IJBPAS, July, 2020, 9(7): 1582-1593

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

HPTLC PROFILING AND ANTIOXIDANT ACTIVITY OF *LEPIDIUM SATIVUM* AND *TRIGONELLA FOENUM GRAECUM* SEEDS

JABEEN A[1*], RANI S[2] AND IBRAHIM M[3]

1: Associate Professor, Department of Pharmacognosy, Nizam Institute of Pharmacy, Deshmukhi (V), Pochampally (M), Behind Mount Opera, Yadadri Bhuvanagiri (Dist)-508284, Telangana, India

2: Associate Professor, Annamalai University, Sadagopan Nagar, Annamalai Nagar, Chidambaram, Tamil Nadu 608002, India

3: Professor and Principal, Prathap Narender Reddy College of Pharmacy, Peddashapur, 509325, Shamshabad, Telangana, India

***Corresponding Author: E Mail: asrajabeendoer@gmail.com**

Received 29th Oct. 2019; Revised 29th Nov. 2019; Accepted 6th Jan. 2020; Available online 1st July 2020

https://doi.org/10.31032/IJBPAS/2020/9.7.5102

ABSTRACT

The objectives of this study were to establish the HPTLC phytochemical profiling and determine the antioxidant activity of ethanolic seed extract blend of *Lepidium sativum* and *Trigonella foenum graecum*. The extract was subjected to assess its antioxidant potential using in vitro system DPPH and NO radical scavenging assay. In the DPPH assay, extract blend has started showing free radical scavenging activity at a concentration of 50 µg/mL (73.18±15%). The free-radical scavenging activity of extract blend was almost near to the standard ascorbic acid (88.2±02% at 50µg/mL concentration). Similarly, the extract blend also has exhibited significant free radical scavenging activity in NO assay from the concentration 100 µg/mL (63.14±0.51%) compared to the standard ascorbic acid (92.78±0.02 % at 100 µg/mL) which clearly indicating that the extract blend is also potent in scavenging free radicals in vitro. The ability of this extract blend to scavenge DPPH and NO could also reflect its ability to inhibit the stress and subsequently the nuero-degenarative diseases related to memory. It is concluded that the ethanolic seed extract blend of *Lepidium sativum* and

Trigonella foenum graecum has strong antioxidant potential. These findings show that the polyphenolic constituents in the extracts are responsible for free radical scavenging capacity

Keywords: *Lepidium sativum*, *Trigonella foenum graecum*, Antioxidant, DPPH, NO, HPTLC

INTRODUCTION

Lepidium sativum Linn (Brassicaceae) is an annual herb locally known as halon in India but commonly known as Garden cress. *Lepidium sativum* is a polymorphous species and its centre of origin is Eritrea and Ethiopia **[1]**. It is a fast-growing edible plant. Seeds, roots and leaves of Gardencress are of economic importance; but the crop is mainly cultivated for seeds. Seven imidazole alkaloids, lepidine B, C, D, E, and F and two new monomeric alkaloids semilepidinoside A and B, sinapic acid and sinapin were reported in seeds of *Lepidium sativum* **[2]**. Benzyl isothiocyanate and benzyl cyanide were reported in colorless volatile oil but α-tocopherol and β-sitosterol were reported in the unsaponifiable matter of *Lepidium sativum* seeds **[3]**. *Trigonella foenum-graecum* (Fabaceae) commonly known as Methi or metha. It is a well-known herb in the Ayurvedic system of medicine. It is small annual herb found in different part of India **[4]**. The seeds contain carbohydrates, some vitamins like carotene, thiamine, riboflavin, nicotinic acid, folic acid, etc. The seed contain two alkaloid trigonelline and choline **[5, 6]**.

HPTLC is coherent for development of chromatographic fingerprints to establish major active constituents of medicinal plants. The degree of separation and resolution can be improved, and the results are much more consistent and reproducible than conventional TLC. In situ qualitative and quantitative measurements by scanning densitometry and the colorful pictorial HPTLC image offers extra, perceptive visible color and/or fluorescence parameters for comparable assessment on the same plate. It also opened a way for a better separation of individual secondary metabolites in plant extracts **[7]**.

