

Efficacy of differently applied tyrosine and tryptophan for modulation of phenolic metabolism in *Trachyspermum ammi* (L.) sprague seedlings

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Abstract: Phenolics are pharmaceutically important molecules. Tyrosine and tryptophan are precursors of phenolic metabolism. It was aimed to investigate the potential of exogenously introduced precursors on the phenolic contents in *Trachyspermum ammi* (L.) Sprague seedlings. The seeds of two local varieties (Chakwal and Desi) were grown in completely randomized design in a growth chamber at $19 \pm 2^\circ\text{C}$ with two amino acids (tyrosine and tryptophan) applied (priming and supplementation in rooting medium) at two treatment levels (0, and 1%). Ten days old seedlings were harvested and subjected for growth (root and shoot length, fresh weight and dry weight) and phenolic estimation was done by HPLC method. Presence of seven phenolic acids including quercetin, chromatotropic acid, gallic acid, chlorogenic acid, sinnapic acid, trans 4 hydroxy 3 methoxy cinamic acid and P-coumaric acid was confirmed in both varieties with dissimilar fraction. Poor growth was observed by “Desi” under controlled conditions that were efficiently enhanced by tyrosine and tryptophan treatments. As precursors both amino acids differed for allosteric regulation of the pathway. That varied from application to application and variety to variety too for a pattern of phenolic accumulation. In conclusion, tyrosine and tryptophan application can be useful for farmers for improved growth of *T. ammi* and for pharmaceutical scientists to modulate metabolites of interest.

Keywords: *Trachyspermum ammi*; seedlings; tyrosine; tryptophan; phenols.

INTRODUCTION

Medicinal plants are gaining attention due to their potential to treat diseases. Therefore, medicinal plants are preferable study tool of agronomists, biochemists, pharmacists and pharmacologists all around the world. *Trachyspermum ammi* belongs to the family Apiaceae. *T. ammi* seeds analysis has revealed it to contain carbohydrates (38.6%), fiber (11.9%), glycosides, tannins, moisture (8.9%), fat (18.1%), protein (15.4%), flavones, saponins and the minor matter (7.1%) containing iron, phosphorus, calcium and nicotinic acid. Fruit of *T. ammi* yields 2% to 4% brownish oil whose major constituent is thymol. The nonthymol fraction (thymene) contains carvacrol, α -terpinene, dipentene, α - and β -pinenes, γ -terpinene and para-cymene. Small amount of α -3-carene, mycene and camphene is also present (Bairwa *et al.*, 2012). It has diverse biological activities against a variety of diseases (Chauhan *et al.*, 2012). *T. ammi* possesses wide varieties of pharmacological and pharmaceutical activities (Velazhahan *et al.*, 2010). It has anti-ulcer (Ramaswamy *et al.*, 2010), anti-septic, anti-tussive and expectorant properties (Ranjan *et al.*, 2012). Phenolics have a wide variety of biological activities (Han *et al.*, 2007). Studies are being conducted to identify the plant

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phenolics (Roy and Mukhopadhyay, 2012). Metabolites

of phenylpropanoids pathways are the phenolics (Maeda and Dudareva, 2012). The key enzyme involved in this pathway is phenylalanine ammonia-lyase (Raes *et al.*, 2003). Comprehensive literatures regarding phenolic acids in *T. ammi* seedlings are inadequate. However, the study to investigate potential of exogenously introduced tryptophan and tyrosine on phenolic contents of its seedlings required to be unrevealed. As a result, it was tried to introduce phenylpropanoid pathway precursors such as tryptophan and tyrosine for possible modulation of phenolics in *T. ammi*.

MATERIALS AND METHODS

Seeds of Chakwal variety and Desi variety were obtained from Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan and local markets respectively. Laboratory experiments were planned in an entirely randomized approach. Sand filled pots were used for sowing of both varieties. In one set, primed and unprimed (soaked for twenty four hours in a 1% solution of tryptophan and tyrosine separately) seeds were sown. In the second set, tryptophan and tyrosine 1% solution was introduced directly @ 5 ml/pot in the rooting medium.

