



E-ISSN: 2278-4136

P-ISSN: 2349-8234

[www.phytojournal.com](http://www.phytojournal.com)

JPP 2020; 9(2): 2024-2033

Received: 20-01-2020

Accepted: 24-02-2020

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## Pharmacological activities of differential parts of selected Essential Indian Spices

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DOI: <https://doi.org/10.22271/phyto.2020.v9.i2ag.11151>

**Abstract**

The role of plants in human health has extensively revealed due to the emergence of numerous advancements in the medicine and nutrition disciplines. The awareness of the benefits of plants in food as wealthy additives poses researchers to pursue for discovering the influence of such ingredients to the health of the human beings. Spices and herbs are well known food ingredients, which enhances the flavour and aroma of the supplemented foods. Botanically, spices are one class of the aromatic plants; they are mainly present in the tropical provinces. Spices play an important role as flavouring agents in the diet and are used throughout the world. Spices refers to dried part of plant that contains volatiles oils or aromatic flavours such as buds (cloves), bark (cinnamon), root (ginger), berries (black pepper), seeds (cumin, coriander). Spices can be used as medicine because they are natural products easily absorbed by our bodies and generally do not have any adverse effects. Herbal remedies are an important source for the discovery of new antibiotics. Highest phenolic content was observed for ethanol extract of *Bunium bulbocastanum* (374.28±0.22 µg/mg GAE) and highest flavonoid content was observed for ethanol extract of *Piper nigrum* (123.58±0.32 µg/mg QE). *Cuminum cyminum* possessed effective inhibition against gram negative bacteria (*Escherichia coli* and *Proteus vulgaris*) and the zone of inhibition were found to be 23 mm at 100 µg/mL concentration respectively. The DPPH<sup>•</sup> radical scavenging activity was found to be higher for *Syzygium aromaticum* among the tested spices with 75.37±0.48% at 120 µg/mL concentration and the IC<sub>50</sub> value was 53.54 µg/mL concentration respectively. The Fe<sup>3+</sup> reduction was found to be higher for *Syzygium aromaticum* among the tested spices with 99.14±0.26% at 120 µg/mL concentration and the RC<sub>50</sub> value was 12.81 µg/mL concentration respectively. The α-amylase enzyme inhibition was found to be higher for *Piper nigrum* among the tested spices with 67.47±0.40% at 120 µg/mL concentration and the IC<sub>50</sub> value was 44.69 µg/mL concentration respectively.

**Keywords:** Indian spices, well diffusion, antioxidant, DPPH radical, Fe<sup>3+</sup> reduction, α-amylase enzyme inhibition

**Introduction**

Free radicals are continuously produced by the body's normal use of oxygen<sup>[1, 2]</sup>. Oxygen is an element indispensable for life. When cells use oxygen to generate energy free radicals are produced by the mitochondria. These by-products are generally reactive oxygen species (ROS) as well as reactive nitrogen species (RNS) that result from the cellular redox process. The free radicals have a special affinity for lipids, proteins, carbohydrates and nucleic acids<sup>[3]</sup>. A free radical is any chemical species (capable of independent existence possessing one or more unpaired electrons, an unpaired electron being one that is alone in an orbital. The simplest radical is the hydrogen atom<sup>[4]</sup>. Oxidative stress is defined as an imbalance between production of free radicals and reactive metabolites, so-called oxidants or reactive oxygen species, and their elimination by protective mechanisms, referred to as antioxidants. This imbalance leads to damage of important biomolecules and cells, with potential impact on the whole organism<sup>[5]</sup>. The harmful effects of ROS are balanced by the action of antioxidants, some of which are enzymes present in the body<sup>[6]</sup>.

Reactive oxygen species can be classified into oxygen-centered radicals and oxygen-centered non radicals. Oxygen-centered radicals are superoxide anion (·O<sub>2</sub><sup>-</sup>), hydroxyl radical (·OH), alkoxyl radical (RO·), and peroxy radical (ROO·). Other reactive species are nitrogen species such as nitric oxide (NO·), nitric dioxide (NO<sub>2</sub>·), and peroxyxynitrite (OONO·). Oxygen centered non-radicals are hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and singlet oxygen (1O<sub>2</sub>), Hypochlorous acid and Ozone<sup>[7, 8]</sup>. The term "antioxidant" refers to any molecule capable of stabilizing or deactivating free radicals before they attack cells. The antioxidants can be endogenous or obtained exogenously e.g, as a part of a diet or as dietary supplements (Figure 1).

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Some dietary compounds that do not neutralize free radicals, but enhance endogenous activity may also be classified as antioxidants. An ideal antioxidant should be readily absorbed and quench free radicals, and chelate redox metals at physiologically relevant levels [9]. Some antioxidants can interact with other antioxidants regenerating their original properties; this mechanism is often referred to as the “antioxidant network” [10].

### Vitamin C-Ascorbic Acid

L-Ascorbic acid is a 6-carbon lactone ring structure with 2,3-enediol moiety. The antioxidant activity of ascorbic acid comes from 2,3-enediol. L-Ascorbic acid first changes to semi-dehydroascorbic acid through donating one hydrogen atom and electron, and then L-dihydroascorbic acid by donating a 2<sup>nd</sup> hydrogen atom and electron. Both L-ascorbic acid and L-dihydroascorbic acid retain the vitamin C activity. Ascorbic acid is highly susceptible to oxidation in the presence of metal ions such as Cu<sup>2+</sup> and Fe<sup>3+</sup>. Oxidation of ascorbic acid is also influenced by heat, light exposure, pH, oxygen concentration, and water activity [11]. The antioxidant mechanisms of ascorbic acid are based on hydrogen atom donation to lipid radicals, quenching of singlet oxygen, and removal of molecular oxygen [12].

