

Medicinal value of most of plants lies in phytochemicals such as phenolics, flavonoids and anthocyanidins. Plant phenolics are a large groups of natural compounds widely distributed in variety of plants and these compounds have antioxidant properties with potential health benefits. Antioxidant compounds play a crucial role in the prevention of chronic diseases such as heart disease and cancer (Subhashini *et al.,* 2010).

Phenolic compounds are frequently occur combined with sugar as glycosides and they are located in cell vacuoles. A successful two-dimensional system to separate phenolic compounds is using solvents, butanol: acetic acid: water; 6:7:3 and sodium formate: formic acid: water; 10:1:100 for acid hydrolysis carried out with 2M HCl for half

an hour, followed by cooling and filtering before the extraction of compounds (Harborne, 1998).

According to previous records, initiation of germination of *Flueggea* seeds has shown very slow rate, begins germination 20 days after sowing (Hanson *et. al.*, 2005). Hence, further experiments are needed to be carried out to establish suitable vegetative propagation methods and to develop seed dormancy breaking methods.

Leaf morphology is of major significance for studies on ecological variation in plant species. Leaf size and shape in the same species may change due to the different environmental conditions (Migalina *et. al.*, 2010).

Quality and quantity of active constituents accumulated in medicinal plants are highly affected by climatic conditions. Climatic changes will results in several stress effects on the plants and amount of the phenolic compounds can rise significantly as multifunctional antioxidants due to the stress effect (Sarosi *et. al.*, 2011; Vallejo *et. al.*, 2003).

Therefore, objectives of this research were to interpret the phenetic variation and determine the phenolic composition in leaf extracts of *F. leucopyrus* in different climatic zones in Sri Lanka and development of suitable propagation system for *F. leucopyrus.*

METHODOLOGY

Questionnaire Survey

A structured questionnaire survey was carried out to identify the medicinal uses and to study the distribution of *F. leucopyrus* in Sri Lanka. This survey was carried-out using a hundred population sample including native doctors, ayurvedic practitioners, general public, drug manufacturers and ayurveda pharmacies in different districts in Sri Lanka (Colombo, Gampaha, Kandy, Anuradapura, Polonnaruwa, Puttalam, Kurunagale, Hambanthota, Kegalle and Ratnapura). The selection of study sites was determined according to the information gathered from the questionnaire survey and also to represent different climatic zones in Sri Lanka.

Phenetic Variation of *F. leucopyrus* in Different Climatic Zones in Sri Lanka

Phenetic variation was assessed using available morphological characters of *F. leucopyrus* samples obtained from districts of Colombo, Kurunegala, Puttalam and Hambantota representing different climatic zones. Eleven morphological characters were selected to interpret phenetic relationships. PAST (PAleontological STatistics) software

package was used for cluster analysis to determine the phenetic variations and relationships of *F. leucopyrus* grown in different climatic zones in Sri Lanka.

Distribution Pattern of Phenolic Composition in Leaves of *F. leucopyrus* in Different Climatic Zones in Sri Lanka

Two-dimensional paper chromatography technique was used to determine the phenolic composition in leaf extracts of *F. leucopyrus* in different climatic zones in Sri Lanka. Phenolic compounds in leaves of each individual sample were extracted to ether and the distribution of compounds was observed using 2-D-Paper chromatography (Figure 1).

