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Antioxidant Activity of Phenolic Compounds Extracted from the Roots and Leaves of *Withania somnifera* (L.) from Different Geographical Locations in India

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ABSTRACT

Phenolic compounds and antioxidant activity were determined in the extracts of *Withania somnifera* root (RE) and leaf (LE) from five different geographical locations like Coimbatore, Gujarat, Kambam, Lucknow and Nagappattinam of India. The highest amount of total phenolic compounds at 29.25 mg/g DW (dry weight) of plant tissue and total flavonoids at 6.3 mg/g DW were found in RE-Lucknow. The maximum amount of kaempferol at 4.472 mg/100 g DW and quercetin at 1.340 mg/100 g DW were found in RE-Lucknow and LE-Lucknow, respectively. Kaempferol was found in all root extracts and LE-Lucknow, while quercetin was found in all leaf extracts and two roots extracts like RE-Coimbatore and RE-Gujarat. RE-Lucknow showed the best radical scavenging activity with the IC_{50} value of 105.06 ± 1.65 µg/mL in the DPPH assay. In addition, RE-Lucknow also showed maximum percentage of ABTS absorption inhibition activity with the IC_{50} value of 204.03 ± 14.88 µg/mL. Therefore, the present study reveals that the *W. somnifera* leaf and root extracts from five geographical locations possess different antioxidant capacities due to various amounts of phenolic compounds.

Keywords: antioxidant activity, flavonoids, leaf extract, phenolic compounds, root extract
Abbreviation: ABTS, 2,2'-azinobis (3-ethylbenzo thiazoline-6-sulfonic acid) diammonium salt; **DPPH**, 2,2-diphenyl-2-picrylhydrazyl hydrate; **DW**, dry weight; **GAE**, gallic acid equivalent; **HPLC**, high-performance liquid chromatography; **IC_{50}**, the amount of antioxidant necessary to decrease the initial radical concentration by 50%; **QE**, quercetin equivalent; **LE**, leaf extract; **RE**, root extract

INTRODUCTION

Free radicals are induced by external sources like food, drugs and environmental pollution and generated by metabolic pathways within body tissues. The production of free radicals is considered the most critical cause of cellular ageing, leading to peroxidation of all macro-molecules including lipids, proteins and nucleic acids (Wilhelm *et al*. 2006; Viňa *et al*. 2007). Reactive oxygen and nitrogen species such as superoxide anions, hydrogen peroxide, hydroxyl, nitric oxide and peroxynitrite radicals, are associated with normal cellular activity, metabolism and signaling (O'Flaherty *et al*. 2006; Bartosz 2009). However, excessive generation of these molecules caused the oxidative stress which is implicated in many diseases and pathological conditions (Wells *et al*. 2005; Afonso *et al*. 2007; Boueiz and Hassoun 2009; Kielland *et al*. 2009). On the other hand, human cells possess comprehensive array of antioxidant defense mechanisms including non-enzymatic and enzymatic antioxidants (Elango *et al*. 2006; Kharrazi *et al*. 2008). These antioxidants are radical scavengers which prevent free radical formation or limit their damaging effect. It is difficult to elucidate that these antioxidants may act independently, because they are chemically related and changes in one antioxidant will affect the activity of others (Lopez-Torres *et al*. 1991). There is a balance between both the activity and intracellular levels of these antioxidants that are essential for the protection to cellular components and their health (Berr *et al*. 2004; Mazur-Kolecka *et al*. 2006; Aybek *et al*. 2007).

Many medicinal plants contain large amounts of antioxidants such as polyphenols which may play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Djeridane *et al*. 2006). The chemistry and nutritional properties of phenolic compounds have been extensively reviewed (Manach *et al*. 2004). Flavonoids are phenolic compounds that exist in plants as secondary metabolites. The investigation has determined correlations between flavonoid structural features and antioxidative and free radical scavenging activities *in vitro* (Pannala *et al*. 2001).

