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# *In vitro* evaluation on antioxidant and antimicrobial activity of spice extracts of ginger, turmeric and garlic

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**ABSTRACT**
Spices like turmeric, ginger, alliums are indispensable for the preparation of our daily food and are reported to possess compounds, which have varied beneficial biological effects and also prevent the microbial spoilage of food. This study evaluates the antioxidant and antimicrobial activity of spice extracts such as ginger, turmeric and garlic by 2, 2'-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Method and also to evaluate their antimicrobial effects by Slant method. The antioxidant activities when compared among ginger, turmeric and garlic the potency of these spices was found to be in the order of Vit C > Ginger > Turmeric ≥ Dry garlic > Fresh garlic. The antimicrobial activity of these spices was found to be in the order of Turmeric > Ginger > Garlic**.** The study indicates that the spices like ginger, garlic and turmeric have antimicrobial and antioxidant activity. Further studies are needed to study the biological effects of antioxidant-rich herbs and spices on oxidative stress related diseases.

**Keywords:** Ginger, Turmeric, Garlic, Antioxidant Activity, Antimicrobial Activity.

## 1. Introduction
Herbs and spices have been found to reduce inflammation, protect against infection, helps to detoxify the liver and cleanse the lungs and other organs and also protect from cell damage that can lead to rheumatoid arthritis, osteoporosis, heart disease and other degenerative diseases [1]. Some common herbs such as cilantro, basil, thyme, onion, ginger, turmeric, garlic etc., offer great health benefits by virtue of their powerful phytochemical and antioxidant properties. Even though there is limited literature on the health effects of herbs and spices or extracts of these, the number of studies investigating the possible health effects of phytochemicals originating from herbs and spices is at large. Most of the products categorized as herbal and traditional plant medicines are also based on antioxidant-rich dietary plants or isolated phytochemicals.
Spice oils have also been reported to prevent microbial spoilage of foods [2, 3]. This preservative property of spices has been attributed to the presence of some antimicrobial principles contained in their oils [4]. The antimicrobial compounds in spices and herbs are mostly in the essential oil fraction.
Ginger (*Zingiber officinale* Roscoe) is one of the most commonly consumed dietary condiments in the world. The main active phytochemicals present in ginger are gingerols, shogaols and paradols, and they have strong antioxidant and chemopreventive properties [6]. The medicinal, chemical and pharmacological properties of ginger has been extensively reviewed [7].Ginger extracts have been extensively studied for a broad range of biological activities including antibacterial, anticonvulsant, analgesic, antiulcer, gastric antisecretory, antitumor, antifungal, antispasmodic, antithrombotic, hypocholesterolemic, antiallergic, antiserotonergic , anticholinergic and other beneficial activities [8]. Many studies have proved that ginger is endowed with strong antioxidant [9,10], antigenotoxic, antimutagenic and anticarcinogenic properties both in *in vitro* and *in vivo* studies Powdered ginger rhizome contains 3.6% fatty oil, 9% protein, 60-70% carbohydrates, 3.8% crude fiber, 8% ash, 9-12% water and other terpenes and terpenoids. Fresh ginger contains 80.9% moisture, 23% protein, 0.9% fat, 1.2% minerals, 2.4% fibre, and 12.3% carbohydrates. Ginger has been shown to be effective against the growth of both gram-positive and gram-negative bacteria including *Escherichia coli, Proteus vulgaris, Salmonella typhi, Staphylococcus aureus* and *Streptococcus viridans* [11].

Turmeric (*Curcuma longa*) is a dietary spice belonging to the family *zingiberaceae*. It is a coloring and flavouring agent in foods, and has been reported to possess antioxidant properties both in *in vitro* and animal studies. Aqueous extracts of turmeric showed antioxidant and antimicrobial activity due to the presence of curcumin (5%), a polyphenolic compound. It is known that the phenolic character of curcumin is responsible for its anti-oxidant properties [12]. Fresh root contains good levels of vitamin-C. Other phytochemicals in turmeric include tumerone, zingiberene, cineole, d-phellandrene, d-sabinene, borneol and other curcuminoids. The majority of the phytochemicals found in turmeric occur in the volatile oil that makes up 7 percent of its weight. Dry rhizomes yield 5.8% essential oil including sesquiterpene (e.g. Zingeberene), sesquiterpene alcohols and ketones, and monoterpenes. Fresh turmeric contains 0.24% oil containing zingiberene. The most significant curcuminoid is curcumin. It has been reported that turmeric has an antimutagenic effect on bacteria *in vitro* [13]. Curcumin is a known bacteriostatic agent whereas the essential oil of turmeric is bactericidal and fungistatic. The active principle, curcumin is known for its inhibitory action on micro-organisms [14].