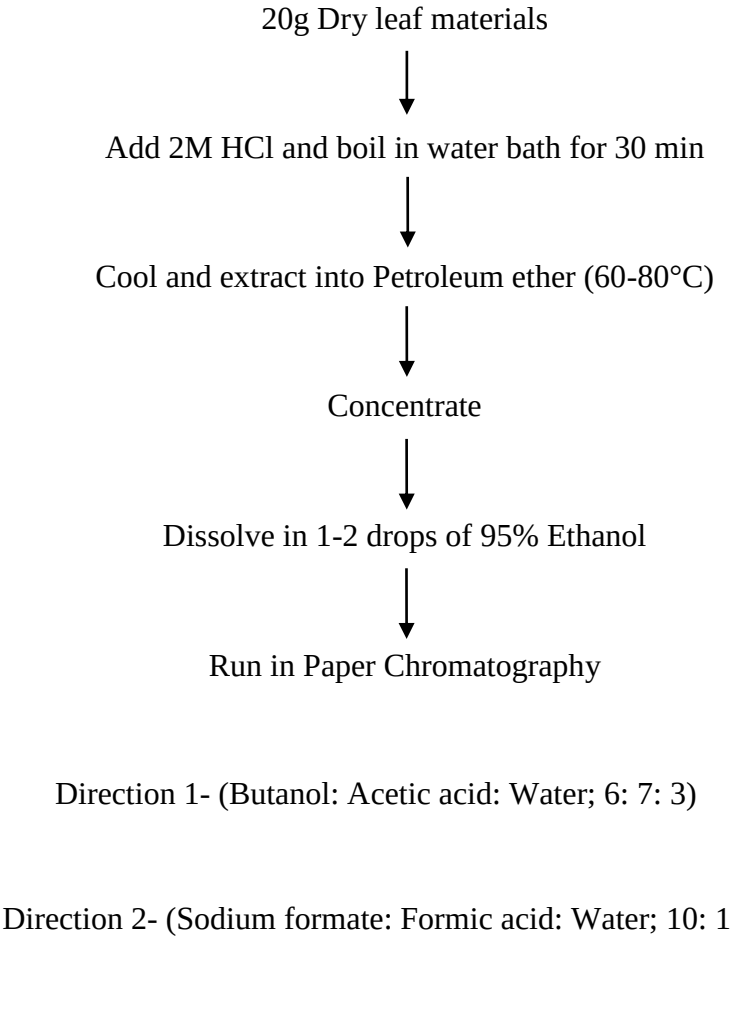

Figure 1: Steps of chemical analysis of the plant material for the phenolic compounds (Harborne, 1998).

The different colour spots developed in chromatography papers were observed under UV (366 nm range) for identification. Color of the spots and Rf values were used in the cluster analysis to evaluate the variation. Phenograms were drawn based on the analysis of the observed characters to interpret the intra specific variations of *F. leucopyrus* in different climatic zones in Sri Lanka.

Establishment of suitable propagation systems for *F. leucopyrus*

Stem cutting method was used to develop suitable propagation system for *F. leucopyrus.* Soft wood, semi hard wood and hard wood cuttings were planted in three different potting media {Coir dust, Sand and Coir dust: Sand (1:1)}. Then establishment of these stem cuttings in different potting media were determined by observing leaf bud formation after one week. One-way analysis of variance (ANOVA; $P < 0{\cdot}05$) was performed to compare the effectiveness of the treatments. If ANOVA has shown significant effects and Turkey's comparison test was used to determine the differences in performance among treatments.

RESULTS AND DISCUSSION

Questionnaire Survey

According to the findings of socio-economical survey, sixty three percent (63%) of individuals (native doctors, general public and ayurvedic practitioners) out of a hundred population sample have shown awareness in therapeutic uses and distribution of *F. leucopyrus* in Sri Lanka. Distribution of *F. leucopyrus* was found to be common in the dry zone compared to the wet zone of Sri Lanka. Most of the users are of the opinion that best ingredient for traditional therapeutic systems can be obtained from the plants grown in the dry zone. Naturally grown *F. leucopyrus* in fifteen different localities in districts of Colombo, Kurunegala, Puttalam and Hambanthota were selected as study sites.

Environmental and climatic conditions, with respect to the data obtained from Meteorology Department of Sri Lanka, of the above selected sites were compared during the period of specimen collection. According to the data, the four districts were experiencing a mean annual temperature around 28°C, and however the mean annual rainfall showed a significant variation (Hambantota in 2012- 107.9 mm, Puttalam in 2013- 50.2 mm, Kurunegala in 2012- 156. 5 mm and Colombo in 2012- 205.4 mm).

