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Research Article

PHYTOCHEMICAL PROFILING, HPTLC FINGERPRINT AND ANTIBACTERIAL, ANTI-FUNGAL, AND ANTIOXIDANT PROPERTIES OF ESSENTIAL OILS EXTRACTED FROM *CUMMINUM CYMINUM*, *ZINGIBER OFFICINALE*, *TRACHYSPERMUM AMMI*, *ALIPNIA GALANGA*, *CEDRUS DEODARA*, AND *ELETTARIA CARDAMOMUM*

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ABSTRACT

Objective: The objective of the study was to carry out the phytochemical profiling of essential oils (EOs) and evaluation of their anti-microbial activity.

Methods: The EOs extracted from *Cumminum cyminum*, *Zingiber officinale*, *Trachyspermum ammi*, *Alipnia galanga*, *Cedrus deodara*, and *Elettaria cardamomum* using clavenger apparatus. Phytochemical analysis and high-performance thin layer chromatography (HPTLC) fingerprinting were carried out for the EO. The antibacterial and antifungal activity were evaluated using agar well-diffusion method against two bacterial strains, *Escherichia coli*, *Staphylococcus aureus* and two fungal strains, *Candida albicans*, and *Aspergillus brasiliensis*. Positive controls ciprofloxacin-30 mg, azithromycin-15 mg, and nystatin NS-50 mg were used. Antioxidant potential of the EOs was investigated by TLC-bioautography method using 1,1-diphenyl-2-picrylhydrazyl derivatization.

Results: The phytochemical analysis reveals presence of various phytochemical such as steroids, terpenoids, and phenylpropanoids. The HPTLC fingerprint is found to be unique for each of the oil. The EO of *Z. officinale* and *T. ammi* showed strong antibacterial activity against *S. aureus*. The EOs of *C. cyminum*, *Tachyspermum ommi* and *A. galanga* displayed prominent antioxidant activity on TLC bioautography. The herbs *Cuminum cynimun*, *T. ammi*, *C. deodara,* and *Ellateria cardamomum* produce reasonable amount of essentials oil, which can be explored for useful their industrial applications.

Conclusions: These EOs can be explored further for their antimicrobial activity. The HPTLC analysis along with derivatization with suitable chromogenic reagents can be a rapid and simple tool for quality control of various EOs.

Keywords: Antimicrobial activity, Bioautography, Essential oils, HPTLC fingerprint, Phytochemical analysis.