Garlic (*Allium moly*), is a species in the onion genus, *Allium. Allium moly*, also known as golden garlic and lily leek. The most important chemical constituents reported from *Alliums* are the sulfur compounds [15]. It has been estimated that cysteine sulfoxides (e.g. alliin) and the non-volatile γ-glutamylcysteine peptides make up more than 82% of the total sulfur content of garlic [16]. The important components of garlic are allicin and sulphur containing compounds like diallyl sulphide (DAS) and diallyldisulphide (DADS) possessing antitumor and antioxidant properties [17, 18].The herb has been used as a carminative in the treatment of dyspepsia, as an aphrodisiac, antipyretic, diuretic, expectorant, and sedative, to treat asthma and bronchitis, and to promote hair growth [19, 20]. Fresh peeled garlic cloves contain moisture (62.8%), protein (6.3%), fat (0.1%), minerals (1%), Fibre (0.8%), carbohydrates (29%), Vit. B (16 mg/100 g), Vit. C (13 mg/100 g). Dehydrated garlic powder contains moisture (5.2%), protein (6.3%), fat (0.6%), minerals (3.2%), fibre (1.9%), carbohydrates (71.4%), Vit A (175IU), Vit C (12 mg/100 g) etc. It is rich in proteins, phosphorous, potassium, calcium, magnesium and carbohydrates. *Allium* has a broad range of antibacterial and antifungal activity. The essential oil, water, and ethanol extracts, and the juice inhibit the *in vitro* growth of *Bacillus* species, *Staphylococcus aureus*, *Escherichia coli*, *Proteus* species, *Streptococcus faecalis*, *Pseudomonas aeruginosa*, *Candida* species, and *Aspergillus niger* [21]. The antioxidant activity depends on the type and polarity of the extracting solvent, the isolation procedures, purity of active compounds, as well as the test system and substrate to be protected by the antioxidant [22].

With the focus to find alternatives for synthetic food ingredients, natural substances having antioxidative, anticarcinogenic, antimicrobial properties need to be further studied [5]. Numerous studies have been done in National Institute of Nutrition, documenting the anticarcinogenic, antimutagenic and antioxidant/anti-inflammatory properties of some selected plant constituents such as turmeric, garlic, onion, ginger etc. It was found necessary to see the antioxidant and antimicrobial activity of these selected plant constituents. Therefore this study was planned to evaluate the antioxidant status and also study the antimicrobial activity of turmeric, garlic, ginger and onion against food borne pathogens like *Salmonella typhi, Staphylococcus aureus*, *Escherichia coli.*

## 2. Materials and Methods

### 2.1. Test material

Ginger, turmeric (and garlic were purchased from the local market. Ginger was peeled, washed, coarsely minced, air dried and pulverized with a blender to a fine powder. Turmeric was air dried and pulverized to a fine powder. Garlic was kept in oven at 60 °C for 2 days for drying and then made into a fine powder. The test organisms such as *Escherichia coli, Salmonella typhi* and *Staphylococcus aureus* were procured from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory (NCL), Pune.

### 2.2. Extraction of the spice material

50 gms of each spice (Ginger, Turmeric and Garlic) dry powder was taken in a conical flask containing 250 ml of distilled water and 250 ml of absolute ethanol. It was kept in mechanical shaker water bath for 6-7 hrs at room temperature. The supernatant was filtered through the whatman filter paper no 1 under vacuum. The residue was again resuspended in 100 mL of ethanol and water and shaken for 6-7 hrs and the procedure was repeated again. The total filtrate was concentrated under vacuum in Rota evaporator at 60-70 °C. The concentrated extract was then lyophilized at -100 to -110 °C with methanol bath temperature between -100 °C under vacuum. The final powder extract was stored in a container and kept in the refrigerator for further analysis.

