Tropical Agricultural Research Vol. 28 (3) : 270– 280 (2017)

Comparative Study on Major Chemical Constituents in Volatile Oil of True Cinnamon (*Cinnamomum verum* Presl. syn. *C. zeylanicum* Blum.) and Five Wild Cinnamon Species Grown in Sri Lanka

T. Liyanage*, T. Madhujith[1] and K.G.G. Wijesinghe[2]

Postgraduate Institute of Agriculture
University of Peradeniya
Sri Lanka

ABSTRACT. *The genus Cinnamomum has 250 species distributed from South, East and South East Asia to Australia. There are eight species of cinnamon grown in Sri Lanka, of which, Cinnamomum verum Pres1 (syn. C. zeylanicum Blume) which is widely cultivated in the country. In addition, seven other species of wild cinnamon are also grown which are endemic to Sri Lanka which are considered wild cinnamon.* In this study, six *Cinnamomum* species were selected namely *Cinnamomum verum*, *Cinnamomum dubium* Nees (Sinhala: *Sewel Kurundu* or *Wal Kurundu*), *Cinnamomum citriodorum* (Sinhala: Pangiri Kurundu), *Cinnamomum rivulorum* Kostermans, *Cinnamomum sinharajense* Kostermans, *Cinnamomum capparu-corende* (Sinhala: Kapuru Kurundu). *Much work has been carried out with Cinnamomum verum, however, work on wild cinnamon varieties is scanty. In this backdrop, this study was carried out to identify and quantify the major chemical constituents of volatile oils obtained from bark and leaves of wild and true cinnamon species cultivated or grown in Sri Lanka. It was revealed that leaf oil of Cinnamomum verum contains significantly ($p<0.05$) higher percentage of volatile oil (3.23%). The highest bark oil content (3.53%) was observed in Cinnamomum sinharajense, the lowest leaf oil (0.41%) and stem bark oil (0.51%) contents were observed in the Cinnamomum rivulorum.* Fifteen major volatile chemical constituents were identified by Liquid Gas Chromatographic analysis in the essential oils obtained from bark and leaf of different cinnamon species. *The highest cinnamaldehyde content (67.57%) was observed in Cinnamomum verum. Cinnamomum sinharajense contained comparatively higher amount of cinnamaldehyde (57.46%) than the other wild cinnamon species. The highest euginol content (87.53%) was observed in Cinnamomum sinharajense leaf.* When compared with the chemical composition of *Cinnamomum dubium* leaf oil, geraniol was the most abundant (24.05%) among the volatile chemical constituents. β-Caryophyllene (41.31%) was identified in *Cinnamomum dubium* stem bark oil. The highest Euginol (22.29%) content was present in essential oil obtained from *Cinnamomum rivulorum* stem bark which was not observed in other cinnamon species.

Keywords: *Cinnamomum*, Essential oil, true cinnamon, wild cinnamon.

[1] Department of Food Science and Technology, Faculty of Agriculture, University of Peradeniya, Sri Lanka
[2] National Cinnamon Research and Training Center, Palolpitiya, Thihagoda, Sri Lanka
* Corresponding Author: thushariphtd@yahoo.com

INTRODUCTION

The genus *Cinnamomum* consists of nearly 250 species distributed in the South, East and South East Asia and Australia (Kostermans, 1995). *Cinnamomum verum* (Pres1) (syn. *Cinnamomum zeylanicum* Blume) also known as true Cinnamon is one of the important spice crops cultivated in Sri Lanka. In addition to *Cinnamomum verum*, genus *Cinnamomum* includes seven other important wild species of Cinnamon (SLS 81, 2010) namely *Cinnamomum dubium* Nees (Sinhala: *Sewel Kurundu* or *Wal Kurundu*), *Cinnamomum ovalifolium* Wight, *Cinnamomum litseafolium* Thwaites (Sinhala: Kudu Kurundu), *Cinnamomum citriodorum* (Sinhala:Pangiri Kurundu), *Cinnamomum rivulorum* Kostermans, *Cinnamomum sinharajense* Kostermans, *Cinnamomum capparu-corende* Blume (Sinhala: Kapuru Kurundu) which are present in Sri Lanka (Sritharan, 1984). *Cinnamomum verum* is an evergreen tree of 10-15 m tall¸ native to Sri Lanka. It is the main commercially cultivated cinnamon species while the other species of *Cinnamomum* are reported to have medicinal and ethno-botanical values (Kumarathilake *et al*, 2010, Abesinghe *et al,* 2014). In many instances, very little distinction has been made between the bark oil of true cinnamon *(Cinnamomum verum* syn. *C. zeylanicum*) obtained from different locations of Sri Lanka (Grurusinghe *et al.*, 1990).

