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Short Communication

Phytochemicals and antioxidant capacity of selected Rutaceae species used in Sri Lanka

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Abstract

Pas-pangiri is a collection of five different Rutaceae species known as *Citrus aurantium* Linn, *Citrus aurantifolia* (Christm. & Panzer) Swingle, *Citrius sinensis* Linn, *Atalantica ceylanica* (Am.) Oliver and *Citrus reticulata* Blanco. *They exhibit many beneficial effects such as anti-bacterial,anti-fungal, antiseptic, anti-inflammatory, etc. Even though these Rutaceae species have extensively used in different medicinal systems, scientific information on phytochemical contents and antioxidant capacity are scattered or lacking. Hence, scientific experiments were done to investigate the phytochemicals and antioxidant potential of these five Rutaceae species grown in Sri Lanka. Both aqueous leaf extracts and leaf oil of all five Rutaceae species were subjected to Thin Layer Chromatographic (TLC) analysis and phytochemical screening using standard protocols. In addition, total phenolic content, total flavonoid content and DPPH free radical scavenging ability were investigated. Preliminary phytochemical screening revealed that presence of phenols, flavonoids, tannin, saponins, steroid glycosides, coumarins, terpenoid and alkaloids in all tested plant species except A. ceylanica. TLC fingerprint profiles of C. sinensis and C. reticulata are similar to each other. However, TLC fingerprint profiles of other tested Rutaceae species were different to each other. Further, C. reticulata exhibited the highest amount of phenols and flavonoids followed by C. aurantifolia, C. aurantium, C. sinensis and A. ceylanica respectively. Accordingly, best free radical scavenging ability was also exhibited with C. reticulata and lowest ability was exhibited with A. ceylanica. Therefore, tested Rutaceae species can be utilized in pharmaceutical and food industries in future.*

Keywords: Antioxidants, Chromatography, *Pas-pangiri*, Phytochemicals, Rutaceae species.

Introduction

Sri Lanka has many medicinal systems including Ayurveda, Traditional, Unani and Siddha which fulfils about 60-70% of the population's health care needs[1]. The Ayurveda system practised in Sri Lanka was introduced along with the Vijayan invasion in the fifth century before Christ[2]. Thereafter, this great knowledge is transmitted from generation to generation with modifications, alternations and so many other developments. Plant based medicines are not only used in Ayurveda, but also used in Siddha, Unani, Deshiya Chikithsa and Traditional medicinal systems of Sri Lanka[3]. Five Rutaceae species are collectively known as *Pas –pangiri* in Sri Lanka (Figure-1). They are *Citrus aurantium* Linn, *Citrus aurantifolia* (Christm. & Panzer) Swingle, *Citriussinensis* Linn, *Atalantica ceylanica* (Am.) Oliver and *Citrus reticulata* Blanco. *Pas –pangiri* is used to cure several kinds of diseases and disorders such as cataract, earaches, cold, headache, stomach ailments, edema, fever, sore throat, chest pain and liver diseases[2].

Scientific investigations have shown that wound healing, antimicrobial, anti-nociceptive, and anti-inflammatory, antioxidantand anthelmintic properties of Rutaceae species[4]. Further many research work have been carried out for Rutaceae species grown in Asian countries including India, Pakistan, and Bangladesh compared to that of Sri Lanka. However, it is clearly understood that phytochemicals and bio-activities of plant/s mainly depends on its growth factors such as soil, climate, nutrients etc. Therefore, experiments were taken to examine the phytochemicals and antioxidant potential of selected five Rutaceae species collectively known as *Pas-pangiri* in Sri Lanka.

Materials and methods

Plant materials: Leaves were taken from previously authenticated plants maintained under same soil and climatic conditions during February 2018 – March 2018. Leaves of the collected Rutaceae species were cleaned with running water separately, cut into small pieces, rinsed with distilled water and air dried for two to three days.

Hot water extracts of Rutaceae species: Leaves (100g) of five Rutaceae species were taken separately, cut into small pieces, kept in a round bottom containing 500mL of water and refluxed (5h). Then filtered, concentrated the filtrate and subjected to freeze drying.

Finally, freeze dried extracts were labelled separately and kept in a refrigerator (at 4°C) until further analysis.

Essential oils of Rutaceae species: Leaves of each plant material (100g) was placed in a round bottom flask containing water (500ml) and subjected to hydro-distillation (5h) using a Clevenger apparatus (EM 0250, Electrothermal.UK) separately. Essential oils accumulated in the apparatus were separated respectively, measured, dried over anhydrous sodium sulphate and stored in a refrigerator in dark until further analysis.

Phytochemical analysis: Distilled water (20mL) was added to each (5g) freeze dried powder and subjected for phytochemical screening as described in Table-1[5].

Development of Thin Layer Chromatography (TLC) fingerprints: TLCs of hot water leaf extract and leaf oil of Rutaceae species were conducted separately. Before spotting, TLC plates were left for activation by drying in the oven at 45 ^{o}C for 2-3h.

Spotting procedure: Each freeze dried powder (1g) of hot water extract was dissolved in distilled water (5ml) and spotted (10µl) on the TLC plate. Each leaf oil of Rutaceae species was directly spotted (10µl) on the TLC plate.