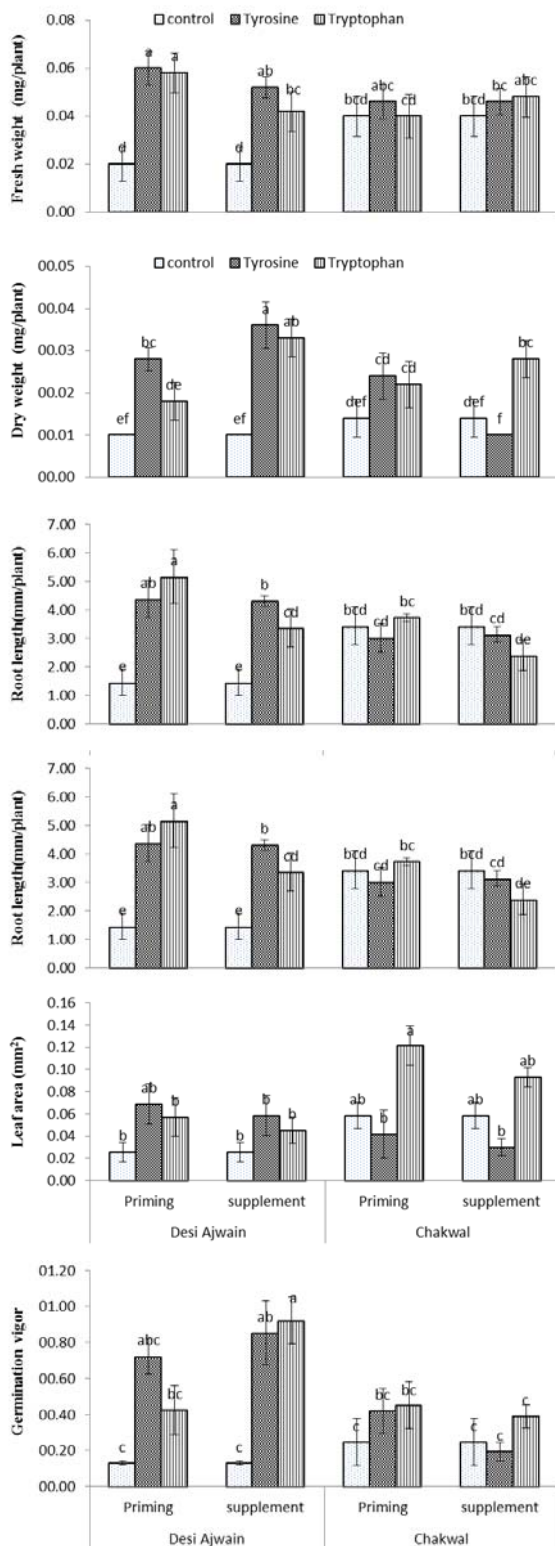


Fig. 1: Comparative growth of *Trachyspermum ammi* L. Seedlings differently treated with tyrosine and tryptophan. Note: Bars indicate \pm standard deviation and means with different letters in each bar are significantly different ($p < 0.05$) according to the DMR test ($n = 5$).

Table 1: Correlation matrix of growth and biochemical parameters

Attributes	Fresh weight	Dry weight	Root length	Shoot length	Germination percentage	Leaf area	Chromatotropic acid	Quercetin	Galic acid	Coumaric acid	Cinnamic acid	Chlorogenic acid	Sinapic acid
Fresh weight		0.60***	0.88***	0.9***	0.7***	0.38*	0.14	-0.7***	0.18	0.03	-0.7***	0.16	0.16ns
Dry weight			0.48***	0.4*	0.62***	0.19	-0.01	-0.26	-0.04	-0.08	-0.46**	-0.3	-0.3
Root length				0.9***	0.85***	0.2	0.18	-0.6***	0.36*	0.2	0.63***	0.03	0.03
Shoot length					0.7***	0.2	0.1	0.74***	0.11	-0.001	-0.7***	0.29	0.29
Germination percentage						0.12	0.12	-0.46**	0.33*	0.06	-0.55***	0.01	0.08
Leaf area							0.6***	-0.33	0.45*	0.54**	-0.24	1***	1***
Chromatotropic acid								-0.36*	0.76***	0.99***	-0.33	-0.16	-0.16
Quercetin									-0.2	-0.27	0.83***	-0.17	-0.17
Galic acid										0.79***	-0.04	-0.3	-0.3
Coumaric acid											-0.24	-0.22	-0.25
Cinnamic acid												-0.1	-0.1
Chlorogenic acid													1***

Note: *, **, *** = significant at 0.05, 0.01 and 0.001 levels, respectively; ns= non-significant.

