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Identification of marker compounds and antioxidant activity of *Terminalia chebula Retz.* fruit pericarps used in selected commercial herbal preparations in Sri Lanka

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Abstract

Medicinal plants contain phyto-constituents which show pharmacological effects. This study is focused on identification and quantification of marker compounds and determination of the antioxidant activity of *T. chebula* Retz. fruit pericarps used in selected commercial herbal preparations in Sri Lanka. Commercial samples were obtained from Sri Lanka (SL_C) and India (IN_C), separately from three different batches of *T. chebula* stocks from raw material quarantine section at Link Natural Products (Pvt) Ltd. As these commercial samples are a mixture of fruits from wider geographical locations, five samples (SL_A) collected from known locations in Sri Lanka were included for comparison. Methanolic extracts (70 % v/v) were prepared from each sample. Chromatographic profiling was done using thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC). Extracts were assayed for gallic acid content, total tannin content and antioxidant activity. Gallic acid and ellagic acid could be used as marker compounds in quality control of *T. chebula* commercial stocks. All samples had a low IC_{50} value than butylated hydroxytoluene (BHT) standard showing that *T. chebula* fruit pericarps have higher antioxidant activity than BHT. Variations in IC_{50} values were observed within and among SL_A, SL_C and IN_C suggesting that both intrinsic and extrinsic factors may lead to the change in antioxidant potential of the fruits. The mean IC_{50} value of SL_C samples was (6.32 ± 2.09) µg/mL whereas that of IN_C samples was (7.42 ± 0.93) µg/mL suggesting that antioxidant activity was higher in SL_C samples over IN_C samples. A variation in antioxidant activity in SL_A samples was observed, depending on the sampling site.

Keywords

Antioxidants, Gallic acid, Polyphenols, Tannin, *Terminalia chebula*.

Introduction

The demand for the herbal drugs is getting popularized day by day all over the world as they are cheap and natural in origin with less side effects (Naik et al., 2004). Several hundred genera of plants have been used in traditional medicine since ancient times (Gupta, 2012). This study is focused on one such plant species, *Terminalia chebula* Retz, belonging to family Combretaceae, and referred to as 'Aralu' in Sinhala. The plant is native to Asia and found mainly in the dry zone of Sri Lanka (Dassanayake & Fosberg, 1981; Gupta, 2012). The dried fruit pericarp is used in the preparation of many ayurvedic drugs such as Thriphala, Arishta, Asava and Churna. It contains numerous phytochemicals such as tannins, flavonoids, sterols, terpenoids, fixed oils and amino acids. Latest studies have reported that *T. chebula* contain more phenolic compounds compared to the plants studied (Gupta, 2012; Saleem et al., 2002). It also possesses a wide spectrum of pharmacological properties such as antimicrobial, antioxidant, antidiabetic, retinoprotective, immunomodulatory, anti-carcinogenic, anti-arthritic and wound healing. leading to non-toxic therapeutic value of the fruit (Gupta, 2012; Juang et al., 2004; Riaz

et al., 2017). *T. chebula* is a key ingredient in herbal drugs used for the treatment of conditions resulting from oxidative stress caused due to excess reactive oxygen species in cellular environments (Chen et al., 2011; Naik et al., 2004; Naik et al., 2005).

Ayurvedic drug manufacturers in Sri Lanka obtain *T. chebula* dried fruits from both local and Indian suppliers for their herbal preparations. These commercial suppliers obtain fruits from different collectors irrespective of the geographical region or the processing conditions. Hence, the *T. chebula* fruits used in herbal preparation is a mixture of fruits only to be identified as Indian and Sri Lankan origin. Although, many previous studies are reported on the antioxidant and other bioactivities of *T. chebula* fruits from a specific origin, herbal drug manufactures cannot totally rely on those data for their quality control purposes, as their raw materials used in herbal preparations are a mixture of fruits of unknown origin. Hence this study aimed at determining and comparing the antioxidant activity of commercial stocks of *T. chebula*.