Reactive oxygen species (ROS)/free radicals have been implicated in causation of more than 100 diseases including diabetes, inflammation, cancer, neurodegenerative disorders, atherosclerosis, liver cirrhosis, nephrotoxicity etc. All aerobic organisms including humans have antioxidant defense mechanisms that protect against oxidative damage. However, the natural antioxidant defense mechanisms can be insufficient and hence dietary intake of antioxidant components is important and recommended **[8]**. It has been suggested that fruits, vegetables and plants are the main source

of antioxidant in the diet. Natural antioxidants may have free-radical scavengers, reducing agents, complexes of pro-oxidant metals, quenchers of singlet oxygen etc. The importance of medicinal plants to prevent or control diseases has been attributed to the antioxidant properties of their constituents, commonly associated with a large number of molecules such as phenols and flavonoids **[9]**.

MATERIALS AND METHODS

Lepidium sativum Linn and *Trigonella foenum-graecum* were procured from the PSSG suppliers. Trivandrum and the seeds were authenticated. 500g of seeds were weighed and allowed to dry under room temperature for duration of one week. The seeds were placed at shaded area without exposing to the sunlight to avoid any evaporation of active constituent such as phenol. The seeds were crushed into powder by using the crusher and collected in the conical flasks. HPTLC fingerprinting was carried out on the CAMAG HPTLC.

Extraction and HPTLC finger printing

HPTLC finger print profiling was carried out according to the method developed by Wagner **[10]** and Harbone **[11]**. The two plant seeds were individually subjected for the successive solvent extraction with various solvents like hexane, chloroform, and ethanol in an increasing polarity order. The final ethanolic extracts of both *Lepidium sativum Linn* and *Trigonella foenum-graecum* were lyophilized in order to produce the solid extracts.

Individual ethanolic extracts were subjected to HPTLC finger printing.The dried extracts and isolated marker compounds were re-dissolved in methanol to a final concentration of 10 mg/mL and 1 mg/mL, respectively, and 2 µL of each sample was spray-applied with a 25 µL Hamilton micro syringe as 8 mm bands, 5 mm from the lower edge of 20 × 10 cm silica gel plates (Silica gel 60 F254, Merck, Germany). The best mobile phase was determined by investigating numerous systems of different polarities. Ascending development was carried out in a 20 × 10 × 4 cm glass twin-trough chamber (CAMAG) up to 75 mm, using the optimized mobile phase ethyl acetate: aceticacid: formicacid: water (100:11:11:27 v/v/v/v) for *Trigonella foenum-graecum* and ethyl acetate: aceticacid: formicacid: water (100:11:15:27 v/v/v/v) for *Lepidium sativum*, after tank saturation for 20 min with 25 mL of the mobile phase at room temperature (25 ± 2 °C) and relative humidity (49 ± 5%). After development and drying for 5 min, the results were viewed and documented under UV light at 254 nm and 366 nm, derivatized with natural product reagent and viewed under white light.

The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The DPPH assay provides an easy and rapid way to evaluate potential antioxidants. The DPPH assay was done according to the standard protocols with slight modifications. Firstly, the optical density (OD) of the DPPH solution was calibrated at 517 nm to between 0.9-1.00. Then, the DPPH solution (160 µL) was added to 40 µL of the extracts at different concentrations (3.125-200 µg/mL). The mixture was incubated in the dark for 30 min, and the absorbance was measured at 517 nm using a micro plate reader (Epoch, Biotek) **[12, 13]**. Lower absorbance of the solution indicated higher free radical scavenging activity. The percentage of scavenging activity was calculated using the formula (1) below: **% scavenging activity = [(A0 −As)/A0] ×100 formula (1)** Where A0 is absorbance of control (DPPH solution without sample), As is absorbance of tested sample (DPPH plus sample). The 50% inhibitory concentration (IC_{50}) values of extracts were determined using a non-linear regression curve of percentage of scavenging activity against the logarithm of concentrations. Ascorbic acid and Trolox were used as positive controls. Each test was done in triplicate and results are presented as mean ± standard deviation (SD) **[14, 15]**.