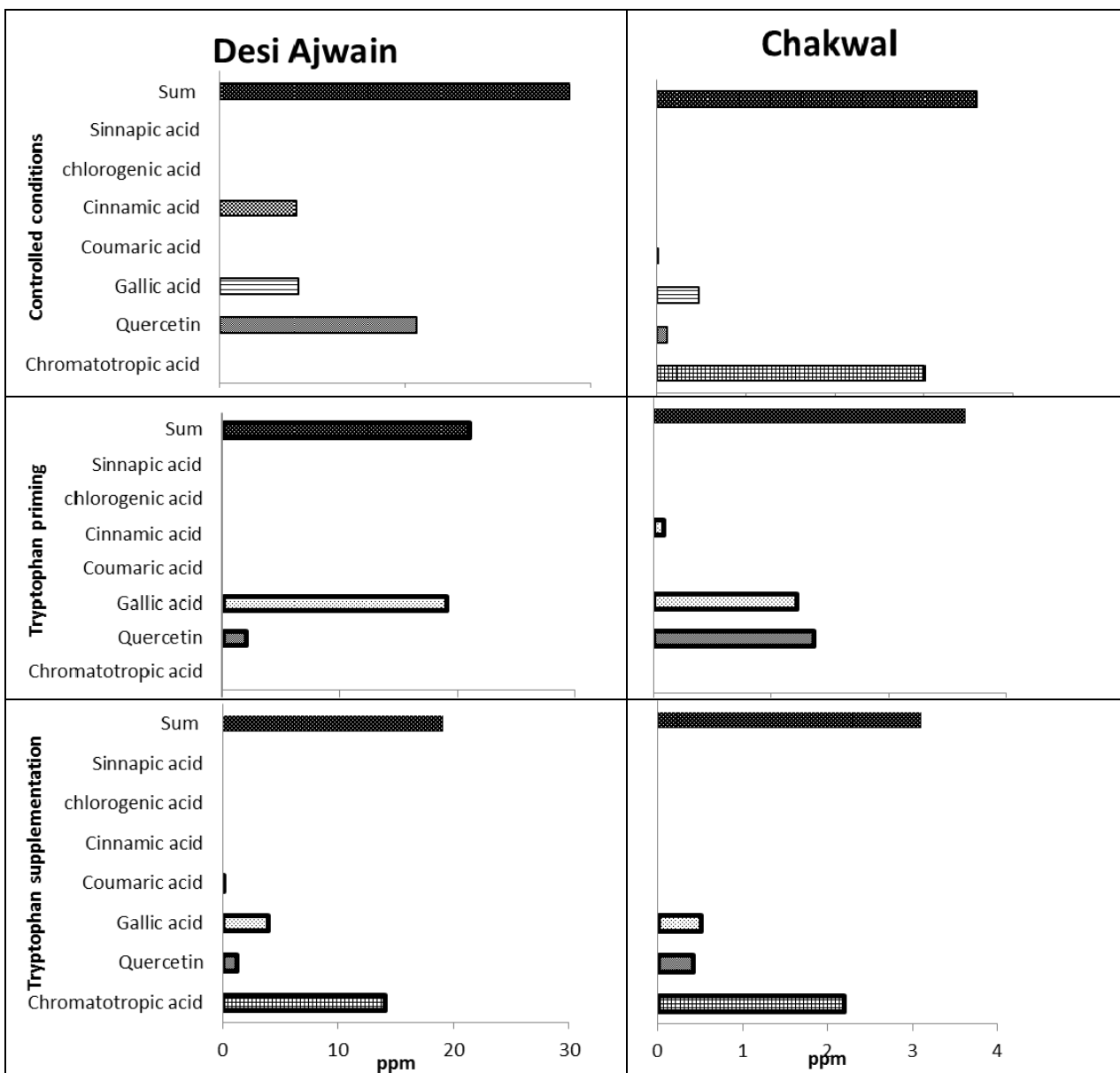


Fig. 3: HPLC chromatogram expressed in terms of ppm fractions of phenolic acids in seedlings of two local varieties of *Trachyspermum ammi* L. sprague grown under controlled and tryptophan treatments.

