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PHYTOCONSTITUENT ANALYSIS OF SUPERCRITICAL FLUID CARBON DIOXIDE EXTRACT OF *NIGELLA SATIVA (*BLACK CARAWAY) SEEDS BY HPLC TECHNIQUE.

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ABSTRACT

Natural products chemistry has experienced explosive and diversified growth, making natural products the subject of much interest and promise in the present day research directed towards drug design and drug discovery. *Nigella sativa* L. (Black cumin) is an annual herbaceous plant, belongs to family Ranunculaceae. Thymoquinone is the major active principle of *Nigella sativa* seeds and is known to have Antimicrobial activities. The present study has been designed to identify Thymoquinone in Supercritical fluid Carbon dioxide extraction (SCFE-CO2) of *Nigella sativa* L. seeds. Preliminary phytochemical analysis revealed the presence of alkaloids, tannins, steroids and terpenoids. Extracts were subjected to Silica gel-G coated TLC plate with Hexane: Ethyl acetate (90:10 v/v) as developing solvents. The RF value for Thymoquinone was found to be 0.81 and the results were compared with Standards. Phytoconstituents analysis and quantification of (SCFE-CO2) was carried out by HPLC on a ZORBAX eclipse plus C18 (4.6 x 250mm, 5µm) column, using isocratic mobile phase of Water:Methonal:2-Proponol (50:45:5% v/v) at a flow rate of 1ml/min. The elution was detected at 254nm. The HPLC analysis confirmed the presence of Thymoquinone (Area 96.84) and other secondary metabolites in SCFE-CO2 Extract of *Nigella sativa* L. seeds.

KEY WORDS: Supercritical fluid Carbon dioxide Extraction, Thin layer chromatography, High performance Liquid chromatography, Retention factor, Retention Time.

1. INTRODUCTION

Mother Nature stands as an inexhaustible source of novel chemotypes and pharmacophores. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs find their origin in nature.[1] Natural products chemistry has experienced explosive and diversified growth, making natural products the subject of much interest and promise in the present day research directed towards drug design and drug discovery.[2] Recently, there has been a renewed interest in natural products research due to the failure of alterative drug discovery methods to deliver many lead compounds in key therapeutic areas such as immunosuppression, anti-infectives and metabolic process.

Nigella sativa seeds, as nutritional and medicinal plant, have traditionally been used for thousands of years as folk medicine and some of its active compounds were reported against many ailments.[3] Different pharmacological effects such as gastric ulcer healing,[4] anti-microbial effect,[5] anti-cancer activity,[6] cardiovascular disorders,[7] gastro protective and antioxidant activity,[8] immunomodulatory, anti-inflammatory and anti-tumour effects,[9] antitussive effect,[10] anti-anxiety effect,[11] anti-asthmatic effect,[12] anti-inflammatory effects in pancreatic cancer cells,[13] anti-helicobacter activity,[14] tumour growth suppression,[15] anti-viral activity against cytomegalovirus,[16] hepatoprotective activity.[17] have been reported for this medicinal plant. Present study has been designed to identify and to estimate Thymoquinone in Supercritical fluid carbon dioxide extract (SCFE-CO_2) of *Nigella sativa* L. seeds by HPLC technique.

2. MATERIALS AND METHODS

2.1. Collection and identification of medicinal plant seeds

Nigella sativa L.seeds was purchased, dried and blended using mechanical blender and packed in air tight container for further use. The plant species was indentified and registered (Reg. No. PARC/2017/3547) by Dr. P. Jayaraman, Director of Plant Anatomy Research Centre.

2.2. Supercritical fluid carbon dioxide extraction (SCFE-CO_2)

SCFE-CO_2 extraction was carried out in Thar SFC extractor, Department of Food Process Engineering, SRM University. Extraction of pulverized *Nigella sativa* L. seeds was done at

120 bar pressure at 40°C. The flow rate of CO_2 and Co-Solvent was 10g/min and 11g/min respectively. Yield of extracts were monitored, dried in rotary evaporator and stored at 4°C until analysis.

2.3. Phytochemical screening

SCFE-CO_2 extract was subjected to preliminary phytochemical screening of Alkaloids, Flavonoids, Saponins, Tannins, Cardiac glycosides, Phenols, Terpenoids and Carbohydrates by adopting the standard protocols described by Sofowora; Trease and Evans.[18-20]

2.4. Thin Layer Chromatography

TLC is a method used to separate wide range of compounds of biochemical interest. The SCFE-CO_2 extract and Thymoquinone standard (Sigma-Aldrich) were spotted on the Silica gel-G coated TLC plate using capillary tube. The spots were carefully dried and eluted using Hexane: Ethyl acetate (90:10 v/v) as developing solvents. The Rf value was interpreted by exposing eluted TLC plates to UV light.

2.5. HPLC analysis

The bioactive compounds in SCFE-CO_2 extract of *Nigella sativa* L. seeds were analysed and quantified using HPLC with following specifications.

Model: Agilent Technologies
Stationary Phase: Silica Gel (Reversed Phase)
Mobile Phase: water: methanol: 2-propanol (50:45:5% v/v)
Main Column: Analytical, ZORBAX Eclipse Plus C18 (4.6 × 250mm, 5µm)
Guard Column: 4.6mm id × 12.5mm PPS Polymer tubes with press-fit 2-micron porosity frits.
Detector: UV – Vis
Flow Rate: 0.5 ml per minute
Injection volume: 20µl
Wavelength: 254, nm

3. RESULTS AND DISCUSSION

Supercritical fluid carbon dioxide extracts of *Nigella sativa* L. seeds are brown and gummy in texture. The preliminary phytochemical screening revealed the presence of Alkaloids, Flavonoids, Tannins, Steroids, Saponins and Terpenoids as shown in **Table 1**. The phytochemicals present in black caraway seeds have many pharmacological applications.[21]

Table 1. Preliminary phytochemical screening of SCFE-CO_2 extracts of *Nigella sativa* L. seeds.