### Vitamin E-Tocopherols

This is a fat-soluble vitamin existing in eight different forms. In humans,  $\alpha$ -tocopherol is the most active form, and is the major powerful membrane bound antioxidant employed by the cell [13]. The main function of Vitamin E is to protect against lipid peroxidation [14] and there is also evidence to suggest that  $\alpha$ -tocopherol and ascorbic acid function together in a cyclic-type of process. During the antioxidant reaction,  $\alpha$ -tocopherol is converted to an  $\alpha$ -tocopherol radical by the donation of a labile hydrogen to a lipid or lipid peroxy radical, and the  $\alpha$ -tocopherol radical can therefore be reduced to the original  $\alpha$ -tocopherol form by ascorbic acid [15]. Antioxidant mechanisms of tocopherols include the transfer of a hydrogen atom at 6-hydroxyl group on the chroman ring, and scavenging of singlet oxygen and other reactive species [16].

### Carotenoids

Carotenoids are a group of tetraterpenoids. The basic carotenoid structural backbone consists of isoprenoid units formed either by head-to-tail or by tail-to-tail biosynthesis. There are primarily 2 classes of carotenoids: carotenes and xanthophylls. Carotenes are hydrocarbon carotenoids and xanthophylls contain oxygen in the form of hydroxyl, methoxyl, carboxyl, keto, or epoxy groups. Lycopene and  $\beta$ -carotenes are typical carotenes whereas lutein in green leaves and zeaxanthin in corn are typical xanthophylls [17]. Carotenoids contain conjugated double bonds and their antioxidant activity arises due to the ability of these to delocalize unpaired electrons [18].

### Polyphenols

Flavonoids, the most important single polyphenol group, are glycosides with a benzopyrone nucleus. The flavonoids include flavones, flavonols, flavanones, flavanonols, and anthocyanins. Antioxidant mechanisms of polyphenolic compounds are based on hydrogen donation abilities and chelating metal ions. After donating a hydrogen atom, phenolic compounds become resonance-stabilized radicals, which do not easily participate in other radical reactions [19].

Phytoconstituents including spices and herbal medicine are also important to manage pathological conditions of diseases caused by free radicals. Exploring the antioxidant principles from natural resources; identification and isolation of phytoconstituents are simultaneously presenting enormous scope for better therapeutic application for treatment of human diseases. In the research study, six different Indian spices such as *Cuminum cyminum*, *Syzygium aromaticum*, *Bunium bulbocastanum*, *Elettaria cardamomum*, *Trigonella foenum – graecum* and *Piper nigrum* were evaluated for specific therapeutic applications.

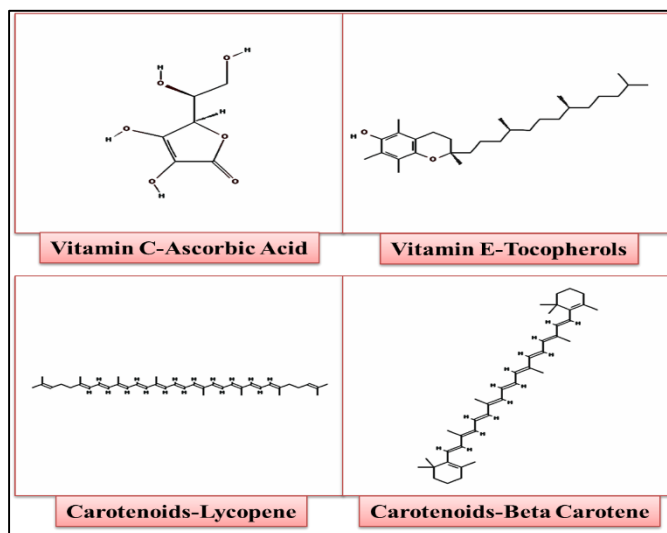


Fig 1: Active structures of important antioxidant molecules

### *Cuminum cyminum* L.

*Cuminum cyminum*, native from East Mediterranean to South Asia belonging to the family Apiaceae-a member of the parsley family. Cumin seeds are oblong and yellow-grey [20, 21].

Cumin seeds are nutritionally rich; they provide high amounts of fat (especially monounsaturated fat), protein, and dietary fibre. Vitamins B and E and several dietary minerals, especially iron, are also considerable in cumin seeds. Cuminaldehyde, cymene, and terpenoids are the major volatile components of cumin [22]. Cumin has a distinctive strong flavour. It's warm aroma is due to its essential oil content. Its main constituent of aroma compounds are cuminaldehyde and cuminic alcohol. Other important aroma compounds of roasted cumin are the substituted pyrazines, 2-ethoxy-3-isopropylpyrazine, 2-methoxy-3-sec-butylpyrazine, and 2-methoxy-3ethylpyrazine. Other components include  $\gamma$ -terpinene, safranal, p-cymene, and  $\beta$ -pinene [23].

### *Syzygium aromaticum* (L.) Merrill & Perry

Cloves are the pink flowering bud of a form evergreen tree (*Eugenia aromatica*), belonging to the family Myrtaceae, are dried until brown and used for medicinal and spicing purposes.

The spice contains many health benefiting essential oils such as eugenol, a phenyl propanoids class of chemical compound, which gives pleasant, sweet aromatic fragrances to the clove-bud. Eugenol has local anesthetic and antiseptic properties, hence; useful in dental treatment procedures. The other important constituents in this spice include: - essential oils: acetyl eugenol, beta-caryophyllene and vanillin, cratogeomyc acid; tannins: gallotannic acid, methyl salicylate (pain-killer); the flavonoids: eugenin, kaempferol, rhamnetin, and

eugenitin; triterpenoids: like oleanolic acid, stigmasterol and campesterol; and several sesquiterpenes <sup>[24]</sup>.

