Research Article

Phytochemical investigations of *Tribulus terrestris* and *Solanum torvum* by FT-IR analysis

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Received: 24 May 2019 Revised: 13 July 2019 Accepted: 14 July 2019

Abstract

Objective: The aim of the present study was to perform preliminary phytochemical screening and methanol and ethyl acetate seed extracts of such as *Tribulus terrestris* (*Tt*) and *Solanum torvum (Sn)* through FT- IR spectroscopy method. **Material and Methods:** The methanol, ethyl acetate extract was screened for its potential antioxidant activity by phenols, tannins, flavonoids and FT-IR spectroscopic studies revealed different characteristic peak values with various functional compounds. **Results:** The total phenolic content of the methanol, ethyl acetate *Tt* extract was (2.71 ±0.17, 2.32 ± 0.18) mg gallic acid equivalent (GAE)/g of extract. The total flavonoid and tannin content (2.15 ± 0.15, 1.98 ± 0.07) mg quercetin equivalent/g of extract and (2.35 ±0.12, 1.75 ±0.06) mg GAE/g of the extract. The total phenolic content of the methanol, ethyl acetate *Sn* extract was (1.63 ± 0.08, 2.28 ±0.18) mg gallic acid equivalent (GAE)/g of extract. The total flavonoid and tannin content (0.96 ± 0.06, 1.56 ± 0.19) mg quercetin equivalent/g of extract and (1.92 ± 0.09, 2.63 ± 0.13) mg GAE/g of the extract. The FT-IR method was performed on a spectrophotometer system, which was used to detect the characteristic peak values and their functional groups respectively. **Conclusion:** The results of the present study preliminary phytochemical screening generated the FT-IR spectrum profile for *Tt* and *Sn*. In conclusion, the obtained results of the tests demonstrated that this plant might be used in the prevention and in the treatment of different diseases related to oxidative stress.

Keywords: *Tribulus terrestris*, *Solanum torvum*, FT-IR, gallic acid, phenolic content, flavonoid and tannin content

Introduction

Medicinal plant research includes much more than the discovery of new drugs. This field has been expanded to also include diverse subjects as negotiation of power based on medicinal plant knowledge (Garro,1986). Plants generally contain both primary metabolites as well as secondary metabolites. The different phytoconstituents present in plants include anthraglycosides, arbutin, bitter drugs, flavonoids, alkaloids, saponins, coumarins, phenol carboxylic acids, terpenes and valepotriates. These phytoconstituents confer specific characteristics and properties of plants. Therefore, the analysis of these constituents would help in determining various biological activities of plants. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug (Parekh and Chanda, 2007). It is one of the most widely used methods to identify the chemical constituents and elucidate the structural compounds and has been used as a requisite method to identify medicines in pharmacopoeia of many countries. However, some adulterants come out in the medicinal market along with the high value medicinal materials. At present, the chromatography is the main tool used to identify the adulterants from the medicinal materials and extract products based on the chemical profile. It is well known that the medicinal materials comprise hundreds of components, and produce their curative effects through mutual effects of many ingredients, so the limited numbers of specific components cannot availably reflect the real qualities of the herbal

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DOI: https://doi.org/10.31024/apj.2019.4.3.4

medicines (Liu et al., 2006). Therefore, an effective and inexpensive analysis method to entirely monitor the whole constituents of the medicinal materials and their corresponding extract products is required. FT-IR has played a vital role in pharmaceutical analysis in recent years (Ellis et al., 2002; Thenmozhi et al., 2011).

The Fourier Transform Infrared (FT-IR) spectroscopy allows the analysis of a relevant amount of compositional and structural information in plants (Kogel-Knaber, 2000). The analysis can be performed both on pure compounds and complex mixtures, without separation into individual components. IR spectrometry is more sensitive and selective than colorimetric methods. Moreover, FT-IR spectroscopy is an established time-saving method to characterize and analyze microorganisms and monitor biotechnological processes (Grube et al., 2008).