Withania somnifera (L.) is a perennial plant belonging to the Solanaceae family. The plant is used not only in the treatment of tuberculosis, rheumatism, inflammatory conditions and cardiac diseases but also as an agent for tonic, anti-stress, antitumor, antibiotic, anticonvulsant and central nervous system-depressant (Sharma and Dandiya 1992). The plants are morphologically indistinguishable among genotypes but differ in their chemical constituents termed as chemotypes. Although there are more than one chemotype of *Withania* spp. in India (Chakraborti *et al*. 1974), the exact number of chemotypes has not yet been studied systematically. Different collections of *W. somnifera* from various geographical regions such as Israel, India and South Africa have been proved as different chemotypes (Lavie 1986). *W. somnifera* have a wide distribution throughout India as both wild and cultivated populations of the species that exhibit enormous diversity in chemical constituents (Singh and Kumar 1998; Dhar *et al*. 2006).

Even though there are numerous studies on *W. somnifera*, no report on phenolic compounds and flavonoids of the plant related with antioxidant properties exists. Therefore, the objective of this work was to quantify phenolic compounds, including flavonoids, using high-performance liquid chromatography (HPLC) and to determine the antioxidant properties by 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH) and 2,2'-azinobis (3-ethylbenzo thiazoline-6-sulfo-

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nic acid) diammonium salt (ABTS) assays of five different geographical locations of *W. somnifera* root and leaf extracts.

MATERIALS AND METHODS

Chemicals

Gallic acid, kaempferol, quercetin dihydrate, DPPH and ABTS were purchased from Sigma-Aldrich Chemicals Co. (St Louis, Mo, USA). Solvents (methanol and water) were of HPLC grade purchased from Fischer Scientific Chemicals Co. (Fair Lawn, NJ, USA). All other chemicals and reagents were of analytical grade and were purchased from standard chemical companies.

Plant materials

The plants were collected and identified by Dr. A. Ganapathi (Professor and Botanist, Bharathidasan University), and a voucher specimen is deposited in the Rabinet Herbarium, Tiruchirappalli, India. The plant parts like root and leaf were selected for this study. Previously, a RAPD study was conducted for the determination of genetic similarities and variations among different locations of *W. somnifera*. The RAPD study showed that the pronounced genetic variations (Udayakumar 2007) among the five different locations (**Fig. 1**) of *W. somnifera* such as Coimbatore (Tamilnadu Agricultural University), Gujarat (Reputed Medicinal Plant Station at Valsad), Kambam (natural source), Lucknow (Central Institute of Medicinal and Aromatic Plants), and Nagappattinam (natural source). Lucknow is in the northern part, Gujarat is in the central part, Coimbatore, Kambam and Nagappattinam are in the southern part of India. Therefore, the five different locations of *W. somnifera* were selected for the analysis of total content of phenolic compounds, flavonoids and individual flavonoids like kaempferol and quercetin and for *in vitro* antioxidant activity.

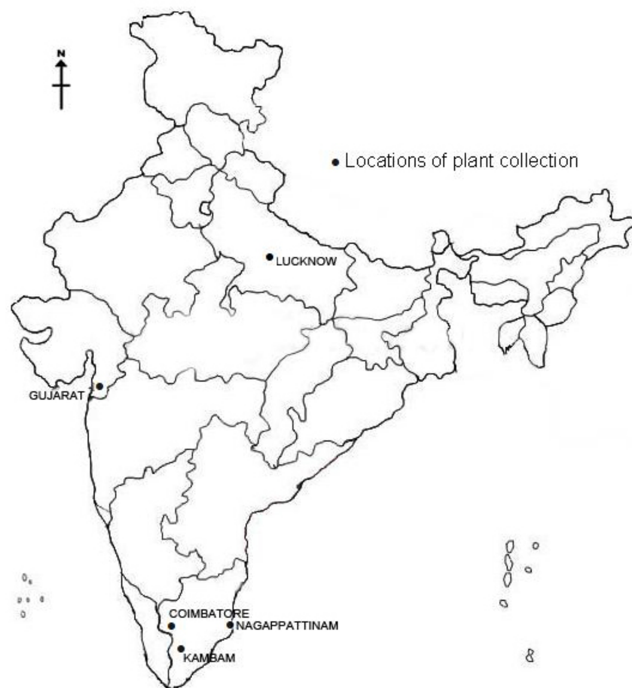

Fig. 1 Different locations of *W. somnifera* sample collection.

