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Screening of Secondary Metabolites and Antibacterial Activity of Some Indian Spices

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ABSTRACT

Current research work deals with screening of secondary metabolites and antibacterial potential of *Rhizoma zingiberis, Cuminum cyminum* L., *Curcuma longa* L., *Piper nigrum* L. and *Cinnamomum tamala* the Indian spices. Ethanol and water were used for preparation of test extracts. The present Indian spices found rich in secondary metabolites and shown presence of significant antimicrobial properties. The ethanolic extract of *Curcuma longa,* aqueous extract of *Cuminum cyminum, Cinnamomum tamala* and *Piper nigrum* yielded more phytochemicals while for *Rhizoma zingiberis* both the solvents found effective. Ethanolic extract of *Piper nigrum* and *Rhizoma zingiberis* found more effective antibacterial among all the spices. The results obtained may support the use of the spices in traditional medicine for the treatment of various diseases and in drug developments.

Keywords: **Indian spices, Secondary metabolites, Antibacterial.**

INTRODUCTION

Plants are the effective source of secondary metabolites used in traditional as well as modern medicines and most of the organisms depend on plants for their existence (Patil *et al,* 2014).

Indian spices include a variety of spices grown across the country and these are used to enhance flavor and aroma of the foods and it also provide antimicrobial properties (Nanasombat *et al.,* 2002). It may also contribute in piquancy of foods and beverages (Praveen and Nazia, 2006). Spices are the most commonly used natural antimicrobial agents in foods. Some of the natural compounds found in various spices possess antimicrobial activity (Indu *et al.,* 2006) and as the evaluation of any drug is based on phytochemical and pharmacological approaches the present research work was undertaken to screen out the qualitative phytochemical content of the Indian spices like *Rhizoma zingiberis, Cuminum cyminum* L., *Curcuma longa* L., *Piper nigrum* L. and *Cinnamomum tamala* using different solvents like water and ethanol along with its antibacterial activity.

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MATERIALS AND METHODS

Collection and Authentication:

Fresh and healthy Indian spices like *Rhizoma zingiberis, Cuminum cyminum* L., *Curcuma longa* L., *Piper nigrum* L. and *Cinnamomum tamala* were brought from the Local market of Gadhinglaj City, Maharashtra, India during April 2015. All spices were authenticated by Prof. R. S. Sawant, Department of Botany, Dr. Ghali College, Gadhinglaj, Kolhapur district, Maharashtra, India.

Preparation of extracts for Phyto-chemical analysis:

150 gm of dried powder of spices was mixed with 500 ml of ethanol for each. After filtration, the filtrate

was dried and used for phytochemical test. For water extract 150 gm of powder were mixed with 1500 ml of distilled water and heat on water bath for 1/3 rd of original concentration and used for further analysis (Godghate and Sawant, 2014).

Identification tests for Phyto-chemical analysis:

The aqueous and ethanolic extracts of the spices were analyzed for the qualitative phytochemicals analysis as shown in table 1 and 2 using standard methods (Damodaran and Manohar, 2012; Patil and Bhise, 2015; Harborne, 1973; Sawant and Godghate, 2013; [a]Patil *et al*, 2015; Sofowora, 1993).

Preparation of test extracts for antibacterial activity:

The aqueous and ethanolic extracts of spices were prepared by addition of 1 gm of respective powder into 10 ml of respective solvents and kept at room temperature for overnight. Sample further used after its centrifugation.

Test organisms:

The standard test microorganisms used in current study were obtained from National Collection of Industrial Microorganisms, Pune, (M.S.) India are as following: *Bacillus subtilis* NCIM 2635, *Proteus vulgaris* NCIM 2813, *Salmonella typhimurium* NCIM 2501 and *Staphylococcus aureus* NCIM 2654.

Preparation of bacterial suspension:

A loop full suspension of the test organisms were aseptically streaked onto nutrient agar slants and incubated (at 37°C for 24 hours). On next day bacterial growth was harvested from the respective slant and suspension was prepared using sterile 1 ml normal saline. The all suspensions stored in the refrigerator at 4°C until used ([b]Patil *et al,* 2015).

Antibacterial activity:

Antibacterial activity of the various spices in aqueous and ethanolic extracts were studied against the various bacteria (Table 3) was determined by using agar well diffusion method on Nutrient agar medium.

Table 1: Phytochemicals of Aqueous (A) and Ethanolic (E) extract of *Rhizoma zingiberis, Cuminum cyminum*, and *Curcuma longa*.