Sri Lanka has the comparative advantage with regard to the cinnamon market due to its intrinsic quality (Gurusinghe *et al.,* 1990). Sri Lanka exports cinnamon to many countries in the world. *viz* Mexico, India, USA, and Europe being the major buyers. The total value of exports of cinnamon was amounted to be Rs. 19099 US$ million in 2015 (Central Bank of Sri Lanka, 2015). Wild cinnamon species are known to carry a secondary gene pool of cultivated cinnamon, therefore, conservation of the secondary gene pool is important. Apart from that, most of these wild cinnamon species have been used for medicinal purposes having high ethno-botanical value. However, the information on the chemical constituents of endemic or wild cinnamon in Sri Lanka is very scanty.

Information on chemical constituents of volatile oil in wild relatives and true cinnamon are very useful for breeding programmes of cultivated cinnamon, identification of economic importance of wild cinnamon species, conservation programmes of wild relatives with the objective of producing value added products from wild cinnamon and sustainable utilization of wild cinnamon species. The volatile constituents of wild relatives of cinnamon grown in Sri Lanka have not been systematically studied to date. In this backdrop, the objective of the current study was to identify and quantify the major chemical constituents of volatile oils obtained from bark and leaves of wild and true cinnamon species cultivated or grown in Sri Lanka.

MATERIALS AND METHODS

Materials
Plant material

Matured leaf and bark samples were collected from randomly selected plants of *Cinnamomum* species, namely *Cinnamomum verum*, *Cinnamomum dubium* Nees (Sinhala: *Sewel Kurundu* or *Wal Kurundu*), *Cinnamomum citriodorum* (Sinhala: Pangiri Kurundu), *Cinnamomum rivulorum* Kostermans, *Cinnamomum sinharajense* Kostermans, *Cinnamomum capparu-corende* Blume (Sinhala: Kapuru Kurundu) cultivated at the National Cinnamon Research and Training Center (NCRTC), Palolpitiya, Matara, Sri Lanka. The plants were selected from among the cinnamon trees cultivated in germplasm collection. Leaf and bark samples were collected from six *Cinnamomum* species. Leaf samples were obtained from all above species while the stem bark samples were obtained from all above species except from *Cinnamomum capparu-corende* due to non-availability of adequate plants at the time of collection.

Chemicals

Toluene (Analytical grade) was purchased from Breckland Scientific Supplies, United Kindom. Authentic standards of cinnamaldehyde, euginol, a-pinene, myrcene, linalool, β caryophyllene, citronellol, geraniol, hydro cinnamic aldehyde, cinamyl acetate, methyl cinnamate, acetyl eugenol, cinnamyl alcohol were purchased from Sigma Chemicals Company Limited, United State of America.

Determination of Moisture Content

The moisture content of cinnamon leaf and bark samples was determined using the distillation method. The dried cinnamon leaf and bark samples were ground and 5.00 g of sample was transferred into a 250 mL round bottom flask containing toluene to immerse the cinnamon sample completely. The flask was kept on a heating mental and the apparatus was assembled and the trap was also filled with toluene until it flowed into the flask. The flask was heated for 4 h until no change occurred in the moisture level (American Spice Trade Association, 1997). The flasks were allowed to cool down to ambient temperature and the volume of moisture in the trap was recorded. Moisture content was calculated as a percentage.