Preparation of extracts of phenolic compounds

Parts (roots and leaves) of the plant were collected from six-months-old field grown plants and brought into the laboratory for phytochemical and *in vitro* antioxidant studies. The roots and leaves were cleaned and dried at room temperature (37°C) for two weeks, then ground well to fine powder. The extracts containing phenolic compounds were obtained based on the method as described by Kahkonen *et al*. (1999). About 500 mg of dry powder into a test tube and 10 mL of 80% aqueous methanol was added in total. The suspension was stirred slightly, sonicated for 5 min, centrifuged for 10 min (1500 × *g*), and then its supernatants were collected. Plant materials were re-extracted twice. Combined supernatants were evaporated, lyophilized and weighed. The five geographical locations of *W. somnifera* root and leaf powder were extracted in the same way and labeled as RE-Coimbatore, RE-Gujarat, RE-Kambam, RE-Lucknow, RE-Nagappattinam, LE-Coimbatore, LE-Gujarat, LE-Kambam, LE-Lucknow and LE-Nagappattinam. The residues were weighed and kept separate in air-tight containers and then stored in a deep freezer until use. The weight of residues was expressed as milligrams per gram of dry weight (mg/g of DW).

Qualitative analysis of phenolic compounds content in different geographical locations of RE and LE

Contents of phenolic compounds of the extracts were analyzed by following standard qualitative methods (Odebiyi and Sofowora 1990). The plant extracts were screened for the presence of boilogically active phenolic compounds like phenols, flavonoids and tannins.

1. Phenols

To 1 mL of herbal extract, 2 mL of distilled water followed by a few drops of 10% aqueous $FeC1_3$ solution was added. The formation of a blue or green precipitate indicates the presence of phenols. Another assay was also performed to confirm the presence of phenols. One mL of herbal extract was diluted to 5 mL with distilled water and to this a few drops of 1% aqueous solution of lead acetate was added. A yellow precipitate indicates the presence of phenols.

2. Flavonoids

In a test tube containing 0.5 mL of herbal extract, 5-10 drops of diluted HCl and a small piece of zinc or magnesium were added and the solution was boiled for a few minutes. The formation of a reddish pink or dirty brown color indicates the presence of flavonoids.

3. Tannins

To 1-2 mL of herbal extract, a few drops of 5% aqueous $FeC1_3$ solution were added. A bluish black color, which disappears on addition of a few mL of dilute H_2SO_4 was followed by the formation of a yellowish brown precipitate indicates the presence of tannins. Another assay was also performed to confirm the presence of tannins. In a test tube containing about 5 mL of herbal extract, a few drops of 1% solution of lead acetate was added. A yellow or red precipitate was formed, indicating the presence of tannins.

Determination of total phenolic compounds content in different geographical locations of RE and LE

The content of total phenolic compounds in methanolic extracts of different geographical locations of *W. somnifera* was determined spectrophotometrically, using the Folin-Ciocalteu method, as described previously (Singleton *et al*. 1999). For the preparation of a calibration curve 1 mL aliquots of 0.024, 0.075, 0.105 and 0.3 mg/mL ethanolic gallic acid solutions were mixed with 5 mL Folin–Ciocaleu reagent and 4 mL (75 g/L) sodium carbonate. The absorption was read after 30 min at 765 nm at 20°C and a calibration curve was drawn. One mL of methanolic extract (10 mg/mL) was mixed with the same reagents as described above. A reagent blank was also prepared using distilled water. After 30 min the absorption was measured for the determination of plant phenolics. All determinations were performed in triplicate. The total content of phenolic compounds was expressed as gallic acid equivalent (GAE) mg/g of DW of plant tissue.

