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EVALUATION OF ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES PRESENT IN COMMON INDIAN SPICES

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ABSTRACT

Extracts of three different spices viz., *clove, big cardamom, star anise* which are widely utilized in Indian kitchen were screened for phytochemicals. They were analyzed for their antioxidant activity (using ***DPPH assay***), total antioxidant capacity (using ***Phosphomolybdate assay***). The total phenolic and total flavonoid content of the spices were determined. They were also examined for antimicrobial property (using ***well diffusion method*** by Kilby Bauer). The spices which were used are **clove** (*Syzygium aromaticum*), **star anise** (*Illicium verum*) and **big cardamom** (*Amomum subulatum*), these spices are indeed very common in Indian food since ancient times so it becomes essential for us to know about its antioxidant & antimicrobial properties. The results indicated that extracts of these spices posses considerable antioxidant activity and antimicrobial activity.

Keywords: *Spices, Antioxidant capacity, DPPH assay, Total Phenolic Content, Antimicrobial properties, well diffusion method.*

I. INTRODUCTION

Indian cuisine is popular for the extensive use of numerous spices. Spices or Masala as it is called in Hindi, may be called the "heartbeat" of an Indian kitchen. The spices are used to flavor the food, making each dish distinct and wonderfully aromatic. Even though spices are also used for many health benefits and medicinal purposes, to prevent diseases and also to preserve food. Spices are defined as "a strongly flavored or aromatic substance of vegetable origin, obtained from tropical plants, commonly used as a condiment. All spices are mostly originated from plants, flowers, fruits, seeds, barks, leaves and roots. Herbs and spices are proved to be salutary to human health for their various attributes such as antibacterial properties, antimutagenic, chemopreventive, antioxidative, anti-inflammatory and immune modulatory effects on cells via action on metabolic, reproductive, neural, gastrointestinal, cardiovascular, respiratory and other systems. These have been used by mankind as food additives.

Numerous studies have been published on the antioxidant capacity and the phenolic constituents of spices (Khatun et al.,2006). Spice extracts contain natural antioxidants that reduce the final microbial load and retard lipid oxidation during storage and contain a varied amount of essential oil compounds, as well as phenolic acids, which can help to inhibit lipid oxidation and control food borne pathogens (Krishnan et al.,2014). The various subcategories of secondary metabolites compounds include phenols, phenolic acids, flavones, flavonoids, flavonols and tannins. These groups usually show antimicrobial effects and serve as plant defense mechanisms against pathogenic microorganisms. They do various functions in plants such as sensorial properties (color, aroma, taste, and astringency), structure, pollination, resistance to pests and predators, germinative processes of seed after harvesting and growth as well as development and reproduction, among others. Antioxidants are the compounds which inhibit the oxidation reactions caused by free radicals. Free radicals which are also called as Reactive Oxygen Species (ROS) or Active Oxygen Species (AOS), are produced during various metabolic cellular processes. ROS includes free radicals such as hydroxyl radicals (–OH) , superoxide ions (O2-), nitric oxide (NO-), alkyl oxide (RO-), alkyl carboxylic acid (ROO-) and non-free radicals such as hydrogen peroxide (H2O2), singlet oxygen (O-), hypo chloride radicals (HClO-) (Halliwell,1995 and Odukoya et al.,2005). I. verum commonly known as Star anise is native to southwest China and Vietnam and is mainly distributed in the tropical and subtropical areas of Asia. Clove or Syzygium aromaticum (Synonym; Eugenia aromatica Kuntz.) belongs to the family of Myrtaceae. The brown, dried, unopened flower buds called cloves, a name coming from French word "clou" meaning nail. Big cardamom

(Amomum subulatum Roxb. Zingiberaceae) commonly known as "***Bari ilaichi***" is a well known plant used in Ayurvedic and Unani medicine. It has been used for the treatment of various diseases and disorders like gastric ulcer.

II. MATERIALS AND METHODS

Chemicals and Reagents:
2,2-Diphenyl-1-picryl hydrazyl (DPPH), methanol, acetone, distilled water, ethanol, sulphuric acid, Sodium phosphate, Ammonium molybdate, Folin Ciocalteu reagent, Sodium carbonate, Ascorbic acid, Aluminium chloride, potassium acetate, Quercetin, Nutrient broth, LB broth, Ampicilin for antimicrobial screening.

