Phytochemical screening and TLC-bioautography detection of antioxidant constituents of some spices of Apiaceae family

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Abstract- Spices have been defined as plant substances from indigenous or exotic origin, aromatic or with strong taste, used to enhance the taste of foods. Herbs and spices have been used during the middle Ages for flavoring, food preservation and medicinal purposes. The present study was carried out on the three spices cumin (*Cuminum cyminum*), fennel (*Foeniculum vulgare*) and caraway (*Carum carvi*) of apiaceae family to determine their phytochemical constituents and were proved to have the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health. The work was designed to study the TLC bio autography antioxidant activity and to identify the main active components of the spices. The results provide evidence that the spices used as a potential source of safe and effective natural antioxidant agents in pharmaceutical and food industries.

Keywords: Phytochemical screening, Antioxidant TLC-Bio autography, *Cuminum cyminum, Foeniculum vulgare and Carum carvi*

Introduction

Spices are the dried parts of aromatic plants, generally used for flavoring, seasoning and imparting aroma in foods. They contain many classes of valuable compounds, which can also exert different biological activities, such as antioxidant and antimicrobial activity. It has been widely accepted that oxidative stress, induced by the over production of free radicals and reactive oxygen species in the human body, plays an important role in the disturbance of health and the pathogenesis of various diseases. Antioxidant compounds have the ability to scavenge these free radicals or reactive oxygen species (ROS) to improve human health, prevent, and even treat diseases. However, some synthetic antioxidants have been reported to elicit side effects. Various

methods have been developed and applied for the screening/evaluation of antioxidant activity *in vitro*, measuring the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•) scavenge capacity **(**Quah et al., 2020).Spices are any of several vegetable substances used to season or flavour food. They are usually dried for use and have distinct flavour and aroma. Common examples Include cumin, fennel and caraway, they stimulate appetite by increasing the flow of gastric juice and are used in most homes and restaurants all over the world (Nwinuka et al., 2005).

Cumin (*Cuminum cyminum*) is known as a small and herbaceous annual plant. It is commonly used as a spice and flavoring agent due to its distinctive aroma. Cumin seeds contain antioxidants and anti-inflammatory compounds that may help reduce inflammation and oxidative stress in the body, which can contribute to chronic diseases. Cumin is used in traditional and veterinary medicines as an astringent, carminative, and stimulant, and to treat diarrhea, flatulence, and indigestion (Al-Snafi , 2015) .

Carum genus has 25 species, which Carum carvi or caraway is the only annual and biennial economical one as spice, aperitif, and carminative in food and pharmaceutical industries. Caraway is widely used in food products due to its pleasant flavor and preservative properties. Caraway fruits are used as remedy to cure indigestion, pneumonia, and as carminative, appetizer, and galactagogue in different traditional systems[1, 2]. According to European Union herbal monograph, caraway is traditionally used for symptomatic relief of digestive disorders (bloating and flatulence). Caraway fruits are used as popular remedy to mask alcoholic breath, anemia, and as antidote agent against venomous beats. Caraway fruits are used for flavoring of rye bread and its infusion is a remedy for colic and digestive disorders, and to fight worms[3]. Caraway fruits possess stimulant, expec-torant and antispasmodic effects and is used for stomach aches, constipation, and nausea. It increases the secretion of gastric juice and promotes the discharge of bile, which increases the appetite and has digestive stimulatory effects[4] Caraway (*Carum carvi*) is the only annual and biennial economical one as spice, and carminative in food and pharmaceutical industries. Caraway is widely used in food products due to its pleasant flavor and preservative properties. Caraway fruits are used as remedy to cure indigestion, pneumonia, and as carminative, appetizer in different traditional systems (Malhotra S, 2006). Caraway fruits are used for flavoring of rye bread and its infusion is a remedy for colic and digestive disorders, and to fight worms (Attokaran M, 2017). It increases the secretion of gastric juice and pro-motes the discharge of bile, which increases the

appetite and has digestive stimulatory effects (Peter K, 2006).