Determination of flavonoids content in different geographical locations of RE and LE

The content of flavonoids in methanolic extracts of different geographical locations of *W. somnifera* was determined spectrophotometrically according to the method of Quettier-Deleu *et al.* (2000) using quercetin as a reference compound. One mL of plant extract in methanol (10 mg/mL) was mixed with 1 mL aluminium trichloride in ethanol (20 g/L) and diluted with ethanol to 25 mL. The absorption at 415 nm was read after 40 min at 20°C. A blank sample was prepared from 1 mL plant extract and 1 drop of acetic acid, and diluted to 25 mL. The absorption of quercetin solutions was measured under the same conditions. Standard quercetin solution was prepared from 0.05 g quercetin (0.05 g/L). All determinations were carried out in triplicate. The amount of flavonoids was expressed as quercetin equivalent (QE) mg/g of DW of plant tissue.

Identification and quantification of kaempferol and quercetin

The analyses of methanolic extracts of different geographical locations of *W. somnifera* were performed using an HPLC system equipped with SPD-10AVP, UV-visible detector and pump LC-10AT (Shimadzu class VP 5, Japan). The mobile phase consisted of 55: 45 (buffer: methanol, v/v). Buffer consisted of 0.3 g H_3PO_4 in 1000 mL with water at pH 2.0 with triethanolamine. The stationary phase consisted of octadecyl silyl silica gel for chromatography R (5 µm) and column: C18 (Gemini). The separation temperature was kept constant at 25°C. Flow rate and sample volume were set to 1.2 mL/min and 20 µL, respectively. All separations were monitored at 370 nm. Peaks were assigned by spiking the samples with authentic samples of kaempferol and quercetin. The amount of kaempferol and quercetin in the samples were calculated using the following formula and the values were expressed as mg/100 g DW of plant tissue.

Kaempferol or quercertin content (mg/100 g DW) =

$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Standard weight}}{\text{Dilution}} \times \frac{\text{Dilution}}{\text{Sample weight}} \times$$

Standard purity × 100.

Antioxidant activity by DPPH radical scavenging assay

As described in our previous study (Choi *et al.* 2002), the hydrogen atom or electron donation abilities of the corresponding extracts were measured from the bleaching of the purple-coloured methanol solution of DPPH. In this spectrophotometric assay, the stable radical DPPH was used as a reagent (Choi *et al.* 2002). Ascorbic acid standard was prepared in deionized water over the range of 11-88 µg/mL (0.063-0.5 mM). Extracts were prepared in different concentrations (50-200 µg/mL) in 80% methanol. Four mL of methanolic solution of DPPH (0.004 mM) were added to 1 mL of various concentrations of extract/standard solutions. One mL of deionized water/methanol serves as a control. The tubes were incubated at room temperature in dark for 20 min. After incubation period the absorbance was read at 517 nm. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the plot of inhibition (%) against extract concentration. Tests were carried out in triplicate. Ascorbic acid standard was used for comparison. Radical scavenging activity was expressed based on the percentage of DPPH radical reduction as IC_{50} (µg/mL). IC_{50} is defined as the amount of antioxidant necessary to decrease the initial DPPH radical concentration by 50%.

Antioxidant activity by ABTS radical cation decolourization assay

This assay assesses the total radical scavenging capacity based on the ability of a compound to scavenge the stable ABTS radical in 6 min (Re *et al.* 1999). For the total antioxidant assay, ABTS was dissolved in deionized water to a 7 mM concentration. The ABTS radical cation ($ABTS^+$) was produced by reacting ABTS stock solution with a 2.45 mM potassium persulfate (final concentration) and incubating the solution in the dark at room temperature for 12-16 h before use. The radical stock solution was diluted with a 5 mM solution of phosphate-buffered saline (pH 7.4) to obtain a spectrophotometric absorbance value of 0.7 at 734 nm. Ascorbic acid standard was prepared in deionized water over the range of 11-88 µg/mL (0.063-0.5 mM). Extracts were prepared in different concentrations (75-200 µg/mL) in methanol. To 40 µL of extract/standard solution, 1.96 mL of $ABTS^+$ solution was added and the tubes were kept in darkness for 6 min and read at 734 nm. This was compared to a control where 40 µL of the solvent was added to 1.96 mL of $ABTS^+$ solution. Assays were performed in triplicate. Antioxidant activity was expressed based on the percentage of ABTS radical reduction as IC_{50} (µg/mL). IC_{50} is defined as the amount of antioxidant necessary to decrease the initial ABTS radical concentration by 50%.