Sr. No.	Test		Results					
			Rhizoma zingiberis		*Cuminum cyminum*		*Curcuma longa*	
			A	E	A	E	A	E
1	Alkaloids:	Wagner's reagent	+	+	+	-	-	+
		Hager's reagent	+	+	+	+	+	-
2	Saponin: Foam test		+	+	+	+	+	+
3	Tannin: $FeCl_3$		-	-	+	-	-	-
4	Steroids		-	-	-	-	+	-
5	Anthocyanin		-	-	-	-	-	+
6	Coumarin		-	+	-	+	-	-
7	Chalcones		-	-	-	-	+	-
8	Protein		-	-	+	+	+	-
9	Amino acids		-	-	-	-	-	-
10	Flavonoids:	Alkaline Reagent Test	+	+	+	+	-	-
		NH_4OH	-	+	+	+	-	-
11	Diterpenes		-	+	+	-	-	-
12	Phytosterol		-	-	-	-	-	+
13	Phenols: $FeCl_3$ test		-	-	+	-	-	+
14	Phlobatannins		+	-	-	-	-	+
15	Leucoanthocyanin		-	-	-	-	-	-
16	Anthroquinone		+	-	-	-	+	+
17	Emodins		-	-	-	-	+	+
18	Cardial Glycosides: Keller-Killani test		-	-	+	+	-	+
19	Carbohydrates: Barfoed's reagent		-	-	-	-	-	+
20	Acid		-	-	-	-	-	-

RESULTS AND DISCUSSION

Phytochemical analysis of aqueous and ethanolic extracts of the spices was carried out and results are presented in Table 1 and 2.

The aqueous extract of *Rhizoma zingiberis* was found with the phytochemicals like alkaloids, saponin, flavonoids, phlobatannin and anthroquinone; the ethanolic extract shown presence of alkaloids, saponin, coumarin, flavonoids and diterpenes.

Aqueous extract of *Cuminum cyminum* L. shown presence of the secondary metabolites like alkaloids, saponin, tannin, proteins, flavonoids, diterpenes, phenol and cardial glycosides and shown its efficiency more than the ethanolic extract, other hand the ethanolic extract shown presence of alkaloids, saponin, coumarin, protein, flavonoids and cardial glycosides.

The aqueous extract with *Curcuma longa* L. shown presence of alkaloids, saponin, steroids, emodins, proteins, anthroquinone and chalcones while, the ethanolic extract shown presence of alkaloids, saponin, anthocyanin, emodins, phytosterol, phlobatannin, phenol, anthroquinone, crdial glycosides and carbohydrates and found more efficient.

The aqueous extract of *Piper nigrum* L. yielded the phytochemicals like alkaloids, saponin, steroids, proteins, flavonoids, phytosterol and phlobatannin while, its ethanolic extract contains only few secondary metabolites like alkaloids, proteins and diterpenes.

Aqueous extract of *Cinnamomum tamala* shown presence of phytochemicals like alkaloids, saponin, coumarin and flavonoids while, its ethanolic extract shown presence of alkaloids, saponin and tannin only.

Flavonoids have anti-inflamatory, antimicrobial (Baez et al, 1999; Ogundipe, 2001; Xu HX and Lee, 2001), antioxidant, vascular activities along with other medicinal properties (Harborne and Willians, 1992). Tannin may be toxic to microorganisms like bacteria, yeast and filamentous fungi (Harborne, 1973). It have

Table 2: Phytochemicals of Aqueous (A) and Ethanolic (E) extract of *Piper nigrum* and *Cinnamomum tamala*

Sr. No.	Test		Results			
			Piper nigrum		*Cinnamomum tamala*	
			A	E	A	E
1	Alkaloids:	Wagner's reagent	+	+	+	-
		Hager's reagent	+	-	+	+
2	Saponin: Foam test		+	-	+	+
3	Tannin: $FeCl_3$		-	-	-	+
4	Steroids		+	-	-	-
5	Anthocyanin		-	-	-	-
6	Coumarin		-	-	+	-
7	Chalcones		-	-	-	-
8	Protein		+	+	-	-
9	Amino acids		-	-	-	-
10	Flavonoids:	Alkaline Reagent Test	+	-	+	-
		NH_4OH	-	-	+	-
11	Diterpenes		-	+	-	-
12	Phytosterol		+	-	-	-
13	Phenols: $FeCl_3$ test		-	-	-	-
14	Phlobatannins		+	-	-	-
15	Leucoanthocyanin		-	-	-	-
16	Anthroquinone		-	-	-	-
17	Emodins		-	-	-	-
18	Cardial Glycosides: Keller-Killani test		-	-	-	-
19	Carbohydrates: Barfoed's reagent		-	-	-	-
20	Acid		-	-	-	-

Key: (+) Positive test, (-) Negative test

Table 3: Antimicrobial activity of Aqueous (A) and Ethanolic (E) extract of *Rhizoma zingiberis* and *Cuminum cyminum*