(a) Puttalam

(b) Colombo

c) Hambantota

(d) Kurunegala

Figure 2: Phenetic variations of *F. leucopyrus* in different climatic zones in Sri Lanka

Cluster Analysis of Phenetic Characters of *F. Leucopyrus* Species in Different Climatic Zones in Sri Lanka

Eleven phenetic characters (Figure 2 and Table 1) of *F. leucopyrus* were used to construct Phenogram I (Figure 3) and it indicated intraspecific variations in individuals of *F. leucopyrus* collected from different climatic zones in Sri Lanka. *F. leucopyrus* species in dry zone were closely similar to the specimens of intermediate zone compared to the wet zone.

Table 1: Phenetic charcters of *F. leucopyrus* in different climatic zones in Sri Lanka

Character	Observations in different districts			
	Colombo	**Kurunegala**	**Puttalam**	**Hambantota**
Habit	Shrub	Shrub	Shrub	Shrub
Stem height (m)	1 to 4	1	1 to 4	4
Number of branches in stem	2	3	5	4
Length of spines (cm)	1	1	2	3
Bark color	Green	Green	Grey	Grey
Leaf shape	Oblong	Oblong	Oblong	Oblong
Leaf base	Round, Emarginate	Round	Round, Emaginate	Round
Leaf margin	Entire	Entire	Entire	Entire
Leaf appearance	Green	Green	Green color with shiny appearance	Green color with shiny appearance
Leaf length (cm)	2	1	1	3
Leaf width (cm)	0.5	0.5	0.5	1.0

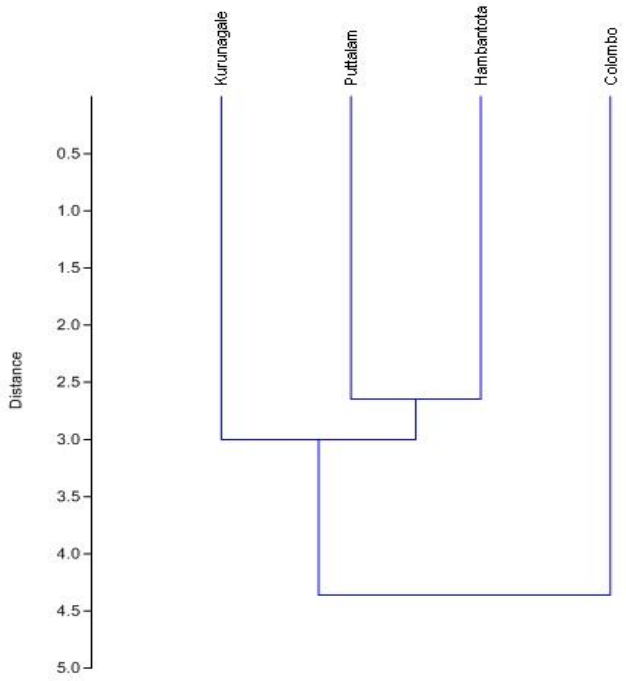

Figure 3: Phenogram I, - Distribution patterns of Phenetic characters of *F. leucopyrus* collected from different climatic zones in Sri Lanka

Distribution Pattern of Phenolic Composition In Leaves of *F. leucopyrus* in Different Climatic Zones in Sri Lanka

Phenolic compounds in leaves of *F. leucopyrus* collected from different climatic zones were used to construct the Phenogram II (Figure 4) and it indicated the presence of intra specific variations in *F. leucopyrus* individuals in different climatic zones in Sri Lanka.

Establishment of Suitable Propagation System for *F. leucopyrus*

One way ANOVA $p<0.05$ and based on Turkey's pairwise comparison with 95% confidence concluded that the semi hard wood and hard wood stem cuttings of *F. leucopyrus* have established well in sand potting medium after one week (Figure 5). Softwood cuttings were not shown any indication of the initiation of establishing in all three media during the period. Medium with only coir dust was also not successful in establishing the three types of cuttings used in the study.

