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HPTLC Profiling and Antimicrobial Studies of Some Commonly Used Indian Spices

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ABSTRACT

Indian medicinal system is one of the most believable and traditional system of medicine in the world where we find importance of spices and condiments in daily life. Spices have been in use as food additives since ancient times. They are used as flavoring agent and also as preservatives. Most of the spices are indigenous in origin with characteristic aroma and strong taste. These spices not only add flavor to dishes but also they have lots of medicinal properties. By considering their polyvalent significance in present investigation we have made an attempt to study antimicrobial potential and HPTLC profiling of *Curicumalongi, Cinnamomumverum, Cuminumcymenium, Piper nigrum.*Chromatographic analysis (HPTLC) showed presence of several phytochemical compounds with variable Rfvalus and concentration. The antibacterial activity showed significant growth inhibition against *Escherichia coli*,*Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa*, *Streptococcus pyogenes,* and*Streptococcus pneumoniae.*The mixture of phytochemical compounds present in the extracts might be responsible for the antibacterial activity against these bacteria. The results obtained support the application of these spices in several traditional ethnomedicinal applications. Furthermore, HPTLC fingerprint developed may be useful in the correct identification of these spices and in detecting adulterations in preparation of commercial spice pockets.

Key Words: HPTLC, Antimicrobial Activity, Spices.

I. INTRODUCTION

Forest resources have been a valuable source of natural products for a long period of time tomaintain human health, especially with more intensive studies in the last decade for natural therapies (Gisleneet al.,2000).Spices and herbs have been long used for thousands of centuries by many cultures to enhance the flavor and aroma of foods. Early cultures also recognized the value of using spices and herbs in preserving foods and for their medicinal value. Scientific experiments since the last 19th century have documented the antimicrobial properties of some spices, herbs and their components (Shelef, 1983; Zaika, 1988).

The spices used in Indian cooking have been used since ages for adding flavor and also for house-hold treatment of infectious diseases. It is imperative to study their antimicrobial activity against the common

human pathogens so that the best spices can be further exploited to determine their active component which can be used for developing drugs (Seema and Anurag, 2015).

TLC is an analytical technique often used both for analysis of phytoconstituents from plant and for plant identification. Also, quality assessment and evaluation of botanical materials can be done by the modern and automated version of TLC(HPTLC) as well as plant-derived foods. Taking into account that spices have botanical origin, this can be used as a rapid method to analyze raw materials, to control their quality andto detect the adulteration (Virgil et.al.2018).By considering the similar view, we are trying to obtain HPLTC profile of four spices most commonly used spices in Indian cooking. In addition to this we are presenting the results of antibacterial potential of these spices against some pyogenic bacteria.

II. MATERIALS AND METHODS

Collection of Plant material:

The plant materials *Curicumalongi*(rhizome),*Cinnamomumverum*(sticks*), Cuminumcymenium*(seeds),*Piper nigrum*(seeds)were collected from local market of Nanded. These material were brought to laboratoryand made in to powder form using mixture grinder.

Extraction of Plant Material:

About 20 gm of powder plants were extracted separately in 250 mlsoxhlet extractorusing 70% ethanol for six hours continuously. The obtained extracts were vaporized under reduced pressure on vacuum evaporator. Finally, the extract was stored at low temperature in refrigerator for further study.

Antibacterial Activity:

The ability to kill or inhibit the growth of pathogenic bacteria was evaluated by antibacterial screening of plant extract. This was performed by agar well diffusion method and the zone of inhibition (ZOI) was measured in mm for each bacteria. The different bacteria used in the study are *Escherichia coli*,*Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Streptococcus pneumoniae*and*Pseudomonas aeruginosa*. The activated bacterial cultures were seeded on sterile nutrient media. Plant extract were poured in the central well and the zone of inhibition were measured against the standard antibiotic ciprofloxacin (Ahuja et. al. 2015).