Nitric oxide radical (NO.) scavenging assay

NO generated from sodium nitroprusside (SNP) was measured according to the method of Marocci. Briefly, the reaction mixture (5.0 ml) containing sodium nitroprusside (5 mM) in phosphate-buffered saline (pH 7.3), with or without the plant extract at different concentrations, was incubated at 25˚C for 180 min in front of a visible polychromatic light source (25W tungsten lamp). The NO. radical thus generated interacted with oxygen to produce the nitrite ion (NO.) which was assayed at 30 min intervals by mixing 1.0 ml of incubation mixture with an equal amount of Griess reagent (1% sulfanilamide in 5% phosphoric acid and 0.1% naphthylethylene-diaminedi-hydrochloride). The absorbance of the chromophore (purple azo dye) formed during the diazotisation of nitrite ions with sulphanilamide and subsequent coupling with naphthyl-ethylene-diaminedi-hydro-chloride was measured at 546 nm. The nitrite generated in the presence or absence of the extracts as well as the standard ascorbic acid at 50, 100 and 250 µg/ml was estimated using a standard curve based on sodium nitrite solutions of known concentrations. Each experiment was carried out in triplicates and the data presented as an average of three independent determinations **[16]**.

RESULTS AND DISCUSSIONS

HPTLC profiling of the extracts

HPTLC fingerprint of *Lepidium sativum* and *Trigonella foenum-graecum* seed extracts under UV 254 nm and 366 before derivatization and under white light after derivatization. The HPTLC fingerprint profile for *Lepidium sativum* extract showed a distinct band pattern, and it is represented in Figure 27-29. The HPTLC analysis for the *Lepidium sativum* extract exhibited eight peaks before derivatization under UV 254 nm. For *Lepidium sativum,* Track 1 (quercetin), Track 2 (kaempferol-3-β-D-(6″-O-trans-p-coumaroyl) glucopyranoside), Track 3 (kaempferol-3–O– β- glucosyl (1 → 2)- glucopyranoside), Track 4 (Compound 1: isoorientin), Track 5 (Compound 2: kaempferol-3-β-D-(6″-O-trans-pcoumaroyl) glucopyranoside), Track 6 (Glucotropoeolin) and Track 7 (Ethanolic seed extract). One unknown fluorescent spot was observed under UV 366 nm with Rf value 0.13.

When the Rf values are compared with the standard spots, it is found that the samples 1(quercetin), 3 (kaempferol-3–O–β-glucosyl(1→2)-glucopyranoside) and 6 (Glucotropoeolin) are comparatively higher in concentration in the given extract.

Antioxidant activity

The extract blend exhibited significant antioxidant activity in this study **(Table 3 and Figure 7)**. All the values are expressed as mean± SEM. The (*) symbol represents the statistical significance done by ANOVA, followed by Dennett's "t" test. The "P" values represented through Dennett's "t" test which are less than 0.05 are considered as statistically significant which means, they are the concentrations where there is a considerable antioxidant activity. In the present study, extract blend has started showing free radical scavenging activity at a concentration 50 µg/mL (73.18±15%). Interestingly, the extract blend is showing the antioxidant activity in a dose dependent manner. At higher concentration 200 µg/mL, the extract blend is showing 90.74±08 % which is having almost near to the inhibition of ascorbic acid which is having 94.54±41% of inhibition.

The free-radical scavenging activity of extract blend was almost near to the standard ascorbic acid (90.74±08% at 0.2 mg/ml concentration) which clearly indicating that the extract blend is also potent in scavenging free radicals in vitro. The ability of this extract blend to scavenge DPPH could also reflect its ability to inhibit the stress and subsequently the neurodegenerative diseases related to memory. Increased oxidative stress is the primary event involved in the pathological abnormalities of AD, including β-amyloid deposition and cholinergic dysfunction.

Oxidative stress occurs when reactive oxygen species (ROS) accumulate to toxic levels in cells, either from excessive production or insufficient degradation, resulting in injury to DNA, lipids, and proteins. Brain tissue is known to be more susceptible to the deleterious effects of ROS because unlike many other tissues, it contained small amounts of protective antioxidant defense systems. The memory impairment may be associated with increased oxidative stress, which results in the degeneration of cholinergic neurons and of other neurotransmitter systems.

