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# Evaluation of antioxidant and antimicrobial activities of Garam Masala, an Indian spice blend, and its' components *In Vitro*

**Tanaya Basu[1], Sauryya Bhattacharyya[1*], Santanu Maitra[2] and Chandan Rai[2]**

*[1]Department of Food & Nutrition, Ramakrishna Vivekananda Mission Sarada Ma Girls' College, Barasat, Kolkata 700126, India*
*[2]Department of Microbiology, Ramakrishna Mission Vidyamandira, Belur, Howrah 711202, India*

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**ABSTRACT**

*The present study demarcates antioxidant and antimicrobial activities of aqueous extracts of garam masala, an Indian spice blend, and its' components viz. clove (Syzygium aromaticum), cinnamon (Cinnamomum verum) and cardamom (Elettaria cardamomum), for efficient combat against detrimental principles like free radicals and microbes. The antioxidant assays performed were DPPH radical decolorization assay, ferric reducing antioxidant potential assay, hydroxyl radical scavenging assay and determination of total polyphenolic contents. Antimicrobial activities were done against common food borne pathogens like Bacillus cereus, Escherichia coli, Staphylococcus aureus and Klebsiella pneumoniae. The major conclusions arising out from the study was that the spice blend showed promising activities in the experimental parameters. It could be postulated that electron transfer mechanism was predominant for the spice blend as was evident from FRAP assay. This might be due to the contribution of the polar polyphenolics in the garam masala as was evident from their higher total phenolics contents. The spice blend also showed promising activity against Staphylococcus aureus and Bacillus cereus. The major contributing factor for its' bacteriostatic activity was mainly due to the presence of cinnamon, as the individual component showed the best antibacterial activity amongst the three.*

**Keywords:** Spice, garam masala, clove, cinnamon, cardamom, antioxidant.

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## INTRODUCTION

Dietary spices are proved to be beneficial to human health for their antioxidative, chemopreventive, antimutagenic, anti-inflammatory and immune modulatory effects on cells via action on gastrointestinal, cardiovascular, respiratory, metabolic, reproductive, neural and other systems [1]. Herbs and spices have long been used by mankind as food additives [2]. Spices are imperative both as functional food ingredients and nutritional supplements as they not only enhance the taste and flavour of foods, but also play a crucial role as supplementary, complementary and synergistic components [3]. In addition, spices like peppers and chilies have also been used for treating several disorders by virtue of their potent medicinal properties [4]. History of Indian spices dates back to 5000 years as *Ayurveda*, the ancient Indian system of medicine used large number of spices, alone or in combination, for preventive as well as curative purposes. Remedial properties of dietary spices described in the *Rigveda* and *Atharvaveda* possibly seem to be the earliest account of the use of herbs in medicine [5]. The heterogenous collection of volatile and non-volatile compounds present in spices, condiments and herbs inarguably contribute significant variety and complexity to the human diet to ensure their therapeutic usage till date.

Garam Masala is a combination of some common ground spices used widely in Indian cuisine as an antioxidant [6]. The spices for garam masala are usually toasted at low temperature to bring out more flavor before the blending. It is usually added to a cuisine near the end of cooking. There's no single garam masala recipe found as the ingredients differ according to region as well as cultural preferences. But for the most, garam masala includes coriander, cumin, cardamom, cloves, black pepper, cinnamon and nutmeg. In the region of the Gangetic plains of West Bengal, garam masala is used to be the combination of clove (*Syzygium aromaticum*), cinnamon (*Cinnamomum verum*) and cardamom (*Elettaria cardamomum*), each of which contains remarkable medicinal properties. Clove, by virtue of its' principal ingredient eugenol and a few flavonoids, shows anti-inflammatory, immunomodulatory, antimicrobial, carminative, anti-diabetic and aphrodisiac properties [7]. Cinnamon principally contains natural oils and other derivatives, such as cinnamaldehyde, cinnamic acid, and cinnamate. In addition to being an good antioxidant, anti-inflammatory, antidiabetic, antimicrobial, anticancer, lipid-lowering, and cardiovascular-disease-protecting substance, cinnamon has also been reported to have activities against neurological disorders, such as Parkinson's and Alzheimer's diseases [8]. Cardamom is a commonly used spice that is popularly known as 'Elaichi' in the Indian households. It is also known as 'Queen of Spices'. The dried cardamom fruits can act as a relief for gastric disorders and heartburn. It increases appetite, and is useful in nausea and vomiting. Cardamom seeds are very effective in treating many genito-urinary disorders, such as gonorrhea, nephritis and frequent micturation. Cardamom has some role as aphrodisiac, and is extremely beneficial in overcoming depression as a mood elevator [9]. Blending of these three spices that forms garam masala would therefore inarguably provide a number of health beneficiary effects due to the presence of wide varieties of bioactives having therapeutic potentials. Indeed, garam masala was found to possess beneficial effects like weight reduction, enhancement in energy metabolism and chemoprevention by modulating hepatic levels of cytochrome $b_5$ and cytochrome $p_{450}$ [5].