Zygophyllaceae (Caltrop family) are a family of approximately 25 genera and 240 species (Kogel-Knaber, 2000) adapted to semidesert and Mediterranean climates. *Tribulus terrestris* (Fiure 1) is a well known and widely distributed species of the genus *Tribulus*. It is known by several common names: puncture vine, caltrop, goat head, bull's head, ground burr nut, devil's thorn (Trease and Evans, 2009) and Arabic names: Al-Gutub, Qutiba, Hasak or Ders El-Agouz (Al-Ali et al., 2003). *Tt* has been used in folk medicine throughout history for conditions such as impotence, rheumatism, edema, hypertension and kidney stones (Kostova and Dinchev, 2005; Akram et al., 2015). Literature showed that *Tt* contains phenolic compounds (Lv et al.,2008), saponins (Wu et al., 1999), sterols (Blios, 1958) and alkaloids (Re et al., 1999).

Solanum torvum (Solanaceae), (Sn) commonly known as Turkey berry is native and cultivated in Africa and West Indies (Adjanohoun et al., 1996). The fruits and leaves are widely used in Camerooninan folk medicine. It also occurs commonly in the moist farms of India. The fruits of *S.torvum* are edible and commonly available in the markets. They are utilized as a vegetable and regarded as an essential ingredient in the South Indian population's diet. A decoction of fruits is given for cough ailments and is considered useful in cases of liver and spleen enlargement (Siemonsma and Piluek, 1994). The plant is sedative and diuretic and the leaves are used as a haemostatic. The ripened fruits are used in the preparation of tonic and haemopoietic agents and also for the treatment for pain (Kala, 2012). It has antioxidant properties (Ndebia et al., 2007). It is intensively used worldwide in the traditional medicine as poison anti-dote and for the treatment of fever, wounds, tooth decay, reproductive problems and arterial hypertension (Ajaiyeoba, 1999). *S. torvum* also possesses immuno-secretory (Israf et al., 2000), antioxidant (Sivapriya and Sriniva, 2007), analgesic and anti-inflammatory, anti-ulcerogenic activities (Nguelefack et al., 2008), Cardiovascular (Mohan et al., 2009), Nephroprotective (Mohan et al., 2010), Antidiabetic (Gandhi et al., 2011), Angiotensin and Serotonin receptor blocking activities (Jaiswal and Mohan, 2012). However, more work needs to be undertaken to fully characterize these compounds, to identify the active molecules with bioactive roles. To fulfill the requirement, the present study was intended to resolve the functional constituents present in the seed of *Tt* and *Sn,* which will be useful for the proper identification of the active compounds and the chemical profile will be used as a pharmacognostic marker to differentiate the adulterant from the commercial samples. Applying metabolomic

Figure 1. *Tribulus terrestris*

Figure 2. *Solanum torvum*

techniques to pharmacognosy as a marker is a new approach, generally used to identify as functional groups. With this knowledge the present study was aimed to identify the functional groups present in crude extract of *Tt* and *Sn* seed through FT-IR spectroscopy.

Materials and methods

Chemicals

Methanol, ethyl acetate was of HPLC grade (Lab-Scan, Dublin, Ireland). All the other reagents were of analytical grade and obtained from Merck (Darmstadt, Germany).

Plant Material

Tt and *Sn* seed were collected in and around Chidambaram, Cuddalore District in the month of January-February 2018. The herbarium of the plant was identified and authenticated by the botanist Dr. V. Venkatesalu and the voucher specimen was deposited to the Department of Botany, Annamalai University, Tamil Nadu, India.

Preparation of sample extract

10 grams of powdered samples were extracted with 50 ml of solvents, such as methanol, ethyl acetate. The samples were kept in the dark for 72 h with intermittent shaking. After incubation, the solution was filtered through filter paper, and the filtrate was collected (crude extracts) and stored in the refrigerator until further use.

Preliminary phytochemicals test

Phytochemical analysis was performed to determine the presence of different phytochemicals as

described by Sadasivam and Manickam (1996).

Estimation of flavonoid content

Total flavonoid content was determined according to the method Chang et al., 2002. A 1ml of *Tt* and *Sn* seed was mixed with 0.1 ml of 10% aluminium chloride and 0.1 ml of 1 M potassium acetate. Methanol (2.8 ml) was added and kept at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm. The flavonoid content was expressed in mg/g, and Rutin was used as a standard compound.