The antioxidant activity may be due to the presence of some potent molecule (s) in the extract blend which is more capable of quenching DPPH radical.

Nitric oxide radical ($NO^{\cdot}$) scavenging assay

Nitric oxide ($NO^{\cdot}$) released from sodium nitroprusside (SNP) has a strong NO^{+} character which can alter the structure and function of many cellular components. The ethanol seed extract blend of *Lepidium sativum* and *Trigonella foenum-graecum* exhibited good $NO^{\cdot}$ scavenging activity, by reducing nitrite concentration in assay medium.

The $NO^{\cdot}$ scavenging capacity was concentration dependent showing 67.78±0.42 % of inhibition at150 µg/mL. The extractsin sodium nitroprusside solution significantly inhibited ($p < 0.05$) the accumulation of nitrite, a stable oxidation product of $NO^{\cdot}$ liberated from sodium nitroprusside in the reaction medium with time compared to the standard Ascorbic acid **(Table 4 and Figure 8)**. The toxicity of $NO^{\cdot}$ increases when it reacts with superoxide to form the peroxynitrite anion ($^{\cdot}ONOO^{-}$), which is a potential strong oxidant that can decompose to produce $^{\cdot}OH$ and NO_2. The present study shows that a *Lepidium sativum* and *Trigonella foenum-graecum* have potent nitric oxide scavenging activity when compared to the standard ascorbic acid.

The extract blend exhibited significant antioxidant activity in this study **(Table 4 and Figure 8).** All the values are expressed as mean± SEM. The (*) symbol represents the statistical significance done by ANOVA, followed by Dennett's "t" test. The "P" values represented through Dennett's "t" test which are less than 0.05 are considered as statistically significant which means, they are the concentrations where there is a considerable antioxidant activity. In the present study, extract blend has started showing free radical scavenging activity at a concentration 100 µg/mL (63.14±0.51%).

Interestingly, the extract blend is showing the antioxidant activity in a dose dependent manner. At higher concentration 150 µg/mL, the extract blend is showing

67.78±0.42 % which is having statistically significant inhibition like ascorbic acid which is having 96.22±0.01% of inhibition, which clearly indicating that the extract blend is also potent in scavenging free radicals in vitro. The ability of this extract blend to scavenge $NO^{\cdot}$ could also reflect its ability to inhibit the stress and subsequently the neurodegenerative diseases related to memory. The ability of this extract blend to scavenge $NO^{\cdot}$ could also reflect its ability to inhibit the stress and subsequently the neurodegenerative diseases related to memory.

Table 1: HPTLC finger printing of*Trigonella foenum graecum* ethanolic seed extract

Sample track	Sample name	Rf value
T1	Quercetin	0.74
T2	(kaempferol-3-β-D-(6″-O-trans-p-coumaroyl) glucopyranoside	0.75
T3	kaempferol-3–O– β- glucosyl (1 → 2)- glucopyranoside	0.75
T4	Glucotropoeolin	0.72
T5	isoorientin	0.75
T6	kaempferol	0.76
T7	Ethanolic extract	0.72
		0.74
		0.75
		0.76
		0.13 (unknown)

Table 2: HPTLC finger printing of *Lepidium sativum* ethanolic seed extract

Sample track	Sample name	Rf value
T1	Sinapic acid	0.56
T2	kaempferol	0.57
T3	Fenugreekine	0.58
T4	Trigonelline	0.56
T5	Sinapine	0.57
T6	Quercetin	0.57
T7	Ethanolic extract	0.56
		0.57
		0.58
		0.72 (unknown)
		0.17 (unknown)