RESULTS AND DISCUSSION

Qualitative analyses of phenolic compounds in different geographical locations of RE and LE

In the present study, qualitative analyses reveal that there are phenolic compounds including phenols, flavonoids and tannins in RE and LE. These phenolic compounds are widely distributed in the plant kingdom at high concentration (Harborne 1993) and known to possess potent antioxidant activity *in vitro* (Lee *et al.* 2004).

Total phenolic content of RE and LE

The total content of phenolic compounds was determined using the Folin-Ciocalteu reagent method in five geographical locations of RE and LE. Though the Folin-Ciocalteu method correlated well with antioxidant capacity analysis procedures, it has a disadvantage because substances such as sugar, ascorbic acid, aromatic amines, sulfur oxide, iron and other compounds can interfere with the Folin-Ciocalteu assay. Such non-phenolic substances and inorganic substances may also interact with Folin-Ciocalteu reagent, thus giving an inaccurate and higher than actual phenolic content (Prior *et al.* 2005). Therefore, we carefully considered this when results are interpreted.

The total phenolic compounds ranged between 10.18-29.25 mg/g DW of plant tissue, depending upon plant part and geographical location (**Table 1**). The highest amounts of phenolic compounds were found in RE-Lucknow at 29.25 mg/g DW and LE-Lucknow at 17.19 mg/g DW in GAE. Numerous factors are known to affect the content of phenolic compounds in plants, including genetic variation, cultural method and environmental factors including soil, weather, and geographical location. In addition, drying of plant samples at room temperature (37°C) will undoubtedly have an impact on phenolic compounds. During early stages, the plant material may have suffered from heat stress, a condition known to induce phenolic accumulation. Recently, it has been reported that the difference in content of phenolic compounds and the ability of antioxidant activity of *W. somnifera* from different geographical locations are due to genotype differences (Scalzo *et al.* 2005). It is logical to hypothesize that the difference in content of phenolic compounds in this study may be due to genetic variation. On the other hand, earlier studies with other plants revealed that the difference in content of phenolic compounds is due to cultural conditions such as conventional and organic production (Lombardi-Boccia *et al.* 2004), climatic conditions (Djeridane *et al.* 2006), or the cultivar and growing conditions (Veberic *et al.* 2008). More recently, the difference in the content of phenolic compounds and its relationship with antioxidant capacity of *Mangifera indica* varieties in Brazil has been reported (Ribeiro *et al.* 2008). Therefore, the difference in content of phenolic compounds among different geographical locations of *W. somnifera* may contribute to the capacity of radical scavenging activity of RE and LE.

Table 1 Total content of phenolic compounds, flavonoids and individual flavonoids like kaempferol and quercetin of five geographical locations of *W. somnifera* root and leaf extracts.

Name of extracts	Yield of extracts (mg/g DW)	Total content of phenolic compounds (mg/g DW of plant tissue in gallic acid equivalents)	Flavonoids (mg/g DW of plant tissue in quercetin equivalents)	Individual compounds of flavonoids (mg/100 g DW of plant tissue)	
				Kaempferol	Quercetin
RE-Coimbatore	150 ± 2.8	23.35 ± 1.2	4.0 ± 0.5	1.014 ± 0.4	0.466 ± 0.5
RE-Gujarat	144 ± 2.2	18.67 ± 0.9	4.4 ± 0.6	0.519 ± 0.7	0.182 ± 0.2
RE-Kambam	156 ± 3.4	25.62 ± 0.9	5.6 ± 0.5	2.319 ± 0.5	n.f
RE-Lucknow	153 ± 2.6	29.25 ± 1.1	6.3 ± 0.8	4.472 ± 0.4	n.f
RE-Nagappattinam	142 ± 3.1	16.03 ± 1.2	4.9 ± 0.4	0.151 ± 0.7	n.f
LE-Coimbatore	146 ± 1.9	15.09 ± 1.3	4.2 ± 0.5	n.f	0.137 ± 0.5
LE-Gujarat	158 ± 2.5	12.88 ± 1.6	4.4 ± 0.3	n.f	0.122 ± 0.2
LE-Kambam	152 ± 2.0	15.80 ± 1.0	5.5 ± 0.4	n.f	0.832 ± 0.2
LE-Lucknow	148 ± 3.1	17.19 ± 0.8	6.0 ± 0.6	4.403 ± 0.2	1.340 ± 0.2
LE-Nagappattinam	154 ± 2.4	10.18 ± 1.1	4.7 ± 0.5	n.f	0.120 ± 0.4