Materials and Methods

Materials

Authentic samples (SL_A) of *T. chebula* fresh fruits were collected from the plant itself by onsite visits to five different localities (Bibila, Buththala, Padiyathalawa, Gampaha and Colombo) of Sri Lanka and authenticated against a voucher specimen (Reference Number: LNP/HB/F-COM/TC-01) available at the Herbarium at Link Natural Products Ltd. (LNP). Commercial samples were obtained separately from three different batches of *T. chebula* from Sri Lanka (SL_C) and India (IN_C) at LNP. All solvents and chemicals used were of AR grade and photochemical standards for chromatographic analysis were supplied by Research and development center at Link Natural Products (Pvt) Ltd.

Methods

Dried fruit pericarps of SL_A, SL_C and IN_C samples were ground and sieved through 710 # mesh to make a homogenized coarse powder and were extracted in 70 % v/v methanol. TLC was developed using ethyl formate, toluene, formic acid, water in the ratio of 30:1.5:4:3 (v/v/v/v) as the mobile phase and visualized after spraying with freshly prepared ferric chloride (1% w/v) (Wagner & Bladt, 1996). High performance liquid chromatography (HPLC) was carried out using 1260 Agilent Infinity II HPLC system with reverse phase. Glacial acetic acid (1% v/v) in milli Q water and acetonitrile were used as mobile phase in a gradient mode. The total tannin content of each dried extract was estimated using Folin-Denis assay (Association of Official Analytical Chemists, 1980) with slight modifications. Briefly Folin-Denis reagent (5.00 mL) and saturated Na_2CO_3 solution (10.00 mL) were added respectively into a 100 mL volumetric flask with aqueous stock solution of crude extract (0.01 % w/v, 10.00 mL). The flask was topped up to the 100 mL mark using distilled water. These solutions were gently mixed and incubated in the dark for 30 min at room temperature. Then the absorbances of the resulting solutions were measured at 760 nm with the UV-Visible spectrophotometer (8453 Agilent). The tannin concentration of prepared sample solution was obtained using the regression equation of the standard calibration curve. Total tannin content of *T. chebula* dried fruit pericarps was calculated as a percentage on dry basis. Antioxidant activity was determined using the DPPH radical scavenging assay described by Chatatikun & Chiabchalard (2013) with slight modifications. Briefly methanolic DPPH solutions (0.634 mM, 40 µL) were added to different concentrations of test solution (1.56,

3.13, 6.25, 12.50, 25.00, 50.00, 100.00 μg/mL) in a 96-well plate. These solutions were gently mixed and incubated in the dark for 20 min at room temperature. BHT was used as the standard. Absorbance of the resulting solutions were measured at 517 nm with the microplate reader (Thermo Scientific™ Multiskan™ FC). The % inhibition was calculated and plotted against the test concentrations to obtain the IC_{50} value. The IC_{50} value was calculated using GraphPad Prism 7.00 Statistic Software.

Results and Discussion

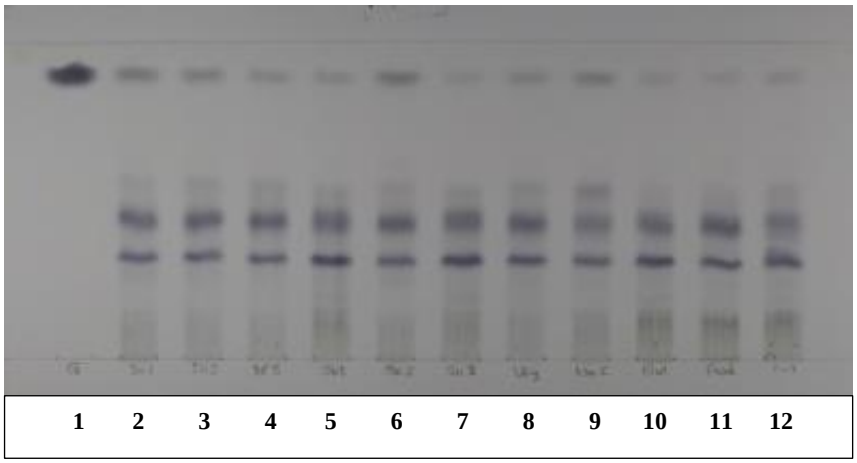


Figure 1. *TLC profile of crude extracts of T. chebula after spraying with 1 % aqueous ferric chloride reagent and observed under white light*

Polyphenolic compounds in *T. chebula* fruits react with ferric chloride and give blackish blue color spots on TLC plate (Figure 1). Gallic acid standard was used to identify marker compound in crude extracts. All the samples contained gallic acid. TLC fingerprints of SL_A, SL_C and IN_C samples showed a similar pattern with slight variation in the intensity of the corresponding spots.