Instrumentation:
Glasswares (conical flasks, beakers, test-tubes, petri plates), grinder, micropipettes, digital balance, spectrophotometer, refrigerator, autoclave, incubator, laminar air flow hood, filter papers, bunsen burner, tripod stand, wire gauge, glass pipettes, cuvette, water bath.

Preparation of sample:
The samples (clove, big cardamom, star anise) were collected from local markets. The samples were checked for dirt or any visible damages prior to the study. The samples were mildly heated for 5-10 mins and were grinded into fine powder. Solvent extracts of these spices were prepared separately.

Microorganisms used:
Bacterial strain that was used was *E.coli*

Extraction of plant materials:
The extraction of spices was carried out according to the method suggested by Brand-Williams, Cuvelie & Berset (1995)

Phytochemical screening:
Phytochemical screening of spices; ***Syzygium aromaticum, Amomum subulatum, Illicium verum*** was carried out according to the methods of Ayoola *et al.* 2008.

Determination of total flavonoid content:
The flavonoids content of different spice extracts of clove, big cardamom, star anise, was measured based on methods described by Ebrahimzadeh *et al.* 2008 with some modifications.

Determination of total phenolic content:
The total phenolic content of different spice extracts of clove, big cardamom, star anise was evaluated using a method described by Kim *et al.* 2003.

Determination of DPPH radical scavenging activity:
The DPPH radical scavenging activity of all the spice extracts was determined using the method proposed by [Brand-Williams, Cuvelie & Berset (1995)] with some modifications.

Total Antioxidant Capacity:Phosphomolybdenum Assay (PM)
Total antioxidant activity was estimated by phosphomolybdenum assay (Aliyu et al, 2013). Ascorbic acid was used as positive reference standard.

ANTIMICROBIAL SCREENING: *Well diffusion method*
The antimicrobial activity of the extracts was determined by ***Kirby–Bauer method*** in agar well diffusion

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III. RESULT AND DISSCUSION

Out of the three spices ***clove*** was found to contain highest amount of phytochemicals than big cardamom and star anise.

Table 1

PHYTOCHEMICAL SCREENING

SPICES	*TERPENOIDS*	*FLAVONOIDS*	*SAPONINS*	*TANNINS*	*PHENOLS*
CLOVE	+	+	+	+	+
BIG CARDAMOM	-	+	-	+	+
STAR ANISE	+	+	-	-	-

The aqueous extracts of clove and big cardamom have shown the higher ***flavonoid content*** whereas methanolic extract of star anise has shown highest total flavonoid content at higher concentrations.

Table 2

TOTAL FLAVONOID CONTENT

SPICES	**EXTRACTS (Conc. µl)**					
	METHANOL			**D/W**		
	25	***50***	***100***	***25***	***50***	***100***
Clove	*2.19±0.02*	*2.54±0.01*	*3.92±0.01*	*2.12±0.04*	*2.54±0.01*	*4.02±0.01*
Big cardamom	*2.08±0.01*	*2.62±0.01*	*3.95±0.01*	*2.08±0.02*	*2.51±0.005*	*3.99±0.01*
Star anise	*1.97±0.01*	*2.59±0.01*	*4.03±0.01*	*2.04±0.005*	*2.54±0.01*	*3.81±0.01*
values represent mean±SD of triplicate; SD= Standard Deviation						

The aqueous extract of clove has higher ***total phenolic content*** followed by big cardamom and star anise than methanolic extracts.

Table 3

TOTAL PHENOLIC CONTENT

SPICES	***ETHANOL EXTRACT***	***HOT AQUEOUS EXTRACT***
CLOVE	0.09±0.02	3.11±0.02
BIG CARDAMOM	1.6±0.173	3.02±0.011
STAR ANISE	1.36±0.03	2.41±0.02
values represent mean±SD of triplicate; SD= Standard Deviation		

The total phenolic content was expressed in mg/g of Gallic acid equivalent, as standard.

