

Preliminary Phytochemical analysis and anti-microbial evaluation of the Cilantro extract

Gayathri P K*, Rithika J, Dhanasree S

Department of Biotechnology, Vel Tech High Tech Dr.Rangarajan Dr.Sakunthala Engineering College, Avadi, Chennai -600 062.

*Corresponding author: E-Mail: k.gayathri@velhightech.com

ABSTRACT

Coriandrum sativum is a potent herb having many therapeutic effects. In the Indian traditional medicine, coriander was used to treat the disorders of digestive, respiratory and urinary systems, as it has diaphoretic, diuretic and stimulant activity. The present study was carried out to investigate the presence of phytochemicals and its respective antibacterial activity. The essential oil from coarsely powdered cilantro seeds was obtained using different solvents (water, ethanol, petroleum benzene, methanol, chloroform) by Soxhlet extraction method. The phytochemical assay was performed for all the extracts and it was observed that the bioactive compounds such as flavonoids, phenols, terpenoids and alkaloids were present. The extract was observed to have inhibitive effect against *Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa* (*P.aeruginosa*) but not against *Staphylococcus aureus* (*S. aureus*). Further studies would involve isolation of specific bio active compound and analyse its anti-proliferative effects.

KEY WORDS: *Coriandrum sativum*, Soxhlet extraction, terpenoids, antimicrobial activity.

1. INTRODUCTION

Coriandrum sativum is an annual herb belonging to the parsley family Apiaceae. It is a slender juicy plant growing in arid zones and is native to the Mediterranean countries. Its seeds are generally small, dry and globular. Now days, these plants are grown all over the countries. They are extensively used in culinary aspects because of their unique aroma. These plants are rich in vitamins such as A, D & K and minerals such as manganese, magnesium, selenium, iron and calcium. In Indian tradition, the plant is used as remedy for digestive, respiratory and urinary problems. It also possesses diuretic, carminative/stimulant properties, anti-microbial, postcoital and anti-fertility activities. Further studies on its hypo-lipidemic, hypoglycaemic and anti-oxidant activities are being studied. It has also been used in heavy metal detoxification. The essential oil obtained from them yield many bioactive components with a variety of efficiencies. Their essential oil is rich in phytochemicals such as tannins, quinones, phenols, terpenoids etc.

The main components of the essential oil include linalool, linalyl acetate, geranial, camphor, limonene, geranyl acetate, γ -terpinene etc. Linalool is the bioactive compound found to occur at high concentration (70%) in the extract. It is used as an additive for processed foods, beverages and as a fragrance ingredient in cosmetics and household detergent. It is the main compound responsible for its anti-microbial and anti-diabetic effects of cilantro seeds. The linalyl acetate (7%) which is the acetate ester of linalool possesses most of the properties of linalool. Other than these compounds, bio-active components such as geranyl acetate (4%) and γ -terpinene(4%) are identified to be the next important compounds which possess activities pertaining to digestive and diuretic ailments. In this study, the presence of terpenoids is desired in the extracts (water, acetone, chloroform, petroleum benzene, ethanol, and methanol) and the anti-microbial activity of the extract showing highest positive result for terpenoids is studied.

2. MATERIALS AND METHODS

Materials required: Acetone, chloroform (Fisher scientific), ethanol, methanol (Medox), petroleum benzene (Emplura agencies), nutrient agar (HiMedia), sterile discs (HiMedia), streptomycin disc (HiMedia). The microbial strains *S. aureus*, *E. coli* and *P. aeruginosa* are procured from MTCC. All chemicals were of analytical grades.

Sample Collection and identification: The seeds of *Coriandrum sativum* were purchased from a local market in Chennai. The seeds obtained were identified by comparing with data from the literature and analysing its microscopic characteristics. The dusts were removed, shade dried for 10 days, coarsely ground and taken for reflux condensation.

Essential oil extraction: For the aqueous extract, about 30g of cilantro seeds were weighed, boiled in 150 mL of water for about an hour and filtered. For other extracts (methanolic, ethanolic, chloroform, acetone and petroleum benzene), 30g of the cilantro seeds were extracted using 130-150 mL of the solvents at 50-60°C for 3-4 cycles in a Soxhlet extractor and the crude extract was concentrated by allowing them for evaporation. All the filtered extracts were stored at -20°C in an air tight container for further analysis.

Phytochemical screening: The extracts were subjected to preliminary phytochemical screening for the detection of various plants constituent using the standard procedure described earlier.

