# Comparative analysis of antioxidant and antibacterial properties of *Aegle marmelos*, *Coriandrum sativum* and *Trigonella foenum graecum*

**Madhura Joglekar, Mangala Mandal, Manasa Paralakoti Somaiah, Shweta Murthy**

Department of Biotechnology, Mount Carmel College, Bangalore, Karnataka 560052, India;
Email: shwetanori@gmail.com

## ABSTRACT

The objective of the present study is to compare the antioxidant and antibacterial property of methanolic extracts of leaves of *Aegle marmelos*, *Coriandrum sativum* and *Trigonella foenum greacum* and estimation of their phenolics and flavonoids. It was observed that *A. marmelos* has the highest phenolic content followed by *T. foenum greacum* and *C. sativum*; similarly the flavonoids contents are high in *T. foenum greacum* followed by *C. sativum* and *A. marmelos*. Antioxidant property was checked by reducing power, NBT assay and $H_2O_2$ scavenging. *A. marmelos* showed the highest reducing power followed by *C. sativum* and *T. foenum greacum* but *T. foenum greacum* showed the highest superoxide and free radical scavenging followed by *C. sativum* and *A. marmelos* respectively. All the extracts were examined for antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* and it was observed that *A. marmelos* has antibacterial activity against *klebsiella pneumoniae* which showed 6 mm of zone of inhibition at a concentration of 500 µg/ml followed by *C. sativum* with 3.5 mm of zone of inhibition against *Staphylococcus aureus* at 500 µg/ml.

**Keywords**: *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, free radical scavenging, *Aegle marmelos*, *Coriandrum sativum*, *Trigonella foenum greacum*.

## INTRODUCTION

Medicinal plants play fundamental role in traditional medicine. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids, which have been found to have medicinal properties. *Aegle marmelos* commonly known as Bael/Bilva belonging to the family Rutaceae has been widely used in indigenous systems of Indian medicine due to its various medicinal properties. Its antidiabetic, antidiarrhoeal, radioprotective, hepatotoxic, antimicrobial, activities have been studied [1]. Coriander (*Coriandrum sativum*), family Apiaceae like many other spices, contains antioxidants, which can delay and prevent the spoilage of food seasoned with this spice. Volatile components in essential oil, from both seeds and leaves, have been reported to inhibit growth of a range of micro-organisms [2]. The antimicrobial and antifungal effects of fenugreek (*Trigonella foenum greacum*) family Leguminosae have been studied [3-7]. The objective of our study was to compare the antimicrobial and antioxidant (reducing power, superoxide anion scavenging and peroxide scavenging activity) property of methanolic extracts of *A. marmelos* and *T. foenum greacum* leaves, and diethyl ether and methanolic extract of *C. sativum.*

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## MATERIALS AND METHODS

All the chemicals used were of analytical grade from Merck and Hi-Media. Optimum amount of leaves were collected, cleaned and washed and then the extracts of the leaves of *A. marmelos, C. sativum* and *T. foenum greacum* were prepared by Soxhlet method [8]. The total phenolic content in all the extracts was determined by Folin Ciocalteau method [9]. The phenolic content of the extracts was quantified by using gallic acid as the standard. The flavonoid content was estimated according to the method described by colorimetric method [10]. The flavonoid content of the plant extract was quantified by using quercetin as the standard. Antibacterial susceptibility testing was done by Kirby-Bauer method [11]. The test was done on pathogenic organisms like *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus*.

The reducing power of plant methanolic extracts was determined by the method of Oyaizu [12] and plant extracts of 1000 to 5000ppm were used. The superoxide anion radical scavenging activity was performed by using the methods of Liu and Ng [13]. The concentration of extracts used was 100 to 600g/ml. The control used in this case was ascorbic acid. The inhibition ratio was calculated from the equation: Percentage inhibition = [(Absorbance of control - Absorbance of test sample) / Absorbance of control] × 100. The ability of the extracts to scavenge peroxide was determined according to the procedure of Nabavi [14] and Hauda [15] and plant extract of 0.2-1.0mg/ml was used. A solution of $H_2O_2$ (40mM) was used as a control. Percentage of $H_2O_2$ scavenged was calculated as follows: Percent scavenged ($H_2O_2$) = [Ao - A1 / Ao] × 100, where Ao - Absorbance of control and A1 - Absorbance in the presence of the sample.