### 2.3. Extraction of fresh garlic

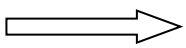
300 gms of fresh garlic was finely chopped into pieces in 400 mL ethanol in ice bath, and was kept in an air tight bottle at 0 °C in a refrigerator for 24 hrs. They were crushed with a motor pestle and were filtered using a whatman filter paper no 1 in cold room. The residue was resuspended in 400 mL of ethanol and kept at 0 °C in a refrigerator for 24 hrs and the procedure was repeated. The filtrate was concentrated under vacuum in Rota evaporator at <50 °C. The final powder extract was stored in a container and kept in the refrigerator.

### 2.4. Preparation of the culture

0.01 ml of test organism was taken and inoculated in 5 ml of nutrient broth and was kept for incubation at 37 °C for 24 hrs. Bacteria were counted by plate count or serial dilution agar plate technique from which $10^{-6}$ concentration was taken for the assay.

### 2.5. Determination of Antioxidant Activity 2, 2'-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Method

The free radical scavenging activity of antioxidants in foods has been substantially investigated and reported in the literature by Miller and Rigelhof *et al.*, (2000) [23]. The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in color which turns to yellow.




The free radical scavenging effect of the all the extracts with different concentrations was assessed by the de-coloration of an ethanol solution of DPPH. Briefly, various extracts of the test compounds at different concentrations were added to an alcoholic

solution of DPPH. Two concentrations of DPPH (0.4 mM & 0.6 mM) working solution was used to determine the inter day and intraday linearity. Vitamin-C was used as a reference standard. The degree of de-coloration indicates the free radical scavenging efficacy by measuring the decrease of DPPH detected at 517 nm. The radical scavenging activity was determined by comparing the absorbance with a blank containing only DPPH and solvent.

$$\%\text{inhibition} = \left(\text{OD of control} - \text{OD of test} / \text{OD control}\right) \times 100$$

### 2.6. Evaluation of antimicrobial activity by slant method

A fixed volume of 0.05 ml of the spice extract was added to a series of test tubes containing 5 ml of sterile nutrient agar media in molten stage (maintained at 45-50 °C) and the concentration of the spice varied from 1-200 mg/ml and this process was done aseptically to avoid contamination and was repeated for each of the spices. The media was then allowed to solidify in the form of a slant. Serial dilution was done and $10^{-6}$ concentration was seeded in each of the tubes. Control tubes were run simultaneously in order to check their growth inhibitory effects. The concentration of the spice at which no growth of the organism was observed was considered as the minimum inhibitory concentration (MIC) of the spice for that organism. The results were expressed in terms of growth of organisms (+) or inhibition of growth (-) after addition of the spices [24].

### 2.7. Statistical analysis

The values were expressed as mean ± SD. The antioxidant activity of nutraceuticals data was analysed by using Graph Pad Prism 5 and linearity was calculated by $r^2$ (regression coefficient) followed by % inhibition.

The percentage inhibition and the intra/inter day variation analysis was done by ANOVA followed by Bonferroni multiple correlation test.

