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SHORT COMMUNICATION

Spray drying of *Tinospora cordifolia* leaf and stem extract and evaluation of antioxidant activity

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Abstract *Tinospora cordifolia* (Guduchi) is widely used in folk medicine/ ayurvedic system of medicine, also in ayurvedic 'Rasayanas' to improve the immune system and used as general tonic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic and anti-diabetic agent. Numerous studies have been reported on the health benefits of individual parts or whole Guduchi plant. However, most of the work has focused on the extracts of *T. cordifolia*. In this study, *T. cordifolia* leaf and stem extract powders were prepared using spray drying at 90 °C outlet temperature of the spray dryer. The powder morphology has also been studied by scanning electron microscopy. The antioxidant activity was followed by DPPH method. The leaf extract powder showed higher retention of antioxidant activity than stem extract powder.

Keywords Spray drying · Antioxidant activity · Polyphenol · Outlet temperatures · DPPH

T. cordifolia is a large, glabrous, deciduous climbing shrub, belongs to the family Menispermaceae, native to India but widely distributed throughout the tropical regions of South Asian countries to an altitude upto 1,000 m (Singh et al. 1984). It is widely used in veterinary folk medicine/ ayurvedic system of medicine in a holistic system of diagnosis and treatment involving nutrition, hygiene and rejuvenation. The plant is used in ayurvedic 'Rasayanas' to improve the immune system and the body resistance against infection. It is used as general tonic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic, anti-allergic and anti-diabetic powerful immune modulator. Guduchi is much useful to enhance the memory (Wealth of India 1976). Chopra et al. (1982) have described the T. *cordifolia* viscous sap has a light yellow colour and nauseating bitter taste and improves metabolic activity, even at a cellular level. So far a variety of constituents have been isolated from *T. cordifolia* plantare well characterized as Alkaloids, Sterols, Lactones, Glycosides etc (Sarma and Khosa 1993; Singh et al. 2003; Chintalwar et al. 1999; Jagetia and Rao 2006). The relative immunomodulating potencies of various phytochemical constituents of *T. cordifolia* were also compared by different mechanisms (Kapil and Sharma 1997). The studies on various extracts of leaf, stem and roots of *T.* c*ordifolia,* viz., hepatoprotctive effect, antioxidant effect, effect against infection, immunomodulatory activity, antistress activity, gastrointestinal protective activity, protection against toxicity of cancer chemotherapy, antineoplastic activity and other properties of therapeutic relevance, like learning memory, antiinflammatory and antiallergic properties, antipyretic, diuretic effect etc have been compiled and categorized (Panchabhai et al. 2008).

Spray drying is a well established method (Masters 1991) for converting liquid feed materials into dry powder form. During the spray drying process, the liquid feed is first atomised and contacted with hot air; evaporation takes place to yield dried particles, which are subsequently separated from the air stream by a variety of methods. Spray drying is widely used to produce foods, such as whey, instant coffee, milk, tea and soups, as well as healthcare and pharmaceutical products, such as vitamins, enzymes and bacteria (Anandharamakrishnan et al. 2007).

In the present report, an attempt has been made to prepare the powder from the water extract of *T. cordifolia*

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leaf and stem. The antioxidant profile and polyphenol content has also been studied to assess the quality of the powder which could be used in preparation of different food products, owing to its medicinal properties. The morphology of the powder has also been studied using Scanning Electron Microscopy.

Materials and methods

The fresh whole plant of *T. cordifolia* was collected from the local garden in the month of January–February. The specimen of *T. cordifolia* has been deposited in the Fruit and Vegetable Technology (FVT) department of Central Food Technological Research Institute, Mysore-570 020. The voucher specimen no. is FVTDH No. LGBCL-TC-7A and & B/2010.

All solvents / chemicals used were of analytical grade. Diphenyl picrylhydrazyl (DPPH) and Butylated hydroxyl Anisole (BHA) have been procured from Sigma Chemical Co. (St. Louis, MO, USA).

The leaves and stem were separated from the whole plant and cleaned thoroughly. 670 g of leaf and 700 g of stems were taken and were partially ground in a mechanical grinder. Leaves were extracted with 6,000 ml of water in autoclave for 60 min at 15 psi. The stem was autoclaved twice with 3,000 and 2,500 ml of water at the same pressure and time as described for leaves. The extracts were filtered using Whatman filter paper (No.1) and kept at 4 °C till further use. The leaf and stem extracts were concentrated in an agitated thin film falling evaporator (Votator Kentucky. USA), followed by flash evaporator (Buchi, Switzerland) and the concentrated samples were stored at 4 °C.