Presence of gallic acid (GA) and ellagic acids (EA) were shown in HPLC fingerprints of all samples (Figure 2 and 3). Hence these two acids can be used as marker compounds in quality control of *T. chebula* commercial stocks. GA was quantified by determining the area under GA peak. GA and total tannin (TT) content is significantly different ($P>0.05$) among SL_A depending on the geographical area (Table 1) and in SL_C and IN_C depending on the batch number (Table 2). IN_C showed higher content of GA than SL_C whereas TT content is higher in SL_C than in IN_C (Table 2). GA content can be increased by improper long storage of the *T. chebula* fruits as other complex polyphenolic compounds can be converted into gallic acid during this storage time. Previous studies have reported *T. chebula* with 32% TT content (Chattopadhyay & Bhattacharyya, 2007; Saha & Verma, 2016). But, our results show higher TT contents than this value for all the samples showing our samples are rich in tannins.

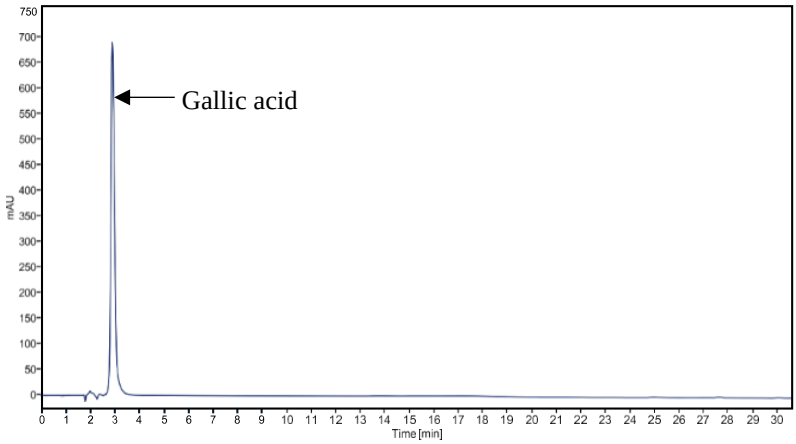
Table 1. *GA and TT content of SL_A*

Sample type	GA % (w/w)* (on dry basis)	Total tannin % (w/w)* (on dry basis)
SL_A - Padhiyathalawa	0.49 ± 0.01d	33.40 ± 0.17d
SL_A - Buththala	0.98 ± 0.01b	43.39 ± 0.41a
SL_A - Gampaha	1.03 ± 0.02b	41.13 ± 0.61c
SL_A - Bibila	0.83 ± 0.02c	42.31 ± 0.23b
SL_A - Colombo	1.86 ± 0.04a	34.12 ± 0.01d

Table 2. *GA and TT content of SL_C and IN_C*

Sample type	GA % (w/w)* (On dry basis)	Total tannin % (w/w)* (On dry basis)
SL_{C1}	1.42 ± 0.02a	51.54 ± 0.14b
SL_{C2}	0.87 ± 0.11c	42.22 ± 0.25c
SL_{C3}	1.10 ± 0.06b	53.67 ± 0.34a
IN_{C1}	$2.97 \pm 0.049a^1$	$43.57 \pm 0.11a^1$
IN_{C2}	$2.18 \pm 0.034b^1$	$42.05 \pm 0.54b^1$
IN_{C3}	$1.59 \pm 0.003c^1$	$42.76 \pm 0.03b^1$
SL_C	1.13 ± 0.28	49.14 ± 6.09
IN_C	2.25 ± 0.69	42.79 ± 0.76

**Results were analyzed statistically at 95 % level (P>0.05) using ANOVA. Each data point represents mean ± SD where n=3; SL_C: mean of SL_{C1-3}, IN_C: mean of IN_{C1-3}.*