Foeniculum Vulgare is usually a perennial, aromatic plant belonging to Apiaceae family with many subspecies and varieties. Fennel (*Foeniculum vulgare*) is an essential aromatic plant with medicinal properties and has well-defined anti-inflammatory and antimicrobial activities (Faudale *et al*., 2008). It has traditionally been regarded as a spice and a medicinal herb. It is a highly valued medicinal crop that is used as an anti-inflammatory, anti-oxidant, intoxicant, gastrointestinal, mucolytic, and spasmolytic agent (Ruberto *et al.,* 2000).

This study was aimed to find out the phytochemicals and antioxidant activity of some spices by TLC guided bio autography. It was hypothesized, that complex compounds can be responsible for the antioxidant action of methanol extracts of cumin, caraway and fennel. A great number of TLC techniques have been developed and successfully applied for qualitative and quantitative analysis of antioxidants (Zhao *et al*., 2010), and the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was often used as a derivatization reagent for this purpose (Olech M, *et al*., 2012). In the screening of antioxidants, the TLC bio autography assay is the method of choice due to several advantages that include flexibility, simplicity and high throughput (Badarinath, A.V, et al., 2010).

Material and Methods

Plant material and extraction

Cuminum cyminum, *Carum carvi* a*nd Foeniculum Vulgare* seeds were collected from Himalaya wellness company, Dehradun. The dried seeds of each spice were crushed separately using a mixer grinder, obtained powder was added with methanol (ie.50g of sample powder in 250ml of solvent) in an iodine flask and kept overnight for 24h. Samples were filtered dried, weighed and stored in refrigerator at 4^0C till use. The dried samples were used with appropriate solvent before study (S Mpofu et al., 2014).

Thin Layer Chromatography

HPTLC finger printing performed for standardization of the drug. CAMAG HPTLC system equipped with Linomat -5 for sample applicator for sample application, Reprostar3 for visualization under UV light at 254nm and 366nm respectively and winCATS software was used to analyse the methanol extract of different spices (Wagner H *et al*., 1984).

TLC DPPH bio-autography for antioxidant activity

TLC Bio autography Assay Thin-layer chromatography (TLC) was used to separate the chemical constituents. The filtrate methanol extract were loaded on to the activated Silica gel G (Merck) plate. The antioxidant compounds were separated using two type of mobile phase of Toluene: Ethyl

acetate (75:25) and also in chloroform: methanol (90:10). Once the run is completed, plates were air dried for 15 min and the plates were sprayed by 0.002% DPPH solution in methanol using a spray gun for 5 sec. The image was observed under visible light at exactly 2 min after spraying using a white light illuminator. The bright yellow bands against the purple background confirm the antioxidant molecule (LI Mensor et al., 2001). The Rf value of the samples were calculated.

Phytochemical Screening

The crude extract was tested for the presence of bioactive compounds by using following standard methods (Hamburger, M. *et al.,* 1991) and (Madhukar. C., 2013).

Test for Carbohydrates

Molish's Tests (general test) To 2ml of the extract solution few drops of molish's reagent and conc. sulphuric acid was added in the test tube. Violet colored ring appears at the junction of two liquid.

Test for Steroid and Triterpenoid

Salkowaski Test - To 2 ml of the extract solution 2 ml of chloroform and 2 ml of conc. H2SO4 was added and shake well. Chloroform layer appears red colour

Test for Flavanoids

Shinoda Tests - To 2 ml of the extract solution, few magnesium turnings, 5 ml 95% ethanol and few drops of conc. HCl was added. Pink to red colour develops.

Test for Alkaloids: Evaporate the aqueous and alcoholic extract separately. To the residue add dil. HCl shake well and filter, perform the following tests.

1. **Dragendorff Test -** To 2 ml of the extract solution, Dragendroff reagent (potassium bismuth iodide solution) was added. Orange brown precipitate is formed.
2. **Mayers Test -** To 2 ml of the extract solution, Mayers reagent (potassium mercuric iodide solution) was added. Precipitate is observed.

Test for Tannins

1. To 2 ml of the extract solutions add 5% ferric chloride solution, deep blue colour is observed.
2. To 2 ml of the extract solution add few drops of 10% lead acetate solution, white precipitate is observed.