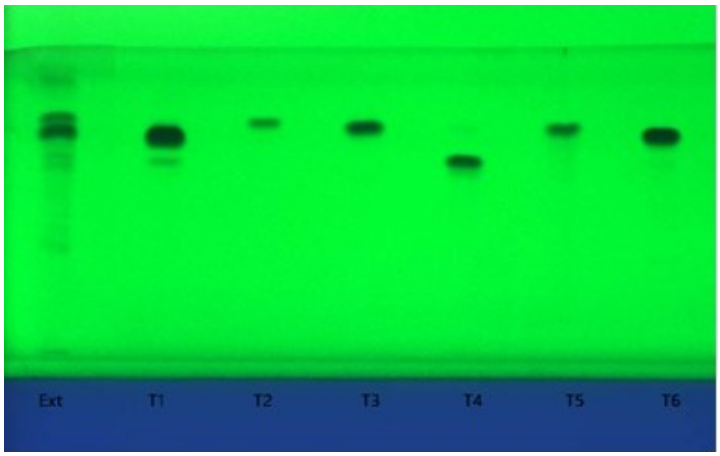

Figure 1: *Lepidium sativum* HPTLC

Figure 2: *Lepidium sativum* HPTLC FLUORESCENT IMAGING

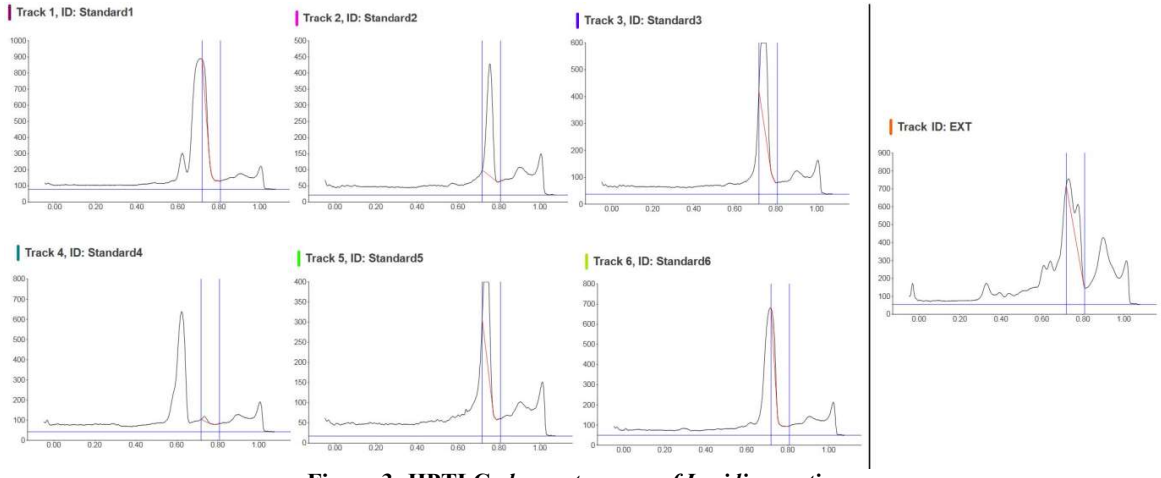

Figure 3: HPTLC *chromatograms of Lepidium sativum*

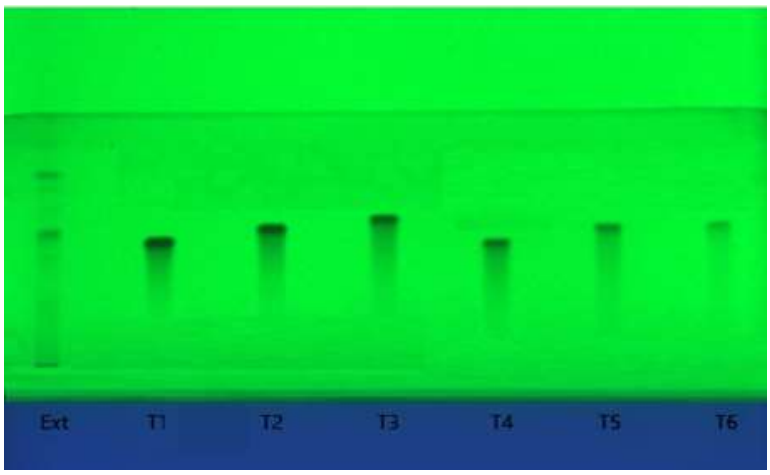

Figure 4: *Trigonella foenum graecum* HPTLC

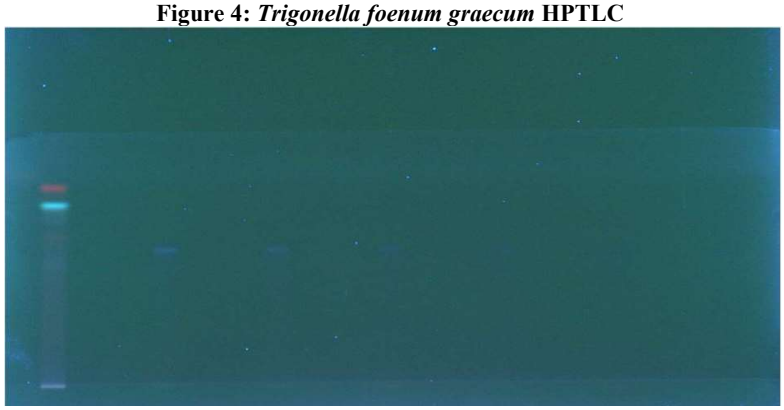

Figure 5: *Trigonella foenum graecum* HPTLC FLUORESCENT IMAGING

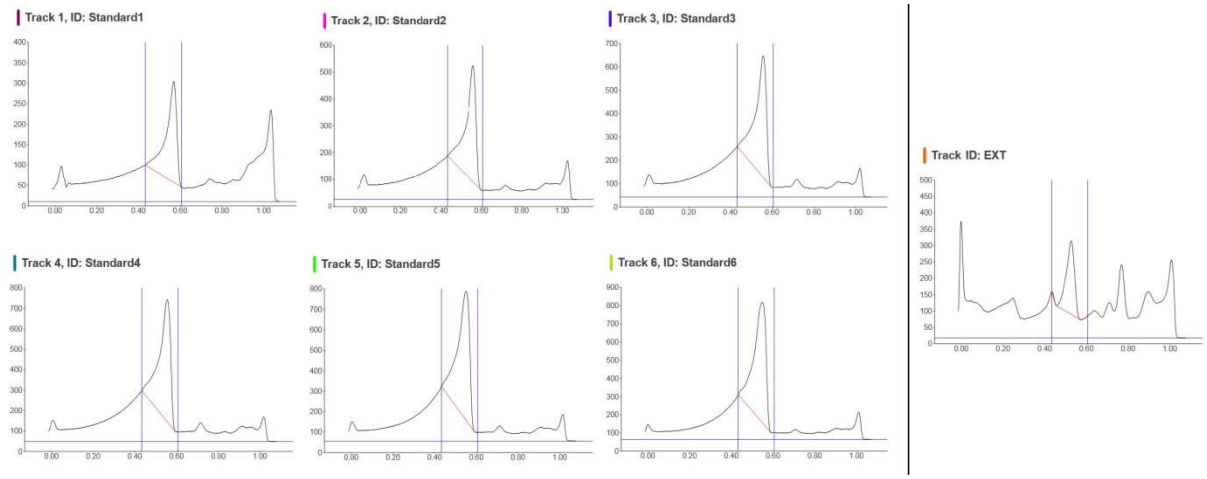

Figure 6: HPTLC *chromatograms of Trigonella foenum graecum*

Table 3: DPPH assay of *Lepidium sativum* and *Trigonella foenum graecum*ethanolic seed extract blend

Concentration (µg/mL)	Percentage of inhibition	
	Ascorbic acid	Extract blend
25	86.21±14	71.04±12
50	88.2±02*	73.18±15*
100	89.4±10*	79.41±21*
150	91.35±03*	82.95±11*
200	94.54±41*	90.74±08*
	IC_{50}=14.99	**IC_{50}=17.59**

Values are expressed as mean± SEM. The symbol represents the statistical significance done by ANOVA, followed by Dennett's "t" test. *P<0.05 is considered as significant

