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A Study on Phytochemical Analysis and Determination of Antioxidant and Antimicrobial Activities of *Coriandrum sativum* (Dhania)

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A B S T R A C T

Dietary phytochemicals are considered as an effective tool to cure various human physiological disorders. Several epidemiological studies have indicated that high intake of natural products is associated with reduced risk of a number of chronic diseases. During recent years consumers have been more concerned about the addition of synthetic additives to food. Therefore, an interest is growing for the search of natural phytoactives with biological activities. Hence, the current study was conducted with the main purpose of phytochemical analysis and determination of antioxidant and antimicrobial activities of *Coriandrum sativum* (Dhania). Leaves of *C. Sativum* was subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with double distilled water. Results delineated the major phytochemical present in the aqueous (Aq.) extract of leaves of *C. sativum* were phenolic compounds/tannins, flavonoids, saponins, alkaloids, proteins and amino acids, glycosides, and reducing sugars. Quantitative estimation of phytochemicals in Aq. extract of leaves of *C. sativum* revealed that that total polyphenol quantity was found to be highest (7.98 GAE) when compared with total flavonoid quantities (4.23 GAE). The IC_{50} values exhibited by Aq. extracts of leaves of *C. Sativum* was found to 3.87 mg/mL. Aq. extracts of leaves of *C. sativum* exhibited antibacterial activities of against pathogenic organisms *viz. Klebsiella pneumonia* (11 mm), *Staphylococcus pneumonia* (15 mm), *Proteus mirabilis* (12 mm), *Eshcherichia coli* (10 mm), *Salmonella typhi* (8 mm), and *Enterobacter cloacae* (13 mm). Furthermore, the antibacterial activity of *Staphylococcus pneumonia* was comparable with that of standard Chloramphenicol. In conclusion, this preliminary study confirms that the *C. sativum* has wide variety of secondary metabolites. Biological activities such as antioxidant and antimicrobial properties of Aq. extract of leaves of *C. sativum* depicted that *C. sativum* could be potential drug agent of folk medicines.

Introduction

Herbs and spices are the most important part of human diet. In addition to boosting flavor, these are also known for their preservative and medicinal value, which forms one of the oldest sciences. It is only in recent years that modern science has started paying attention to the properties of spices (DeSouza *et al.,* 2005). Dietary phytochemicals are considered as an effective tool to cure various human physiological disorders.

Several epidemiological studies have indicated that high intake of natural products is associated with reduced risk of a number of chronic diseases such as artherosclerosis and cancer (Ajmal *et al.,* 2006). During recent years consumers have been more concerned about the addition of synthetic additives to food and the two most commonly used antioxidants, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), have shown DNA damage induction.(Sasaki *et al.,* 2002) Therefore, an interest is growing for the search of natural antioxidants for the public perception that natural and dietary antioxidants are safer than synthetic analogues (Dastmalchi *et al.,* 2008; Nadeem *et al.,* 2010).

Due to the side effects of conventional medicine, the use of natural products as an alternative way in healing of various diseases has been reported in the last few decades. From the safety point of view, one of the important sources for the search of natural antioxidants are herbs and spices.

Among them, coriander has much importance due to its versatile use as a spice as well as an herb. In Indian and Central Asian recipes, coriander leaves are used in large quantities. The dry fruits are known as coriander seeds. The seeds have a lemony citrus flavor when crushed, due to terpenes linalool and pinene. The roasted coriander seeds are called as dhana dal used as snacks. It is the main ingredient of the two south Indian dishes includes sambhar and rasam (Pathak and Kasture Sanjay, 2011).

The plant *Coriandrum sativum* (Dhania), belonging to the family Apiaceae, locally known as Dhania', is a medicinal herb. All parts of the plant are edible, but the fresh leaves and the dried seeds are the most common parts used in cooking.