Growth chamber was used for placement of seed pots at $19 \pm 2^\circ\text{C}$. Data was collected carefully for determination of percentage of germination. Harvesting of 10 days old seedlings were done and data for leaf width, shoot and root length, dry weight and fresh weight was recorded. Calculation of leaf area was done using the formula (Leaf area = Leaf width \times Leaf length \times 0.75). Biochemical analysis was done on harvested samples.

Determination of Phenolic acids

HPLC analysis

HPLC reverse phase chromatography analysis was used for high performance liquid chromatography, performed

in isocratic conditions with RP C-18 HPLC column (Luna 5μ C-18, particle size $5\mu\text{m}$, $250 \times 4.6\text{ mm}$ id (2), Phenomenex, USA) at temperature 25°C . During the path of injection $5\mu\text{l}$ was volume, mobile phase was methanol-0.4 % acetic acid (80:20, v/v), rate of flow was one ml/min and at 290 nm, detection was carried out. However, 0.03 AUFS was the attenuation response. Samples after filtration from organic solvent compatible membrane filters (pore size $0.45\mu\text{m}$) were introduced into sample loop. Cinnamic acid, chlorogenic acid, ferulic, vanillic, caffeic, gallic and tannic acids were used as external and internal standards. Retention time (Rt) and areas of the peaks were used for comparison.