We can illustrate that aqueous and acetone extracts of three spices viz., clove, big cardamom, and star anise have greatest scavenging activities on DPPH radicals (Free radical-scavenging activity 91.1%, 92.5% and 90.23% respectively) but scavenging activity of aqueous extract big cardamom was significantly higher than that of a clove

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and star anise. Methanol extracts of big cardamom and star anise shown significantly higher radical-scavenging activity(Free radical-scavenging activity 87.8% and 86.1% respectively) than the methanolic extract of clove.

Table 4

DPPH scavenging activity of spices

Spice extracts	*conc. in µl*				
Clove	**5**	**10**	**15**	**20**	**25**
Methanol	65.73±1.35	67.4±2.56	73.63±3.38	81.4±4.72	80.9±2.76
D/W	85.03±0.50	87.57±0.58	88.61±0.42	90.3±0.68	91.1±0.41
Acetone	80.3±0.781	84.3±0.75	88.01±0.6	87.5±1.50	89.3±0.50
Big cardamom					
Methanol	76.7±3.20	80.1±2.05	82.9±1.21	87.4±3.75	87.8±1.57
D/W	85.9±1.24	88.9±1.24	91.3±0.81	91.3±0.50	92.5±0.70
Acetone	77.5±0.97	80.1±1.76	81.2±1.91	84.7±1.27	85.4±1.51
Star anise					
Methanol	70.5±1.62	73±4.01	77.2±4.30	88.6±2.23	86.1±2.81
D/W	83.9±1.15	86.6±0.83	89.1±0.41	91.9±0.41	90.2±0.65
Acetone	73.1±1.75	76.8±3.01	80.8±2.93	83.9±0.94	85.1±0.57

values represent mean±SD of triplicate; SD= Standard Deviation

The results are shown in table 5 and are compared with standard graph plot of Ascorbic acid µg/ml .

Table 5

TOTAL ANTIOXIDANT CAPACITY

SPICES	EXTRACTS	*Concentration in µl*		
		S1	**S2**	**S3**
CLOVE	*METHANOL*	12.26±0.66	21.53±0.05	24.2±0.65
	D/W	12.53±0.11	20.9±0.08	23.3±0.1
	ACETONE	10.63±0.05	22.7±1.01	24.3±0.1
BIG CARDAMOM	*METHANOL*	11.9±0.08	20.4±1.02	24.66±1.36
	D/W	15.2±2.17	17.3±0.15	23.7±0.1
	ACETONE	15.4±0.1	19.2±0.2	24.2±0.1
STAR ANISE	*METHANOL*	10.9±1.12	19.4±0.15	23.73±0.15
	D/W	12.46±0.11	21.4±0.2	24.2±0.11
	ACETONE	16.77±3.96	21.58±0.07	23.8±0.20

values represent mean±SD of triplicate; SD= Standard Deviation; s=conc.

The aqueous extracts of clove and star anise shown the broadest antibacterial activity by inhibiting growth of all bacterial strain tested (the diameter of inhibition zone, 14.3-23.3 mm). However the methanolic extract of big cardamom performed high antibacterial activity than other methanolic extracts of clove and star anise against the tested bacteria. In general, the inhibitory activity of water extracts of both clove and star anise was greater than that of its methanol and acetone extracts.

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Table 6

ANTIMICROBIAL SCREENING OF SPICES			
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SPICES	*INHIBITION ZONES(mm/50µl)*		
	EXTRACTS		
	METHANOL	***D/W***	***ACETONE***
CLOVE	14	14.3	9
BIG CARDAMOM	15.4	12	14.3
STAR ANISE	8	23.3	7

Statistical analysis
The results are represented as mean of three replicates followed by the standard deviation, that is, mean ± standard deviation.

IV. CONCLUSION

Antioxidant activity assessment may require a combination of different methods, and the results in this study confirm the antioxidant activity and antimicrobial property of spice extracts. The evaluation for antioxidant activity using different methods shown that the spice extracts used in the present study can be considered good sources of natural compounds with significant antioxidant activity. The preliminary results obtained from this study seem to justify the use of *spices* in dealing with different health disorders in terms of cost-effectiveness and easy of availability, especially for the people in remote area. The present study may provide a scientific support for the traditional use of these spices and may endow with the valuable idea for a complete study on the bioactive compound that contributes to these biological properties and also their possible mechanism of action are suggested. The spices have been screened for phytochemical constituents seemed to have the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health

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