Critical review of the literature indicated that *in vitro* antioxidant studies with clove, cardamom and cinnamon were mainly performed with the aqueous alcoholic extracts of the substances. In view of the fact that the substances comprise garam masala, which is used in cuisines, the antioxidant profiling should be done with their aqueous extracts to decipher their actual beneficial roles in human nutrition. In the light of above discussion, the present study was designed to analyze antioxidant potential of an experimental mixture of garam masala having the ingredients in 1:1:1 weight-to-weight ratio using some common *in vitro* tests. The study also was probably the first to adjudicate the effect of aqueous extracts of garam masala and its' components against some food borne microorganisms like *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae*.

## MATERIAL AND METHODS

### Chemicals

2,2′-Diphenyl-1-picryl hydrazyl (DPPH) were obtained from Himedia, India. Analytical grade of 2-Deoxy-D-ribose was obtained from Loba Chemie; thiobarbituric acid (TBA), ascorbic acid, gallic acid, Folin-Ciocalteau's solution, sodium hydroxide and sodium carbonate were obtained from Merck, India. Muller Hinton Agar and broth were purchased from HiMedia, India. All other reagents and chemicals used were of analytical grade procured from local sources. Deionized distilled water was used in the entire study.

### Preparation of samples

The samples (clove, cinnamon, cardamom) were procured from local markets of Barasat, Kolkata. The samples were checked for dirt or any visible damages prior to the study. Such samples were discarded. 1 gms each of the samples were taken in 10 ml solvent (40% aqueous ethanol) separately, for the preparation of extract. A sample of garam masala was prepared by mixing the three spices in 1:1:1 weight-to-weight ratio and extracted in same manner. After extraction, the samples were heated for 2-3 mins and then centrifuged at 8000 rpm for 5 mins. The clear supernatants were used for *in vitro* antioxidant assays.

### DPPH radical decolorization assay

The DPPH assay was performed using a previously described procedure [10]. 1 ml DPPH solution (0.1 mM) was mixed with 0.5 ml sample solution and the decrease in absorbance of the mixture after 20 minutes of incubation in the dark was monitored at 517 nm in a Systronics spectrophotometer (model – 2202). The concentration that causes a decrease in the absorbance of initial oxidants by 50% is defined as $IC_{50}$ of the samples. Gallic acid was used as positive control and comparing with its' $IC_{50}$ and the results were expressed as Gallic acid equivalents (µM/gm sample).

**Ferric reducing antioxidant power (FRAP)**
Ferric reducing potentials of the samples were estimated with a previously established procedure with minor modifications [11]. Briefly, a maximum of 100 µl of extract solution or standard was mixed with 1.9 mL of FRAP reagent and incubated at 37$^{o}$C for 30 mins. FRAP reagent was prepared by mixing 0.1 M acetate buffer (pH 3.6), 10 mM TPTZ solution and 20 mM $FeCl_3$ solution. After the stipulated time period, absorbance was measured at 593 nm in a UV-Vis spectrophotometer (model – Systronics 2202). Gallic acid is used as standard. Results are expressed as Gallic acid equivalents (GAE).

**Estimation of total phenolics content**
Total phenolic compound contents were determined by the Folin-Ciocalteau method [12]. The samples (0.5 ml) were mixed with Folin-Ciocalteau reagent (5 ml, of 1:10 diluted sample with distilled water) for 5 min and aqueous sodium carbonate (4 ml, 1 M) was then added. The absorbance of the reaction mixture was then measured at 765 nm in a UV-Vis spectrophotometer (model – Systronics 2202). Gallic acid was used as standard. The results were expressed in terms of µg gallic acid equivalent/gm sample.