In the Indian traditional medicine, coriander is used in the disorders of digestive, respiratory and urinary systems, as it has diaphoretic, diuretic, carminative and stimulant activity (Benjumea *et al.,* 2005; Maghrani *et al.,* 2005). However, Pharmacological studies have demonstrated the Hypoglycemic,(Gray and Flatt, 1999) Hypolipidemic, (Chithra and Leelamma, 1999) Antimutagenic, (Cortés-Eslava *et al.,* 2004) Antihypertensive, (Medhin *et al.,* 1986) Antioxidant,(de Almeida Melo *et al.,* 2003) Antimicrobial,(Kubo *et al.,* 2004) and postcoital antifertility (Al-Said *et al.,* 1987) activity of *C. sativum*. It has also been used in heavy metal detoxification (Karunasagar *et al.,* 2005). Hence, in the present study we aimed for phytochemical analysis and determination of antioxidant and antimicrobial activities of *C. sativum* (Dhania).

Materials and Methods

Collection of plant material

The leaves *C. sativum* were purchased from the local market of Chikkaballapura, Karnataka, India and washed with tap water for several times and then once with double distilled water. After washing, it was dried at room temperature and then crushed with the help of electric grinder. Powdered sample was stored for further use.

Extraction

Approximately 50 g of dried and coarsely powdered leaves of *C. sativum* were subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with 550 mL of double distilled water.

All the extracts were concentrated by distilling the solvent in a rotary flash evaporator. The extracts were preserved in airtight containers and stored at room temperature until further use.

Phytochemical Screening

Chemical screening was carried out on the aqueous (Aq.) extracts of leaves of *C. sativum* by using standard procedure to detect phytoconstituents as described by sofora (1993); Trease and Evans (1989) and Harborne (1973).

Test for Alkaloids

Approximately 0.2g of Aq. extract of leaves of *C. sativum* was warmed with 2% H_2SO_4 (2.0ml) for two minutes. The reaction mixture was filtered and few drops of Dragendrof's reagent was added to the filtrate. Orange red precipitation showed the presence of alkaloids moiety.

Test for Tannins and Phenolic Compounds

The Aq. extract of leaves of *C. sativum* in **s**mall quantity was mixed with water and heated on water bath and filtered. To the filtrate, few drops of ferric chloride ($FeCl_3$) was added. A dark green colouration indicate the presence of tannins.

Test for Glycosides

About 0.6g of Aq. extract of leaves of *C. sativum* was hydrolyzed with HCl and neutralized with NaOH solution and few drops of Fehling's solution A and B were added. Formation of red precipitate indicates the presence of glycosides.

Test for Reducing Sugars

The Aq. extract of leaves of *C. sativum* was shaken with distilled water and filtered. Few drops of Fehling's solution A and B were added and boiled for few minutes. Formation of an orange red precipitate confirms the presence of reducing sugar.

Test for Saponins

About 0.2g of Aq. extract of leaves of *C. sativum* was shaken with 5 mL of distilled water and then heated to boil. Frothing (appearance of creamy miss of small bubbles) showed the presence of saponins.

Test for Flavonoids

0.2g of Aq. extract of leaves of *C. sativum* was dissolved in diluted 10% NaOH and few drops of 2M HCl was added. A yellow solution that turns into colorless indicate the presence of flavonoids.

Test for Steroids

2 mL of acetic anhydride was added to 0.5g of Aq. extract of leaves of *C. sativum* and then added 2 mL of H_2SO_4. The change of color from violet to blue or green or red showed the presence of steroids.

Test for Terpenoids

0.3g of Aq. extract of leaves of *C. sativum* was mixed with 2 mL of chloroform ($CHCl_3$) and 3 mL of concentrated 6M H_2SO_4 was carefully added to form a layer. Reddish brown coloration at the interface was formed which indicate positive results for the presence of terpenoids.