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INTRODUCTION

Essential oils (EOs) are the concentrated and complex liquid mixtures of volatile compounds originating from various plants. Each EOs from the different plant possess characteristic and unique aromatic odor [1,2]. They are extracted from various parts of the aromatic plants such as flowers, leaves, stalks, fruits, seeds, buds, rhizomes, roots, barks, and resins [3,4]. The EOs have wide commercial applications such cosmetics, aromatherapy, as flavors in food and beverages, food preservation, home care, hygiene products, disinfectants, agriculture, and fragrance industries [5,6]. The EOs are reported for various pharmacological properties such as anti-inflammatory, analgesic [7], antifungal, antibacterial [8], antioxidant [9], antiviral [10] and immune boosting action [11], and anproperties. EOs are traditionally used for various purposes such in aromatherapy, anxiolytic, body relaxing, stress relief, respiratory related ailments, central fatigue [12], in dental care [13,14], antispasmodic, local anesthetic, antispasmodic [16], and anticancer properties [17]. Some of the EOs have reported to be effective in relief of migraine symptoms [15]. The essentials oils are extracted from various parts of the plant by using various techniques such as steam distillation, hydrodistillation, hydrodiffusion, super critical fluid extraction, organic solvent extraction, and cold pressing [1,4,18]. EOs are complex mixture of various low molecular weight molecules such as saturated and unsaturated hydrocarbons, alcohols, aldehydes, esters, ethers, ketones, phenol oxides, monoterpenoids, sesquiterpenoids, phenylpropanoids, and aromatic compounds, which attribute to their characteristic odors [5,6,19]. Some of the well-known compounds of EOs are eucalyptol, beta-pinene, d-limonene, l-menthol, carvacrol, citral, citronellol, camphor, cinceole, eugenol, thymol, and chavicol [4,8]. The herbs *Cumminum cyminum*, *Zingiber officinale*, *Trachyspermum ammi*, *Alipnia galanga*, *Cedrus deodara*, and *Elettaria cardamomum* are reported to contains EOs and used as common spice in foods. Conventionally, *C. cyminum* is used as carminative, digestive, and galactagogue. The EO of cumin has analgesic properties [18,19]. *Z. officinale* is used for the treatment of colds, nausea, arthritis, pain relief, migraines, abdominal disorders, and hypertension [20-22] and ginger EO is used for food preservation [20]. *T. ammi* is commonly known as ajwain or caraway and is widely used spice across the world. It is traditionally used as antispasmodic, carminative, appetite stimulant and in treatment of diarrhea, gastrointestinal problem and bronchial ailments [23]. The rhizomes of *A. galanga* traditionally used in the treatment of stomach problems [24,25]. The heartwood of *C. deodara* is traditionally used in India for inflammation and rheumatoid arthritis [26]. The aromatic pods of *E. cardamomum* are used for asthma, dental infections, cataracts, nausea, diarrhea, cardiac, digestive, and kidney disorders and used as a flavored spice in food [27-29]. Currently, the most common method used for analysis of the EOs is gas chromatography (GC), GC-mass spectrometry [30,31]. In the present study, we have explored application of high-performance thin layer chromatography (HPTLC) fingerprint and TLC derivatization as a supporting tool for analysis of EOs. The antioxidant activity of EOs was checked by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) derivatization with TLC-autobiography. Antibacterial activity was evaluated for the EOs on pathogenic bacteria *Escherichia coli, Staphylococcus aureus* and pathogenic fungus *Candida albicans and Aspergillus brasiliensis* using agar well-diffusion method.

METHODS

All the chemicals and reagents used were of analytical grade. The microbiology media, broth and antibiotic test-discs used were purchased from HiMedia. The autoclave and incubator was from Technico Industries Ltd and digital colony counter was from Sensel. Antibiotic Zonescale from HiMedia was used for measuring zone of inhibition.

Plant materials

Alpinia galanga (dried roots), *Z. officinale* (dried rhizomes), *C. cyminum* (dried fruits), *T. ammi* (dried fruits), *E. cardamomum* (dried fruits), and *C. deodara* (heartwood) were collected from the local source. The raw materials were identified and verified by botanist Mr. Patchaimal.

EO extraction

The EO from the herbal materials (fruit, rhizome, seed, and wood) was obtained by hydrodistillation method using clavenger apparatus. The dried seeds of *Cuminum cynimun* (98 kg) were placed in a distillation apparatus with 800 l of distilled water and hydro-distilled for 8 h to collect 5.7 l (1.5%) of EO. Similarly, 4.0 l (3.7%) of EO was obtained from 108 kg of seeds of *T. ammi*, 600 ml (0.2%) from 250 kg of *A. galanga* rhizomes, and 350 ml (0.1%) from 250 kg of *Z. officinale.* EO from yield was 2% from each of *C. deodara* (500g) and *Ellateria cardamomum* (25 g) obtained by hydrodistillation in a clevenger apparatus.

HPTLC analysis

HPTLC analysis of EOs was done using precoated TLC plate (Merck-Germany) of silica gel $60F_{254.}$ For HPTLC fingerprinting EO was dissolved in hexane at 50 mL/mL concentration and 10 mL of the sample was applied on the plate with 6 mm band length. The sample was applied by Hamilton syringe using Camag Linomat-V applicator. The plate was run in the Camag twin trough chamber using the mobile phase consist of hexane-diethyl ether [8:2; v/v]. The plat was visualized in CAMAG ultraviolet (UV) cabinet under UV 254 nm and 366 nm. After chromatographic development derivatization of the plate was done with anisaldehyde-sulfuric acid reagent. The scanning was done using Camag Scanner-III equipped with winCATS software version 1.4.3 [32,33].