Test for Tannins: To 1 mL of the extract, 1mL of the 5% FeCl₃ was added. The formation of greenish black pigment indicates the presence of Tannins.

Test for Flavonoids: To 1 mL of extract add 1 mL of 2N NaOH and few drops of Conc. HCl. The appearance of yellow colour indicates the presence of Flavonoids.

Test for Quinones: To 1 mL of extract, 1 mL of Conc. H₂SO₄ was added. The formation of red colour indicates the presence of Quinones.

Test for Glycosides: To 1 ml of extract, 1 ml of chloroform and few drops of 10% ammonium solution were added. The formation of pink colour indicates the presence of glycosides.

Test for Cardio glycosides: To 1 mL of extract, 1 mL of glacial acetic acid was added along with few drops of 15% FeCl₃. Then 1 ml of Conc. H₂SO₄ was added. The formation of brown ring indicates the presence of Cardio glycosides.

Test for Terpenoids: To 1 mL of extract, 1 mL of chloroform along with 4-5 drops of Conc. H₂SO₄ was added. The formation of a reddish brown interface indicates the presence of Terpenoids.

Test for Phenols: To 1 mL of extract, 1 mL of Na₂CO₃ along with Folin's reagent was added. The formation of blue/green colour indicates the presence of Phenol.

Test for Coumarins: To 1 mL of extract, 1 mL 10% NaOH was added. The formation of yellow colour indicates the presence of Coumarins.

Anti-microbial Screening: The Agar disc diffusion method (Bauer *et al.*, 1966) was employed for the determination of anti-microbial activity with slight modification. The nutrient agar was poured onto 90mm diameter Petri plates until the thickness of the agar was 4mm. 0.1 ml of each bacterial solution was uniformly inoculated onto the plates by means of sterile swabs. Plates were allowed to stand for 15 minutes. At the same time, 6 mm diameter sterile discs were soaked with the chloroform cilantro extracts at different concentrations (20 µL, 30 µL and 40 µL respectively). The disks were symmetrically placed onto the medium by means of sterile tweezers. Disc soaked with chloroform was used as a negative control. The plates were incubated for 24±2h at 37°C under anaerobic conditions. The results were evaluated by measuring the areas around the disc with no bacterial growth. The experiments were made in triplicates. The results were obtained using the following formula:

$$\text{Inhibition value} = \text{Inhibition diameter (mm)} - (\text{Disk diameter (6mm)} / 2)$$

Statistical Analysis: Conventional statistical methods were used to calculate means and standard deviations of three simultaneous assays for the antibacterial activity.

3. RESULTS AND DISCUSSION

Phytochemical analysis: The phytochemical investigations for 8 different bioactive compounds were tested against 6 different solvents. Totally 48 tests were performed and the results of the same are listed in Table 1. Out of these, 28 tests gave positive results whereas the remaining 20 tests were observed to be negative. Quinones, flavonoids and phenols were present in almost all the extracts whereas the rest of the phytochemicals were absent in all the extracts.

The results of the phytochemical analysis of various solvents like chloroform, acetone, petroleum benzene, ethanol and methanol are depicted in Fig 1, 2, 3, 4 and 5 respectively. Among the 6 solvents used chloroform extract showed the presence of 6 phytoconstituents (flavonoid, quinone, glycoside, cardioglycoside, terpenoid and phenol) followed by petroleum benzene, ethanol and methanol extracts which showed the presence of 5 phytoconstituents. The bioactive compound of our interest terpenoids (due to its antioxidant property) was present in chloroform, petroleum benzene and aqueous extracts. Among the 3 best solvents, chloroform extract was taken for further analysis since it showed the presence of maximum number of phytoconstituents. The other antioxidant rich compounds like flavonoids, phenols and quinones were also present in the chloroform extract which shall enhance the medicinal value of the extract.

The presence of terpenoids were found be very strong in the chloroform extract. The high amount of terpenoids indicates strong potential of the extract to act as an anti-oxidant agent. The terpenoids were widely used in the drug discovery for cancer since it has a potent anti-proliferative activity. Mono-terpenes obtained from lavender species were found to be active against the liver cancer cell lines. These evidences suggest that the chloroform extract shall be used for further studies dealing with antiproliferative and anticancerous activity.