S. No.	Phytochemical components	Inference
1	Alkaloids	+
2	Flavonoids	+
3	Phenol	-
4	Tannins	+
5	Glycosides	-
6	Steroids	+
7	Saponins	+
8	Terpenoids	+

+ Positive, - Negative.

Table 2. Thin layer chromatography.

S. No.	Extract	Mobile phase	No. of spots	Rf value
1	Standard	Hexane: Ethyl acetate (9:1 v/v)	2	0.81 0.07
2	SCFE-CO_2	Hexane: Ethyl acetate (9:1 v/v)	4	0.81 0.47 0.38 0.07

Rf – Retention factor

Thymoquinone is a bioactive constituent of *Nigella sativa* L. seeds which possesses considerable pharmacological properties.[22] Thin layer Chromatographic elution of SCFE-CO_2 revealed the separation of four spots with Rf value 0.81, 0.47, 0.38 and 0.07 as shown in **Table 2.** The Rf value of Thymoquinone was found to be 0.81 which is equivalent to Thymoquinone standard.

Table 3. HPLC analysis of SCFE-CO_2 extract of *Nigella sativa* L. seeds.

Peak	Retention time	Area %
1	1.152	0.2559
2	1.272	0.8903
3	1.329	1.0496
4	1.828	0.1055
5	7.019	0.8582
6	9.915	96.8405

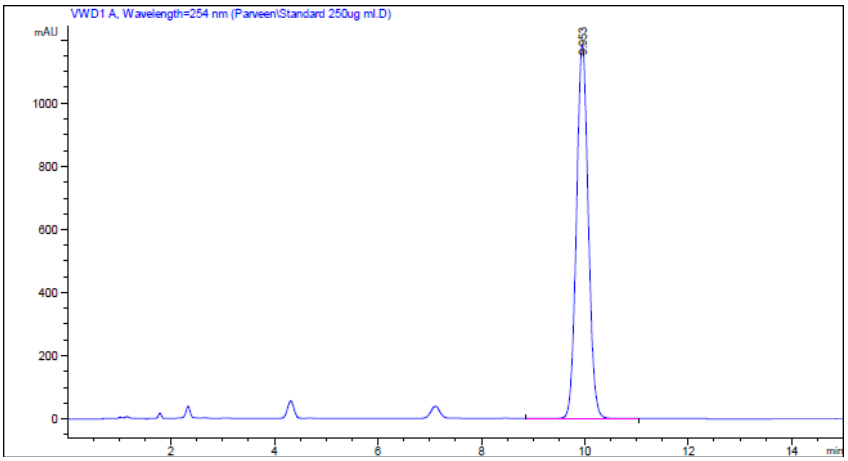

Figure 1. HPLC chromatogram of Thymoquinone standard.

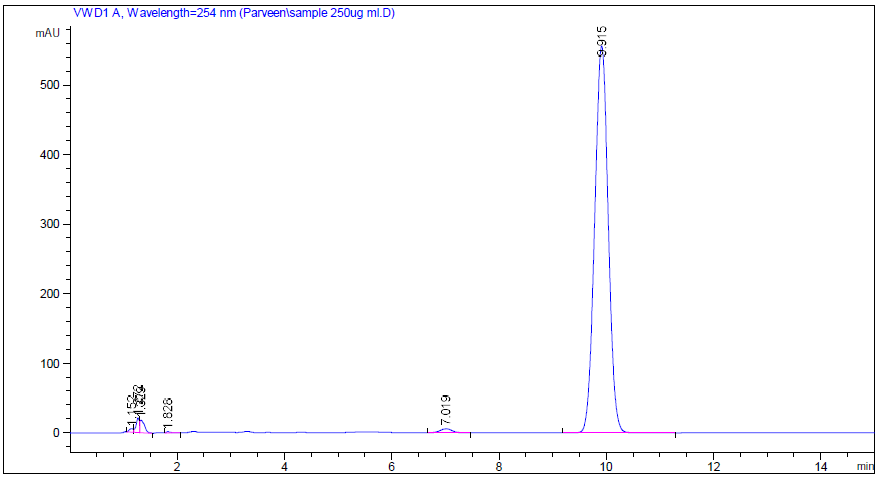

Figure 2. HPLC chromatogram of SCFE-CO_2 extract of *Nigella sativa.*

The HPLC Analysis of SCFE-CO_2 extract of *Nigella sativa* L. seeds revealed 6 peaks with retention time and peak area as shown in **Table 3 and Fig. 1.** Presence of Thymoquinone in extract was confirmed by analysing the peak retention time of Thymoquinone standard (**Fig-2**). The peak at 9.915 retention time revealed the presence of Thymoquine (TQ) in extract with the area percentage of 96.8405. This is in agreement with the observation noted in previous studies.[23] SCFE-CO_2 extract had higher Thymoquinone content when compared to other extracts which in agreement with the study conducted by Erkan *et al*. (2008).[24]

4. CONCLUSION

HPLC analysis of Supercritical fluid extract of *Nigella sativa* L. seeds revealed the presence of 6 compounds with the area percentage of 96.8405 for Thymoquinone. Thus our present study shows that the Supercritical fluid carbon dioxide extract of *Nigella sativa* L. seeds at 120bar/40°C has maximum recovery of Thymoquinone without degradation.

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