Tests for Phenol

Methanol Extract To 2mL of extract, 5% ferric chloride solution was added. Deep blue black colour indicates the presence of phenol.

Tests for Saponins

The extract was diluted with distilled water and shaken in graduated cylinder for 15 minutes. The formation of layer of foam indicates the presence of saponins.

Tests for Glycosides

To 2 ml of test solution, 3 ml of glacial acetic acid and 1 drop of 5% ferric chloride were added in a test tube. 0.5 ml of concentrated sulphuric acid was carefully added by the side of the test tube. Formation of blue color in the acetic acid layer indicates the presence of glycosides.

TLC profiling of Methanol extracts gave an impressive results that directing towards the presence of number of phytochemicals. Different phytochemicals gave different Rf values in different solvent system. This variation in Rf values of the phytochemicals provides a very important clue in understanding of their polarity and also helps in selection of appropriate solvent system for separation of pure compounds by column chromatography. Mixture of solvents with variable polarity in different ratio used for separation of pure compound from plant extract. The selection of appropriate solvent system for a particular plant extracts was achieved by analyzing the Rf values of compounds in different solvent system.

Result and Discussion

In phytochemical screening of the present study carried out in the spices revealed the presence of medicinal active constituents

Table-1 Results of phytochemical screening of methanol seed extract of three spices:

Phytochemical Test	*Cuminum cyminum* (Methanol extract)	*Carum carvi* (Methanol extract)	*Foeniculum Vulgare* (Methanol extract)
Tannins	+	+	-
Flavanoids	+	+	-
Terpenoids	-	-	-
Saponins	+	-	-
Steroids	+	+	+
Carbohydrates	+	+	+
Glycosides	+	+	+
Alkaloids	+	+	+
Phenol	+	+	+

Key: + indicates presence of the Phytoconstituents
- indicates absence of the Phytoconstituents

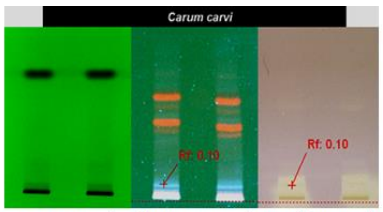

Fig 1: TLC Bio autography of *Carum carvi* in Toluene: ethyl acetate (75: 25)

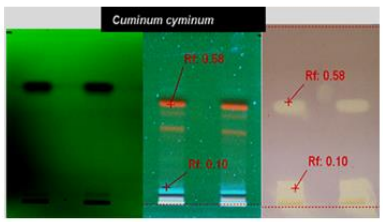

Fig 2: TLC Bio autography of *Cuminum cyminum* in Toluene: ethyl acetate (75: 25)

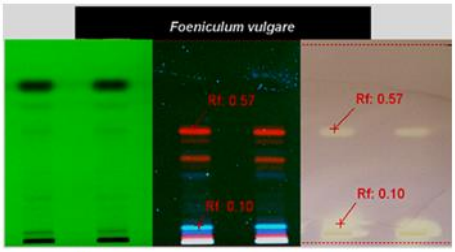

Fig 3: TLC Bio autography of *Foeniculum vulgare* in Toluene: ethyl acetate (75: 25)

TLC-DPPH assay

TLC-DPPH test was applied to the plant extracts to identify radical scavenging activities of separated compounds. DPPH bioautograms of plant extracts were compared UV_{254} and UV_{366} visualization of TLC plate developed by using toluene: ethyl acetate: (75:25 v/v) mobile phase. TLC analysis was carried out for separation of more than six standard compounds including alkaloids, flavanoids, terpenoids, Phenol and steriods was detected in all samples. (**Figuer-1**) shows the DPPH activity *of Carum carvi* at Rf between 0.01 to 0.20. In (**Figure-2**) *Cuminum cyminum* shows strong activity at Rf 0.58 and also some constituent shows antioxidant activity between Rf 0.01-0.20. In (Fig -3) *Foeniculum vulgare* shows two distinct spots are visible, labelled with Rf values of 0.57 and 0.10.The spot with Rf 0.57 is likely a more polar compound, interacting more strongly with the stationary phase. The spot with Rf 0.10 is less polar and interacts more with the mobile phase.