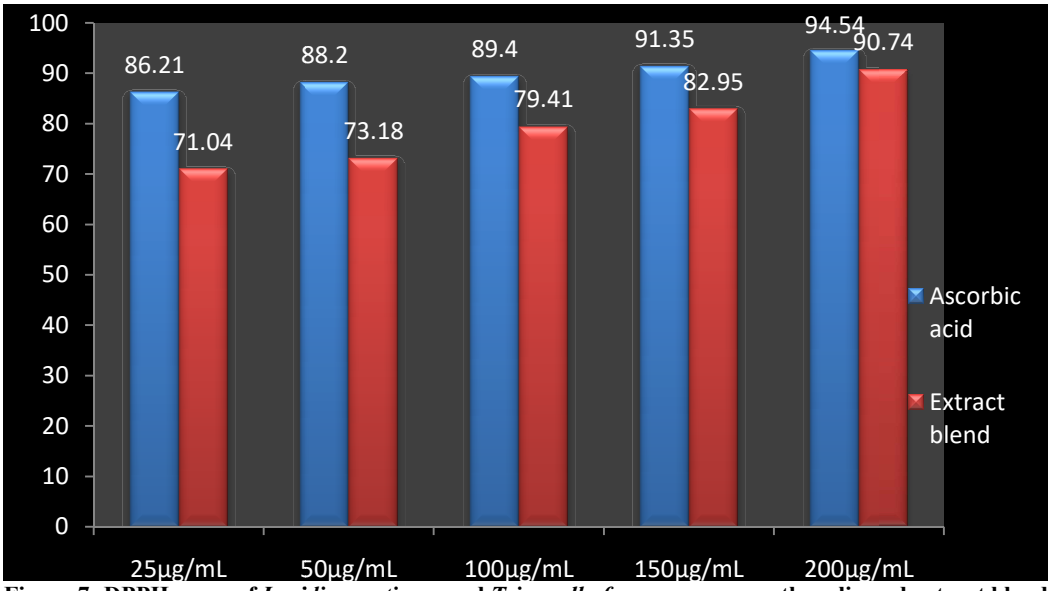

Figure 7: DPPH assay of *Lepidium sativum* and *Trigonella foenum graecum*ethanolicseed extract blend

Table 4: Scavenging of Nitric oxide by *Lepidium sativum* and *Trigonella foenum-graecum*ethanolic seed extract blend

Concentration in µg/ml	Extract blend	Ascorbic acid
50	51.47±1.1	78.32±0.65
100	63.14±0.51*	92.78±0.02
150	67.78±0.42*	96.22±0.01
	IC_{50}=48.57	**IC_{50}=31.92**

Values are expressed as mean± SEM. The symbol represents the statistical significance done by ANOVA, followed by Dennett's "t" test. *P<0.05 is considered as significant

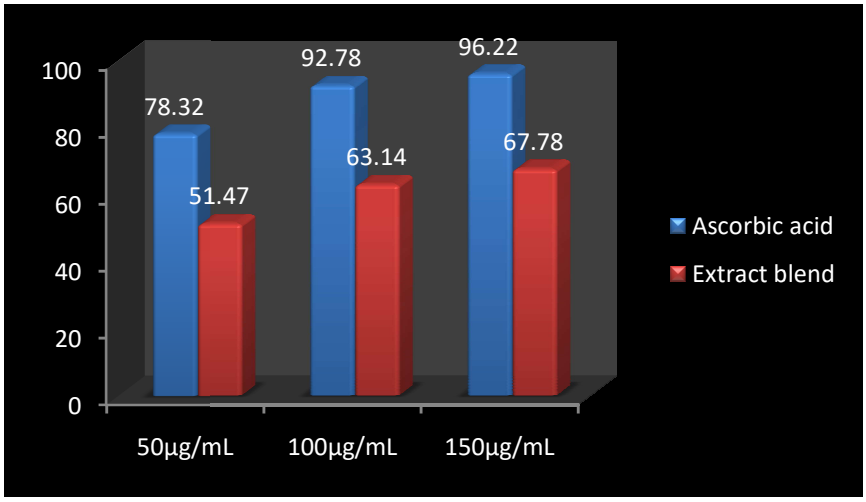

Figure 8: Scavenging of Nitric oxide by *Lepidium sativum* and *Trigonella foenum-graecum*ethanolic seed extract blend

CONCLUSION

In conclusion, the HPTLC fingerprinting profile of the extracts provided a good qualitative identification method for phytochemical investigation by direct band comparison and can clearly confirm the presence of Sinapic acid, kaempferol, Fenugreekine, Trigonelline, Sinapine and Quercetin in *Trigonella foenum-graecum* seed. Simlarly, presence of Quercetin, kaempferol-3-β-D-(6″-O-trans-p-coumaroyl) glucopyranoside, kaempferol-3–O– β- glucosyl (1 → 2)-glucopyranoside, Glucotropoeolin, isoorientin and kaempferol in *Lepidium sativum* seeds.