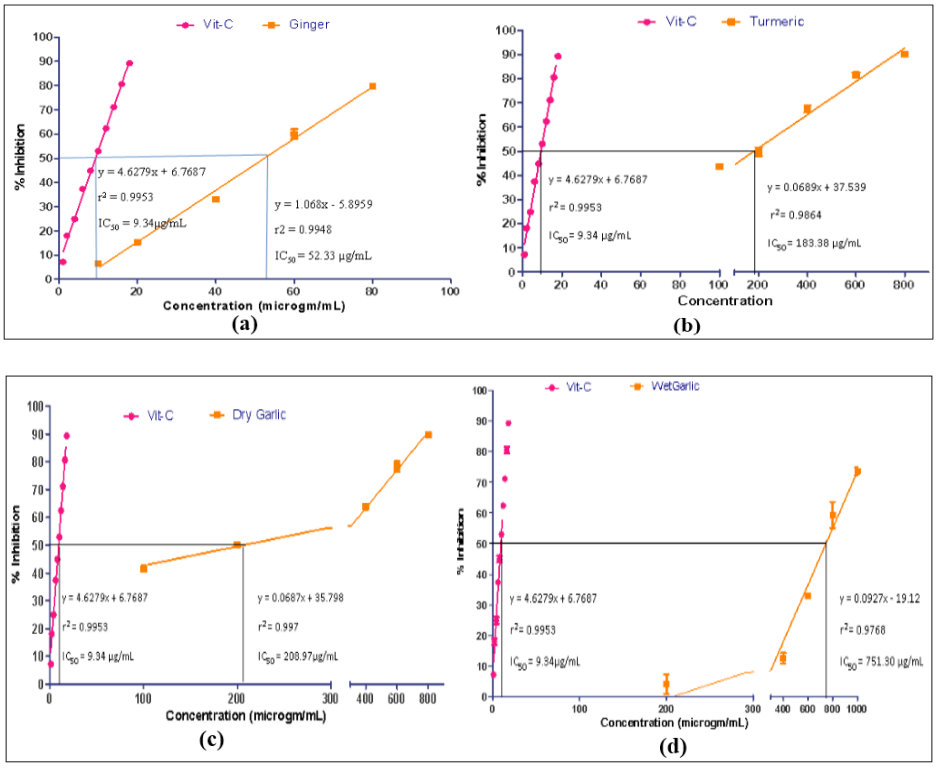
## 3. Results

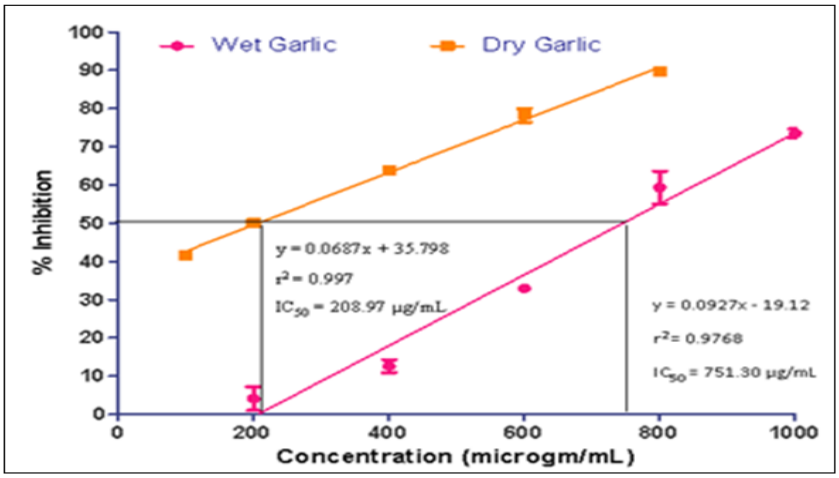
The percent yield of the spice extracts is shown in shown in Table 1.

The $IC_{50}$ on the intra/inter day was found to be ranging between 8-10 µg/ml with both concentrations of 0.4 mM and 0.6 mM. No significant difference was found in $IC_{50}$ of 0.4 mM and 0.6 mM of DPPH (Intra/inter day) (Table 2).

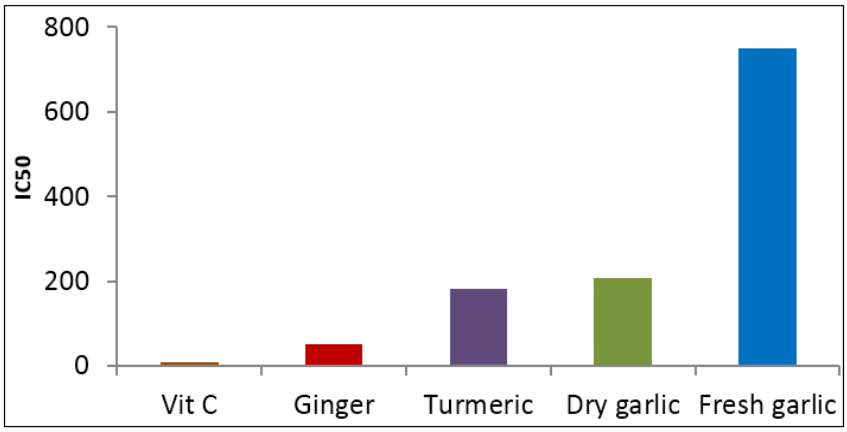
The % inhibition of Vit C was between 7 to 90% at different concentrations ranging from 1-20 µg and was linear ($r^2$= 0.9953) The $IC_{50}$ was found to be 9.34 µg/ml (Table 3). The % inhibition by ginger was between 6 to 89 % with different concentrations ranging from 10-100 µg. The response was found to be linear ($r^2$= 0.9948) and the $IC_{50}$ was 52.33 µg/ml (Fig 1a). The % inhibition by turmeric was 43% to 91% with different concentrations ranging from 100-1000 µg and it was found to be linear ($r^2$= 0.9864) and the $IC_{50}$ was 183.38 µg/ml (Fig 1b). The % inhibition by dry garlic was 41% to 91% with different concentrations ranging from 100-1000 µg. It was found to be linear ($r^2$= 0.9997) and the $IC_{50}$ was 208.97 µg/ml. The potency of dry garlic and turmeric was almost equal (Fig.1c).The % inhibition by fresh garlic was between -2% to 73% of concentrations ranging from 100-1000 µg and was found to be linear ($r^2$= 0.9768) the $IC_{50}$ was 751.30 µg/ml (Fig 1d). Fresh garlic was less potent than dry garlic by nearly 50% (Fig.1e).The antioxidant activities when compared among ginger, turmeric and garlic. The potency of these spices was found to be in the order of Vit C > Ginger > Turmeric ≥ Dry garlic > Fresh garlic (Fig 1f).