TLC plate spotted with hot water extracts of Rutaceae species was kept in a glass chamber containing a solvent mixture of dichloromethane, ethyl acetate and cyclohexane (8:1.5:2.3v/v) and TLC plate spotted with leaf oils of Rutaceae species was kept in a glass chamber containing a solvent mixture of dichloromethane and cyclohexane (4:0.5v/v).

Quantification of phenols and flavonoids: Quantification of phenols[6] and flavonoids[7] were estimated according to spectrophotometric methods.

DPPH scavenging assay: A 100µl of DPPH solution (200mg in 100mL methanol) was mixed with 50µl of each sample at five various concentrations and 90µl of methanol was added to each well, shaken, incubated in the dark for 10 minutes at room temperature (25±2^{o}C) and absorbance (at 517nm) was taken using a spectrophotometer (Spectramax plus 384, Sunnyvale, USA). Same procedure was followed to the control (without any plant extract). Finally, % of radical scavenging activity and IC_{50} values were calculated.

Statistical comparison: Data were presented as Mean ± Standard Error Mean. Statistical analysis were done by one-way variance (ANOVA), following Tukey's HSD post hoc test ($p \leq 0.05$).

Results and discussion

Results of preliminary phytochemical analysis revealed that except *A. ceylanica* all other tested spices of plant family Rutaceae presence of several bioactive compounds such as phenols, tannis, flavonoids, steroid glycosides, coumarins, alkaloids, terpenoids and saponins. However, steroid glycosides, alkaloids, terpenoids and saponins were absent in *A. ceylanica* (Table-2)[9]. The presence or absence of phytochemicals can be varied from plant to plant even within same species.

Analysis of Rutaceae species grown in Pakistan revealed the presence of phenols, flavonoids, and terpenoids. However, phytochemicals such as alkaloids, saponins, and glycosides and steroids were not detected in *C. aurantifolia*[10]. These variations may be due to the differences in climatic conditions, soil conditions and geographical location[11]. This may be reason for the dissimilarities of the findings of Rauf et al.[10] with present work. Generally, phytochemicals are known to exhibit many therapeutic activities such as anti- inflammatory, antimicrobial, antihypertensive and anti-diabetic effects[12,13]. Therefore, present study has revealed that tested Rutaceae species are rich in therapeutic activities.

TLC is used to identify plant ingredients in herbal medicines as it is a simple, low cost, versatile and specific method. The TLC fingerprint is unique to particular plant or plant based product and has the potential to clarify authenticity of chemical constituent/s. TLC fingerprints of hot water extracts of Rutaceae species are given in Figure-2. Interestingly, TLC fingerprint profiles (both hot water extracts and essential oils) of *C. sinensis* and *C. reticulata* are similar to each other. However, TLC fingerprint profiles of other tested Rutaceae species were different to each other. Among the tested Rutaceae species, *C. reticulata* contained highest amounts of phenols and flavonoids followed by *C. aurantifolia, C. aurantium, C. sinensis* and *A. ceylanica* (Table-3) respectively.

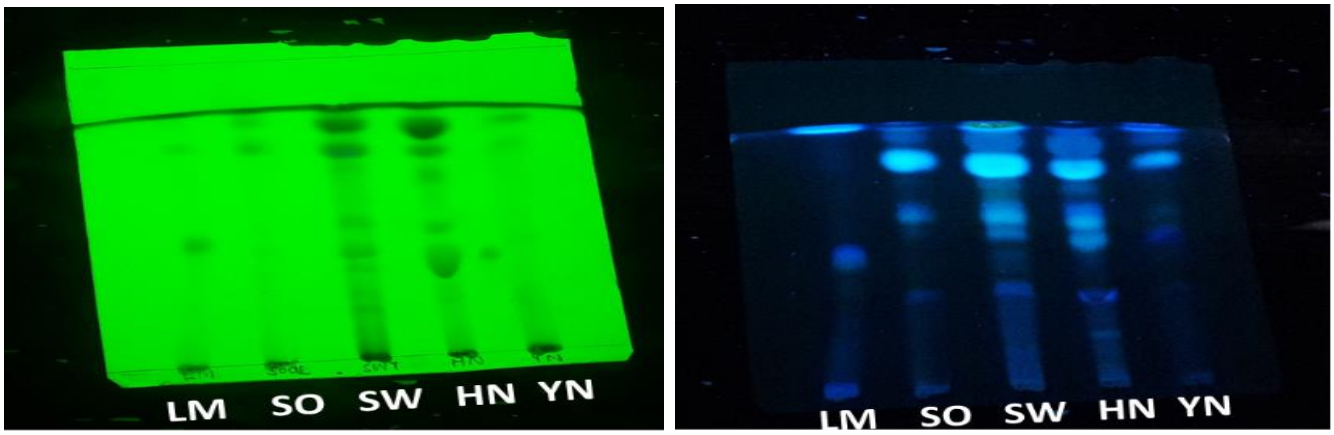
Phenols and flavonoids are found to be important chemical constituents for plant growth, reproduction, as hormone modulators, cardiovascular diseases, certain types of cancers, diabetes, inflammation, etc)[14,15]. A stable yellow colour 1,1-diphenyl-2-picrylhydrazine is formed when DPPH radical reacts with a free radical scavenger or hydrogen donor. The values of % radical scavenging activity and IC_{50} obtained for DPPH assay are given in Table-4.