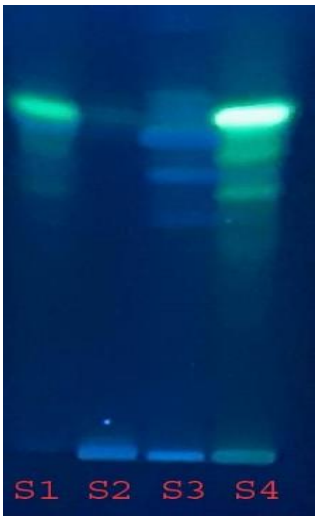
III. HPTLC ANALYSIS OF EXTRACTS

HPTLC fingerprinting of extracts were carried out as per the method described by Monaet.al.(2012). Two microliters of the ethanolic extract was applied (band length –8.0 mm) on a precoated TLC aluminium sheets of silica gel G60 F254 of 200 µm thickness plate- 05 x10cm (Merck, Mumbai) using LinomatV TLC applicator (Camag, Muttenz, Switzerland) equipped with a 100-µL syringe. Prior application, the plate was pre-washed with methanol AR and dried at 60°C. TLC plates were developed using the mobile phase Tolune: ethyl acetate: diethyl amine (8:1:1) in a Camag HPTLC twin-trough chamber (10 x10cm). The chamber was saturated with filter paper for 15 minutes and plate equilibrium was carried out for 10 minutes. Plate was developed upto85.0 mm and dried under stream of air. Separated bands were quantified by HPTLC densitometric scanning using Camag TLC Scanner 4 in the absorption mode operated by WinCATS software (version 1.4.8). After scanning the spectra and tables thus obtained were analyzed to interpret the results.

IV. RESULTS AND DISCUSSION

Development of standard procedure through HPTLC is a new approach which may lead to proper standardization of different spices and ayurvedic drugs based on fingerprinting characteristics (Meena, et.al.2013).In India, the trend of consumption of spices and herbs in food or using them as medicine aims to maintain proper sanitation, health, and hygiene and to increase longevity of life. Several spices such as ajowan, clove, ginger, black pepper, cumin, and asafetida are commonly used in the Indian diet (RomikaDhiman, et. al. 2015)Spices have been used for not only flavor and aroma of the foods but also to provide antimicrobial properties (Nanasombat,et al., 2002).

Antimicrobials are agents that kill microorganisms or inhibit their growth. The antimicrobial effects of the plant extracts are sufficient in a way to cater the healing effect. The antimicrobial effect of spices extracts also helps to prevent diseases in many forms (Bhawana, et.al. 2014).



HPTLC Plate at 366 nm

[Tracks S1-*Cinnamomumverum*;S2-*Cuminum cymenium*;S3 *Piper nigrum* ;S4*Curcuma longi*]

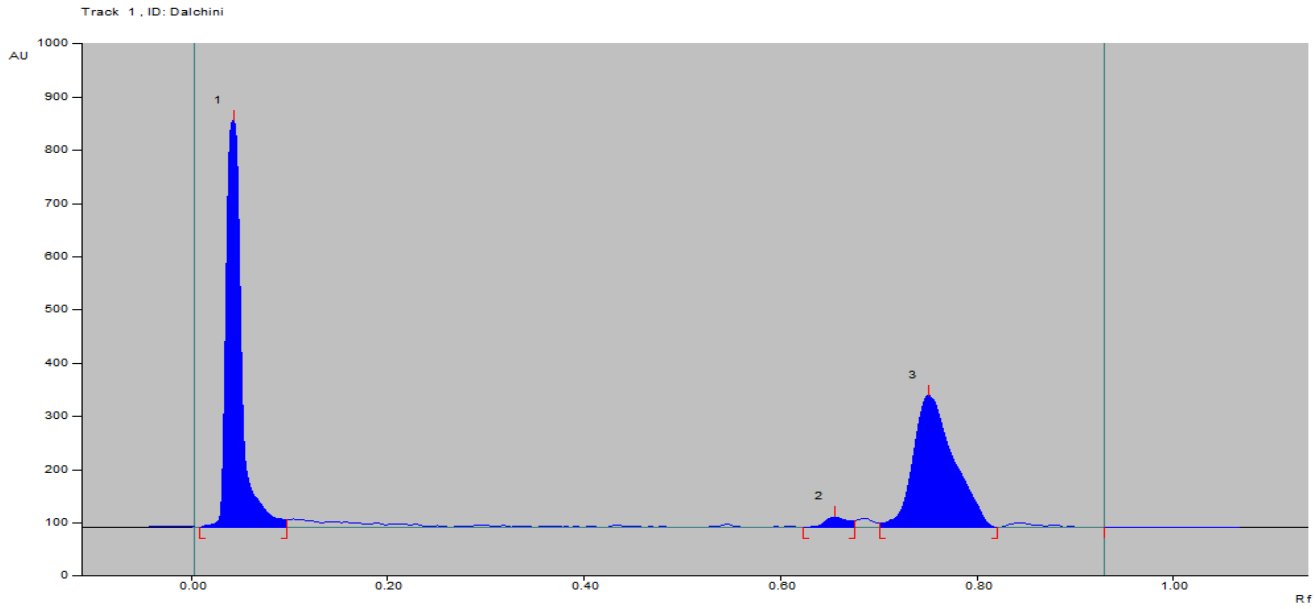


Fig1: HPTLC profile (Peak Display) of *Cinnamomumverum*sticks.