#### ***Bunium bulbocastanum* L.**

*Bunium bulbocastanum* is a plant species in the family Apiaceae. It was once used as a root vegetable in parts of western Europe, and has been called great pignut or earthnut <sup>[25]</sup>. *B. bulbocastanum* (Irani Zeera) is a perennial plant that grows to about 0.6 m (2ft) by 0.3 m (1ft) <sup>[26]</sup>. It is found primarily in northern India, Kashmir, Afghanistan, Tajikistan and Persia, though they do grows wild as far as South Eastern Europe. *B. bulbocastanum* has been used for edible purposes for years. Its seeds can be used either in raw or cooked form to enhance food flavors or to improve its taste. Medicinally it is also used as an astringent <sup>[27]</sup>.

#### ***Elettaria cardamomum* L. Maton**

The term Cardamom has been applied to the aromatic capsules of plants. Most of them from India, belongs to the family Zingiberaceae. The small cardamom, described as a queen of spices, is a rich spice called from the seeds of *Elettaria cardamomum*. The average chemical content of Indian cardamom seeds is as follows: moisture (8.3%), volatile oil (8.3%), non-volatile ether extracts (2.9%), vitamin C (12%) and vitamin A (175 international units) per 1 gm seeds. Cardamom is used for flavouring various food preparations, confectionery, perfumes, beverages, liquors and preparation of medicine in India and other countries. In medicine, it is used as a powerful aromatic, stimulant, carminative, stomachic and diuretic, but rarely used alone. It also checks nausea and vomiting, helps in combating digestive ailments. Herbal lores on this spice suggest that it can be used to freshen breath and support smooth digestion <sup>[28]</sup>.

#### ***Trigonella foenum - graecum* (Linn.)**

*Trigonella foenum - graecum* belonging to the family Papilionaceae commonly known as Fenugreek is a aromatic, 30-60 cm tall, annual herb, cultivated throughout the country <sup>[29, 30, 31]</sup>. The endosperm of the seed is rich in galactomannan, young seeds mainly contain carbohydrates and sugar. Mature seeds content amino acid, fatty acid, vitamins, and saponins. The seeds of fenugreek contain a large quantity of folic acid (84 mg/100 g). It also contents disogenin, gitogenin, neogitogenin, homorientin saponaretin, neogigogenin, and trigogenin <sup>[32, 33]</sup>. The main chemical constituents of *T. foenum-graecum* are fibers, flavonoids, polysaccharides, saponins, flavonoids and polysaccharides fixed oils and some identified alkaloids viz., trigonelline and choline <sup>[34, 35]</sup>.

#### ***Piper nigrum* L.**

*Piper nigrum* L. (black pepper), famous as the king of spices, is a flavoring vine of the family Piperaceae that is cultivated for its fruit <sup>[36, 37]</sup>, which is usually dried and used as spice and seasoning. In dried form the fruit is referred to as peppercorn. Piperine exhibits diverse pharmacological activities like antihypertensive and antiplatelets <sup>[38]</sup>, antioxidant, antitumor <sup>[39]</sup>, anti-asthmatics <sup>[40]</sup>, antipyretic, analgesic, anti-inflammatory, anti-diarrheal, antispasmodic, anxiolytic, antidepressants <sup>[41]</sup>, hepato-protective <sup>[42]</sup>, immunomodulatory, antibacterial, antifungal, anti-thyroids, antiapoptotic, anti-metastatic, antimutagenic, anti-spermatogenic, anti- Colon toxin, insecticidal and larvicidal activities etc. Piperine has been found to enhance the therapeutic efficacy of many drugs, vaccines and nutrients by increasing oral bioavailability by inhibiting various metabolizing enzymes. It is also known to enhance cognitive action and fertility <sup>[43]</sup>.



**Fig 2:** Habitat of selected Indian spices

## Materials and Methods

### Spices Collection and Extraction process

The selected Indian spices such as *Cuminum cyminum*, *Syzygium aromaticum*, *Bunium bulbocastanum*, *Elettaria cardamomum*, *Trigonella foenum-graecum* and *Piper nigrum* (Figure 2) were collected from Indian herbal market. Different parts of the selected spices were preferred for the pharmacological evaluation. Cumin seeds, Dried clove flower buds, Wild cumin seeds, Fresh green Cardamomum pods, Fenugreek seeds and Black peppercorn dried fruits were carefully cleaned and were made semi-powdery using sterile mortar and pestle. The selected spices were individually soaked in ethanol for 72 hours [44, 45]. Then the supernatant was filtered and condensed in room temperature devoid of heat supply, which produces colour variation depending upon the extractability of the spices.

### Determination of total phenols

Folin-Ciocalteu reagent method was used to determine the total phenolic compounds with slight modifications. Each of the ethanol extract such as *Cuminum cyminum*, *Syzygium aromaticum*, *Bunium bulbocastanum*, *Elettaria cardamomum*, *Trigonella foenum-graecum* and *Piper nigrum* (1 mg/mL) of about one hundred  $\mu\text{L}$  was mixed with 900  $\mu\text{L}$  of methanol and 1 mL of Folin Ciocalteu reagent (1:10 (v/v) diluted with distilled water). After 5 min, 1 mL of 20%  $\text{Na}_2\text{CO}_3$  (w/v) solution was added. The mixture was then allowed to stand for 30 min incubation in dark at room temperature. The absorbance was measured using UV-Vis Spectrophotometer at 765 nm [46] and the total phenolic content was expressed in terms of gallic acid equivalent ( $\mu\text{g}/\text{mg}$  of extract), which is a common reference compound.

### Determination of total flavonoids

The total flavonoid content of each of the ethanol extract such as *Cuminum cyminum*, *Syzygium aromaticum*, *Bunium bulbocastanum*, *Elettaria cardamomum*, *Trigonella foenum-graecum* and *Piper nigrum* was determined using aluminium chloride colorimetric method with slight modifications. One mL of each of the ethanol extract of different selected spices (1 mg/mL) was mixed with 0.5 mL of 5% (w/v) sodium nitrite solution and incubated for 5 min at room temperature. Then, 0.5 mL 10% (w/v) aluminium chloride solution was added and incubated for further 5 min at room temperature followed by the addition of 1 mL of 1 M NaOH solution. The absorbance was measured using UV-Vis Spectrophotometer at 510 nm [47] and the total flavonoid content was expressed in terms of quercetin equivalent ( $\mu\text{g}/\text{mg}$  of extract), which is a common reference compound.