Anti-microbial activity: The anti-microbial activity of the cilantro extract against the test micro-organisms was qualitatively and quantitatively accessed by the absence or presence of zones and diameter of the zones. Results of the disc diffusion assay (Table 2) indicated that the extracts showed very strong effects against the gram negative bacteria. The gram positive bacteria were resistant to the extract since they showed no zone around them. Among the gram negative bacteria used *E.coli* showed more sensitivity than *P.aeruginosa*. This activity was carried out with different concentration of the extract i.e., 20 µL, 30 µL, 40 µL. Streptomycin; a standard antibiotic was used as positive control which showed a good activity against them. The chloroform was used as the negative control which showed no zone against these micro-organisms. When 40µL of the extract was used, the plates showed complete growth inhibition of the micro-organisms. When 30µL extract was used in the plate, the *E.coli* showed a zone of 27 mm growth whereas *P.aeruginosa* showed a zone of 25 mm. When tested with 20 µL of extract, the *P.aeruginosa* and *E.coli* showed 17 mm and 20 mm respectively as zone of inhibition. All the experiments were done in triplicates and the results obtained from the phytochemical analysis of the chloroform extract, which showed compounds

responsible for anti-bacterial activities, they showed a potential action against the microbes. This potent action shows their activity against food spoilage and attack of bacteria on the foods.

Table.1.Phytochemical screening of *Coriander Sativum* extracts.

| Active constituents | Aqueous extract | Ethanol extract | Methanol extract | Pet. benzene Extract | Acetone extract | Chloroform extract |
|--------------------------|-----------------|-----------------|------------------|----------------------|-----------------|--------------------|
| Tannins | ++ | - | - | - | - | - |
| Flavonoids | - | +++ | + | ++ | ++ | + |
| Quinones | ++ | ++ | + | ++ | + | + |
| Glycosides | - | - | - | - | - | + |
| Cardio glycosides | - | ++ | + | + | - | + |
| Terpenoids | +++ | - | - | +++ | - | ++++ |
| Phenols | - | + | + | + | + | ++ |
| Coumarins | - | ++ | + | - | - | - |

Table.2.Antimicrobial assay of chloroform extract

| Name of the test organism | Zone of inhibition \pm SD (mm) | | | |
|---------------------------|----------------------------------|---------------------------|---------------------------|---------------|
| | 20 μ l of the extract | 30 μ l of the extract | 40 μ l of the extract | Streptomycin |
| <i>E.coli</i> | 20 \pm 1.25 | 27 \pm 0.76 | Complete inhibition | 30 \pm 0.6 |
| <i>P.aeruginosa</i> | 17 \pm 0.57 | 25 \pm 0.45 | Complete inhibition | 35 \pm 0.32 |
| <i>S.aureus</i> | - | - | - | 27 \pm 0.87 |

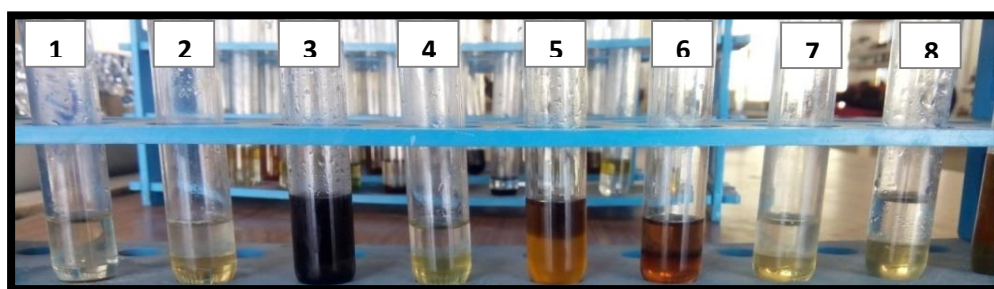


Figure.1. Results of phytochemical analysis of the chloroform extract; Negative for tannins (1,8), Positive for flavonoids, quinines, glycosides, cardio glycosides, terpenoids, phenols (2-7)

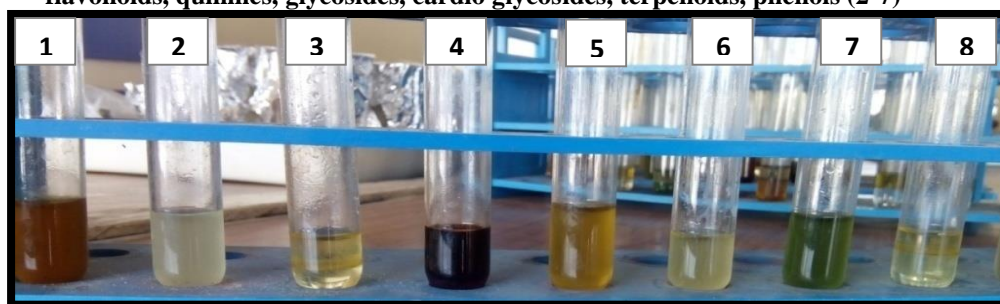


Figure.2. Results of phytochemical analysis of the acetone extract; Negative for tannins, glycosides, cardio glycosides, terpenoids, coumarins(1,4-6,8); Positive for flavonoids, quinines, phenols (2,3,7)