Track 1, ID: Dalchini

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.1 AU	0.04 Rf	765.2 AU	74.05 %	0.10 Rf	13.7 AU	10158.2 AU	53.49 %
2	0.63 Rf	0.2 AU	0.66 Rf	19.8 AU	1.92 %	0.68 Rf	12.8 AU	394.3 AU	2.08 %
3	0.70 Rf	7.6 AU	0.75 Rf	248.3 AU	24.03 %	0.82 Rf	0.1 AU	8438.9 AU	44.44 %

Fig 2: HPTLC profile (Peak Table) of *Cinnamomumverum*sticks

The results from HPTLC finger print scanned at wavelength 254 nm for ethanol extract of *Cinnamomumverum*sticks showed threephytochemical compoundwith corresponding ascending order of Rf values start from 0.04 to 0.75 in which highest concentration of the compound found to be 74.05% and its corresponding Rf value is 0.04.This is recorded in Figure1 and 2 respectively.

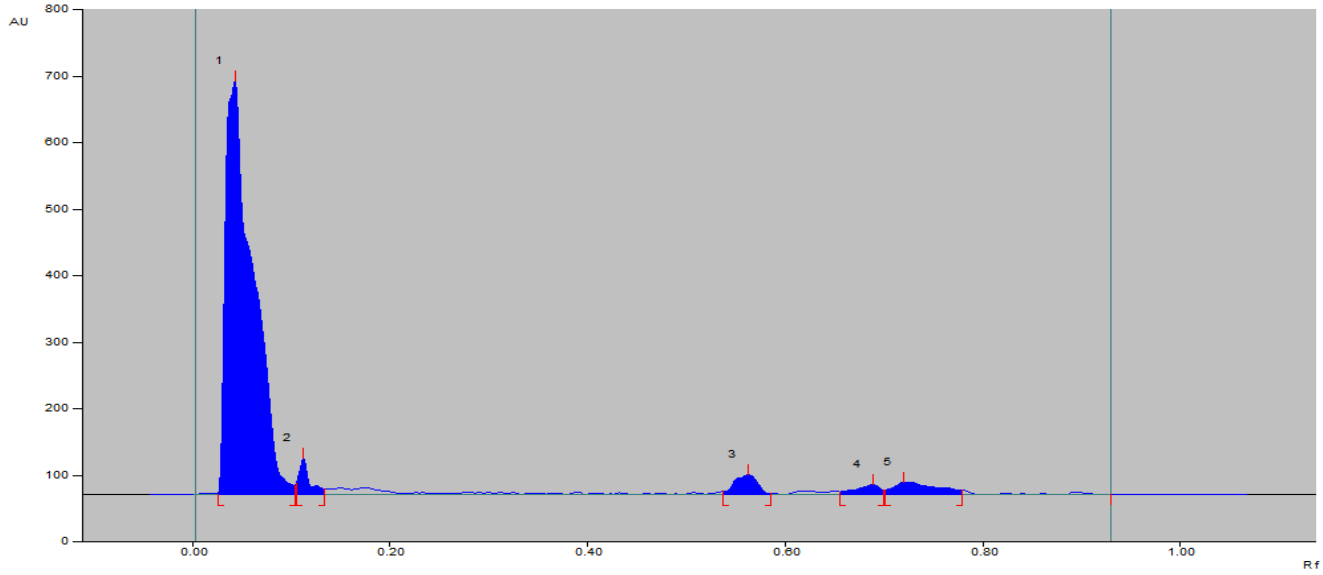


Fig 3: HPTLC profile (Peak Display) of *Cuminumcymenium*seed

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.03 Rf	0.4 AU	0.04 Rf	622.0 AU	83.99 %	0.11 Rf	13.3 AU	14472.6 AU	87.64 %
2	0.11 Rf	17.1 AU	0.11 Rf	55.0 AU	7.43 %	0.13 Rf	8.2 AU	464.1 AU	2.81 %
3	0.54 Rf	4.2 AU	0.56 Rf	29.8 AU	4.03 %	0.59 Rf	2.2 AU	589.2 AU	3.57 %
4	0.66 Rf	4.1 AU	0.69 Rf	15.1 AU	2.04 %	0.70 Rf	6.6 AU	303.9 AU	1.84 %
5	0.70 Rf	6.7 AU	0.72 Rf	18.6 AU	2.52 %	0.78 Rf	5.9 AU	684.0 AU	4.14 %