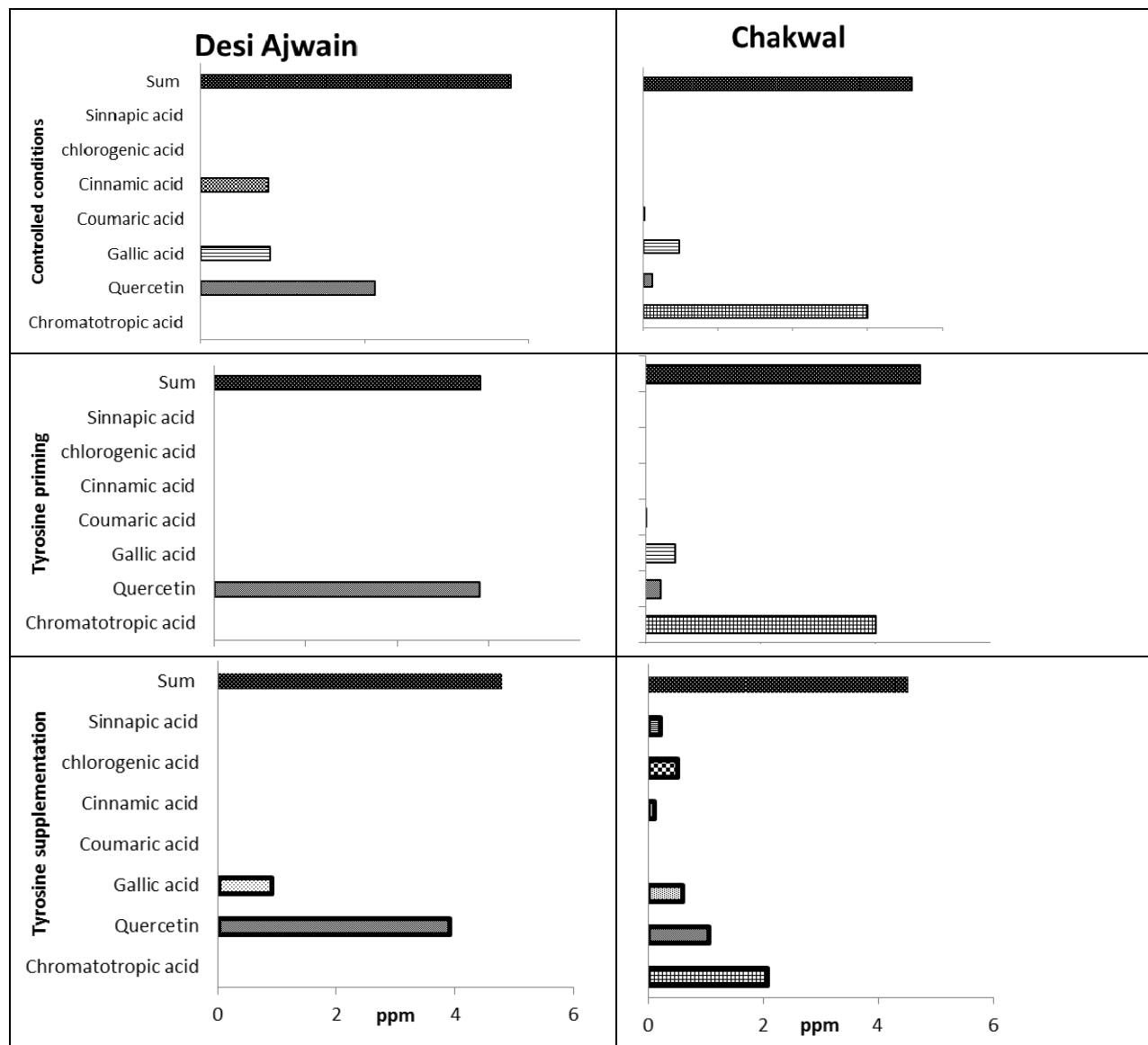


Fig. 2: HPLC chromatogram expressed in terms of ppm fractions of phenolic acids in seedlings of two local varieties of *Trachyspermum ammi* L. sprague grown under controlled and tyrosine treatments.

RESULTS

Both varieties differed for their growth. For all growth parameters Chakwal was better than Desi. Both amino acid treatments improved growth of Desi variety. Whereas, in Chakwal variety, effect of treatments was positive only for dry weight that was increased by tryptophan supplementation (fig. 1 and table 1). In a current study, 7 phenolic contents, including, cinnamic acid, chromatotropic acid, three hydroxycinnamic acids (sinapic acid, chlorogenic acid and p-coumaric acid) one flavonol (quercetin) and one hydroxybenzoic acids (gallic acid) were detected (table 1). Under controlled conditions, Chakwal showed accumulation of Chromatotropic acid (30 ppm), p-coumaric acid (0.03 ppm), gallic acid (4.7

ppm) and quercetin (1.16 ppm). In contrast, Quercetin (5.3 ppm), Gallic acid (2.1 ppm) and Cinnamic acid (2.06 ppm) were determined in a Desi variety (figs. 2 and 3).

DISCUSSION

Similar findings in intra specie difference of growth have also been observed in maize (Mahmood *et al.*, 2010) and wheat (Hussain and Sajjad, 2001). A wide range of pharmaceutical and pharmacological activities has been related to phenolics. Chlorogenic acid has been reported for anti-bacterial, hypoglycemic and hypolipidemic (Bassoli *et al.*, 2008) and supportive activity against diabetes, fatness, liver problems, heart diseases, and tumor (Kopelman, 2000; Rodriguez *et al.*, 2006). Sinapic

acid is known for its anti-cancer (Senawong *et al.*, 2013) and cinnamic acid for its helicobacter pylori inhibition potential (Siddaraju *et al.*, 2009). In the present study, the correlation of these detected phenolics was significant with leaf area and other phenolics (table 1). In the present research, response of differently supplied tyrosine and tryptophan has been explored at the seedling stage of *T. ammi*. Both amino acids are linked with plastids for their biosynthesis. But tyrosine with one benzene ring and tryptophan with double ringed indole structure have different entrance into phenylpropanoid metabolism. Therefore, the effect of both amino acids upon phenolic quantity and fractions was different in each mode of application and variety (fig. 2 and 3). Differential phenolic accumulation in treated seedlings pointed out the difference of allosteric regulation induced by both substrates (tyrosine and tryptophan) of phenylpropanoid pathway. Tyrosine treatment positively affected fraction of sinapic acid, chlorogenic acid and cinnamic acid in Chakwal variety. Tryptophan application affected the accumulation of coumaric acid, gallic acid, and chromatotropic acid exclusively in a Desi variety.