n.f - not found. Values are expressed as mean ± SD of three determinations.

Flavonoids content of RE and LE

The amount of flavonoids varied from 4.0 to 6.3 mg/g DW of plant tissue in QE among five geographical locations of RE and LE (**Table 1**). The highest amount of flavonoids in QE was found in RE-Lucknow (6.3 mg/g DW) and LE-Lucknow (6.0 mg/gDW). It is known that a certain structure of flavonoids, particularly the hydroxyl position in the molecule, determines the antioxidant properties (Magnani *et al*. 2000; Yang *et al*. 2001); in general these properties depend on the ability to donate hydrogen, or electrons to a free radical. Many flavonoids have shown strong antioxidant properties and quercetin has been established as a strong antioxidant principle and used as a standard in antioxidant experiments (Choi *et al*. 2002). It was reported that flavonoids could remove the $O_2^{\bullet-}$ in human bodies, strengthen the natural disease-fighting system, improve blood circulation and lower blood pressure (Liu and Li 2002). Comparison of the concentration of flavonoids in *W. somnifera* samples of five geographical locations showed differences.

Identification and quantification of kaempferol and quercetin

The amounts of individual compounds of flavonoids like kaempferol and quercetin in different geographical locations of RE and LE were determined using HPLC analysis (**Table 1**). Kaempferol and quercetin standards were used and standard peaks obtained at particular retention times i.e. kaempferol - 6.427 min and quercetin - 21.162 min. These peaks with retention times were used for the determination of kaempferol and quercetin in root and leaf extracts of different geographical locations of *W. somnifera* (**Fig. 2**). Kaempferol was found in all geographical locations of RE, among those the maximum amount of kaempferol was found in RE-Lucknow (4.472 mg/100 g DW). However, it was absent in all geographical locations of LE except LE-Lucknow (4.403 mg/100 g DW). In the case of quercetin, it