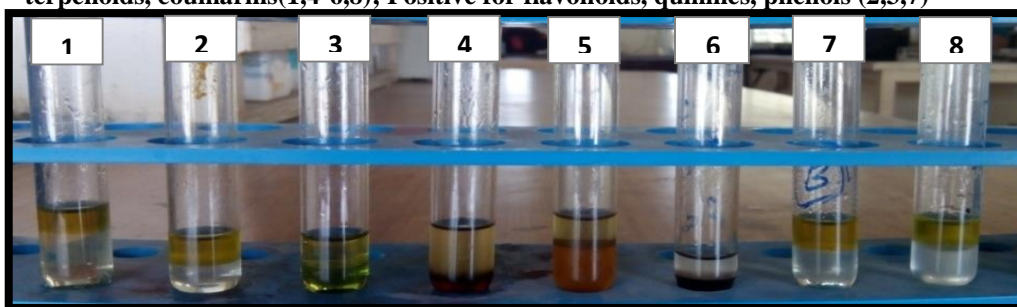


Figure.3. Results of phytochemical analysis of the petroleum benzene extract; Negative for tannins, glycosides, coumarins (1,4,8); Positive for flavonoids, quinines, cardio glycosides, terpenoids, phenols (2,3,5-7)

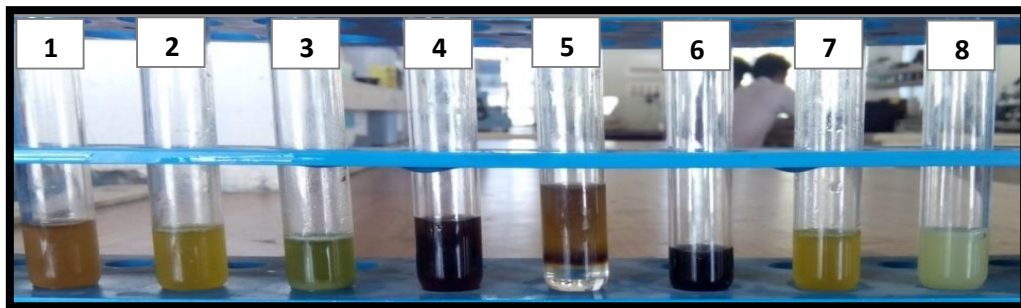


Figure.4. Results of phytochemical analysis of the ethanol extract; Negative for tannins, glycosides, terpenoids (1,4,6); Positive for flavonoids, quinines, cardio glycosides, phenols, coumarins (2,3,5,7,8)

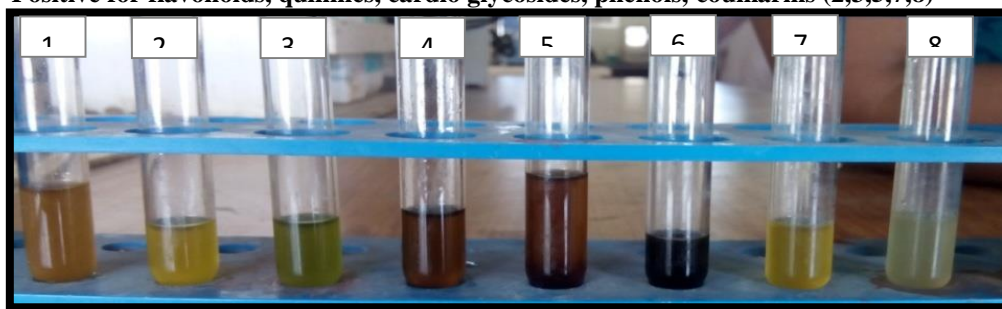


Figure.5. Results of phytochemical analysis of the methanol extract; Negative for tannins, glycosides, terpenoids (1,4,6); Positive for flavonoids, quinines, cardio glycosides, phenols, coumarins (2,3,5,7,8)

4. CONCLUSION

From the study, it was known that the chloroform extracts showed activity against gram negative bacteria than the gram positive bacteria.

5. ACKNOWLEDGEMENT

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