Fig 4: HPTLC profile (Peak Table) of *Cuminumcymenium*seed

The results from HPTLC finger print scanned at wavelength 254 nm for ethanol extract of *Cuminumcymenium*seed showed five phytochemical compoundsand corresponding ascending order of Rf values are from 0.04to0.72 in which highest concentrationof compound was found to be 83.99 % and its

corresponding Rf value is 0.04.This is recorded in Figure4. The corresponding HPTLC chromatogram is presented in Figure 3 which shows five peaks of phytoconstituents

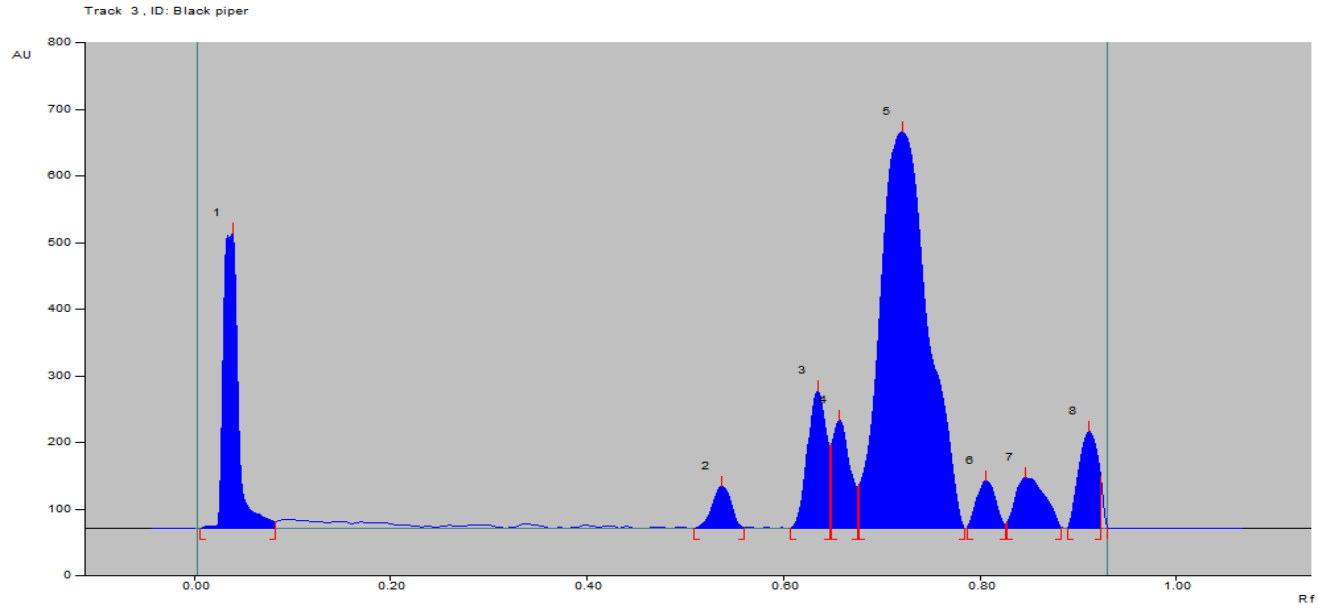


Fig 5: HPTLC profile (Peak Display) of *Piper nigrum*fruits.