### Antibacterial activity by Agar well diffusion method

Nutrient agar was prepared and poured in the sterile Petri dishes and allowed to solidify. 24 hours grown bacterial pathogens were swabbed on nutrient agar plates [48]. Then, each of the ethanol extract such as *Cuminum cyminum*, *Syzygium aromaticum*, *Bunium bulbocastanum*, *Elettaria cardamomum*, *Trigonella foenum-graecum* and *Piper nigrum* in a fixed concentration (100  $\mu\text{g}/\text{mL}$ ) was loaded in the clean lawns made using sterile cork borer. Tetracycline (30  $\mu\text{g}$ ) was used as standard. The plates were then incubated at 37°C for 24 hours and after incubation, the inhibition diameter was measured and recorded.

## Antioxidant activities

### DPPH<sup>•</sup> radical scavenging activity

The antioxidant activity of the ethanol extracts of *Cuminum cyminum*, *Syzygium aromaticum*, *Bunium bulbocastanum*, *Elettaria cardamomum*, *Trigonella foenum-graecum* and *Piper nigrum* was measured on the basis of the scavenging activity of the stable 1, 1-diphenyl 2-picrylhydrazyl (DPPH) free radical. One mL of 0.1 mM DPPH solution was mixed with 1 mL of various concentrations (20-120  $\mu\text{g}/\text{mL}$ ) of each of the ethanol extract such as *Cuminum cyminum*, *Syzygium aromaticum*, *Bunium bulbocastanum*, *Elettaria cardamomum*, *Trigonella foenum-graecum* and *Piper nigrum*. The mixture was then allowed to stand for 30 min incubation in dark condition. One mL of methanol and one mL of DPPH solution was used as the control. The decrease in absorbance was measured using UV-Vis Spectrophotometer at 517 nm [49] and the  $\text{IC}_{50}$  was calculated. Ascorbic acid was used as the standard reference. The percentage of DPPH<sup>•</sup> radical inhibition was calculated as:

$$\% \text{ of DPPH}^{\bullet} \text{ radical inhibition} = \left[ \frac{\text{Control} - \text{Sample}}{\text{Control}} \right] \times 100$$

### Ferric ( $\text{Fe}^{3+}$ ) reducing power activity

The reducing power of the ethanol extracts of *Cuminum cyminum*, *Syzygium aromaticum*, *Bunium bulbocastanum*, *Elettaria cardamomum*, *Trigonella foenum-graecum* and *Piper nigrum* was determined by slightly modified method [50]. One mL of each of the ethanol extract such as *Cuminum cyminum*, *Syzygium aromaticum*, *Bunium bulbocastanum*, *Elettaria cardamomum*, *Trigonella foenum-graecum* and *Piper nigrum* in different concentrations (20-120  $\mu\text{g}/\text{mL}$ ) was mixed with phosphate buffer (1 mL, 0.2 M, pH 6.6) and potassium ferricyanide [ $\text{K}_3\text{Fe}(\text{CN})_6$ ] (1 mL, 1% w/v). The mixtures were then incubated at 50 °C in water bath for 30 min. 0.5 mL of trichloroacetic acid (10% w/v) was added to all test tubes. Then to the mixture 0.1 mL of ferric chloride (0.01%, w/v) was added and the absorbance was measured using UV-Vis Spectrophotometer at 700 nm and the  $\text{RC}_{50}$  was calculated. Ascorbic acid was used as the standard reference. The percentage of  $\text{Fe}^{3+}$  reduction was calculated as:

$$\% \text{ of } \text{Fe}^{3+} \text{ reduction} = \left[ \frac{\text{Sample} - \text{Control}}{\text{Sample}} \right] \times 100$$

### Antidiabetic activity by Starch-iodine method

$\alpha$ -amylase enzyme inhibition activity was carried out based on the starch-iodine test. The total mixture was composed of various concentrations (20-120  $\mu\text{g}/\text{mL}$ ) of ethanol extracts of *Cuminum cyminum*, *Syzygium aromaticum*, *Bunium bulbocastanum*, *Elettaria cardamomum*, *Trigonella foenum-graecum* and *Piper nigrum*. 10  $\mu\text{L}$  of alpha amylase enzyme prepared in 0.02 M sodium phosphate buffer (pH 6.9 containing 6 mM sodium chloride) and was incubated at 37°C for 10 min. Then soluble starch (1%, w/v) was added to each reaction set and incubated at 37°C for 60 min. One hundred  $\mu\text{L}$  of 1 M Hydrochloric acid was added to stop the enzymatic reaction and followed by 200  $\mu\text{L}$  of iodine reagent (5 mM Iodine and 5 mM Potassium Iodide) was added. The colour

change was noted and the absorbance was measured using UV-Vis Spectrophotometer at 595 nm<sup>[51]</sup> and the IC<sub>50</sub> was calculated. The control reaction representing 100% enzyme activity did not contain any of the ethanol extracts. A dark-blue colour indicates the presence of starch; a yellow colour indicates the absence of starch, while a brownish colour indicates partially degraded starch in the reaction mixture. In the presence of inhibitors from the extract, the starch added to the enzyme assay mixture is not degraded and gives a dark blue colour complex, whereas no colour complex is developed in the absence of the inhibitor, indicating that starch is completely hydrolyzed by  $\alpha$ -amylase. Acarbose was used as the standard reference. The percentage inhibition of alpha amylase enzyme was calculated as:

$$\% \text{ inhibition of alpha amylase enzyme} = \left[ \frac{\text{Sample} - \text{Control}}{\text{Sample}} \right] \times 100$$