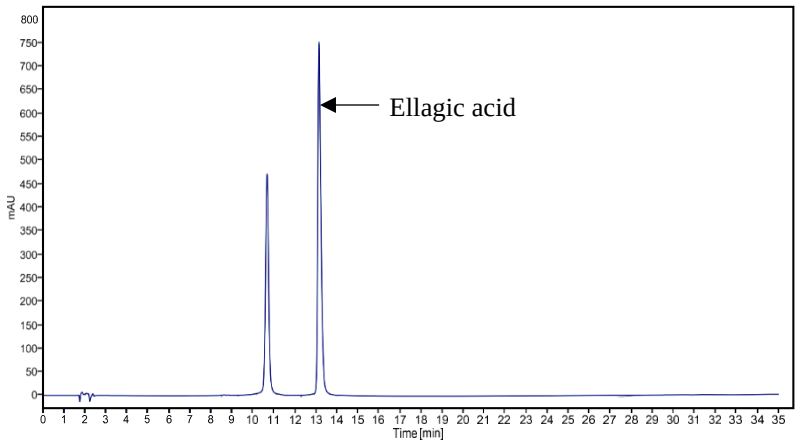


Figure 2. *HPLC fingerprint of GA and EA*

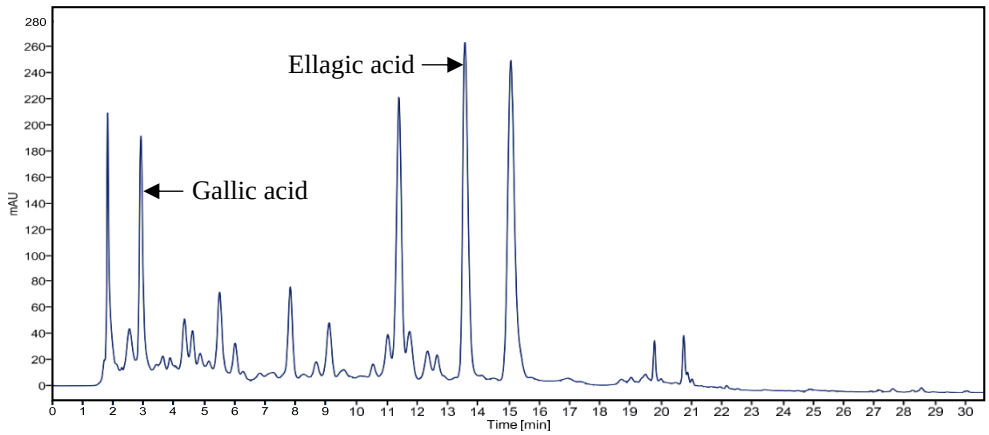


Figure 3. *HPLC fingerprint of IN_{C1}*

Tannins are phenolic compounds which contribute to the antioxidant properties [12]. Hence, the radical scavenging activity of *T. chebula* was tested.

Table 3. *IC_{50} of SL_A, SL_C and IN_C*

Sample	**IC_{50} (µg/mL)***
BHT	39.28 ± 1.68
SL_A - Padhiyathalawa	6.26 ± 0.42
SL_A - Buththala	9.42 ± 0.66
SL_A - Gampaha	7.36 ± 0.54
SL_A - Bibila	8.04 ± 0.68
SL_A - Colombo	7.45 ± 0.49

Sample	**IC_{50} (µg/mL)***
BHT	39.28 ± 1.68
SL_{C1}	8.73 ± 0.53
SL_{C2}	5.18 ± 0.48
SL_{C3}	5.06 ± 0.49
IN_{C1}	8.11 ± 0.59
IN_{C2}	7.78 ± 0.59
IN_{C3}	6.36 ± 0.51
SL_C	6.32 ± 2.09
IN_C	7.42 ± 0.93