The scavenging potentials of the antioxidants present in the extracts indicate the degree of discoloration of the reaction mixture. The results indicate that hydrogen donating ability of Rutaceae species were comparatively high. These values are in agreement with previous research work[16,17]. As shown in Table-4, the highest percentage free radical inhibition has been expressed by *C. reticulata* followed by *C. aurantifolia, C. aurantium* and *C. sinensis* while *A. ceylanica* has shown comparatively less percentage inhibition. Presence of high amounts of phenolic and flavonoids compounds in *C. reticulata* may be the reason for this. Similar results were reported with *C. reticulata* grown in India[11].

Conclusion

In the present study, attempts were made to reveal the phytochemical composition and antioxidant capacity of Rutaceae species grown in Sri Lanka. The findings of this study clearly demonstrate that Rutaceae species possess pharmacologically important phytochemical compounds and potent antioxidant properties which validate the therapeutic value of them in traditional medicine. Among the tested Rutaceae species, *C. reticulata* can be selected as a promising candidate for pharmaceutical and food industry.

Figure-1: Five selected Rutaceae species found in Sri Lanka: (A) *Citrus aurantifolia*, (B) *Citrus aurantium*, (C) *Citrus sinensis*, (4) *Citrus reticulata*, (5) *Atalantia ceylanica*.



(a) Under 254nm (b) Under 366nm

Figure-2: Thin Layer Fingerprint Profies of hot water extracts of Rutaceae spiecies (a) under 254nm and (b) 366nm *Citrus aurantifolia* (LM) *Citrus aurantium* (SO) *Citrus sinensis* (SW) *Citrus reticulata* (HN) *Atlantica ceylanica* (YN).

Table-1: Tests for qualitative phytochemical analysis.

Phytochemical compound	Name of the test performed	Observation
Phenols	Ferric chloride (5%) test	Blue/green colour precipitate
Tannins	Ferric chloride (3%) test	Blackish blue precipitate
Flavonoids	Alkaline reagent test	Intense yellow colour formation
Steroid glycosides	Concentrate H_2SO_4 test	Brown ring colouration at the interphase
Coumarins	NaOH test	Yellow colour formation
Alkaloids	Mayer's test	White colour precipitate
	Wagner's test	Brown/reddish colour precipitate
	Tannic acid test	Yellow crystalline precipitate
Terpenoids	Salkowski test	Appearance of reddish brown colour
Saponins	Foam test	Stable persistent froth

Table-2: Phytochemical screening of selected Rutaceae species.

	C.aurantifolia	*C.aurantium*	*C.sinensis*	*C.reticulata*	*A.ceylanica*
Phenols	+	+	+	+	+
Tannins	+	+	+	+	+
Flavonoids	+	+	+	+	+
Steroid glycosides	+	+	-	+	-
Coumarins	+	+	+	+	+
Alkaloids	+	+	+	+	-
Terpenoids	+	+	+	+	-
Saponins	+	+	+	+	-

(+) denotes the presence and (–) denotes the absence of respective class of compounds.

Table-3: Quantification of phenols and flavonoids of selected Rutaceae species.

Plant species	Total phenolic content expressed as µg of Gallic Acid Equivalents /mg of dry weight	Total flavonoids content expressed as µg of Quercetin Equivalents /mg of dry weight
C. aurantifolia	138.17 ± 4.56^{b}	64.26 ± 2.14^{b}
C. aurantium	129.23 ± 2.94^{c}	56.98 ± 1.92^{c}
C. sinensis	56.43 ± 3.78^{d}	41.43 ± 2.87^{d}
C. reticulata	189.11 ± 2.45^{a}	82.16 ± 1.24^{a}
A. ceylanica	14.87 ± 1.89^{e}	16.48 ± 1.63^{e}

Data expressed as Mean ± SEM, n=4, ^{a,b,c,d,e} Significant differences (in terms of phenolic content and flavonoid content) between the each plant species were indicated by alphabetical superscripts.

Table-4: Antioxidant activity of selected Rutaceae species.

Plant species	% Radical scavenging activity	IC_{50} value (µg/mL)
C. aurantifolia	52.1^{b}	108.3 ± 2.96^{c}
C. aurantium	51.6^{c}	131.2 ± 3.61^{d}
C. sinensis	48.0^{d}	145.5 ± 5.84^{e}
C. reticulata	64.0^{a}	83.6 ± 4.88^{b}
A. ceylanica	20.9^{e}	238.17 ± 4.65^{f}
Trolox	-	8.68 ± 0.78^{a}

Data expressed as Mean ± SEM, n=4, ^{a,b,c,d,e,f} Significant differences (in terms of radical scavenging activity and IC_{50} value) between the each plant species were indicated by alphabetical superscripts.

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