Preliminary phytochemical analysis

Preliminary phytochemical analysis was done for presence of steroids, phytosterols, alkaloids, and phenolics in the EO. The plates were derivatized with various reagents such as Liebermann-Burchard, anisaldehyde-sulfuric acid reagent, vanillin-sulfuric acid reagent, ferric chloride, and Dragendorff reagents [34-36].

TLC bio-autographic DPPH assay

TLC bioautography is an simple, rapid, reliable, and comparatively in-expansive technique of chromatography separation and *in situ* identification of bioactive compounds on the TLC plates [35]. The reaction between DPPH radical and an active compound is a method commonly used to determine its antioxidant activity, which has been used to directly locate those types of compounds on TLC plates. The characteristic reaction of this technique produces a pale yellow on the spots with the compounds having antioxidant activity. The plate was developed using hexane-diethyl ether (8:2) as mobile phase was used sprayed with 0.2% methanolic solution of DPPH and incubate in the dark. Then the photograph of the TLC was recorded in gap of 30 min. The images where the spots showed maximum color intensity were considered [36,37].

Antimicrobial activity

Lyophilized cultures pellets of Gram-negative *E. coli*-ATCC-8739™, Gram-positive *S. aureus*-ATCC® 6538™, and fungus *C. albicans*-ATCCC 10231 and *A. brasiliensis*-ATCC 16404 used in antimicrobial assay. All the strains were purchased as kwik-stik from HiMedia. The bacteria and fungus culture were rejuvinated in Tryptone Soya Broth at 37°C for 18 h and then stocked in soyabean casein digest agar and Sabouraud dextrose Emmons agar, respectively. The subculture was prepared from the stock for antimicrobial assay.

Antimicrobial activity using well-diffusion technique

The agar well-diffusion method was employed to determine antibacterial and antifungal activity of EOs. The bacterial and fungus inoculum were spread over the nutrient-agar plate using a sterile cotton swab to provide an uniform microbial growth. The holes of 6 mm diameter were made on the agar plates and 75 ml of each of the EOs are added to wells in triplicate to evaluate antibacterial activity. For antifungal activity 20 ml of EOs was used. The plates were incubated for 48 h at 37°C. The antimicrobial activity was evaluated by measuring the diameter of inhibition zone in millimeters using zone scale. Further, TLC-bioautography was used for identification antimicrobial compounds of the EOs.

RESULTS

Extraction of EO

The EO collected from the herbs was analyzed for different physicochemical parameters (Table 1). Among the oils *T. ammi* was having highest yield followed by *C. deodara and E. cardamomum*.

HPTLC analysis

In the present work, suitable method was developed for simultaneous HPTLC fingerprint of the EO. HPTLC chromplate under UV 254 and derivatized plate are shown in the Fig. 1. The characteristic HPTLC fingerprint is shown in Table 2.

Track 1: *C. cyminum*, Track 2:*Z. officinale*, Track 3:T. *ammi,* Track 4:*A. galanga*, Track 5: *C. deodara*, and Track 6: *E. cardamomum.*

Preliminary phytochemical screening

The EOs were screened for the presence of phytosterols, terpenoids, alkaloids, phenolics, etc. Derivatization of TLC with various derivatization showed grey, brown, pink, violet, and green color zones. This indicates presence of various phytochemicals, for example, terpenoids, steroids, and phenylpropanoids. The major spots visible on the plates are shown in Fig. 2 summarized in Table 3.