Organism used	Zone of inhibition (in mm)											
	Rhizoma zingiberis (10 %)						*Cuminum cyminum* (10 %)					
	A		E		Control		A		E		Control	
	50µl	100µl	50µl	100µl	50µl	100µl	50µl	100µl	50µl	100µl	50µl	100µl
Bacillus subtilis NCIM 2635	-	-	15.0± 1.00	18.6± 0.57	-	-	-	-	-	-	-	-
Proteus vulgaris NCIM 2813	-	-	-	-	-	-	-	16.6± 0.57	18.0 ±1.0 0	22.0± 1.00	-	-
Salmonella typhimurium NCIM 2501	-	-	20.0± 1.00	18.0± 1.00	-	-	-	-	-	-	-	-
Staphylococcus aureus NCIM 2654	-	-	14.6± 0.57	18.0± 1.15	-	-	-	-	-	-	-	-

Note: Each value is the mean of three readings ± SD.

Table 4: Antimicrobial activity of Aqueous (A) and Ethanolic (E) extract of *Curcuma longa* and *Piper nigrum*

Organism used	Zone of inhibition (in mm)											
	Curcuma longa (10 %)						*Piper nigrum* (10 %)					
	A		E		Control		A		E		Control	
	50µl	100µl	50µl	100µl	50µl	100µl	50µl	100µl	50µl	100µl	50µl	100µl
Bacillus subtilis NCIM 2635	-	-	16.0± 1.00	12.6± 0.57	-	-	-	-	16.0± 1.00	21.0± 1.00	-	-
Proteus vulgaris NCIM 2813	-	-	-	-	-	-	-	-	-	-	-	-
Salmonella typhimurium NCIM 2501	-	-	-	-	-	-	-	-	12.0± 1.00	22.0± 1.00	-	-
Staphylococcus aureus NCIM 2654	-	-	-	-	-	-	-	-	14.0± 1.00	16.0± 1.00	-	-

potential antiviral (Lin *et al,* 2004; Krishna et al, 2014) and antibacterial activity (Akiyama et al, 2001; Funatogawa et al, 2004; Venkanna Lunavath and Estari Mamidala, 2013).

The data (Table 3, 4 and 5) revealed about antibacterial activity of the spices. Only the ethanolic extract of *Rhizoma zingiberis* shown antibacterial activity against all the bacteria, except *Proteus vulgaris* and its 10 % of 50 µl concentration found to be more effective. The aqueous (100 µl) and ethanolic (50 µl) extract of *Cuminum cyminum* L. found effective against *Proteus vulgaris* only. Ethanolic extract from turmeric shown zone of inhibition against *Bacillus subtilis* only and its 50 µl of 10 % concentration found effective. Aqueous extract of *Piper nigrum* L. doesn't show any inhibition zone against any test organisms but its ethanolic extract shown effective inhibition zone against all the bacteria except *Proteus vulgaris.* Only the ethanolic extract of *Curcuma longa* L. was found effective against *Baccilus subtilis* and *Salmonella typhimurium*

and its 50 µl of 10 % concentration was most effective. The results obtained may support the use of the mentioned spices in traditional medicine for the treatment of Rheumatism, Nausea, Diarrhea, Diuretic diseases, digestive system related diseases, in Flatulense, muscle spasma, Anti-inflammatory, in some autoimmune diseases, etc. and in drug developments.

CONCLUSION

The research concludes that present Indian spices are the rich source of valuable secondary metabolites and shown presence of significant antimicrobial properties. Specifically ethanolic extract of *Curcuma longa,* aqueous extract of *Cuminum cyminum, Cinnamomum tamala* and *Piper nigrum* yielded more phytochemicals while both solvents found effective for *Rhizoma zingiberis*. Ethanolic extract of *Piper nigrum* and *Rhizoma zingiberis* found most effective antibacterial among all.

Conflict of interests:

The authors declare that there is no conflict of interests regarding the publication of this paper.

Table 5: Antimicrobial activity of Aqueous (A) and Ethanolic (E) extract of *Cinnamomum tamala*

Organism used	Zone of inhibition (in mm)					
	Cinnamomum tamala (10 %)					
	A		E		Control	
	50µl	100 µl	50µl	100 µl	50µl	100 µl
Bacillus subtilis NCIM 2635	-	-	18.0± 1.00	20.6 ±0.5 7	-	
Proteus vulgaris NCIM 2813	-	-	-	-	-	
Salmonella typhimurium NCIM 2501	-	-	17.0± 1.00	14.0 ±1.0 0	-	
Staphylococcus aureus NCIM 2654	-	-	-	-	-	

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