**Hydroxyl radical scavenging assay**
Hydroxyl radical scavenging potentials of the samples were estimated with a previously described procedure with minor modifications [13]. Briefly, 10 mM each of $FeSO_4.7H_2O$, EDTA, 2-deoxy-D-ribose and $H_2O_2$ solutions were prepared in water. 0.2 ml each of above four and 0.2 ml sample and/or standard solution was mixed in a test tube and incubated at 37$^{o}$C for 90 mins. $H_2O_2$ solution was added last. After the incubation, 1 ml of 2.8% (w/v) aqueous TCA solution and 1 ml of 1% (w/v) aqueous TBA solution were added to the reaction mixture and kept at boiling water bath for 20 mins. Development of the pink chromophore was measured at 532 nm in a UV-Vis spectrophotometer (model – Systronics 2202). Gallic acid is used as standard. Results were expressed as Gallic acid equivalents (GAE).

**Bacterial strains used**
The bacterial strains used in this study included *Bacillus cereus* (MTCC 1272), *Escherichia coli* (MTCC 1610), *Staphylococcus aureus* (MTCC 9542) and *Klebsiella pneumoniae* (MTCC 9544). The strains were obtained from IMTECH, Chandigarh, India and preserved at Department of Microbiology, Ramakrishna Mission Vidyamandira, Howrah.

**Antibacterial activity assay**
The antibacterial activity was measured by agar well diffusion method. Each bacterial isolates was previously grown on sterile Muller Hinton Agar (HiMedia M173) plate at 35°C for 24 hours. Single colony of each of the isolates was grown in Muller Hinton broth (HiMedia M391) for 3 hours at 35°C. After that, each of the isolates was inoculated with 100 µl of standardized inoculums of each bacterium (in triplicates) and spread with sterile cotton swabs. Wells are 6 mm sizes were made with sterile borer into agar plates containing the bacterial inoculums. Different working dilutions of extracts of clove, cardamom, cinnamon and a 1:1:1 weight-to-weight mixture of the above three were prepared in sterile water in concentrations of 25 mg/ml, 50 mg/ml, 100 mg/ml and 200 mg/ml. From these different dilutions, 50 µl solution was poured into the wells of the respective culture plates. The plates thus prepared were left at room temperature for ten minutes allowing the diffusion of the extract into the agar. After incubation for 24 hours at 35$^{o}$C, the plates were observed. If antibacterial activity was present on the plates, it was indicated by an inhibition zone surrounding the well containing the different dilutions of extract and nanoparticles. The zone of inhibition was measured and expressed in millimetres. Antibacterial activity was recorded if the zone of inhibition was greater than 6 mm. The antibacterial activity results were expressed in term of the diameter of zone of inhibition and <9mm zone was considered as inactive; 9-12mm as partially active; while13-18mm as active and >18mm as very active [14].

## RESULTS AND DISCUSSION

**DPPH radical decolorization assay**
DPPH assay was used to determine the scavenging potential of antioxidant extracts based on their capabilities as hydrogen donor in tandem with electron transfer. The results of DPPH radical scavenging assay of garam masala (GM) and was almost equivalent to its' components except for cardamom (Fig. 1). A very low value of cardamom might be due to its' lesser extraction of non-polar bioactives, which play crucial role in this assay. It was already established that DPPH radical scavenging was mostly done by the non-polar bioactives of the extracts [10]. Presence

of cardamom thus provided lesser amounts of non-polar bioactives in GM, which could have some pharmacological implications systemically.

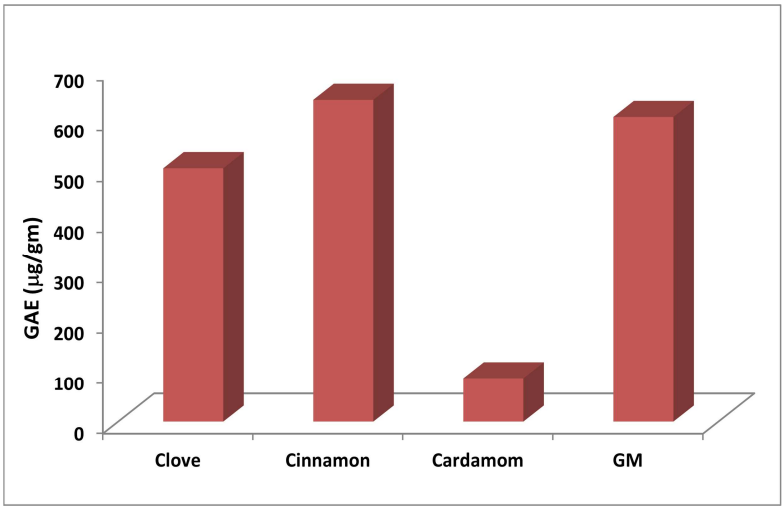


**Fig. 1: DPPH radical scavenging activities of GM and its' components. Results are expressed as gallic acid equivalent (GAE, mean of *n*=3).**