## Results and Discussion

### Total phenols and flavonoids determination

The phenol and flavonoid compounds quantified in the ethanol extracts of *Cuminum cyminum*, *Syzygium aromaticum*, *Bunium bulbocastanum*, *Elettaria cardamomum*, *Trigonella foenum-graecum* and *Piper nigrum* seemed to be responsible

for the antioxidant activity (Table 1). These results provide a comprehensive profile of the antioxidant activity of the ethanol extracts of *Cuminum cyminum*, *Syzygium aromaticum*, *Bunium bulbocastanum*, *Elettaria cardamomum*, *Trigonella foenum-graecum* and *Piper nigrum* with respect to their phenols and flavonoids content. Highest phenolic content was observed for ethanol extract of *Bunium bulbocastanum* and highest flavonoid content was observed for ethanol extract of *Piper nigrum*. The difference in amounts of phenols is probably related to geographical and environmental factors, processing methods and other intrinsic factors (genetic, extracting solvent) and extrinsic (environmental, handling and development stage) which may play role in such a large variation<sup>[52]</sup>. Also, the phenol content of a plant depends on a number of as well as the Folin-Ciocalteu assay gives a crude estimate of the total phenolic compounds present in an extract/fraction. It is not specific to polyphenols, but many interfering compounds may react with the reagent, giving elevated apparent phenolic concentrations<sup>[53]</sup>. Oxidative stress is considered to be substantial, if not crucial, in the initiation and development of many current conditions and diseases, including: inflammation, autoimmune diseases, cataract, cancer, parkinson's disease, arteriosclerosis and aging<sup>[54]</sup>.

**Table 1:** Quantitative estimation of ethanol extracts of different Indian species

S. No	Phytochemicals Value ( $\mu\text{g}/\text{mg}$ )	<i>C.cyminum</i>	<i>S.aromaticum</i>	<i>B.bulbocastanum</i>	<i>E.cardamomum</i>	<i>Trigonella foenum-graecum</i>	<i>P.nigrum</i>
1	Phenols (GAE)	105.33 $\pm$ 0.25	319.06 $\pm$ 0.48	374.28 $\pm$ 0.22	179.98 $\pm$ 0.34	141.12 $\pm$ 0.40	311.39 $\pm$ 0.13
2	Flavonoids (QE)	92.24 $\pm$ 0.14	68.80 $\pm$ 0.36	78.71 $\pm$ 0.19	41.36 $\pm$ 0.39	95.50 $\pm$ 0.28	123.58 $\pm$ 0.32

(\*Average value of 3 replicates)

### The antioxidant properties of phenolic and flavonoid compounds are mediated by the following mechanisms

1. Scavenging radical species such as ROS/ reactive nitrogen species (RNS)
2. Suppressing ROS/RNS formation by inhibiting some enzymes or chelating trace metals involved in free radical production;
3. Up regulating or protecting antioxidant defense<sup>[55]</sup>.

The reduction activity of phenolic and flavonoid compounds depends on the number of free hydroxyl groups in the molecular structure, which would be strengthened by steric hindrance<sup>[56]</sup>.

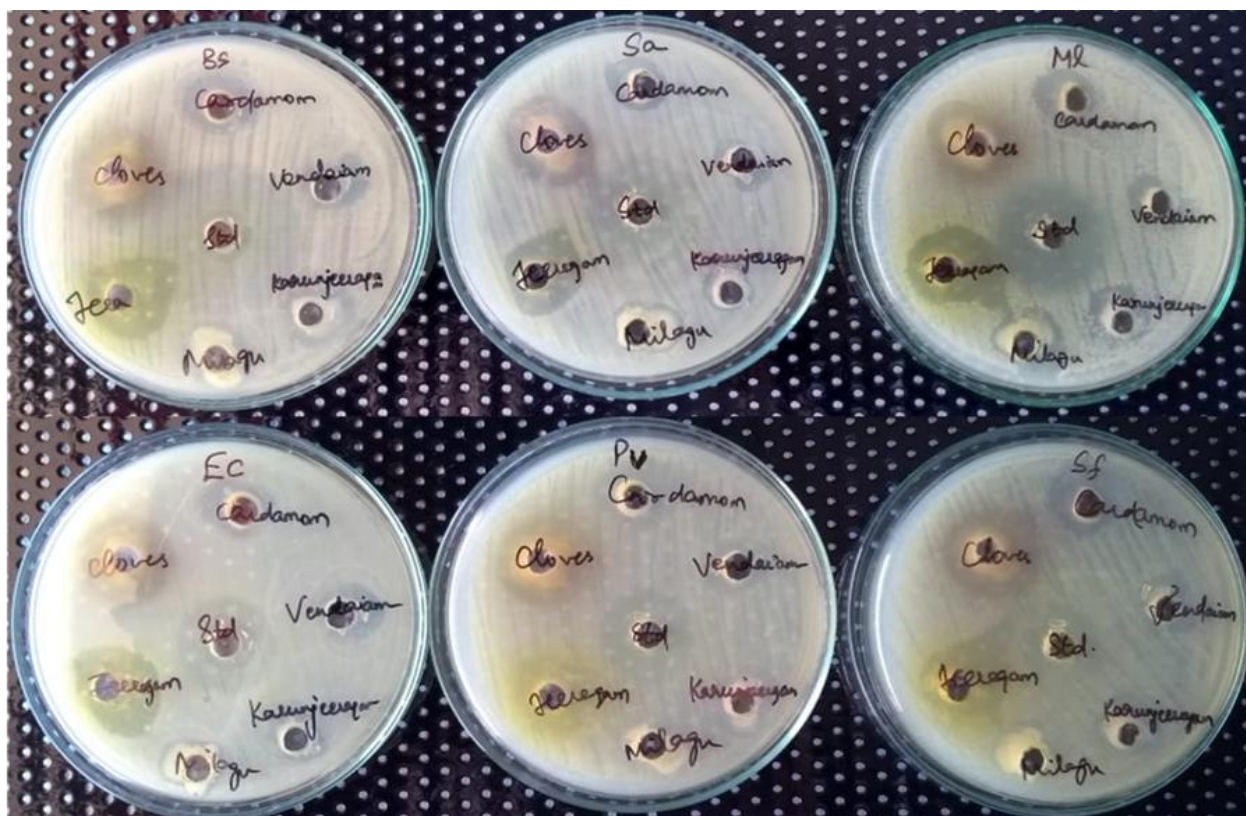
### Antibacterial activity by Agar well diffusion method