TLC-bioautography assay

The antioxidant compounds were visualized by appearance of yellow zones (Fig. 3) against violet background of the TLC plates. DPPH assay is one of the most popular and frequently used methods of antioxidant assay. All the EOs showed visible antioxidant activity as yellowish zone

Table 1: Physicochemical parameters of the essential oils

*Essential oils	Description	Refractive index	Weight/ml	Yield (%w/w on dry basis)
CCEO	Yellow color oil with characteristic odor	1.4987	0.9123	1.50
ZOEO	Dark Yellow oil with characteristic odor	1.5184	0.9163	0.1
TAEO	Light yellow oil with characteristic odor	1.4859	0.8733	3.70
AGEO	Dark yellow oil with characteristic odor	1.4701	0.9109	0.2
CDEO	Colorless transparent liquid with slightly viscous nature and with characteristic odor	1.4962	0.8261	2.0
ECEO	light yellow color oil with characteristic odor	1.4730	0.9251	2.0

CCEO: **Cumminum cyminum* essential oil, ZOEO: *Zingiber officinale* essential oil, TAEO: *Trachyspermum ammi* essential oil, AGEO: *Alipnia galangal* - essential oil, CDEO: *Cedrus deoda*-Deodara essential oil, ECEO: *Elettaria cardamomum* - essential oil