Track 3, ID: Black piper

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.2 AU	0.04 Rf	442.2 AU	25.10 %	0.08 Rf	9.6 AU	5680.8 AU	13.62 %
2	0.51 Rf	0.1 AU	0.54 Rf	63.1 AU	3.58 %	0.56 Rf	1.5 AU	985.6 AU	2.36 %
3	0.61 Rf	0.2 AU	0.64 Rf	206.3 AU	11.70 %	0.65 Rf	24.2 AU	3253.6 AU	7.80 %
4	0.65 Rf	125.7 AU	0.66 Rf	162.7 AU	9.23 %	0.68 Rf	62.7 AU	2402.0 AU	5.76 %
5	0.68 Rf	64.3 AU	0.72 Rf	595.1 AU	33.77 %	0.79 Rf	0.7 AU	23956.6 AU	57.46 %
6	0.79 Rf	1.3 AU	0.81 Rf	71.6 AU	4.07 %	0.83 Rf	6.9 AU	1116.6 AU	2.68 %
7	0.83 Rf	8.2 AU	0.85 Rf	76.1 AU	4.32 %	0.88 Rf	1.6 AU	1898.6 AU	4.55 %
8	0.89 Rf	0.8 AU	0.91 Rf	145.1 AU	8.23 %	0.93 Rf	77.1 AU	2400.7 AU	5.76 %

Fig 6: HPTLC profile (Peak Table) of *Piper nigrum*fruits

The results from HPTLC finger print scanned at wavelength 254 nm for ethanol extract of *Piper nigrum*fruitsshowedeight polyvalent phytoconstituentsand corresponding ascending order of Rf values start from 0.04 to 0.91 in which highest concentration of the phytoconstituent was found to be 33.77 % with its corresponding Rf value 0.72.This is recorded in Figure6. The corresponding HPTLC chromatogram is presented in Figure 5 which shows eight peaks of phytoconstituents