Test for Proteins and Amino acids

To the 0.3 g of Aq. extract of leaves of *C. sativum* few drops of 0.2% ninhydrin solution was added and heated for 5 minutes. Blue colouration indicate the presence of proteins.

Quantitative Estimation of Phytochemicals

Total phenolics

The concentration of total phenolics in the Aq. extract of leaves of *C. sativum* was determined by the Folin-Ciocalteu assay that involves reduction of the reagent by phenolic compounds, with concomitant formation of a blue complex, its intensity at 725nm increases linearly with the concentration of phenolics in the reaction medium (Singleton *et al.,* 1999). The phenolic content of the extract was determined from calibration curve and were expressed in mg gallic acid equivalent/g of extract powder.

Total flavonoid

Aluminum chloride colorimetric method was used for flavonoids determination in Aq. extract of leaves of *C. sativum* (Ordonez *et al.,* 2006). The content was determined from extrapolation of calibration curve which was made by preparing gallic acid solution (0-0.8 mg/ml) in distilled water. The concentration of flavonoid was expressed in terms of mg gallic acid equivalent/g of extract powder.

Antioxidant Assay

The modified literature protocol of Blois was used for antioxidant assay (Blois, 1958; Uddin *et al.,* 2012). Briefly 2, 2-diphenyl-1-picrylhydrazyl (DPPH) solution (1mL;1mM) was prepared in methanol and mixed with sample solution (3mL, containing 20-100ug) in double distilled water. The control was also run which contains only double distilled water. The hydrogen atom or electron donation abilities of extract and standards were measured from the bleaching of the purple-colored methanol solution of 2, 2-diphenyl1-picrylhydrazyl (DPPH). The absorbance was measured at 517 nm after 30 min incubation. Decreasing of the DPPH solution absorbance indicates an increase of the DPPH radical-scavenging activity. Scavenging of free radicals by DPPH as percent radical scavenging activities (% RSA) was calculated by using the formula; DPPH% = (Control abs – Extract abs / Control) × 100. The IC_{50} value was determined by using linear regression equation *i.e.*, Y = Mx + C; Here, Y = 50, M and C values were derived from the linear graph trendline.

Evaluation of Antibacterial Activity

Pathogenic Microorganisms

The pathogenic and multiple antibiotic-resistant isolates *viz. Klebsiella pneumonia, Staphylococcus pneumonia, Proteus mirabilis, Eshcherichia coli, Salmonella typhi,* and *Enterobacter cloacae* were isolated from clinical samples of local hospital in and around Chikkaballapura and confirmed by various microscopic evaluation like Gram's staining (Gram, 1884). Motility, capsule and spore formation was confirmed as per the procedure prescribed by Collins and Lyne (1970). All the bacterial pathogens were further confirmed by suitable biochemical tests,(Barrow and Feltham, 1993) and used for antimicrobial activity studies.

The direct colony suspension method is the most convenient method for inoculum preparation. The inoculum was prepared by making a direct broth or saline suspension of isolated colonies selected from an agar plate. The suspension was adjusted to achieve a turbidity equivalent to a 0.5 McFarland standard. This results in a suspension

containing approximately 1 to 2 × 10^8 colony-forming units (CFU)/mL. To perform this step accurately, used adequate light to visually compare the inoculum tube and the 0.5 McFarland standard against a card with a white background and contrasting black lines. Optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension, the pathogenic bacteria culture was inoculated into culture plates to screen for antibacterial properties.

Determination of Antibacterial Activities

Antibacterial activities of plant extracts were tested by agar well diffusion method.[40] The culture plates were prepared by pouring 20 ml of sterile Muller Hintonagar (MHA).1 ml inoculums suspension was spread uniformly over the agar medium using a sterile glass rod to get uniform distribution of bacteria.