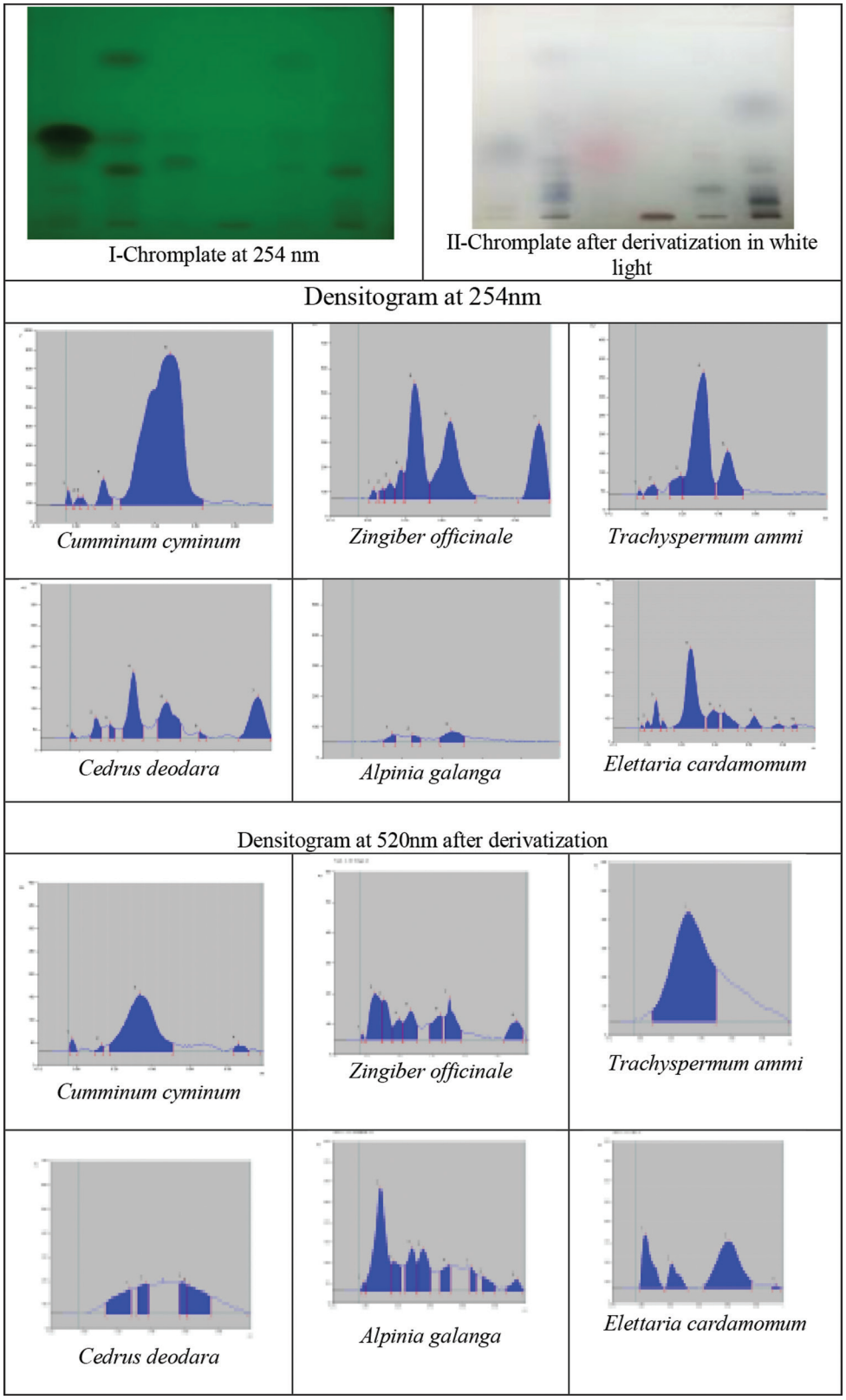

Fig. 1: High-performance thin-layer chromatography chromatogram of essential oils

on the TLC plates after interaction with DPPH reagent. Among the oils *C. cyminum*, *Trachyspermum ammi* and *A. galanga* showed the visible zone quickly indicating the strong antioxidant activity while for other it develops only after 2 h that infers weaker antioxidant activity in comparison to other EOs of the study.

Antibacterial and antifungal activity of EO

All the above EOs were tested for their antibacterial and antifungal activity. *C. cyminum* and *T. ammi* EOs showed promising antifungal activity against two tested pathogenic fungus and bacteria. Ciprofloxacin and azithromycin were used as positive control in the antibacterial

Table 2: Rf value of essential oils at 254 nm

Plant name	R_f at 254 nm	R_f at 520 nm after derivatization
Cumminum cyminum essential oil-CCEO	0.11, 0.16, 0.20, 0.26, 0.34, 0.53, 0.98	0.42, 0.94
Zingiber officinale essential oil-ZOEO	0.11, 0.16, 0.20, 0.26, 0.34, 0.53, 0.98	0.13, 0.18, 0.28, 0.35, 0.53, 0.59, 0.98
Trachyspermum ammi essential oil - TAEO	0.13, 0.28, 0.40, 0.53	0.40
Alipnia galanga- essential oil-AGEO	0.23, 0.34, 0.53	0.35, 0.45, 0.67, 0.70
Cedrus deoda - Deodara essential oil -CDEO	0.16, 0.24, 0.36, 0.53, 0.98	0.18, 0.26, 0.37, 0.44, 0.59, 0.74, 0.82, 0.98
Elettaria cardamomum-essential oil -ECEO	0.13, 0.17, 0.34, 0.47, 0.54, 0.71	0.11, 0.29, 0.69

Table 3: Spray reagent and color zone of various secondary metabolite using TLC derivatization for essential oils

Sample description	Color of the spot with reagent in visible light			
	Vanillin sulfuric acid	Anisaldehyde sulfuric acid	Liebermann-Burchard	DPPH
CCEO	Grey, pink	Grey, violet	Grey, blue	Yellow color zones
ZOEO	Violet, blue and grey	Violet, blue	Grey	Yellow color zones
TAEO	pink color	brick red	-	Yellow color zones
AGEO	violet, blue	Violet, blue and pink	Grey	Yellow color zones
CDEO	violet and blue	violet, grey	Grey, pink	Yellow color zones
ECEO	Grey, pink	Grey, pink	Grey	Yellow color zones

CCEO: **Cumminum cyminum* essential oil, ZOEO: *Zingiber officinale* essential oil, TAEO: *Trachyspermum ammi* essential oil, AGEO: *Alipnia galanga*- essential oil, CDEO: *Cedrus deoda* - Deodara essential oil, ECEO: *Elettaria cardamomum* - essential oil