The ethanol extracts of *Cuminum cyminum*, *Syzygium aromaticum*, *Bunium bulbocastanum*, *Elettaria cardamomum*, *Trigonella foenum-graecum* and *Piper nigrum* were investigated for *in vitro* antibacterial activity against

microorganism including Gram-positive bacteria (*Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*, *Shigella flexneri*, *Proteus vulgaris*). The antibacterial sensitivity of the ethanol extracts and their potency were assessed quantitatively by measuring the diameter of clear zone in cultures in petriplates. The inhibitory effect was well observed for the ethanol extracts of *Cuminum cyminum*, *Syzygium aromaticum*, *Bunium bulbocastanum*, *Elettaria cardamomum*, *Trigonella foenum-graecum* and *Piper nigrum* against tested bacterial pathogens. *Cuminum cyminum* possessed effective inhibition against gram negative bacteria (*Escherichia coli* and *Proteus vulgaris*) and the zone of inhibition were found to be 23 mm at 100  $\mu\text{g}/\text{mL}$  concentration respectively (Table 2 and Figure 3). The antibacterial activity of each of the ethanol extract could be correlated as due to the presence of secondary metabolites such as flavonoids, phenolic compounds, terpenoids, tannins and alkaloids that adversely affect the growth and metabolism of microbes.

**Table 2:** Antibacterial activity of ethanol extracts of different Indian spices

Bacterial pathogens	Zone of inhibition (mm) at 100 $\mu\text{g}/\text{mL}$						Standard (Tetracycline) 30 $\mu\text{g}/\text{mL}$
	<i>C.cyminum</i>	<i>S.aromaticum</i>	<i>B.bulbocastanum</i>	<i>E.cardamomum</i>	<i>Trigonella foenum-graecum</i>	<i>P.nigrum</i>	
<i>B. subtilis</i>	22	18	12	14	17	10	25
<i>S. aureus</i>	19	15	10	12	10	10	14
<i>M. luteus</i>	20	16	10	12	-	12	19
<i>E. coli</i>	23	19	13	14	13	14	22
<i>P. vulgaris</i>	23	21	12	14	10	14	21
<i>S. flexneri</i>	22	21	12	13	11	13	13



**Antibacterial activity of ethanol extracts of *Elettaria cardamomum*, *Trigonella foenum-graecum*, *Bunium bulbocastanum*, *Piper nigrum*, *Cuminum cyminum* and *Syzygium aromaticum* -100 µg/mL Standard-(Tetracycline)-30 µg/mL**

**Fig 3:** Antibacterial activity of ethanol extracts of different Indian spices

Tannins bind to proline rich proteins and interfere with the protein synthesis [57]. Flavonoids are hydroxylated phenolic substance known to be synthesized by plants in response to microbial infection and it should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls [58]. Antimicrobial property of saponins is due to its ability to cause leakage of proteins and certain enzymes from the cell [59]. Steroids have been reported to have antibacterial properties, the correlation between membrane lipids and sensitivity for steroidal compound indicates the mechanism in which steroids specifically associate with membrane lipid and exerts its action by causing leakages from liposomes [60].

#### Antioxidant activities

##### DPPH<sup>•</sup> radical scavenging activity

DPPH<sup>•</sup> radical scavenging method is a decolorization assay

that will measure the capacity of antioxidants to directly scavenge DPPH<sup>•</sup> radicals by monitoring its absorbance using spectrophotometer at wavelength of 517 nm [61]. The DPPH method provided rapid and an easy way to evaluate the antioxidant activity of most of the plant extracts. The ethanol extracts of *Cuminum cyminum*, *Syzygium aromaticum*, *Bunium bulbocastanum*, *Elettaria cardamomum*, *Trigonella foenum-graecum* and *Piper nigrum* to scavenge free radicals was assessed by using DPPH<sup>•</sup> radical as the substrate, which measures the hydrogen or electron donating ability of the selected spices. The ethanol extracts of *Cuminum cyminum*, *Syzygium aromaticum*, *Bunium bulbocastanum*, *Elettaria cardamomum*, *Trigonella foenum-graecum* and *Piper nigrum* reducing the stable purple colour DPPH (1,1-diphenyl-2-picryl hydrazyl) free radical to the yellow coloured 1,1-diphenyl-2-picryl hydrazine and the reduction capacity increases with increasing concentration of the selected spices. The DPPH<sup>•</sup> radical scavenging activity was found to be higher for *Syzygium aromaticum* and lower inhibition for *Elettaria cardamomum* (Table 3 and Figure 4).