A sterile cork borer (6 mm) was used to make wells in each plate for extracts. The Aq. Extract of leaves of *C. sativum* (400 µl) was added into the well and allowed to diffuse in the agar medium. Also, chloramphenicol (10 µg) was used as standard antibiotic for each culture. Plates containing drug were left for one hour in order to diffuse properly in media and to get dry. Then the plates were incubated for 24h at37°C during which the activity was evidenced by the presence of a zone of inhibition surrounding the well. Each test was repeated three times and the antibacterial activity was expressed as the mean of the diameter of the inhibition zones (mm) produced by the plant extracts.

Results and Discussion

The major phytochemicals found in Aq. extract of leaves of *C. sativum* were found to be phenolic compounds/tannins, flavonoids, saponins, alkaloids, proteins and amino acids, glycosides, and reducing sugars (Table 1).

Quantitative estimation of phytochemicals in Aq. extract of leaves of *C. sativum* was represented in Table 2. Results revealed that total polyphenols quantity was found to be highest (7.98 GAE) phytochemicals found in Aq. extract of leaves of *C. sativum* when compared with total flavonoid quantities (4.23 GAE).

The results of antibacterial activities of Aq. extracts of leaves of *C. sativum* exhibited against pathogenic organisms *viz. Klebsiella pneumonia, Staphylococcus pneumonia, Proteus mirabilis, Eshcherichia coli, Salmonella typhi,* and *Enterobacter cloacae* was plotted in Figure 1. Results depicted that standard antibiotic i.e., chloramphenicol exhibited zone of inhibition of 21 mm. The highest zone of inhibition of Aq. extract of leaves of *C. sativum* was found to be 15 mm against *Staphylococcus pneumonia* followed by13 mm, 12 mm, 11 mm, 10 mm, and 8 mm against *Enterobacter cloacae, Proteus mirabilis, Klebsiella pneumonia, Eshcherichia coli, and Salmonella typhi* respectively.

The IC_{50} values exhibited by Aq. extracts of leaves of *C. Sativum* was found to 3.87mg/mL (Table 3).

Due to the biologically advantageous effects of plant-based products resulting from the antioxidant activities of phenolic phytochemicals, active research on plant-based products has been driven in recent years. Because they have no harmful effects on people, and hence plant products are preferred to synthetic compounds in the treatment of diseases.

In India, there are many different traditional medical systems that rely heavily on local plant species for their raw drug materials. As a result, it's important to consider traditional medicines as potential new therapeutic agents (Uddin *et al.,* 2012). Therefore, in this study

we aimed for phytochemical analysis and determination of antioxidant and antimicrobial activities of *C. sativum* (Dhania). Different phytochemicals have various protective and therapeutic effects which are essential to prevent diseases and maintain a state of well-being. Our study results on the qualitative analysis of the Aq. extract of leaves of *C. sativum* revealed the presence of phenolic compounds/tannins, flavonoids, saponins, alkaloids, proteins & amino acids, glycosides, and reducing sugars. Moreover, quantitative estimation of phytochemicals in Aq. extract of leaves of *C. sativum* revealed that that total polyphenol quantity was found to be highest (7.98 GAE) phytochemicals found in Aq. extract of leaves of *C. sativum* when compared with total flavonoid quantities (4.23 GAE). Furthermore, the IC_{50} values exhibited by Aq. extracts of leaves of *C. Sativum* was found to 3.87 mg/mL.

Literature reports evidenced that phenolic compounds and flavonoids have been reported to be associated with antioxidative action in biological systems, acting as scavengers of singlet oxygen and free radicals.(Rice-Evans *et al.,* 1997) Vinson *et al.,* (1998) conclusively shown close relationship between total phenolic content and antioxidative activity of the fruits and vegetables (Vinson *et al.,* 1998). Flavonoids are versatile bioactive secondary metabolites present in almost all plant species. Most representative family members include flavones, flavanes, flavonols, catechins, and anthocyanidins.