Extraction of leaf and bark essential oil

The leaf samples of *Cinnamomum* species were separately dried under shade over 5 days. The air-dried 50 g of leaf sample (50 g) was placed in a 500 mL round bottom flask, distilled water was added into the flask up to 50% of its volume and positioned on a heating mantel. The flask was connected to Clevenger apparatus and hydro-distillation process was carried out for 5 h to obtain cinnamon leaf oils.

Cinnamon bark samples were chopped into small pieces and allowed to air dry under shade over 5 days. The dried cinnamon bark samples were ground using an analytical cooling grinder and approximately 30 g of ground sample was placed in a 250 mL round bottom flask. The cinnamon bark oil was extracted through hydro-distillation as explained above.
Distilled cinnamon leaf and bark oils were separately collected into pre-weighed glass vials and the weight of oil collected was calculated. Essential oil content of each sample was determined on dry weight basis using the moisture content (ASTA Method No 5.0, 1997). The volatile oils obtained as explained above were stored in glass vials at 5 ^{0}C until analysis.

Determination of colour and odour characteristics of cinnamon bark and leaf oils

The colour and odour of cinnamon oils obtained were determined by 30 untrained panelists and the colour and odour characteristics were recorded.

Determination of the chemical composition of essential oils of cinnamon species

Shimadzu GC -8A Gas liquid Chromatograph equipped with Flame Ionization Detector (FID) carrying 3 × 3 mm stainless steel column packed with 10% Carbowax 20M on Chromsorb WAW 40/80 mesh with argon carrier gas was employed for the determination of volatile chemical constituents. The injector and detector temperatures were set at 60 °C and 230 °C, respectively. Extracted essential oil sample of 0.3 µl of was injected using a manual syringe with a programme rate of 4 ^{0}C min^{-1}. Area percentages for the components were obtained with a data recorder (Shimadzu cooperation, 1987). The determinations were carried out in triplicate.

The essential oil samples were injected 0.5 µL in duplicates and peak were recorded using different stationary phases, retention times for the individual components were first obtained and compared with these of authentic samples. Peak enrichment techniques were used to identify the constituents. The data collected at each particular time period were analyzed using the software "SAS" system LSD was used to compare means at significance level of 5%.

RESULTS AND DISCUSSION

Comparative leaf and stem bark oil content of true cinnamon and wild cinnamon species

The yield of leaf and stem bark oils of true and wild cinnamon species was calculated on dry weight basis. It was observed that the cinnamon species varied significantly in their stem bark oil as well as leaf oil content.

Table 1: Average leaf and bark oil contents of cinnamon species

Species	Percentage leaf oil content on dry weight basis	Percentage bark oil content on dry weight basis
Cinnamomum verum	*3.26^{a}*	3.06^{b}
Cinnamomum dubium	0.86^{d}	1.55^{c}
Cinnamomum rivulorum	0.43^{e}	0.51^{e}
Cinnamomum sinharajense	2.41^{b}	3.53^{a}
Cinnamomum citriodorum	0.92^{d}	0.82^{d}
Cinnamomum capparu- corende	1.49^{c}	n.d.

n.d.- Not detected
Means with the same letters are not significantly different at $p<0.05$ (LSD = 0.063 and LSD = 0.1146).

The highest leaf oil yield (3.26%) was observed with *Cinnamomum verum* (Table 1). *Cinnamomum rivulorum* leaves showed the lowest oil yield (0.43%) ($p<0.05$). Among investigated wild relatives of cinnamon, *Cinnamomum sinharajense* exhibited higher leaf and stem bark oil. The significantly ($p<0.05$) highest content of stem bark oil (3.53%) was observed in *Cinnamomum sinharajense* while the lowest stem bark oil yield (0.51%) was observed in *Cinnamomum rivulorum*.