**Each data point represents mean ± SD where n=3; SL_C: mean of SL_{C1-3}, IN_C: mean of IN_{C1-3}*

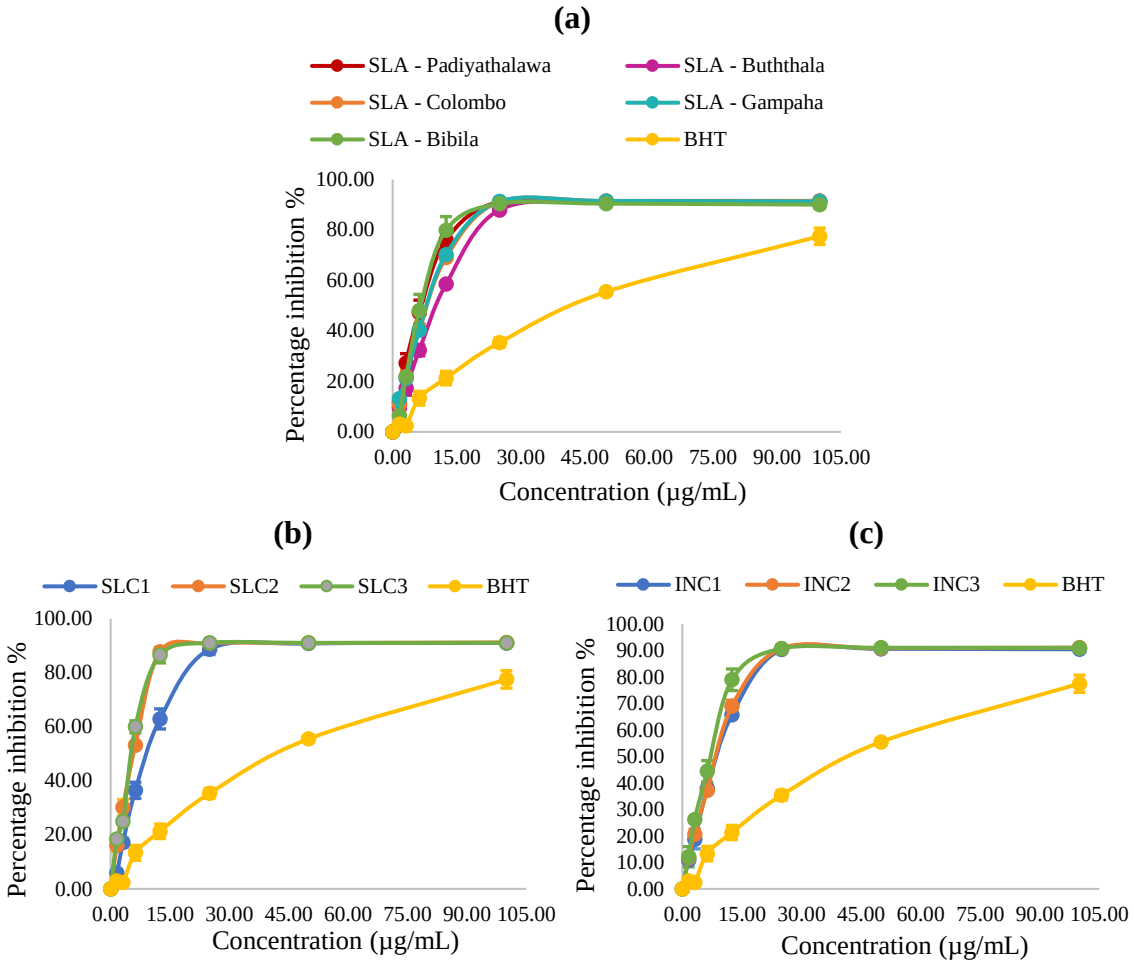


Figure 4. *DPPH radical scavenging activity of (a) SL_A (b) SL_C and (c) IN_C*

All the samples of *T. chebula* showed higher radical scavenging activity than BHT. Slight variations in IC_{50} values were seen among SL_A, SL_C and IN_C samples (Figure 4). The mean IC_{50} value of SL_C samples was lower than the mean IC_{50} value of IN_C samples, showing a higher antioxidant activity in *T. chebula* than in Indian varieties. Previously *T. chebula* fruits showing IC_{50} of 14 ± 0.05 µg/mL is reported (Saha & Verma, 2016). Our results showed lower IC_{50} values for all the samples than this value showing a higher antioxidant activity. Variation of antioxidant activity among themselves and within SL_A, SL_C and IN_C could be attributed to both intrinsic and extrinsic factors. Genetic factors, maturity stage, growth conditions, soil conditions, geographical location, climatic zone (localities in Colombo and Gampaha are in wet zone and Padiyathalawa, Bibila and Buththala are in the dry zone of Sri Lanka), climatic conditions and raw material processing conditions (drying process) affect the ultimate composition of phytoconstituents. In addition, the raw material storage time may also be a contributing factor towards the variations observed in SL_C and IN_C.