**Ferric reducing antioxidant power (FRAP)**

In this assay, the reduction power of the sample extracts were indicated by their abilities to transfer electrons towards the FRAP reagent. The result showed that GM scored well over its' ingredients regarding their reducing abilities (Fig. 2). It was also observed that the GAE value of GM was more than its' DPPH scavenging values, indicating that electron transfer to be the foremost phenomena for their antioxidant potentials.

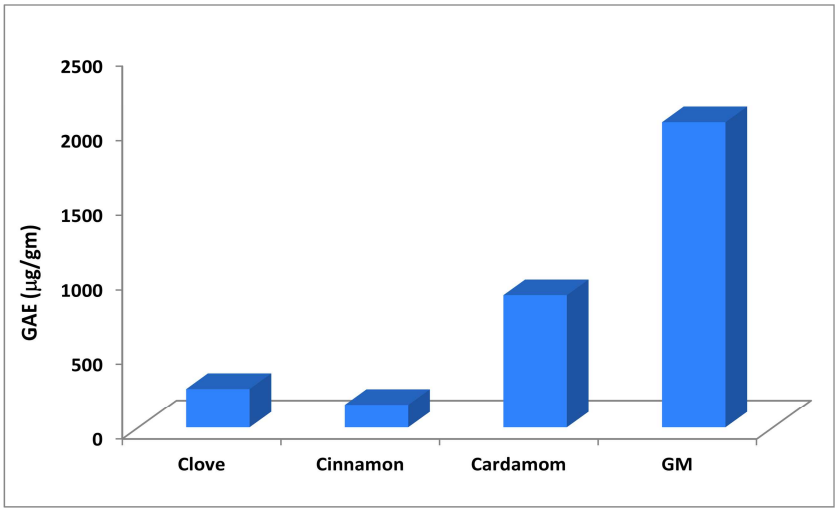


**Fig. 2: Ferric reducing potential (FRAP) of GM and its' components. Results are expressed as gallic acid equivalent (GAE, mean of *n*=3).**

**Total phenolics content**

Total phenolic contents of GM and its' three components were very close as depicted in Fig. 3 with a marginal improvement in the GM. This assay is performed in aqueous medium and in alkaline conditions, which indicates contribution of the polar polyphenolics in their anionic states towards antioxidant capacities of the extracts. The

results obtained from the present assay thus indicated that polar polyphenolics were the predominant antioxidants in GM when it is extracted with water, as in cooking.

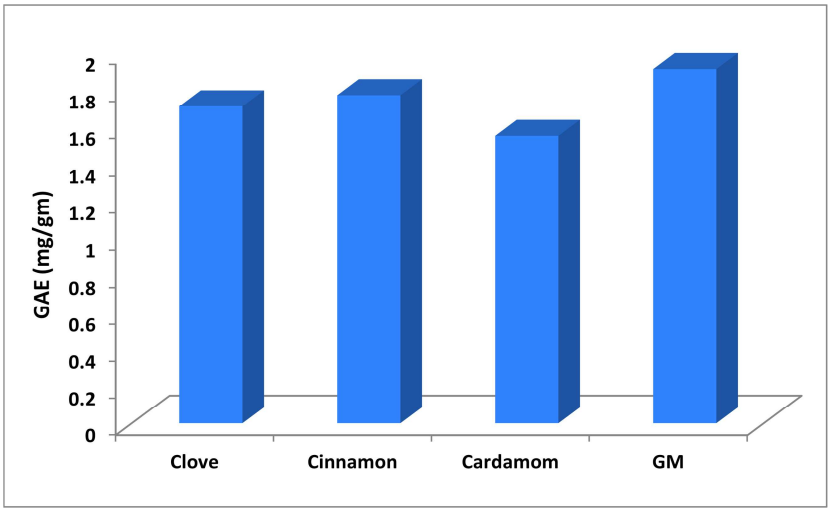


**Fig. 3: Total phenolic contents of GM and its' components. Results are expressed as gallic acid equivalent (GAE, mean of *n*=3).**

### Hydroxyl Radical Scavenging assay

The scavenging activity for the most deleterious radical was highest for cinnamon (Fig. 4). The activity was reduced in GM. Even cardamom showed better hydroxyl radical scavenging than GM. All these observations indicated that hydroxyl radical scavenging might be accomplished by the non-polar bioactives of the extracts, as the results largely resembled to that of the DPPH radical scavenging potentials of the extracts.