Colour and odour characteristics of bark and leaf oil of *Cinnamomum* species

Hydro-distillation of the bark and leaf of *Cinnamomum* species gave different colour and odour characteristics. *Cinnamomum verum* leaf and bark oil were golden in colour while the colour of other species was pale yellow. Pleasant cinnamon like odour was observed with *Cinnamomum verum* as well as with *Cinnamomum citriodorum* bark oil. *Cinnamomum citriodorum* leaf oil exhibited a pleasant strong citronella grass oil like odour. Pleasant geranium like odour was observed in *Cinnamomum dubium* leaf oil (Table 2).

Table 2: Odour characteristics of stem bark and leaf oils of different *Cinnamomum* species

Species name	Leaf oil	Bark oil
Cinnamomum verum	Pleasant Clove like odour	Pleasant Cinnamon like odour
Cinnamomum dubium	Pleasant geranium like odour	Pleasant
Cinnamomum rivulorum	Slightly unpleasant clove like	Pleasant Clove like odour
Cinnamomum sinharajense	Slightly unpleasant	Pleasant
Cinnamomum citriodorum	Pleasant strong Citronella grass oil like odour	Pleasant Cinnamon like odour
Cinnamomum capparu-corende	Pleasant	--

Cinnamaldehyde and Euginol content in stem bark oil of C*innamomum* species

Most important chemical constituents in cinnamon industry are cinnamaldehyde and euginol. The cinnamaldehyde and euginol content in stem bark oil of different cinnamon species are shown in Fig. 1.The highest cinnamaldehyde content (67.57%) was found in *Cinnamomum verum.* This observation is quite comparable with Paranagama *et al.* (2001). The highest euginol content (22.29%) was detected in *Cinnamomum rivulorum* bark oil while the highest cinnamaldehyde content (57.46%) was detected in *Cinnamomum sinharajense*. The lowest euginol content (0.7%) was observed in *Cinnamomum sinharajense* among the four wild cinnamon species. *Cinnamomum dubium* was shown lowest cinnamaldehyde content (0.86 %) (Fig. 1).

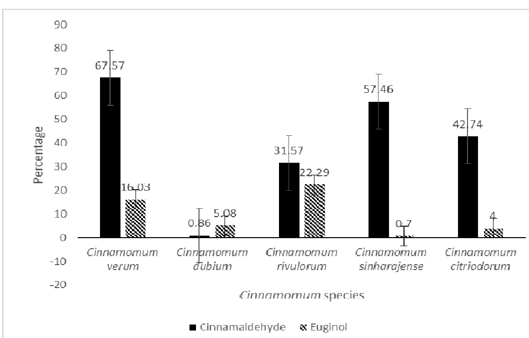

Fig. 1. Cinnamaldehyde and Euginol content in stem bark oil of five different *Cinnamomum* species

Cinnamaldehyde and Euginol content of leaf oil of *Cinnamomum* species

Euginol and cinnamaldehyde content of *Cinnamomum* leaf oil are presented in Fig. 2. The highest euginol content of leaf oil was observed in *Cinnamomum sinharajense* (87.53%). The euginol content of true cinnamon leaf (86.28%) was comparable with the values reported by Paranagama *et al.* (2001). Cinnamaldehyde percentage of leaf oil was comparatively less than (<3%) bark oil of any *Cinnamomum* species tested.

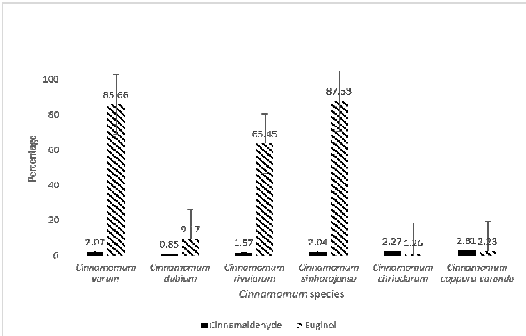

Fig. 2: Euginol and cinnamaldehyde content in leaf oil of six *Cinnamomum* Species

Other major constituents in *Cinnamomum* species of bark and leaf oils

The major constituents, identified by GC analysis in the essential oils of cinnamon bark and leaf oils are listed in Table 3. Fifteen major volatile chemical constituents were identified in cinnamon bark and leaf oil of different species.