The results indicated that ginger, turmeric and garlic were found to be antimicrobial. MIC's of ginger for *E. coli* (175 mg/ml), *Staphylococcus aureus* (125 mg/ml) and *Salmonella* (150 mg/ml) was observed. MIC's of turmeric for *E. coli* (125 mg/ml), *Staphylococcus aureus* (100 mg/ml) and *Salmonella* (150 mg/ml) was observed. MIC's of garlic for *E. coli* (175 mg/ml), *Staphylococcus aureus* (150 mg/ml) and *Salmonella bacteria* (200 mg/ml) were noted (Table 4). The antimicrobial activity of these spices was found to be in the order of Turmeric > Ginger > Garlic**.**






**(e)**




**(f)**

**Fig 1:** % inhibition of DPPH (0.6 mM) free radicals by a) ginger b) turmeric c) dry garlic d) fresh (Wet) garlic e) Comparison of dry garlic v/s fresh (wet) garlic f) $IC_{50}$ of Vit C and spices.

**Table 1:** Spices yield after extraction

| **Test material** | solvent (ratio) | % yield |
|---|---|---|
| **Ginger** | Ethanol: water (1:1) | 4% |
| **Turmeric** | Ethanol: water (1:1) | 8% |
| **Dry garlic** | Ethanol: water (1:1) | 74% |
| **Fresh garlic** | Ethanol | 17.2% |

**Table 2:** Intra/Inter day variation of Vit C

| **$IC_{50}$ (µg/ml) of Vit C (DPPH (0.4 mM))** | |
|---|---|
| **Intraday** | |
| **Morning** | 9.19±0.141 |
| **Afternoon** | 8.32±0.303 |
| **Interday** | |
| **Morning** | 9.03±0.1 |
| **Afternoon** | 9.42±0.21 |
| **$IC_{50}$ (µg/ml) of Vit C (DPPH (0.6 mM)** | |
| **Intraday** | |
| **Morning** | 9.58±0.114 |
| **Afternoon** | 10.80± 0.313 |
| **Interday** | |
| **Morning** | 9.91±0.07 |
| **Afternoon** | 10.36±0.134 |

Values are mean±SD

**Table 3:** Percentage inhibition of DPPH (0.6mM) by Vit C

| Vit C (µg) | % inhibition | |
|---|---|---|
| 1 | 7.223±0.159 | |
| 2 | 18.11±1.516 | |
| 4 | 24.94±1.596 | |
| 6 | 37.35±0.798 | y = 4.6279x + 6.7687 |
| 8 | 44.86±1.516 | |
| 10 | 52.99±1.197 | $r^2 = 0.9953$ |
| 12 | 62.35±0.718 | |
| 14 | 71.10±0.0 | $IC_{50} = 9.34$ µg/mL |
| 16 | 80.58±1.596 | |
| 18 | 89.27±0.478 | |
| 20 | 90.12±0.239 | |