A tall-form twin-fluid atomizer co-current pilot scale spray dryer (Bowen Engineering, INC. Somerville, New Jersey, USA) was used for spray drying. The atomisation was performed by a twin-fluid nozzle, using compressed air at 45 psi as the atomising gas. The inlet temperature was brought to the desired temperature by directly heat in a burner using LPG gas, allowing control of the inlet air temperature. The spray drying operation was started by feeding distilled water and the outlet temperature was set at 90 °C by adjusting the liquid feed rate. Once the required outlet temperature was obtained, the concentrated extract solution was fed into the drying chamber. The inlet temperature was used as the second variable as it could be set more easily and reproducibly than the liquid feed flow rate for a given outlet temperature. The liquid feed rates were measured for each experiment by measuring the change in volume of feed in the feed vessel over time. The measured liquid feed flow rates and inlet and outlet air temperatures are given in the Table 1. The particles were separated by a cyclone and collected in a receiving vessel. The final products were sealed immediately in polythene air sealed bags and stored at 4 °C.

The average moisture content of spray dried powders was determined by (American Association of Cereal Chemists 2000) method. 50 g of both leaf and stem extract were completely dried at 130 °C for 1 h to find out the moisture content. The analysis was done in triplicates and the average value was reported on wet weight basis.

Radical scavenging activity (RSA) of the leaf and stem extract of *T. cordifolia* and spray dried powder was determined as described by Blios (1958). Different concentrations (10 and 40 ppm) of the dried powder and BHA (25 ppm) were taken and the volume was adjusted to 100 μL with MeOH. 5.0 ml of 0.1 mM methanolic solution of DPPH was added to these tubes and shaken vigorously. The tubes were allowed to stand at 27 °C for 20 min. The control was prepared as above without any extract and MeOH was used for the baseline correction. The changes in the absorbance of the samples were measured at 517 nm using Genesys-5 UV-visible spectrophotometer (Milton Roy, USA). RSA was expressed as the inhibition percentage and was calculated using the following formula, $\%\,\text{radical scavenging activity} = (\text{Control OD} - \text{sample OD}/\text{Control OD}) \times 100$.

The concentration of phenolic compounds of the two spray dried powder samples was determined according to the method of Heinonen et al. (1998) and results were expressed as tannic acid equivalents. The spray dried powders were dissolved in a mixture of methanol and water (6:4 *v/v*). Samples (0.2 mL) were mixed with 1.0 mL 10-fold-diluted Folin-Ciocalteu reagent and 0.8 mL of 7.5% sodium carbonate solution. The mixture was allowed to stand for 30 min at room temperature and the absorbance was measured at 760 nm using a Genesys-5 UV-visible

Table 1 Experimental conditions used in the spray drying and characteristics of the spray dried powder

Sample	Drying temp. (°C)		Flow rate (mL/sec)	Moisture content (wb)	% Retention of polyphenols
	Inlet	Outlet			
Leaf	145	90	0.9	3.0±0.1	87.9±0.6
Stem	145	90	1.0	4.9±0.1	82.4±0.5