Table 3:Major volatile Chemical constituents of bark and leaf oils of *Cinnamomum species*

	Percentage of chemical constituents											
	Cinnamomum verum		*Cinnamomum dubium*		*Cinnamomum rivulorum*		*Cinnamomumsinharajense*		*Cinnamomumcitriodorum*		*Cinnamomum capparu-corende*	
Compound Name	Leaf oil	Bark oil	Leaf oil	Bark oil	Leaf oil	Bark oil	Leaf oil	Bark oil	Leaf oil	Bark oil	Leaf oil	Bark oil
α -Pinene	0.37	5.76	4.04	nd	3.17	nd	nd	nd	nd	nd	nd	na
Myrcene	0.76	1.38	nd	nd	nd	nd	nd	nd	nd	nd	nd	na
Linalool	0.97	3.78	nd	nd	nd	nd	nd	nd	30.71	8.94	4.35	na
β Caryophyllene	1.08	3.66	5.60	41.31	1.24	8.21	1.04	4.54	1.46	3.56	17.31	na
Citronellol	nd	nd	nd	nd	nd	nd	nd	nd	2.44	nd	nd	na
Geraniol	nd	nd	24.05	3.86	2.06	7.76	nd	nd	0.92	19.95	5.54	na
Unknown	nd	nd	nd	nd	nd	nd	nd	nd	38.05	nd	15.63	na
Hydro cinnamic aldehyde	nd	nd	4.30	7.70	nd	nd	nd	nd	nd	nd	nd	na
Cinnamaldehyde	2.07	67.57	0.85	0.86	1.57	31.57	2.04	57.46	2.27	42.74	2.81	na
Cinamyl acetate	nd	nd	nd	nd	nd	nd	nd	13.69	3.20	1.07	2.50	na
Methyl cinnamate	nd	nd	2.48	1.94	nd	nd	nd	nd	nd	3.52	4.49	na
Eugenol	85.66	16.03	9.17	5.08	63.45	22.29	87.53	0.7	1.26	4.0	2.23	na
Acetyl eugenol	6.07	nd	nd	1.02	nd	nd	nd	nd	nd	nd	nd	na
Cinnamyl alcohol	nd	nd	15.65	8.61	nd	nd	1.50	0.58	5.36	nd	5.76	na

nd: Not detected na: Not available

When compared to chemical composition of *Cinnamomum dubium* leaf oil, geraniol was the most abundant (24.05%) among the volatile chemical constituents (Table 3). β - Caryophyllene (41.31%) was observed in *Cinnamomum dubium* stem bark oil. (Table 3). β-caryophyllene, is a natural bicyclic sesquiterpene which is a constituent of many essential oils. Several biological activities are attributed to β -caryophyllene, such as anti-inflammatory, antibiotic, antioxidant, anti-carcinogenic and local an aesthetic activities. Therefore, *Cinnamomum dubium* stem bark and leaf oil chemical composition is different from other wild species.

The odour and chemical composition of essential oil of *Cinnamomum sinharajense* leaf and stem bark much close to true cinnamon stem bark and leaf oils. (Table 3). The Cinnamyl acetate content of essential oil of *Cinnamomum sinharajense* stem bark was 13.69%. (Table 3). The highest Euginol (22.29%) content was determined in essential oil of *Cinnamomum rivulorum stem* bark. It is not observed in other cinnamon species. (Table 3). *Cinnamomum citriodorum* oil odour is much close to Lemongrass oil or Citronella oil odour. One of the major chemical constituent (38.05%) was not identified in leaf oil of *Cinnamomum citriodorum.* Linalool (30.71 %) and Citronellol (2.44 %) were determined as other major chemical compounds in leaf oil of *Cinnamomum citriodorum*. Geraniol (19.95 %) was observed in stem bark oil of *Cinnamomum citriodorum* (Table 3). *Cinnamomum capparu-corende* this species leaf oil odour was much closed to clove oil or nutmeg oil odour. Other major chemicals are β- Caryophyllene (17.31 %), Geraniol (5.54 %), Unknown (15.63 %) (Table 3).