Conclusion

Commercial *T. chebula* stocks used in selected herbal preparations are rich in polyphenolic compounds and possess very high antioxidant activity. It may benefit consumers beyond the targeted therapeutic value of these herbal preparations. Commercial samples of Sri Lanka possess higher antioxidant activity than those from India. Gallic acid and ellagic acid could be used as marker compounds in the quality

control of *T. chebula* commercial stocks. However, the study could be expanded to identify and quantify other marker compounds of *T. chebula* dried fruit pericarps. Biological assays are of paramount importance to evaluate the pharmacological properties of medicinal plants. Hence, more biological assays such as anti-microbial, anti-inflammatory, anti-cancer are also recommended to *T. chebula* fruits.

References

Association of Official Analytical Chemists. (1980). *Official method of analysis of the AOAC* (13th ed.). Washington, D.C., 158.

Chatatikun, M., & Chiabchalard A. (2013). Phytochemical screening and free radical scavenging activities of an orange baby carrot and carrot (*Daucus carota* Linn.) root crude extracts. *Journal of Chemical and Pharmaceutical Research*, *5*(4), 97-102.

Chattopadhyay, R. R., & Bhattacharyya, S. K. (2007). Plant review *Terminalia chebula*: an update. *Pharmacognosy Reviews*, *1*(1), 151-156.

Chen, X., Sun, F., Ma, L., Wang, J., Qin, H., & Du, G. (2011). In vitro evaluation on the antioxidant capacity of triethylchebulate, an aglycone from *Terminalia chebula* Retz fruit. *Indian Journal of Pharmacology*, *43*(3), 320-323.

Dassanayake, M. D., & Fosberg, F. R. (1981). *A revised handbook to the flora of Ceylon* (Vol. 2, pp. 38-42). Amerind Publishing Co.

Gupta, P. C. (2012). Biological and pharmacological properties of *Terminalia chebula* Retz. (Haritaki) - an overview. *International Journal of Pharmacy and Pharmaceutical Science*, *4*(3), 62-68.

Juang, L., Sheu, S., & Lin, T. (2004). Determination of hydrolysable tannins in the fruit of *Terminalia chebula* Retz. by high-performance liquid chromatography and capillary electrophoresis. *Journal of Separation Science*, *27*(9), 718-724.

Naik, G. H., Priyadarsini, K. I., Naik, D. B., Gangabhagirathi, R., & Mohan, H. (2004). Studies on the aqueous extract of *Terminalia chebula* as a potent antioxidant and a probable radio protector. *Phytomedicine*, *11*(6), 530-538.

Naik, G. H., Priyadarsini, K. I., Bhagirathi, R. G., Mishra, B., Mishra, K. P., Banavalikar, M. M., & Mohan, H. (2005). In vitro antioxidant studies and free radical reactions of triphala, an ayurvedic formulation and its constituents. *Journal of Phytotherapy Research*, *19*(7), 582-586.

Riaz, M., Khan, O., Sherkheli, M. A., Khan, M. Q., & Rashid, R. (2017). Chemical constituents of *Terminalia chebula*. *Natural Products Indian Journal*, *13*(2), 112.

Saha, S., & Verma, R. J. (2016). Antioxidant activity of polyphenolic extract of Terminalia chebula Retzius fruits. *Journal of Taibah University for Science*, *10*(4), 805-812.

Saleem, A., Husheem, M., Harkonen, P., & Pihlaja, K. (2002). Inhibition of cancer cell growth by crude extract and the phenolics of *Terminalia chebula* Retz. fruit. *Journal of Ethnopharmacology*, *81*(3), 327-336.

Wagner, H., & Bladt, S. (1996). *Plant drug analysis - a thin layer chromatography atlas* (2nd ed.). Germany.