Estimation of tannin content

Total tannin content was determined according to the method Julkunen-Titto (1985). Briefly, 50 l of *Tt* and *Sn* seed was mixed with 1.5 ml of 40% vanillin (prepared with methanol), and then 750 l of HCl was added. The solution was shaken vigorously and left to stand at room temperature for 20 min in darkness. Absorbance against a blank was read at 500 nm. Catechin was used as standard.

Estimation of phenol content

The total phenol content was measured using the Folin–Ciocalteu method Taga et al.,1984. *Tt* and *Sn* seed (100 ml) was mixed with 2 ml of 2% Na_2CO_3 and allowed to stand for 2 min at room temperature. Then, 100 ml of 50% Folin–Ciocalteu phenol reagent was added. After incubation for 30 min at room temperature in darkness, the absorbance was read at 720 nm. The total phenol content of the samples was expressed as mg Gallic acid per gram.

FTIR Spectroscopic Analysis

All spectra were obtained with the aid of an OMNI-sampler attenuated total reflectance (ATR) accessory on a Nicolet FT-IR spectrophotometer (Thermoscientific Nicolet is10, USA) followed by previous methods with some modifications (Kim et al., 2004). A small amount of powdered seed was respectively placed directly on the germanium piece of the infrared spectrometer with constant pressure applied and data of infrared absorbance, collected over the wave number ranged from 4000 cm^{-1} to 675 cm^{-1} and computerized for analyses by using the Omnic software (version 5.2). The reference spectra were acquired from the cleaned blank crystal prior to the presentation of each sample replicate. All spectra were collected with a resolution of 4-1 cm and to improve the signal-to-noise ratio, 256 scans were co-added and averaged. Samples were run in triplicate and all of them were undertaken within a day period.

Results and discussion

To study the phytochemical composition of the extracts prepared from *Tt* and *Sn*, a phytochemical screening was performed allowing to consider the possible medical uses that may have this plant as several studies have demonstrated the positive correlation between the phytochemical composition of plants and their medicinal uses (Farhan et al., 2012).

The results obtained from the phytochemical screening show that *Tt* and *Sn*, in various secondary metabolites at different concentrations. Indeed, we note the presence of proteins, saponins, flavonoids, phenolic compounds, reducing sugars, carbohydrates, amino acids, glycosides, steroids and tannins in the methanol and ethyl acetate seed extract (Table 1). Consequently, *Tt* and *Sn* by its richness in different secondary metabolites may have several medical importances such as anti-tumor especially the ethanol extract due to the presence of flavonoids (Kandaswami et al., 2005) and antioxidant due to its richness in phenolic compounds (Rammal et al., 2012). The secondary

Table 1. Qualitative phytochemical analysis different extracts of *Tribulus terrestris* (*Tt*) and *Solanum torvum (Sn)*

Chemical constituents	*Tribulus terrestris* (*Tt*)		*Solanum torvum (Sn)*	
	Methanol	Ethyl acetate	Methanol	Ethyl acetate
Alkaloids	--	++	+	+
Terpenoids	+	++	+	--
Tannins	+	++	--	+
Saponins	--	--	--	--
Flavonoids	+	++	++	++
Phenols	+	++	--	++
Amino acid	++	++	+	++
Aromatic acid	--	--	--	--
Glycosides	--	+	--	+
Steroids	--	--	--	--
Carbohydrates	+	+	+	++
Essential oil and Resins	+	++	--	+
Pholabatanins	+	--	++	--
Xantho protein	--	++	--	+
Anthroquinones	--	--	--	--
Phytosterols	--	+	--	--

++: Intensely present, +: Present, -- : Absent

Table 2. Quantitative phytochemical analysis different extracts of *Tribulus terrestris* (*Tt*) and *Solanum torvum (Sn)*

Solvents	*Tribulus terrestris* (*Tt*)		*Solanum torvum (Sn)*	
	Methanol	Ethyl acetate	Methanol	Ethyl acetate
Total Phenolics (mg GAE/g dry wt)	2.71 ±0.17	2.32 ± 0.18	1.63 ± 0.08	2.28 ±0.18
Total Flavonoids (mg RUE/g dry wt)	2.15 ± 0.15	1.98 ± 0.07	0.96 ± 0.06	1.56 ± 0.19
Total Tannins (mg CAE/g dry wt)	2.35 ±0.12	1.75 ±0.06	1.92 ± 0.09	2.63 ± 0.13

Values are means of three analyses of the extract ± Standard deviation (n=3). (GAE: Gallic acid equivalent, RUE: Rutin equivalent, CAE: Catechin equivalent)

metabolites were quantified, and the total phenol, total tannin, total flavonoid, *Tt* and *Sn*, methanol and ethyl acetate, for good antioxidant activity (Table 2).