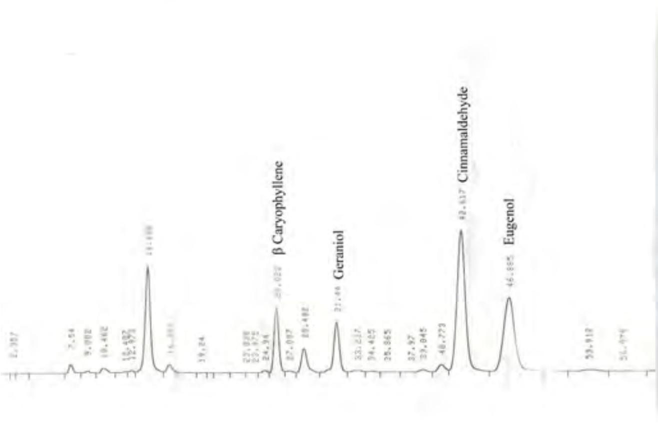

Fig. 4. GLC analysis of *Cinnamomum rivilorum* bark oil

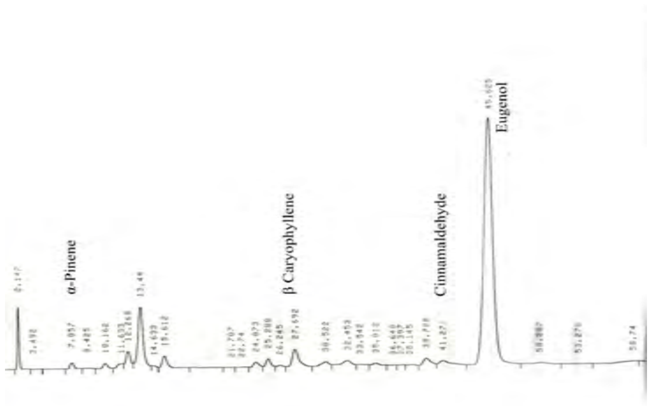

Fig. 5. GLC analysis of *Cinnamomum rivilorum* leaf oil

CONCLUSION

The highest leaf oil content was found in the *Cinnamomum rivulorum (*3.26 %) while the highest bark oil content was observed in the *Cinnamomum sinharajense* (3.53%). The lowest leaf oil (0.43%) and stem bark oil content (0.51%) were observed in the *Cinnamomum rivulorum*. The highest cinnamaldehyde content was observed in *Cinnamomum verum* (67.57%). The lowest Cinnamaldehyde content was observed in *Cinnamomum dubium* (0.86%). *Cinnamomum sinharajense* has comparatively high amount of cinnamaldehyde (57.46%) than other wild cinnamon species. Highest Euginol content (87.53%) was detected in *Cinnamomum sinharajense* leaf. Comparatively Euginol content (85.66%) of true Cinnamon leaf was less than *Cinnamomum sinharajense* leaf. When compared to chemical composition of *Cinnamomum dubium* leaf oil, geraniol was the most abundant (24.05%) among the volatile chemical constituents. β-Caryophyllene (41.31%) was observed in *Cinnamomum dubium* stem bark oil. The highest Euginol (22.29%) content was determined in essential oil of *Cinnamomum rivulorum stem* bark. It is not observed in other cinnamon species.The essential oil content and chemical composition of stem bark as well as leaf oil is widely different among the *Cinnamomum* species available in Sri Lanka.

ACKNOWLEDGEMENT

The authors wish to express their sincere thanks to Dr. H.A. Sumanasena (Director, Research), Mr. E.D.K. Edirisinghe (Senior Technological Officer) and A.D.D Roshika (Research and Development officer) of The Central Research Station, Department of Export Agriculture, Sri Lanka.