## RESULTS AND DISCUSSION

On comparing the extracts of diethyl ether and methanol of *C. sativum*, we can interpret that diethyl ether has greater flavonoid content than methanolic extract. While in methanolic extracts, *A. marmelos* showed highest phenolic content followed by *T. foenum greacum*, while *C. sativum* had the least phenolic content. On comparing the diethyl ether and methanolic extracts of *C. sativum*, methanolic extract had shown more phenolic content of 292.8 µmol/ml (Table 1). It has long been known that phenolic compounds are effective antioxidant and have been used for decades. The ability of phenolic compound to quench free radicals arises because of their acidity (ability to donate protons) and their delocalized electrons (ability to transfer electrons while remaining relatively stable) characteristics of benzene rings [16].

Table 1. Phenolic and flavonoid levels in the leaf extracts of *A. marmelos*, *C. sativum* and *T. foenum greacum*.

| Plant extract | Phenolics (µmol/ml) | Flavonoids (µg/ml) |
|---|---|---|
| *A. marmelos* (methanolic) | 504.9 | 0.25 |
| *C. sativum* (methanolic) | 292.8 | 0.60 |
| *T. foenum greacum* (methanolic) | 414.0 | 0.70 |
| *C. sativum* (diethyl ether) | 262.5 | 1.35 |

Flavonoids are the most common group of polyphenolic compounds found ubiquitously in plants. Flavonoids are most commonly known for their antioxidant property. Siddique [17] reported the flavonoid content of methanolic extract of *A. marmelos* (leaf 8.24mg/kg and stem 1.4 mg/kg of Quercetin equivalents) and also reported total phenolic content in *A. marmelos* (methanolic extract of leaf 9.836mg/kg and that of stem 7.439mg/kg). Dixit [6] reported antioxidant activity in germinated fenugreek which is due to the presence of polyphenols and flavonoids. Wangensteen [2]

reported that leaves of coriander had more phenolic content than the seeds. All the extracts were studied for antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Of these, *A. marmelos* methanolic extract showed zone of inhibition of 4.5mm and 6mm at concentration of 250µg/ml and 500µg/ml respectively against *Klebsiella pneumoniae* while *C. sativum* diethyl ether extract showed zone of inhibition of 2.5mm and 3.5mm at a concentration of 250µg/ml and 500µg/ml respectively against *Staphylococcus aureus.*

Hence coriander has strong antibacterial activity. Research suggests that the volatile oils found in the leaves of the coriander plant may have antimicrobial properties against food borne pathogen [18]. The reducing power increased with an increase in extract concentration (Table 2). The increasing absorbance is indicative of increasing reducing power. On comparing the methanolic extracts of *A. marmelos*, *T. foenum greacum* and *C. sativum*, the reduction of Fe(III) to Fe(II) is rapid in case of *A. marmelos* indicated by rapid increase in absorbance followed by *T. foenum-greacum*. *C. sativum* shows very slow reduction and on comparing the diethyl ether and methanolic extract of *C. sativum*, the methanolic extract showed greater reducing power as compared to the diethyl ether extract. Reducing power is to measure the reductive ability of antioxidant and it is evaluated by the transformation of Fe(III) to Fe(II) in the presence of sample extracts [15]. *A. marmelos* is reported to have DPPH scavenging activity while *C. sativum* is found to be potent antioxidant.