**Table 3:** DPPH<sup>•</sup> radical scavenging activity of ethanol extracts of different Indian spices

S. No	Concentration (µg/mL)	Percentage of inhibition*					
		<i>Cuminum cyminum</i>	<i>Syzygium aromaticum</i>	<i>Bunium bulbocastanum</i>	<i>Elettaria cardamomum</i>	<i>Trigonella foenum-graecum</i>	<i>Piper nigrum</i>
1	20	14.07±0.12	30.65±0.35	22.61±0.38	11.05±0.18	11.6±0.26	20.85±0.32
2	40	20.10±0.38	37.68±0.15	38.94±0.10	17.83±0.19	33.66±0.25	22.86±0.33
3	60	25.62±0.11	56.03±0.18	45.47±0.19	19.84±0.36	41.45±0.21	31.15±0.37
4	80	36.18±0.19	61.30±0.36	53.76±0.22	30.65±0.28	47.73±0.28	41.70±0.12
5	100	40.20±0.22	69.34±0.25	64.32±0.32	36.68±0.14	54.27±0.30	45.72±0.28
6	120	46.98±0.43	75.37±0.48	70.10±0.47	42.71±0.25	55.02±0.17	56.28±0.14
IC <sub>50</sub> values		124.37 µg/mL	53.54 µg/mL	65.97 µg/mL	140.48 µg/mL	83.80 µg/mL	109.36 µg/mL

(\*Average value of 3 replicates)

**Ferric (Fe<sup>3+</sup>) reducing power activity**

The reducing power activity was carried out by the reduction of Fe<sup>3+</sup> to Fe<sup>2+</sup> by the ethanol extracts of *Cuminum cyminum*, *Syzygium aromaticum*, *Bunium bulbocastanum*, *Elettaria cardamomum*, *Trigonella foenum-graecum* and *Piper nigrum* and the subsequent formation of ferro-ferric complex. Fe (III) reduction is often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action [62]. The reducing ability of a compound

generally depends on the presence of reductones (antioxidants), which exert the antioxidant activity by breaking the free radical chain by donating a hydrogen atom or by neutralizing the free radicals by donating an electron and become lone pair of electrons instead of odd electron. The reduction ability increases with increase in concentration of the ethanol extracts. The Fe<sup>3+</sup> reduction was found to be higher for *Syzygium aromaticum* and lower reduction for *Trigonella foenum-graecum* (Table 4 and Figure 4).

**Table 4:** Ferric (Fe<sup>3+</sup>) reducing power activity of ethanol extracts of different Indian spices

S. No	Concentration (µg/mL)	Percentage of reduction*					
		<i>Cuminum cyminum</i>	<i>Syzygium aromaticum</i>	<i>Bunium bulbocastanum</i>	<i>Elettaria cardamomum</i>	<i>Trigonella foenum-graecum</i>	<i>Piper nigrum</i>
1	20	72.59±0.25	78.01±0.21	48.84±0.16	71.02±0.30	19.04±0.19	71.99±0.37
2	40	73.46±0.31	80.08±0.17	65.73±0.25	71.90±0.42	39.28±0.23	75.65±0.22
3	60	79.06±0.35	80.70±0.19	76.49±0.32	72.54±0.41	40.97±0.36	77.90±0.13
4	80	88.51±0.11	82.86±0.38	78.88±0.34	81.30±0.36	56.29±0.31	79.88±0.24
5	100	88.81±0.28	84.60±0.15	80.63±0.47	90.54±0.27	59.23±0.48	83.26±0.19
6	120	89.41±0.47	99.14±0.26	82.47±0.12	90.56±0.25	62.47±0.10	90.41±0.41
RC <sub>50</sub> values		13.77 µg/mL	12.81 µg/mL	20.47 µg/mL	14.08 µg/mL	71.06 µg/mL	13.89 µg/mL

(\*Average value of 3 replicates)

**Antidiabetic activity by Starch-iodine colour method**

Diabetes mellitus (DM) is a chronic disease characterized by a deficiency in insulin production and its action or both. That leads to prolonged hyperglycemia with disturbances in most metabolic processes inside the human body [63]. The most popular methods for diagnosis of diabetes include the measuring of fasting plasma glucose level (FPG), which is done in the early morning. Patients with FPG below 100 mg/dl are considered normal; those between 100 and 125 mg/dl indicate pre-diabetic while those individuals with glucose levels above 125 mg/dl are considered diabetic [64]. The alpha-glucosidase inhibitors "starch blockers" inhibit certain enzymes responsible for the breakdown of

carbohydrates in the small intestine. They act mainly by decreasing the rate of carbohydrate absorption in the body. Moreover, acarbose, an important example in this class, reversibly inhibits both pancreatic  $\alpha$ -amylase and  $\alpha$ -glucosidase enzymes by binding to the carbohydrate-binding region and interfering with their hydrolysis into mono-saccharides. This results in a slower absorption together with a reduction in postprandial blood-sugar levels [65, 66]. The ability of for  $\alpha$ -amylase enzyme inhibition was assessed by starch-iodine method. The  $\alpha$ -amylase enzyme inhibition was found to be higher for *Piper nigrum* and lower inhibition for *Elettaria cardamomum* (Table 5 and Figure 4).