FT-IR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The Infra-red spectroscopic (IR) analysis of study *Tt,* in a bandwidth ranging from 400 to 4000 cm-1, revealed the presence of different functional groups (Figure 3). The peaks showed that the seed extract of *Tt* may have the compounds like OH group, Alkanes, C-H group, C-H stretching, Carbonyl group, CH bending, Aliphatic amines, Carboxylic acid compounds (Table 3). The Infra-red spectroscopic (IR) analysis of studied *Sn,* in a bandwidth ranging from 400 to 4000 cm^{-1}, revealed the presence of different functional groups (Figure 4). The peaks showed that the seed extract of *Sn* may have the compounds like OH group, Alkanes, C-H group, C-H stretching, C=O Carbonyl group, CH bending, Aliphatic amines, Carboxylic acid compounds (Table 4). The above result was analyzed with interpretation of infrared spectra, a practical approach (John Coates, 2000). This analytical method is rapid, highly effective, visual and accurate for pharmaceutical research.

Analysis of the seed extract of *Tt* and *Sn* sample under FT-IR technique showed that the presence of phenolic compound and flavonoid which can be isolated and further screened for different kind of biological activities depending their therapeutic uses. Further research will be needed to find out the structural analysis of flavonoid compound by the use of different analytical methods such as NMR, HPLC, UPLC and Mass spectrophotometer.

Table 3. FT-IR spectral peak values and functional groups obtained for the methanol seed extract of *Tribulus terrestris*

S. No	Peak values	Functional groups
1	3205.27	OH group
2	2997.52	Alkanes
3	2945.47	C-H group
4	2840.93	C-H stretching
5	1642.93	Carbonyl group
6	1477.18	CH bending
7	1449.78	CH bending
8	1407.78	CH bending
9	1112.31	Aliphatic amines
10	1013.63	Carboxylic acid

Table 4. FT-IR spectral peak values and functional groups obtained for the ethyl acetate seed extract of *Solanum torvum*

S. No	Peak values	Functional groups
1	3331.49	OH group
2	2986.56	Alkanes
3	2946.95	C-H group
4	2835.22	C-H stretching
5	1657.05	C=O Carbonyl group
6	1449.40	CH bending
7	1409.31	CH bending
8	1112.55	Aliphatic amines
9	1015.21	Carboxylic acid