Table 2. Absorbance of different concentration of leaf samples of *A. marmelos*, *C. sativum* and *T. foenum greacum.*

| Concentration (ppm) | *A. marmelos* (methanolic) | *C. sativum* (methanolic) | *T. foenum greacum* (methanolic) | *C. sativum* (diethyl ether) |
|---|---|---|---|---|
| 1000 | 0.086 | 0.225 | 0.122 | 0.008 |
| 2000 | 0.425 | 0.418 | 0.165 | 0.026 |
| 3000 | 0.581 | 0.558 | 0.245 | 0.058 |
| 4000 | 0.642 | 0.626 | 0.306 | 0.072 |
| 5000 | 0.947 | 0.782 | 0.354 | 0.104 |

Superoxide anionic radicals ($O_2^-$) are formed by activated phagocytes such as monocytes, macrophages, eosinophils and neutrophils and the production of oxygen is an important factor in the killing of bacteria by phagocytes. In the PMS-NADH-NBT system, superoxide anion, derived from dissolved $O_2$ from the coupling reaction of PMS-NADH, reduces NBT. The decrease in absorbance at 560nm with antioxidant indicates the consumption of superoxide anion in the reaction mixture. On comparing the methanolic extracts the percentage inhibition increased with increase in concentration of the extracts. *T. foenum greacum* showed maximum % inhibition even at lower concentration of around 100 g/ml. But the increase in % inhibition is not very steep with concentration. Methanolic extract of *C. sativum* showed gradual increase in % inhibition with increase in concentration. *A. marmelos* does not show very high superoxide scavenging activity. Therefore there is no significant rise in percentage inhibition with respect to concentration. On comparing the *C. sativum* extracts of methanol and diethyl ether, % inhibition gradually increase with increase in concentration in both the cases. *C. sativum* (methanol) extract showed percentage inhibition of 58 % and *C. sativum (*diethyl ether) extract showed 52 % inhibition while *T. foenum greacum* showed 64.3 % inhibition (Table 3).

Hydrogen peroxide is a weak oxidizing agent that inactivates a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. It can cross cell membranes rapidly; once inside the cell, it can probably react with $Fe^{2+}$ and possibly $Cu^{2+}$ ions to form hydroxyl radicals and this may

be the origin of many of its toxic effects [19]. $H_2O_2$ converts into singlet oxygen and OH radical, which then becomes very powerful oxidizing agent. $O_2$, $OH^-$, $H_2O_2$ can cross membranes and may oxidize a number of compounds [20]. On comparing the methanolic extracts with standard ascorbic acid at a concentration of 0.2 mg/ml, standard ascorbic acid showed 27.53 % scavenging while the extract of *T. foenum greacum* showed greater scavenging of peroxide ion of 73.07 % followed by *A. marmelos* which scavenged 56.08%. *C. sativum* (diethyl ether) has 55.63% scavenging.

Table 3. Percentage inhibition of superoxide radical shown by leaf extracts of *A. marmelos, C. sativum* and *T. foenum greacum*.

| Concentration of sample (µg/ml) | *A. marmelos* (methanolic) | *C. sativum* (methanolic) | *T. foenum greacum* (methanolic) | *C. sativum* (diethyl ether) |
|---|---|---|---|---|
| 100 | 6.0 | 2.7 | 50.0 | 7.5 |
| 200 | 4.4 | 25.0 | 52.0 | 32.0 |
| 300 | 5.2 | 33.5 | 51.0 | 35.0 |
| 400 | 5.8 | 30.0 | 53.0 | 37.6 |
| 500 | 6.6 | 39.0 | 56.8 | 45.0 |
| 600 | 7.6 | 58.0 | 64.3 | 52.0 |

Thus, the extracts showed higher antioxidant activity as compared to ascorbic acid. On comparing the diethyl ether extract of *C. sativum* and ascorbic acid, at a concentration of 0.2 mg/ml diethyl ether extract of *C. sativum* showed better scavenging of peroxide ion of 55.63 % than the ascorbic acid that scavenged peroxide ion of 27.53 %. The methanolic extracts of *A. marmelos*, *T. foenum greacum* and diethyl ether extract of *C. sativum* scavenged $H_2O_2$ (peroxide ion) which may be attributed to the presence of phenolics, which could donate electrons thereby neutralizing it into water [21].

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