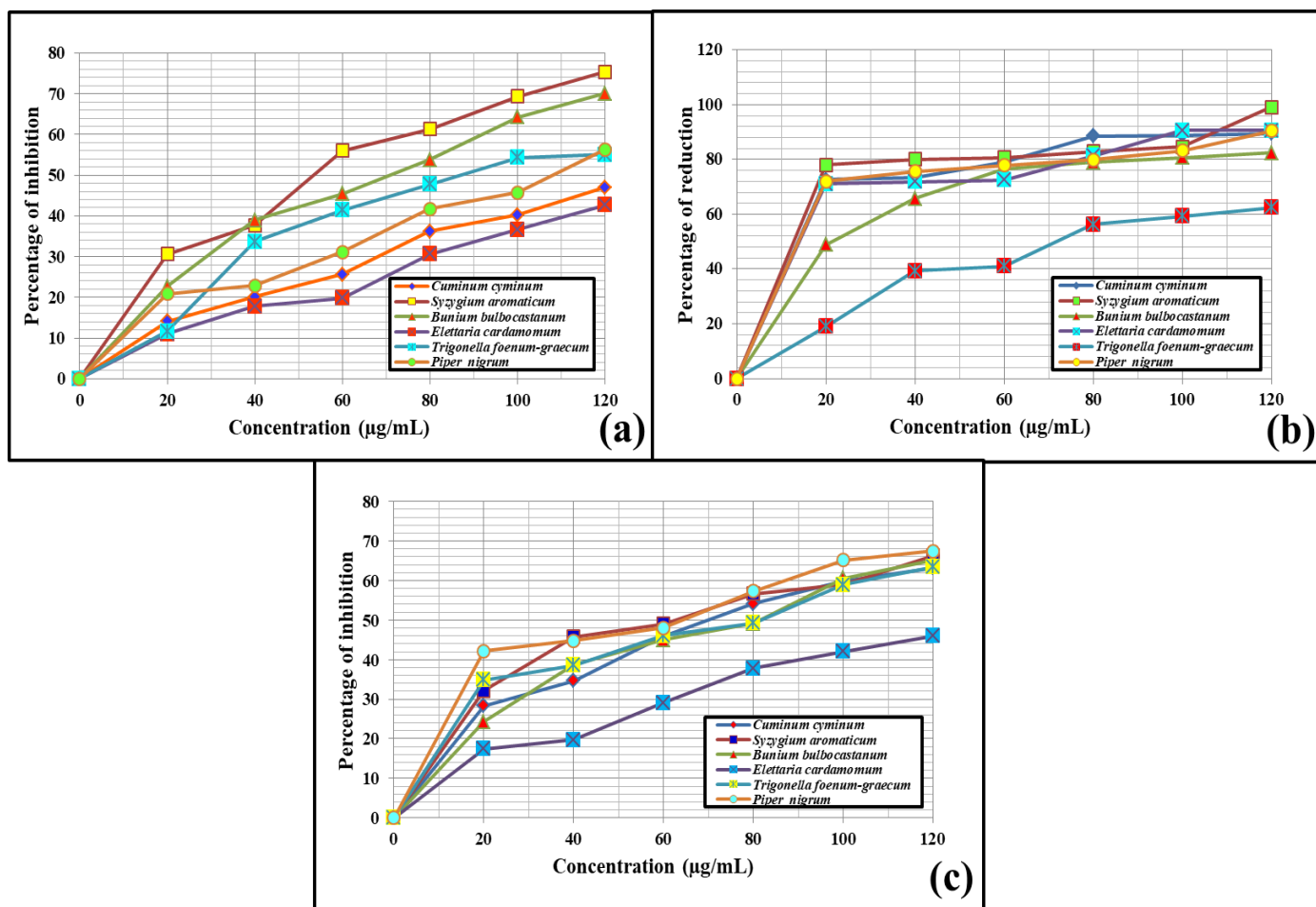
**Table 5:** Alpha-amylase enzyme inhibition of ethanol extracts of different Indian spices

S. No	Concentration (µg/mL)	Percentage of inhibition*					
		<i>Cuminum cyminum</i>	<i>Syzygium aromaticum</i>	<i>Bunium bulbocastanum</i>	<i>Elettaria cardamomum</i>	<i>Trigonella foenum-graecum</i>	<i>Piper nigrum</i>
1	20	28.33±0.14	32.15±0.26	24.26±0.22	17.38±0.43	34.91±0.38	42.19±0.19
2	40	34.61±0.23	45.82±0.39	38.73±0.13	19.67±0.14	38.62±0.29	44.75±0.42
3	60	45.87±0.38	49.03±0.30	45.02±0.28	29.05±0.23	46.11±0.27	48.03±0.41
4	80	54.20±0.42	56.71±0.45	49.14±0.37	37.78±0.26	49.27±0.16	57.32±0.21
5	100	59.92±0.11	59.03±0.41	60.56±0.12	42.07±0.31	58.92±0.10	65.16±0.33
6	120	63.18±0.32	66.21±0.16	65.08±0.40	45.94±0.39	63.45±0.32	67.47±0.40
IC <sub>50</sub> values		65.40 µg/mL	43.64 µg/mL	66.63 µg/mL	118.84 µg/mL	65.06 µg/mL	44.69 µg/mL

(\*Average value of 3 replicates)

Higher concentrations of Magnesium and lower concentrations of Potassium play a vital role in diabetes management [67, 68]. Hence the significant antidiabetic potential of selected spices could be due to the high concentration of Magnesium along with Calcium. The Ca<sup>2+</sup> ion activates insulin gene expression via CREB (Calcium

Responsive Element Binding Protein) and is responsible for exocytose of stored insulin [69]. Pro-inflammatory cytokines and/or abnormally high blood glucose promote the development of oxidative stress in pancreatic islets to aggravate beta-cell death by apoptosis [70].



(a) - DPPH radical scavenging activity, (b) - Ferric (Fe<sup>3+</sup>) reducing power activity, (c) - Alpha-amylase enzyme inhibition method

**Fig 4:** Therapeutic applications of ethanol extracts of different Indian spices

## Conclusion

Spices have been widely used as condiments for thousands of years because of their flavour, taste and colour. Several spices have been used as medicinal plants in folk medicine for the treatment of various diseases because they contain many bioactive compounds and possess a lot of beneficial health effects. For example, some antioxidants from spices, such as Curcumin (turmeric), Eugenol (clove), and Capsaicin (red pepper), were experimentally evidenced to control cellular oxidative stress due to their antioxidant properties and their capacity to block the production of reactive oxygen species and interfering with signal transduction pathways. Spices and herbs have been extensively studied in different countries because of the high antioxidant activity in certain spices and their beneficial effects on human health. As part of our diet, spices in addition to fruits and vegetables, could provide us with additional sources of natural antioxidants. Antioxidants from spices are a large group of bioactive compounds which consist of flavonoids, phenolic compounds, sulphur-containing compounds, tannins, alkaloids, phenolic, diterpenes, and vitamins.

## Acknowledgement

The authors are thankful to Armats Biotek Training and Research Institute for providing facilities to carry out research work.

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