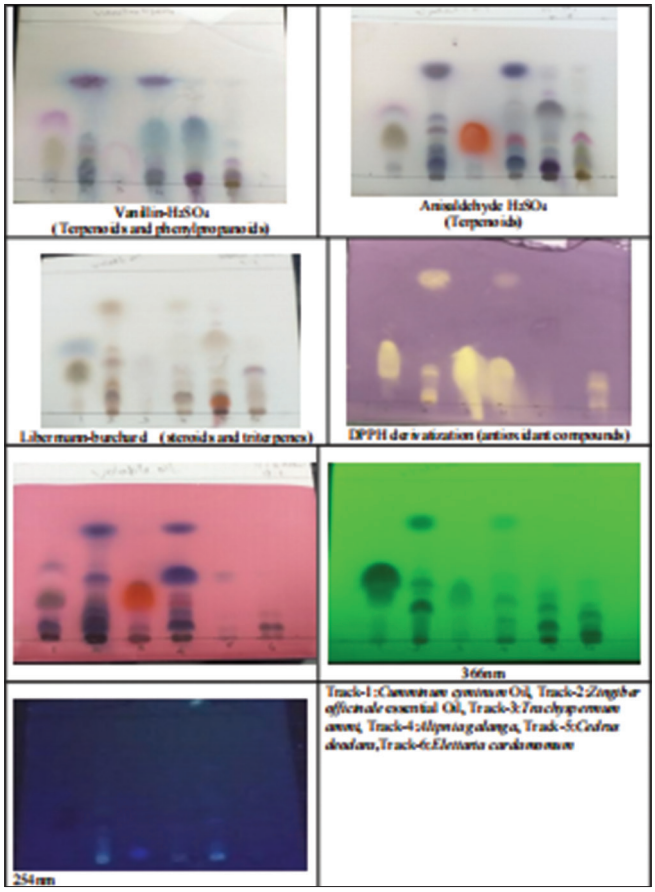

Fig. 2: The thin-layer chromatography derivatization with various derivatizing reagents

activity and itraconazole and ketoconazole was used as positive control in antifungal activity. The antimicrobial activity and antibiogram are presented in Table 4 and Fig. 4, respectively.

Antimicrobial activity by TLC-bioautography

On the TLC the EOs of *Z. officinale*, *A. galanga*, and *E. cardamomum* showed prominent inhibition zone in *C. albicans, A. brasilensis,* and *S. aureus*, and while the oils *Z. officinale, T. ammi, and A. galanga*, showed a clear zone in *E. coli.*

DISCUSSION

Out of all, the *T. ammi* yield maximum EOs of 3.7%. The HPTLC analysis showed a unique fingerprint for each oil and derivatization of TLC with various chromogenic reagents demonstrated a unique array of colors that can be used as a diagnostic characteristic for all the oils. The EOs of *C. cyminum* and *T. ammi* found to be having promising anti-bacterial and antifungal activity *in vitro* assay. Cumin seed oil contains various hydrocarbon and oxygenated monoterpenes and sesquiterpenes, for example, cuminaldehyde, limonene, α- and β-pinene, 1,8-cineole, o-and p-cymene, α- and γ-terpinene, ρ-Mentha-1,3-dien-7-al, and ρ-Mentha-1,4-dien-7-al [18,19]. The EO of *Z. officinale* has been reported to contain more than 115 phytochemicals with gingerols and shogaols are the major components [20-22]. The major component of *T. ammi* EO is thymol, p-cymene and gama-terpinine [23]. The major component of *Alpinia galanga* oil is Its contents include 1,8-cineole, α-fenchyl acetate, camphor, methyl cinnamate, and guaiol [24]. The compounds identified in *C. deodara* EO are beta-himachalene, alpha-himachalene, and gamma-himachalene [26]. The EOs of *E. cardamomum*is rich in oxygenated monoterpenes, 1.8-cineole, α-terpinyl acetate, linalool acetate, sabinene, and linalool [27-29]. These phytochemical might attribute to the observed activity of the tested EOs.