Values are average of three repeats

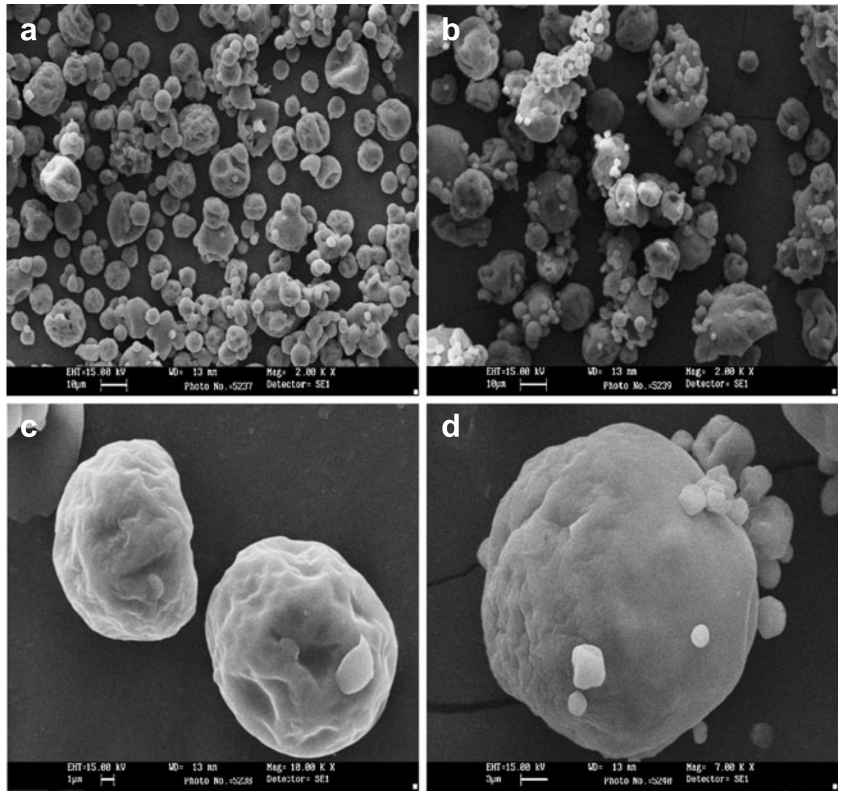

Fig. 1 Scanning Electron Microscopy of *Tinospora cordifolia* leaf and stem extracts; **a** Spray dried leaf extract powder, **b** Spray dried stem extract powder, **c** Spray dried leaf extract particles (x 10000), **d** Spray dried stem extract particle (x 7000)

spectrophotometer (Milton Roy, USA). The estimation of phenolic compounds was carried out in triplicate, and the results were averaged.

Morphology of spray dried *Tinospora cordifolia* powder samples were studied using Scanning Electron Microscopy. Spray dried samples were mounted on the specimen holder and sputter-coated with gold (2 min, 2 mbar). Finally, each sample was transferred to the microscope, where it was observed at 15 kV and a vacuum of 9.75×10^{-5} torr. A scanning electron microscope (Leo 435 VP, Leo Electronic Systems, Cambridge, U.K.) was used to scan the images.

Results and discussion

The moisture content of spray dried powders of leaf and stem are shown in Table 1. The leaf powder showed lower moisture content and also less hygroscopic than the stem power. During the falling rate period, a permeable crust was formed and the droplet temperature rose towards the dry-bulb temperature of the air. If the partial pressure of moisture vapour at the droplet centre exceeded ambient pressure and thus resulted in a bubble formation and increase in the temperature of droplet inflated to an outer radius and finally yields in irregular random shaped particle (Etzel et al. 1996). In the case of stem, the strong crust restricted the water vapour diffusion as well as bubble inflated thus, resulted in high moisture content. Moreover, the high moisture content and hygroscopic nature of stem extract favours the particle agglomeration (Fig. 1b). Hence, the sting crust and agglomeration resulted in low degree of heat and mass transfer during drying of stem extract.

The % RSA of both leaf and stem powders spray dried at 90 °C outlet temperature has been determined by DPPH RSA evaluation method (Table 2). This showed that the leaf powder exhibited higher activity than that of stem powder

Table 2 % Radical scavenging activity (RSA) of *Tinospora cordifolia* leaf and stem extract and their of spray dried powder

Samples	10 ppm		40 ppm		100 ppm	
	Leaf	Stem	Leaf	Stem	Leaf	Stem
Aqueous Extract	15.4±0.4	12.1±0.3	26.6±0.5	18.8±0.4	54.1±0.4	41.3±0.4
Spray dried powder	13.1±0.2	10.5±0.2	25.3±0.3	17.9±0.4	49.8±0.5	38.6±0.4

Values are average of three repeats

at comparative concentrations. This may be attributed to the higher polyphenol content of leaf powder than the stem powder and also the type of compounds present in both parts which contribute towards RSA. Overall the RSA of both activity leaf and stem powder was lower than BHA at comparative concentration which showed 82.16% RSA at 25 ppm.

The total polyphenol content of spray dried powder of leaf and stem at 90 °C outlet temperatures are shown in Table 1. It was noticed that the total % polyphenols of spray dried powder of leaf at 90 °C outlet temperature was higher than of stem.

The scanning electron micrographs spray dried powder of leaf and stem water extract at 90 °C are shown in Fig. 1a and b respectively and the magnified structure of single particle of leaf (x 10000) and stem (x 7000) spray dried powder are shown in Fig. 1c and d respectively. Micrographs of Fig. 1a and b show distributed spherical particles with smooth surface and lower degree of aggregation in case of leaf extract powder whereas the stem sample showed the higher degree of aggregation of the particles. This may be due to hygroscopic nature of material. During spray drying, it was noticed that stem powder was more hygroscopic than the leaf powder. Moreover, Fig. 1c depicts more shrinkage on the surface of the leaf powder particle probably due to higher heat and mass transfer during drying than the stem powder.

T. cordifolia leaf and stem water extracts were successfully spray dried at 90 °C outlet temperatures of the spray drier. The leaf powders dried at 90 °C have lower moisture content than the stem powder. Leaf extract shown higher % retention of total polyphenols as well as antioxidant activity. Owing to various beneficial and medicinal properties of *T. cordifolia* and high solubility of the spray dried powder samples from both leaf and stem, the preparations may be used in the formulation of different food products with various health benefits.

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