Their antioxidant potential toward ROS depends on structural characteristics such as the number and substitution pattern of hydroxyl groups and the extent at which these groups are glycosylated (Amic *et al.,* 2003). Therefore, reported antioxidant activities of Aq. extract of leaves of *C. sativum* could be accredited to the total phenolic and flavonoid content present in the Aq. extract of leaves of *C. sativum.* Our study results revealed that Aq. extracts of leaves of *C. sativum* exhibited antibacterial activities of against pathogenic organisms *viz. Klebsiella pneumonia* (11 mm), *Staphylococcus pneumonia* (15 mm), *Proteus mirabilis* (12 mm), *Eshcherichia coli* (10 mm), *Salmonella typhi* (8 mm), and *Enterobacter cloacae* (13 mm). Furthermore, the antibacterial activity of *Staphylococcus pneumonia* was comparable with that of Chloramphenicol. These findings are comparable with the findings of Ratha V. Bai and Kanimozhi (2012) wherein authors reported the antibacterial potential of different solvent extracts of *C. sativum* against pathogenic microorganisms (Ratha V. Bai and Kanimozhi, 2012).

The results obtained in the present study are encouraging as this study evidenced the vide variety of secondary metabolites present in the Aq. extracts of leaves of *C. sativum*. *C. sativum* shown considerable antioxidant and antimicrobial properties. Hence this study supplies as evidence-based study for leaves of *C. sativum* could be exploited in the management of various human ailments.

This preliminary study confirms that *C. sativum* has wide variety of secondary metabolites *viz.* phenolic compounds, flavonoids, saponins, alkaloids, proteins and amino acids, glycosides, and reducing sugars.

Furthermore, total polyphenol quantity was found to be highest when compared with total flavonoid quantities. Biological activities such as antioxidant and antimicrobial properties of Aq. extracts of leaves of *C. sativum* depicted that *C. sativum* could be potential drug agent of folk medicines. However further studies are recommended to be conducted to elucidate the exact mechanism of action of particular secondary metabolites present in *C. sativum* against various ailments.

Table.1 Photochemical screening of Aq. extract of leaves of *C. sativum*

Phytochemical Components	Aq. Extract of leaves of *C. sativum*
Alkaloids	+
Flavonoids	+
Glycosides	+
Proteins and Amino acids	+
Reducing sugar	+
Saponins	+
Steroids	-
Phenolic compounds	+
Tannins	+
Terpenoids	+
Carbohydrates	-

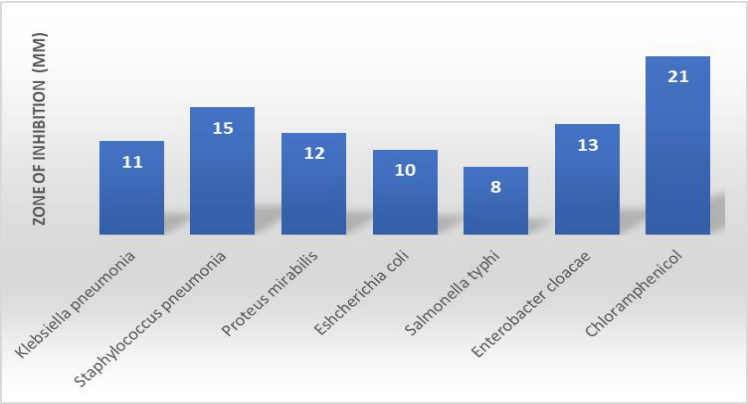
Table.2 Quantitative analysis of phytochemicals present in Aq. extract of leaves of *C. sativum*

Chemical Components	Aq. extract of leaves of *C. sativum*
Total flavonoids	4.23 GAE
Total phenolics	7.98 GAE

Table.3 Antioxidant activities of Aq. extract of leaves of *C. sativum*

S. No.	Aq. extracts of leaves of *C. Sativum*	IC_{50} (mg/mL)
1	Leaves	3.87

Fig.1 Determination of antibacterial activities of Aq. extracts of leaves of *C. Sativum*



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