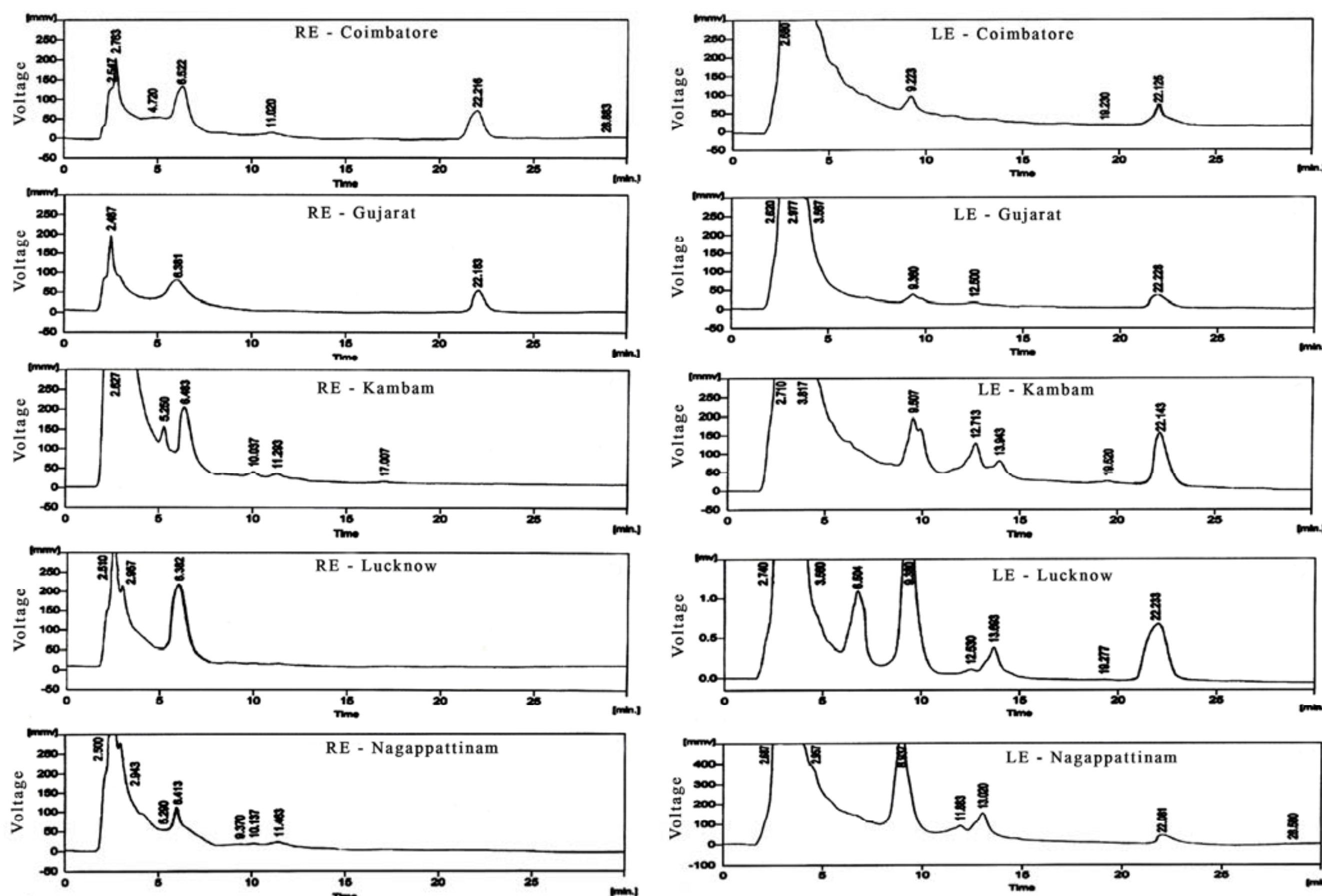

Fig. 2 HPLC chromatogram of RE-Coimbatore, RE-Gujarat, RE-Kambam, RE-Lucknow, RE-Nagappattinam, LE-Coimbatore, LE-Gujarat, LE-Kambam, LE-Lucknow and LE- Nagappattinam.

was found in all geographical locations of LE and the maximum amount was found in LE-Lucknow (1.340 mg/100 g DW). Quercetin was also found in RE-Coimbatore and RE-Gujarat, but it was absent in RE-Lucknow, RE-Kambam and RE-Nagappattinam. The results showed that the phenolic and flavonoid compounds were found in different quantities in different tissues. Both HPLC and spectrophotometric analyses confirmed that the five geographical locations of RE and LE possess phenolic compounds and flavonoids. In addition, the HPLC peaks in this study showed the presence of some other unknown compounds.

Total antioxidant activity: DPPH radical scavenging assay and ABTS radical cation decolourization assay

The phenolic compounds and flavonoids in some medicinal plant extracts are related with antioxidant properties (Choi *et al*. 2002; Djeridane *et al*. 2006). In this study, the individual flavonoid compounds like kaempferol and quercetin in the methanolic extracts may also be responsible for the part of antiradical and antioxidant activities of *W. somnifera* along with other phenolic compounds. The total antioxidant activity of five geographical locations of RE and LE was determined by the method of DPPH radical scavenging assay and ABTS radical cation decolourization assay. The result of $DPPH^{\bullet}$ and $ABTS^{+\bullet}$ inhibition by the different geographical locations of RE and LE are shown in **Table 2**. All the 10 investigated methanolic extracts almost completely inhibited DPPH absorption.