REFERENCES

Abeysinghe, P.D., Samarajeewa, N.G.C.D., Li G., Wijesinghe K.G.G. (2014). Preliminary investigation for the identification of Sri Lankan *Cinnamomum* species using randomly ampliedpolumorphic DNA (RAPD) and sequene related amplified polymorphic (SRAP) markers *Journal of National Science .Foundation of Sri Lanka 42(3): 201-208*

Official Analytical Methods of the American Spices Trade Association (1997), ASTA Method No.2.0, Fourth Edition (Revised. 2011).

Official Analytical Methods of the American Spices Trade Association (1997), ASTA Method No 5.0, Fourth Edition, 1997. (Revised 2013)

Central Bank, Annual Report (2015), Central Bank of Sri Lanka, 30, Janadhipathi Mawatha, Colombo 01, Sri Lanka: Statistical appendix table 73.

Grurusinghe, P. de A. Senanayake, M., Perera, K. P. and Premakumara, K.V. S. (1990) Study on quality characteristics of cinnamon grown in Sri Lanka. *Journal of Agricultural Sciences*. 27: 22-27.

Indrasena, I.K., Induruwa, I.V.A.D.C.S., Liyanage, T., Wijesinghe, G. and Edirisinghe, E. D. K. (2010). Comparative assessment of oil and coumarin content in bark of cassia (*Cinnamomum cassia*) and true cinnamon (*Cinnamomum zeylanicum*) Prodeeding of International symposium University of Ruhuna Sri Lanka. part 1: 26.

Kostermans, A.J.G.H. (1995). Lauraceae *A Revised Handbook to the Flora of Ceylon* 112-129. (Eds. M.D. Dasanayake, F. Fosberg and W.D. Clayton) volume ix Amerind Publishing Co. Pvt. Ltd. New Delhi, India.

Kumarathilake, D.M.H.C., Senanayake, S.G.J.N., Wijesundera, D.S.A., Wijesekara, G.A.W. and Ranawaka, R.A.A.K. (2010). Extinction Risk Assessments at the Species Level: National Red List Status of Endemic Wild Cinnamon Species in Sri Lanka. *Tropical Agricultural Research*. *21(3):* 247 - 257

Paranagama, P. (1990). "Analysis of Sri Lankan essential oils by Gas Chromatography & Mass Spectroscopy," A Thesis Submitted to the University of Kelaniya, Published by U.M. Senanayake Industrial Technology Institute, pp. 23-24.

Paranagama, P.A., Wimalasena, S., Jayathilake, G.S., Jayawardena, A.L., Senanayake, U.M. and Mubarak, A.M. (2001). A comparison of essential oil constituents of bark, leaf, root and fruit of Cinnamon (*Cinnamomum zeylanicum* (Blum.)) grown in Sri Lanka. *Journal of National Science Foundation, Sri Lanka 29(3 and 4):* 147-153.

Purseglove, J.W., Brown, E.G., Green, C.L. and Robins, S.R.J. (1981). Longman Inc., New York, Spices, vol. 1:

Senanayake, U.M., Edwards R.A. and Lee T.H., (1976). Cinnamon. *Food technology in Australia. 28, No.9*: 333-338

Sri Lanka standard Institute (2010). SLS 81: 2010. Specification for Ceylon Cinnamon, Fourth Revision: 3-3.

Sritharan, R. (1984). The study of genus Cinnamon, (Unpublished postgraduate report), Post graduate Institute of Agriculture, Peradeniya, Sri Lanka pp. 2-25.

Wijesinghe, K.G.G. and Pathirana, R. (2000). Germplasm screening and varietal development of cinnamon (*Cinnamomum verum* Presl.) *In:* Senarathne, R. and Dayathilake, D.A. (Eds). Revitalization of Cinnamon Industry in Sri Lanka, Premadasa Printers, Matara, Sri Lanka: 15 - 17.