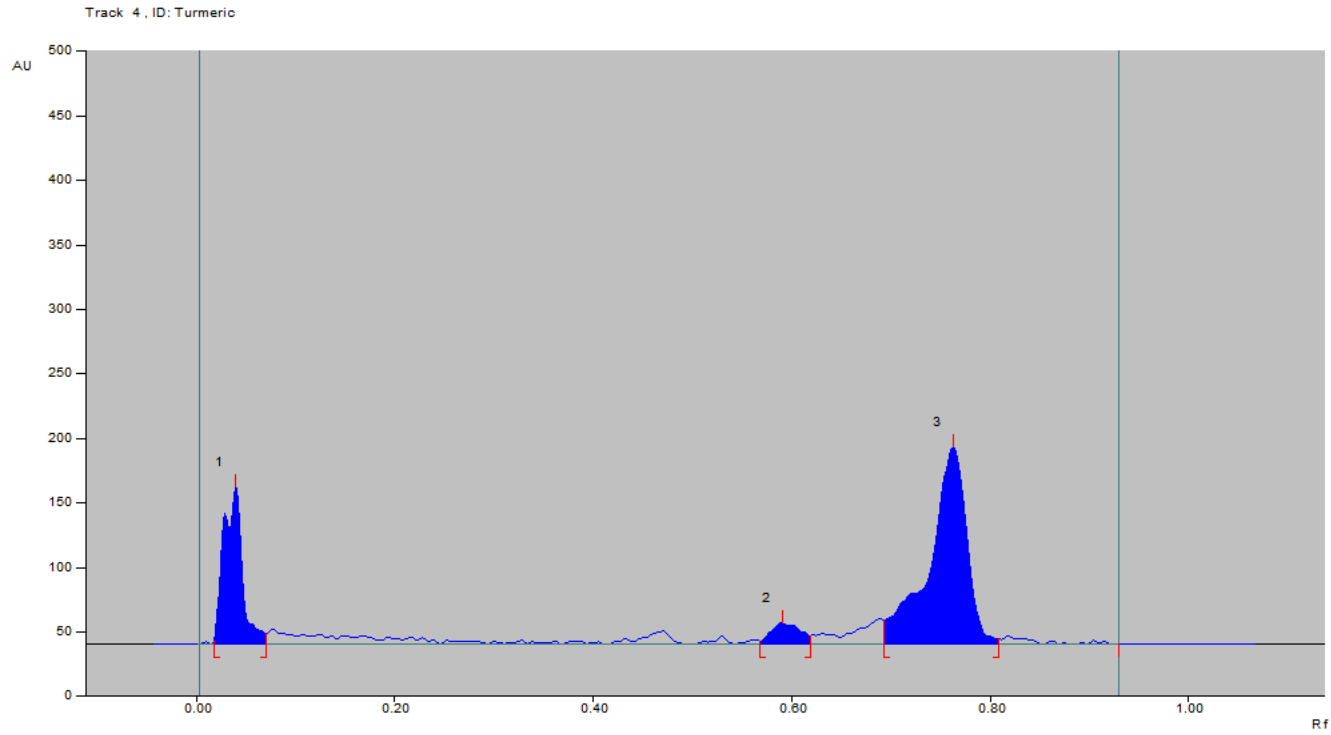


Fig 7: HPTLC profile (Peak Display) of *Curcuma longi*powder

Track 4, ID: Turmeric

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	2.0 AU	0.04 Rf	121.6 AU	41.84 %	0.07 Rf	8.3 AU	1940.2 AU	27.37 %
2	0.57 Rf	2.4 AU	0.59 Rf	16.4 AU	5.64 %	0.62 Rf	6.6 AU	407.8 AU	5.75 %
3	0.69 Rf	18.8 AU	0.76 Rf	152.7 AU	52.53 %	0.81 Rf	3.7 AU	4740.3 AU	66.88 %

Fig 8: HPTLC profile (Peak Table) of *Curcuma longi*powder

The results from HPTLC finger print scanned at wavelength 254 nm for ethanol extract of *Curcuma longi*powder showed three polyvalent phytoconstituentsand corresponding ascending order of Rf values are from 0.04to0.76 in which highest concentration of the phytoconstituent was found to be 52.53 % with its corresponding Rf value 0.76.This is recorded in Figure8. The corresponding HPTLC chromatogram is presented in Figure 7 which shows three peaks of phytoconstituents

Table 1: Antimicrobial activity against pyogenic bacteria showing zone of inhibition in mm.

Sr. No	Test Organism	Plant Extracts				Ciprofloxacin (Control)
		Curicumalongi	*Cinnamomumverum*	*Cuminumcymenium*	*Piper nigrum*	
1	*Escherichia coli*	14	12	10	14	20
2	*Staphylococcus aureus*	12	10	09	11	19

3	*Staphylococcus epidermidis*	15	11	08	10	21
4	*Pseudomonas aeruginosa*	14	12	07	10	17
5	*Streptococcus pyogenes*	14	12	08	11	22
6	*Streptococcus pneumoniae*	10	11	05	12	20

The results of antibacterial activity of selected spices on some pyogenic bacteria are presented in table 1. From the results it is clear that extracts of these spices has shown significant antibacterial activity in comparison with standard antibiotic ciprofloxacin. The extracts of *Curicumalongi*found to be more inhibitory to*Staphylococcus epidermidis* (15 mm)flowed by *Pseudomonas aeruginosa, Escherichia coli* and*Streptococcus pneumoniae*(14 mm).Similarly *Piper nigrum*shows maximum zone of inhibition against*Escherichia coli* (14) followed by *Streptococcus pneumoniae*(12 mm*) Streptococcus pyogenes*and *Staphylococcus aureus* (11 mm). Likewise ethanoic extracts of *Cinnamomumverum*was found to be more toxic to*Escherichia coli, Pseudomonas aeruginosa* and *Streptococcus pyogenes* (12 mm). The extracts of *Cuminumcymenium*was found to be less inhibitory as compared to other spices tested. The results obtained supports the earlier findings of Abhishekh Seth, (2010). The obtained resultsjustifies scientific andtraditional inclusion of these spices in ayurvedicmedicine preparation.This preliminary study can be further extended in determining the active component of the spices so that effective medicinal preparations can be made. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments.

Similarly, HPTLC is a powerful analytical method equally suitable for qualitative and quantitative analytical tasks. HPTLC is playing an important role in today's analytical world, not in competition to HPLC but as a complementary method. It combines fascinating art of chromatography with accuracy and quickness with better separation and resolution (Jadhav, 2018). HPTLC method deals with qualitative and quantitative analytical applications such as herbal and dietary supplements, nutraceuticals, and various types of medicines. It is used in quality control and in purity checks, in the detection and identification of pharmaceutical raw materials, drugs and their metabolites in biological media. HPTLC method is also a very powerful tool for identification of the presence of adulterants in herbal products based on the characteristic image produced and much useful for determining the presence and the quantification of both unwanted substitution as well as intentional adulteration in the formulation (Chidambaram et al. 2015).Therefore, the present piece of work finds its application in detecting different types of adulteration in preparation and marketing of spices

V. CONCLUSION

From the above study it is clear that the spices which are being used in Indian cooking process are having remarkable antimicrobial potentials. Since pathogens are developing resistance against present medicines, their antibacterial potential can be used as alternative to existing therapies. HPTLC data generated in the above study will be useful in finding unwanted mixing in spices.

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