Among the five geographical locations of *W. somnifera*, RE-Lucknow and LE-Lucknow showed the best radical scavenging activity with IC_{50} values of 105.06 ± 1.65 µg/mL and 174.39 ± 10.25 µg/mL respectively. RE-Kambam and LE-Kambam also showed good radical scavenging activity with the IC_{50} values of 161.79 ± 8.23 µg/mL and 238.39 ± 23.25 µg/mL, respectively. DPPH absorption in varying concentrations (75-200 µg/mL) of the different locations of both RE and LE decreased continuously up to 200 µg/mL and further increases in the concentration did not change the absorbance. The concentration at which it scavenges 50% of DPPH radical is given as the IC_{50} value of the extract. RE-Gujarat, LE-Gujarat, RE-Nagappattinam and LE-Nagappattinam had considerably less radical scavenging activity when compared to other geographical locations of *W. somnifera* extracts, which may be due to lower presence of radical scavenging compounds like phenolic compounds including flavonoids.

The decolorization assay of ABTS radical cation showed quite similar results compared to those obtained in the DPPH reaction. In the present study, three different concentrations of extracts (75, 150 and 200 µg/mL) were used in the ABTS absorption inhibition (%) study. Among these three concentrations, 200 µg/mL of extract had more absorption inhibition activity in all five geographical locations of *W. somnifera* extracts than other concentrations. RE-Lucknow and LE-Lucknow showed maximum percentage of ABTS absorption inhibition activity with the IC_{50} values of 204.03 ± 14.88 µg/mL and 209.05 ± 16.42 µg/mL, respectively. The extracts from Kambam location (RE and LE) and Coimbatore location (RE and LE) were the most active, when compared to other geographical locations like Gujarat (RE and LE) and Nagappattinam (RE and LE).

In this study, the investigation of five different locations of *W. somnifera* showed that the content of phenolic compounds varies with the geographical locations. In the content of phenolic compounds, Lucknow geographical location is the highest and Nagappattinam geographical location is the lowest among the tested geographical locations. In addition, the antioxidant properties of five geographical locations of RE and LE varied widely, which may be due to the variations in the phenolic compounds, including flavonoids. It also indicates that an increase in the content of phenolic compounds from the extracts resulted in an increase in the antioxidant activities assayed. In all five geographical locations RE showed more phenolic compounds, including flavonoids, and more antioxidant activities than LE. The results confirmed that the antioxidant activity of extracts of *W. somnifera* roots and leaves may be due to the presence of phenolic compounds including kaempferol and quercetin were identified and quantified from the extracts for the first time.

Table 2 Mean total antioxidant activity (IC_{50}) of five geographical locations of *W. somnifera* root and leaf extracts using DPPH radical scavenging and ABTS radical cation decolourisation assays.

Name of extracts	DPPH radical scavenging assay-IC_{50} (µg/mL)	ABTS radical cation decolourisation assay-IC_{50} (µg/mL)
RE-Coimbatore	184.82 ± 12.04	217.32 ± 14.76
RE-Gujarat	243.67 ± 35.41	234.11 ± 18.48
RE-Kambam	161.79 ± 8.23	210.53 ± 16.44
RE-Lucknow	105.06 ± 1.65	204.03 ± 14.88
RE-Nagappattinam	270.67 ± 31.44	240.27 ± 24.54
LE-Coimbatore	256.07 ± 27.86	220.51 ± 22.92
LE-Gujarat	267.91 ± 30.95	237.81 ± 26.51
LE-Kambam	238.39 ± 23.25	214.25 ± 20.11
LE-Lucknow	174.39 ± 10.25	209.05 ± 16.42
LE-Nagappattinam	302.81 ± 41.18	267.56 ± 32.44
Vitamin C	45.73 ± 2.28	58.36 ± 3.14

Values are expressed as mean ± SD of three determinations. Vitamin C was used as a reference compound. The IC_{50} value is defined as the amount of extract necessary to decrease the initial DPPH or ABTS radical concentration by 50%.

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