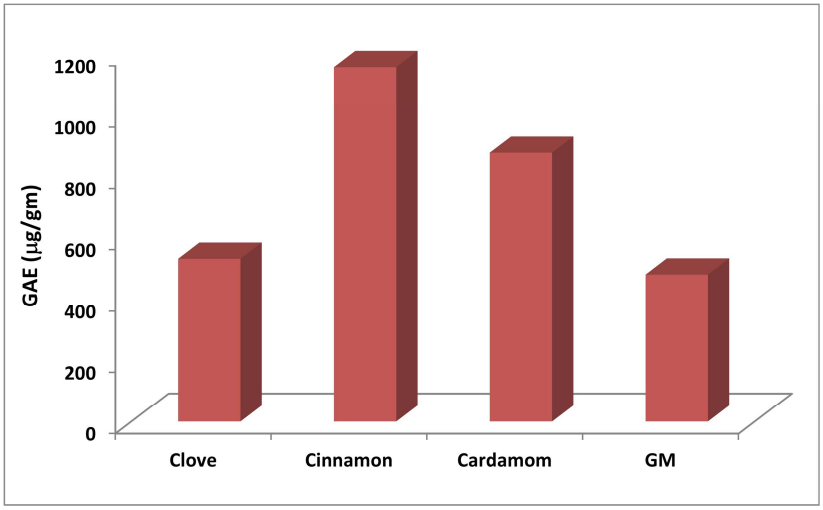


**Fig. 4: Hydroxyl radical scavenging activities of GM and its' components. Results are expressed as gallic acid equivalent (GAE, mean of *n*=3).**

### Antibacterial Activity Assay

The antibacterial properties of aqueous extracts of GM and its' components against four common food borne bacteria were assessed quantitatively by determining the diameter of inhibition zones as shown in Table 1. All individual peels extracts and their mixtures in concentrations of 200 µg/ml showed significant inhibition towards all the selected bacteria (both gram-positive and gram-negative) except in case of cardamom against *E. coli*. Among the individual components, clove showed maximum effectiveness against all selected bacteria.

**Table 1: Bacteriostatic activities of GM and its' components**

| Sample | Concentration of the extracts (μg/ml) | Diameter of zone of inhibition (mm) | | | |
|---|---|---|---|---|---|
| | | *S. aureus* | *E. coli* | *B. cereus* | *K. pneumoniae* |
| Clove | 25 | 14 | - | 11 | 10 |
| | 50 | 17 | 9 | 15 | 16 |
| | 100 | 19 | 12 | 18 | 18 |
| | 200 | 23 | 14 | 22 | 21 |
| Cinnamon | 25 | - | - | 13 | 9 |
| | 50 | 11 | 9 | 19 | 16 |
| | 100 | 16 | 10 | 26 | 18 |
| | 200 | 17 | 13 | 30 | 21 |
| Cardamom | 25 | - | - | - | - |
| | 50 | - | 11 | - | 8 |
| | 100 | 9 | 14 | - | 12 |
| | 200 | 12 | 21 | - | 13 |
| GM | 25 | 10 | - | 8 | - |
| | 50 | 15 | 10 | 14 | - |
| | 100 | 16 | 14 | 16 | 12 |
| | 200 | 18 | 16 | 20 | 14 |

## CONCLUSION

The present study elaborated comparison of the antioxidant and antimicrobial activities of a common Indian spice blend – garam masala, and it's three components, viz. clove, cinnamon and cardamom. The study delved into the beneficial activities of the spice and the individual components to get an understanding about their efficient combat against detrimental principles like free radicals and microbes. The major conclusions arising out from the study was that the spice blend showed promising activities in the experimental parameters. It could be postulated that electron transfer mechanism was predominant for the spice blend as was evident from FRAP assay. This might be due to the contribution of the polar polyphenolics in the garam masala as was evident from their higher total phenolics contents. The spice blend also showed promising activity against *Staphylococcus aureus* and *Bacillus cereus*. The major contributing factor for its' bacteriostatic activity was mainly due to the presence of cinnamon, as the individual component showed the best antibacterial activity amongst the three.

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