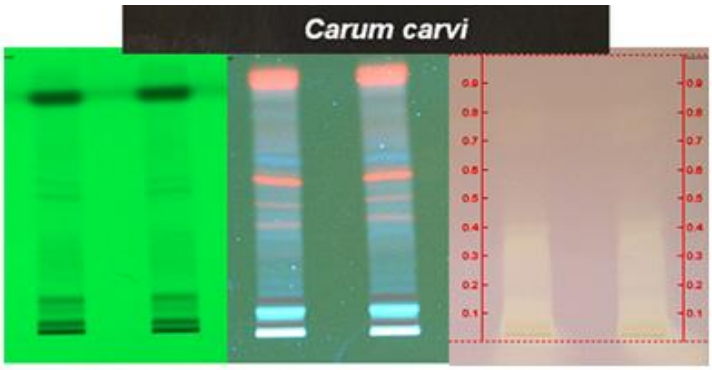

Fig 4: TLC Bio autography of *Carum carvi* in Chloroform :Methanol (90: 10)

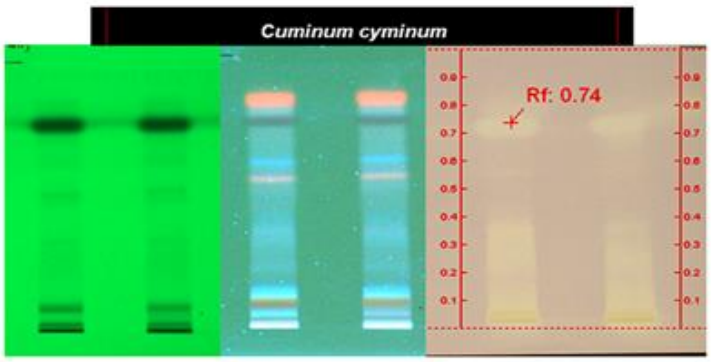

Fig 5: TLC Bio autography of *Cuminum cyminum* in Chloroform :Methanol (90: 10)

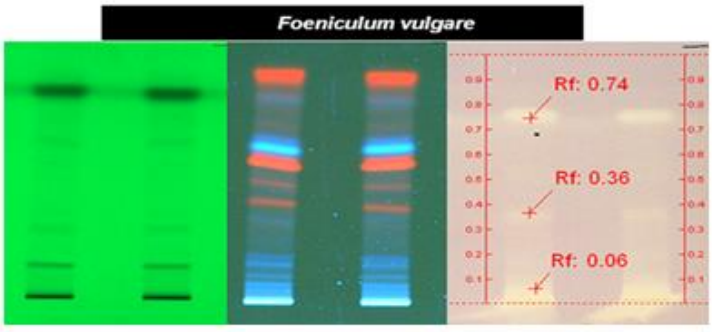

Fig 6: TLC Bio autography of *Foeniculum vulgare* in Chloroform :Methanol (90: 10)

DPPH assay was used to determine the free radical-scav-

The plant extract was applied to a TLC plate and developed using mobile phase chloroform: methanol (90:10). The TLC plate shows several bands, suggesting the presence of multiple compounds of *Carum carvi*. It shows the strong antioxidant activity at Rf value (0.01 to- 0.40) (**fig-4**). In (**fig- 5**) *Cuminum cyminum* shows one distinct band is visible with an Rf value of 0.74. This suggests the presence of a compound with a specific polarity that may have antioxidant activity. In *Foeniculum vulgare* the extract shows multiple zones of inhibition with Rf values around 0.74, 0.36 and 0.06. This indicates the presence of multiple antioxidant compounds in the extract (**Fig-6**).

Conclusion

Spices are indeed effective sources of antioxidants. Antioxidants are compounds that help protect cells from damage caused by harmful molecules called radicals. Free radicals can contribute to various health problems, including chronic diseases like heart disease, cancer, and neurodegenerative disorders. TLC bioautography provides a valuable tool for understanding the antioxidant profiles of cumin, fennel and caraway. By identifying and characterizing these bioactive compounds, we can connect the power of nature to promote human health and well-being. Considering the problems, we can say that bio-autographic detection technique would create a new era in separation science.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

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