CONCLUSIONS

Both treatments can be used to enhance agronomic yield of *T. ammi*. Further, it has been confirmed that the substrates of phenylpropanoid pathway that vary in structure and their entry in pathway differ for allosteric regulation of the pathway thus variable pattern of individual fractions is manifested. Potential of tyrosine and tryptophan application can be used for modulation of phenolic metabolism to overproduce metabolites of interest. Therefore, therapeutic potential of *T. ammi* associated with above mentioned phenolic can be modified by simple applications used in the current study.

REFERENCES

- Bairwa R, Sodha R and Rajawat S (2012). *Trachyspermum ammi*. *Pharmacogn. Rev.*, **6**: 56-60.
- Bassoli B, Cassolla P and Borba G (2008). Chlorogenic acid reduces the plasma glucose peak in the oral glucose tolerance test: effects on hepatic glucose release and glycaemia. *Cell Biochem. Func.*, **26**: 320-328.
- Chauhan B, Kumar G and Ali M (2012). A Review on phytochemical constituents and activities of *T. ammi* (L.) sprague fruits. *Am. J. PharmTech. Res.*, **2**: 329-340
- Han XZ, Shen T and Lou H (2007). Dietary polyphenols and their biological significance. *Int. J. Mol. Sci.*, **8**: 950-988.
- Hussain S and Sajjad A (2001). Growth and yield response of three wheat varieties to different seeding densities. *Int. J. Agric. Biol.*, **3**: 228-229.
- Kopelman P (2000). Obesity as a medical problem. *Nature.*, **404**:635-643.
- Liu R (2003). Health benefits of fruits and vegetables are from additive and synergistic combination of phytochemicals. *Am. J. Clin. Nutr.*, **78**: 517-520.
- Maeda H and Dudareva N (2012). The shikimate pathway and aromatic amino acid biosynthesis in plants. *Ann. Rev. Plant Biol.*, **63**: 73-105.
- Mahmood S, Parveen A, Hussain I, Javed S and Iqbal M (2014). Possible involvement of secondary metabolites in the thermotolerance of maize seedlings. *Int. J. Agric. Biol.*, **16**: 1075-1082.
- Raes J, Rohde A, Christensen J, Van Y and Boerjan W (2003). Genome-wide characterization of the lignification tool box in Arabidopsis. *Plant Physiol.*, **133**: 1051-1071.
- Ramaswamy S, Sengottuvelu S, Sherief S, Jaikumar S, Saravanan R and Prasadkumar C (2010). Gastroprotective activity of ethanolic extract of *Trachyspermum ammi* fruit. *Int. J. Pharm. Bio, Sci.*, **1**: 1-15.
- Ranjan B, Sodha R and Rajawat B (2012). *Trachyspermum ammi*. *Pharmacogn. Rev.*, **6**: 56-60.
- Rodriguez D, Sotillo M, Hadley S and Sotillo J (2006). Insulin receptor exon 11+/- is expressed in Zucker (fa/fa) rats, and chlorogenic acid modifies their plasma insulin and liver protein and DNA. *J. Nut. Biochem.*, **17**: 63-71.
- Roy D and Mukhopadhyay S (2012). Enhanced rosmarinic acid production in cultured plants of two species of *Mentha*. *Ind. J. Exp. Bot.*, **50**: 817-825.
- Senawong T, Misuna S, Khaopha S, Nuchadomrong S, Sawatsitang P, Phaosir C and Sripan B (2013). Histone deacetylase (HDAC) inhibitory and antiproliferative activities of phenolic-rich extracts derived from the rhizome of *Hydnophytum formicarum* Jack.: sinapinic acid acts as HDAC inhibitor. *BMC Compl. Alt. Med.*, **13**: 1-6.
- Siddaraju M, Annaiah H and Shylaja M (2009). Gastroprotective effect of ginger rhizome (*Zingiber officinale*) extract: Role of gallic acid and cinnamic acid in H⁺, K⁺-ATPase/H.pylori inhibition and anti-oxidative mechanism. *Evid. Based Complement. Alternat. Med.*, **6**: 1-13.
- Velazhahan R, Vijayanandraj S, Vijayasamundeeswari A, Paranidharan V, Samiyappan R and Iwamoto T (2010). Detoxification of aflatoxins by seed extracts of the medicinal plant, *Trachyspermum ammi* (L.) Sprague ex Turill – structural analysis and biological toxicity of degradation product of aflatoxin G1. *Food Contr.*, **21**: 719-725.