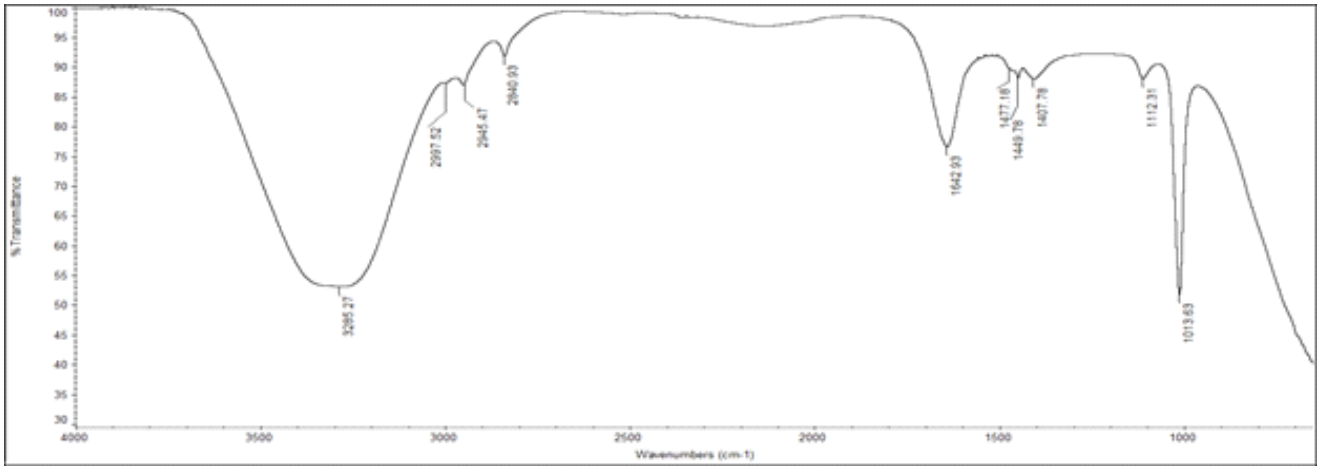

Figure 3. FT-IR spectral peak values and functional groups obtained for the methanol seed extract of *Tribulus terrestris*

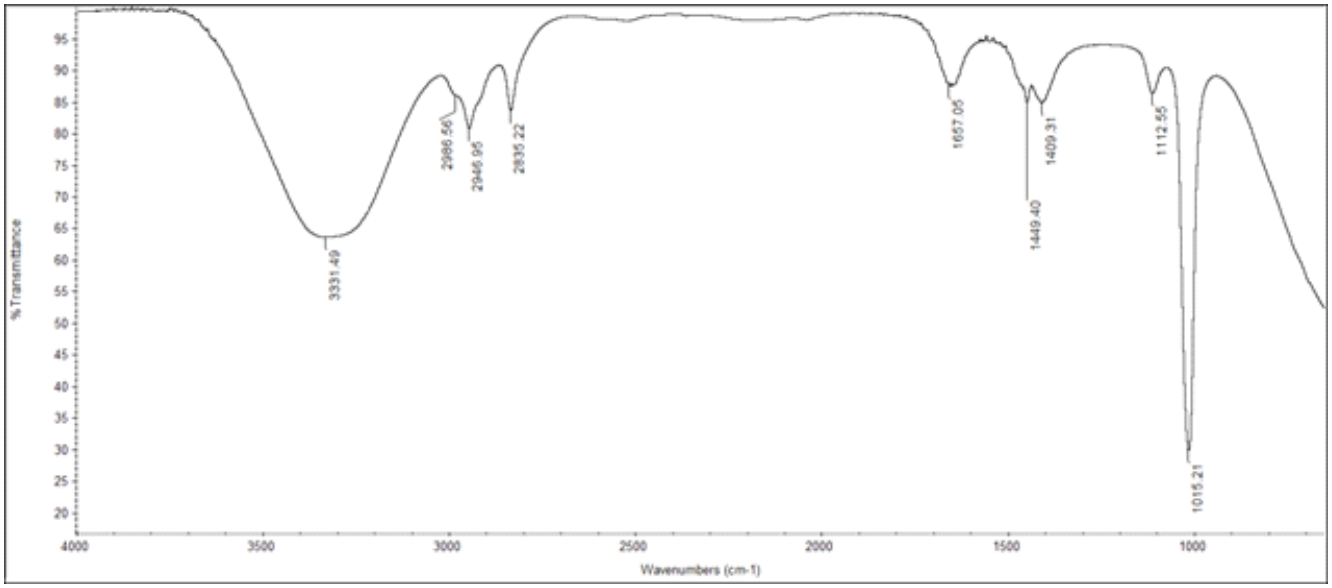

Figure 4. FT-IR spectral peak values and functional groups obtained for the ethyl acetate seed extract of *Solanum torvum*

Conclusion

The present study reveals that *Tt* and *Sn* seed is a rich source of natural antioxidants which could be extracted efficiently with methanol and ethyl acetate. This may be related to the high amount of phenolic compounds and flavonoids in this plant extract. The data clearly indicated that the extracts methanol and ethyl acetate of *Tt* and *Sn* showed good antioxidant activity. The results of our work demonstrated the importance of this plant thus highlighting the possibility of its medical use, particularly its antioxidant activity due its richness in different secondary metabolites, especially the phenolic compounds and therefore can be used in the prevention of several diseases associated with oxidative stress. Further research will be needed to find out the bioactive class of compounds which may be subjected to subsequent target isolation.

Conflicts of interest: Not declared.

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