CONCLUSIONS

The present work demonstrates use of HPTLC finger print is a simple and rapid method that can be used for quality control and characterization of the EO. Each of the EOs showed a characteristic pattern upon derivatization with the reagents. The TLC-Bioautography methods were found to be a rapid, consistent, low cost and simple method for preliminary evaluation of bioactivity of the herbal sample and can be explored for evaluation of other biological activities due to its simplicity and ease of handling. The EOs showed varied level of antioxidant and antimicrobial activity. These results substantiates the wide application EOs in treatment of microbial infections and as well as a natural food preservative in traditional practice. The study highlighted potential of EOs as promising alternatives for the treatment of microbial infections with current rise in antimicrobial resistant species to convention antibacterial and antifungal drugs.

Table 4: Antibacterial activity of various essential oils

S. No	Name of the essential oil	*E. coli*	*S. aureus*	*C. albicans*	*A. brasilensis*
1	*Alpinia galanga*	14	14	10	20
2	*Zingiber officinale*	14	14	20	18
3	*Cumminum cyminum*	12	20	30	20
4	*Trachyspermum ammi*	12	32	26	22
5	*Cedrus deodara*	Nil	14	16	14
6	*Elettaria cardamomum*	Nil	11	12	16
7	Ciprofloxacin	37	32	-	-
8	Azithromycin	16	28	-	-
9	Ampicillin 2 mcg	Nil	12	-	-
10	Itraconazole-IT-30cg	-	-	32	40
11	Ketoconazole-KT-50 mcg			28	24
12	Nystatin-50 mcg	-	-	Nil	Nil
13	Amphotericin-B-50 mcg			20	Nil

E. coli: *Escherichia coli*, *S. aureus*: *Staphylococcus aureus*, *C. albicans*: *Candida albicans*, *A. brasilensis*: *Aspergillus brasiliensis*

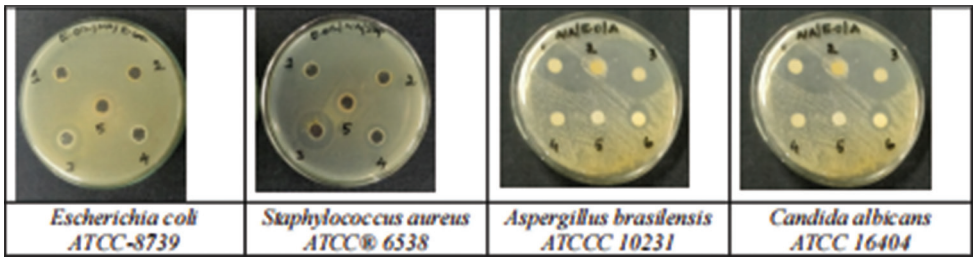

Fig. 3: Antibiogram of antimicrobial activity

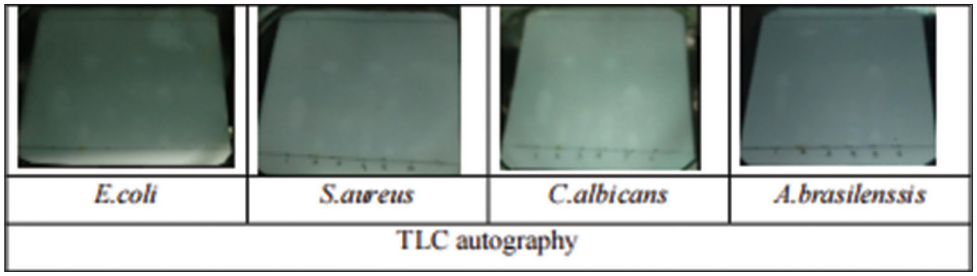

Fig.4: Thin-layer chromatography autobiography of essential oils

AUTHORS' CONTRIBUTION

The concept design and experiment support was kindly provided Dr Ramesh Raghava Varrier the managing director of the company AVN Ayurveda Formulation Pvt Ltd. The antimicrobial study was done by first author Mr. Guruvaur appan. Corresponding author Mr. Manas did experiment design and planning the protocol and manuscript writing. The HPTLC and TLC analysis report was done by Mrs. Anithakumari and Mrs Maheswari. The extraction of EO was done by Mr. Ramesh. The manuscript editing and correction was done by Dr. Srikrishna.

CONFLICT OF INTEREST

The authors declares no conflict of interest.

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