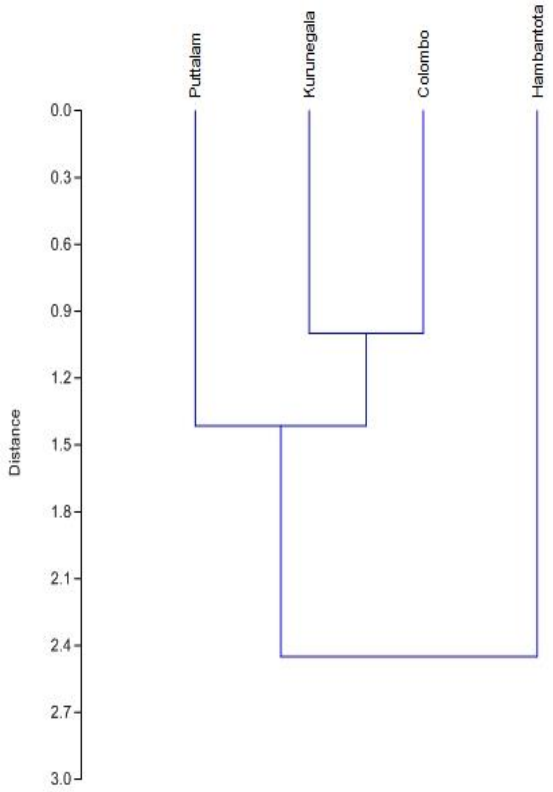

Figure 4: Phenogram II, - Distribution patterns of phenolic compounds in leaves of *F. leucopyrus* collected from different climatic zones in Sri Lanka

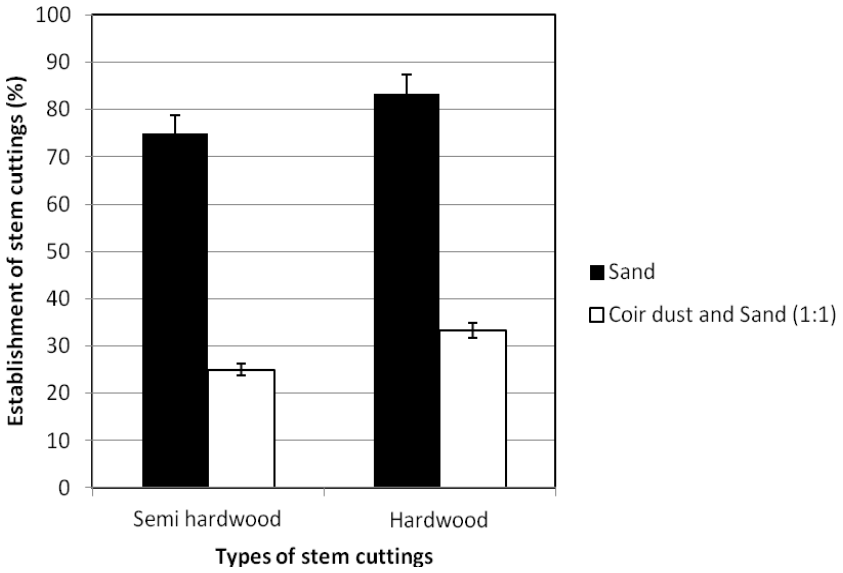

Figure 5: Establishment of stem cuttings of *F. leucopyrus* in different potting media after a week

CONCLUSIONS

Phenograms developed using the morphometrics and leaf phenolic composition of individuals of *F. leucopyrus* representing different climatic zones have clearly shown intra specific variations in the morphology and the distribution of phenolics in leaves. Further elucidation of phytochemicals is needed to determine the effectiveness of ecotypic variation in the efficacy of leaves of *F. leucopyrus* used in preparation of therapeutic systems.

Semi-hardwood and hardwood cuttings of *F. leucopyrus* established well within a period of one week in sand potting medium. This information can be used to establish suitable propagation system for *F. leucopyrus* for commercial cultivation.

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