Values are mean±SD

**Table 4.** Effect of ginger, turmeric and garlic on growth of test organisms

| Spice | Dose (mg/ml) | Test organism | | | Spice | Dose (mg/ml) | Test organism | | | Spice | Dose (mg/ml) | Test organism | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *E. coli* | *S. aureus* | *S. typhi* | | | *E. coli* | *S. aureus* | *S. typhi* | | | *E. coli* | *S. aureus* | *S. typhi* |
| Ginger | 1 | + | + | + | Turmeric | 1 | + | + | + | Garlic | 1 | + | + | + |
| | 15 | + | + | + | | 15 | + | + | + | | 15 | + | + | + |
| | 25 | + | + | + | | 25 | + | + | + | | 25 | + | + | + |
| | 50 | + | + | + | | 50 | + | + | + | | 50 | + | + | + |
| | 75 | + | + | + | | 75 | + | + | + | | 75 | + | + | + |
| | 100 | + | + | + | | 100 | + | - | + | | 100 | + | + | + |
| | 125 | + | - | + | | 125 | - | - | + | | 125 | + | + | + |
| | 150 | + | - | - | | 150 | - | - | - | | 150 | + | - | + |
| | 175 | - | - | - | | 175 | - | - | - | | 175 | - | - | + |
| | 200 | - | - | - | | 200 | - | - | - | | 200 | - | - | - |

+ indicates growth - indicates no growth

## 4. Discussion

The antioxidant activity was exhibited by ginger, turmeric and garlic and it was comparable to a well-known antioxidant vitamin C. Ginger was found to have the highest anti-oxidant potency followed by turmeric, dry garlic and the fresh garlic. Owing to its good antioxidant potency ginger is also known to be a good chemoprevention [25].

In last few decades research on spices has been directed to understand their medicinal, antioxidant, antimutagenic and anticarcinogenic properties. Thus spices like ginger, turmeric and garlic can protect the human body against cellular oxidation reactions, bacterial infections and other metabolism related disorders. The extracts of many spices and herbs have become popular in recent years for their antimicrobial and antioxidant properties and attempt to characterize their bioactive principles have gained momentum for varied pharmaceutical and food processing applications [26].

In this study the antioxidant activity of these selected spices is indicated by a higher antioxidant index as determined by the DPPH method. This assay has the ability to measure the extracts by donating hydrogen to the DPPH radical, resulting in bleaching of the DPPH solution.

Spices are rich sources of polyphenolic compounds having strong antioxidant capacities and could potentially replace the synthetic antioxidants in food systems and offer additional health benefits. Consumption of spices has been implicated in the prevention of many chronic diseases such as cardiovascular diseases, cancer and inflammation etc. [27]. In our present study different antioxidant capacities was observed related to different extracts of the spices and their activities were comparable to the well-known antioxidant Vit C. Ginger was found to have the highest antioxidant activity compared to turmeric and garlic.

In this study, the antimicrobial activity was found to be highest in turmeric, followed by ginger and garlic against *Escherichia coli, Salmonella typhi* and *Staphylococcus aureus*. Extensive research has proved that antimicrobial activity of spices can prevent growth of pathogens in foods. Many studies reported the inhibitory activities of spices and herbs against food – borne pathogens. It was shown that gram negative bacteria are more resistant than gram positive bacteria to the essential oils present in spices, which can act as antimicrobial agents [28].

The antimicrobial activities of commonly used herbal spices form the basis for many applications including raw and processed food preservation, pharmaceuticals, alternative medicines and natural therapies [29]. The organo sulphur compounds present in garlic is thought to scavenge free radicals and also inhibit bacterial growth via interaction with sulphur containing enzymes. Inhibitory effects of garlic against other bacterial strains of *Salmonella typhi, Escherichia coli, and Staphylococcus aureus* have been studied and reported [30]. The results demonstrated in this study is in agreement with the findings of previous workers [31]. By and large, all tested extracts exhibited antimicrobial activity against these bacteria

Based on our observations on antimicrobial activities of spices it can be mentioned that spices can prevent spoilage of foods to certain extent due to their antimicrobial property. Generally the amounts of spices and herbs added to foods are generally too low to prevent spoilage by microorganisms. Even though traditionally, spices are used as food preservatives and antiseptics, it is necessary to establish their antimicrobial properties. The differences in the antimicrobial activity of the extracts that have been reported by various investigators might be due to the microorganisms used and method of extractions [32] reported that out of 46 spices and herbs

extracts, 12 spices exhibited high antibacterial activities against the five food borne bacteria (*E. coli, B. cereus, L. monocytogenes, S. aureus, S. anatum*).

## 5. Conclusion

The study indicates that the spices like ginger, garlic and turmeric have antimicrobial and antioxidant activity. Further research may be needed to understand the in-depth mechanisms through which these effects are exerted and also study the biological effects of antioxidant-rich herbs and spices on oxidative stress related diseases.

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