This investigation also provides evidence which suggests that the *Lepidium sativum* and *Trigonella foenum graecum* ethanolic seed extract blend has anti-oxidant activity through DPPH and NO scavenging antioxidant assays, that might offer some promising effects for the treatment of memory impairment conditions like amnesia and Alzheimer's disease.

ACKNOWLEDGEMENT

Authors are thankful to the management of Nizam college of pharmacy, Hyderabad for providing the facilities required to carry out the present work.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

REFERENCES

[1] Al-Yahya Mossa JS, Ageel AM, Rafatullah S, Pharmacological and safety evaluation studies on Garden cress *(Lepidium sativum L.)* seeds, Phytomedicine, 1994, 1(2), 155-159.

[2] Doke, Snehal, Guha, Manisha, Garden cress (*Lepidium sativum L.*) Seed - An Important Medicinal Source, A Review, Journal of natural product and plant resources, 2014, 4, 69-80.

[3] Lee J, Koo N, Min DB, Reactive oxygen species, aging and antioxidative neutraceuticals. Comp Rev Food Sci Food Safety, 2004, 3(1), 21-33.

[4] Acharya SN, Thomas JE, Basu SK, Fenugreek- an old-world crop for the new world, Biodiversity 2006,7(3-4), 27–30.

[5] Naidu MM, Shyamala BN, Naik PJ, Sulochanamma G, Srinivas P, Chemical composition and antioxidant activity of the husk and endosperm of fenugreek seeds, Food Sci. Technol., 2010, 44, 451-456.

[6] Wani SA, Kumar P, Fenugreek- A review on its nutraceutical properties and utilization in various food products, J saudi society agri science 2018,17(2),97–106.

[7] Senguttuvan J, Subramaniam P, HPTLC Fingerprints of Various Secondary Metabolites in the Traditional Medicinal Herb *Hypochaeris radicata* L, Journal of Botany, 2016, 1–11.

[8] Markesbery WR,Oxidative stress hypothesis in Alzheimer's disease, Free Radical Biology and Medicine, 1997, 23(1), 134–147.

[9] Holttum JR, Gershon S,The cholinergic model of dementia, Alzheimer type, progression from the unitary transmitter concept, Dementia 1992, 3, 174–185.

[10] Wagner H, Baldt S, Plant Drug Analysis, A Thin Layer Chromatography Atlas, Springer, Berlin, Germany, 2nd edition, 1996.

[11] Harborne JB, Phytochemical Methods, Chapman and Hall, London, UK, 3rd edition, 1998.

[12] Moreira PI, Santos MS, Oliveira CR, Shenk JC, Nunomura A, Smith MA, Zhu X, Perry G, Alzheimer disease and the role of free radicals in the pathogenesis of the disease, CNS Neurological Disorders and Drug Target, 2008, 7(1), 3–10.

[13] Tabet N, Mantle D, Orrell M,Free radicals as mediators of toxicity in Alzheimer's disease, a review and hypothesis, Adverse Drug Reaction and Toxicol Review, 2000, 19(2), 127–152.

[14] Awika JM, Rooney LW, Sorghum phytochemicals and their potential impact on human health, Phytochem., 2004, 65,1199–1221.

[15] Liu R, Gao M, Qiang GF, Zhang TT, Lan X, Ying J et al,, The anti-amnesic effects of luteolin against amyloid β25–35 peptide-induced toxicity in mice involve the protection of neurovascular unit,

Neuroscience, 2009,162, 1232–1243.

[16] Marcocci L, Maguire JJ, Droylefaix MT, Packer L, The nitric oxide-scavenging properties of *Ginkgo biloba* extract EGb 761, Biochem. Biophys. Res. Commun., 1994,201, 748–755.