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Phytochemical profiling of essential oils extracted from *Myristica fragrans*, *Illicium verum*, *Syzygium aromaticum*, *Foeniculum vulgare*, and *Cinnamomum cassia* using TLC, HPTLC, GC techniques and evaluation of their antimicrobial activities

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Abstract
Background: Essential oils constitute a major class of natural products. They possess various biological activities like anti-inflammatory, anti-oxidant, analgesic, and antimicrobial activities and have immense application in food, cosmetics and pharmaceutical industries. In the current study, the essential oils were extracted from six medicinal plants including *Myristica fragrans* (endosperm of the seed or karnel and the mace), *Illicium verum*, *Syzygium aromaticum*, *Foeniculum vulgare*, and *Cinnamomum cassia.* The phytochemical profiling was carried out by using TLC derivatization, HPTLC fingerprinting, and GC analysis. The antibacterial and anti-fungal activity was evaluated by agar-well diffusion method. The antioxidant activity was evaluated by DPPH based TLC-bioautograph method.
Results: Each of the essential oils showed a different pattern of fingerprint on TLC, HPTLC and GC chromatogram due to their unique phytochemical composition. All of the above essential oils demonstrated promising antimicrobial activity against the tested pathogenic bacteria *(E. coli* and *S. aureus*) and fungus (*C. albicans* and *A. brasiliensis*) in various extents. The essential oil extracted from *M. fragrance*, *C. cassia* and *S. aromaticum* showed prominent antioxidants as per the TLC visualization after DPPH derivatization.
Conclusion: TLC, HPTLC and GC showed unique fingerprints of each of the essential oils. The promising antimicrobial activity and antioxidant activity infer that these essential oils could be explored further for their therapeutic potential as new antimicrobial and antioxidant leads and as a natural food preservative in substitution to various synthetic preservatives.

Keywords: Essential Oils, medicinal plants, HPTLC, TLC, DPPH-antioxidant activity, antimicrobial activity, phytochemical analysis

Introduction
Essential oils are concentrated liquids that consist of a complex blend of volatile organic produced by various plant parts. Each of the essential oils possesses characteristic odour and flavour depending on their source. They are extracted by means of expression, stem distillation, solvent extraction, microwave extraction, supercritical fluid extraction, and ultrasound-assisted extraction methods from a variety of plant parts such as flowers, seeds, leaves, rhizomes, fruits, woods, bark, etc. [1]. Till date more than 3000 essential oils reported and approximately 300 essential oils are extensively used in food, pharmaceutical, agrochemical, cosmetic, sanitary, home care, and insect repellent products due to their unique aromatic odour and pharmacological properties [2]. The majority of phytochemicals in essential oils belong to classes of monoterpenes and sesquiterpenes and their oxygenated derivatives such as aldehydes, alcohols, phenols, ketones, ethers, esters, peroxides, and lactones. A few essential oils also contain compounds containing nitrogen and sulphur groups, fatty acid esters, and phytochemicals of the class phenylpropanoids [2]. The essential oils have important role in plant ecology such as adaption to external stress, inter and intra-plant signaling, defence against pathogens and herbivores...etc [4]. The essential oils containing unique molecular scaffold possess various biological activities like anti-inflammatory, antioxidant, antimicrobial, antiviral, wound healing, and anxiolytic effects. Because of their characteristic aromatic odour they possess insect repellent properties.

Essential oils are part of various traditional systems of medicine like Ayurveda, Traditional Korean Medicine and European traditional medicine for aromatherapy, anxiolytic, pain relief, dental care, antispasmodic, spasmolytic, and carminative usage [4-6]. Some of the well-known essential oils such as peppermint oil, lavender oil, thyme oil, clove oil, eucalyptus oil, and dill oil are popularly used in various oral care, antiseptic, stress relief, analgesic and digestive products [7-9]. The plants *Myristica fragrans, Illicium verum, Syzygium aromaticum, Foeniculum vulgare*, and *Cinnamomum cassia* have been used as spices in various food preparations. They have been used in various Ayurveda and other traditional medicines across the world for thousands of years for treatment and prevention of a wide range of ailments. The plant *Myristica fragrans* commonly known as nutmeg belongs to the Myristicaceae family. The commonly used parts of it are nutmeg karnel (endosperm of the dried seed) and the mace (the red colored soft cover around kernel) are called as Jatiphala and Jatipatri respectively in Ayurveda. They are used in traditional medicines for the treatment of various tumours, gastrointestinal troubles, for improving digestion, kidney disease, rheumatoid arthritis, sleep disorder, wound healing and infections [10-11]. *Illicium verum* commonly known as star anise belongs to the family of Illiciaceae. Traditionally it is used as an herbal medicine in traditional Chinese and Ayurveda formulations for treatment of skin inflammation, rheumatoid arthritis, asthma, and bronchitis [12-13]. *Syzygium aromaticum* commonly known as clove is an aromatic herb belongs to the family of Myrtaceae. Traditionally it is used as an analgesic for headache, joint pain, toothaches and dental pain. Modern research has reported various biological activities for clove essential oils such as antimicrobial, antioxidant, insecticidal, antiviral, anti-inflammatory, wound healing, anaesthetic, and anticancer activities [14]. The plant *Foeniculum vulgare* commonly known as Fennel, belongs to the family Umbelliferae having a pleasant aroma and flavour. It is used in traditional medicines for the treatment of wide range of ailments like gastrointestinal disorders such as abdominal pains, stomachache, diarrhea, constipation, and irritable colon…etc, as a digestive, galactogogue, leucorrhoea and kidney related ailments [15]. *Cinnamon cassia* is a popular spice belongs to the Lauraceae family. In traditional medicines it is used for treatment of various diseases like stomach pain, menstrual pain, diabetes, toothache, bad breath, arthritis, and gastrointestinal disorders, etc [16-17]. In the current study the essentials oils from these plants were analyzed by extensive TLC derivatization, HPTLC fingerprint and GC analysis. The antimicrobial activities of the essential oils extracted from the plant were evaluated against human pathogenic bacteria and fungus.

Materials and Methods

All the chemicals and reagents used were of analytical grade. The microbiology media, broth and standard antibiotic discs used were purchased from HiMedia. DPPH was purchased from SRL Company. The refractive index of the essential was measured using an Abbe's refractometer (Advance Research Instruments Company, New Delhi, India).

Plant materials

The dried plant materials of *Illicium verum* (Flower), for *Myristica fragrans* the outer red arill of seed called mace and the inner brown karnel called nutmeg (endosperm of the seed) is collected, *Syzygium aromaticum* (flower bud), *Foeniculum vulgare* (seed), *Cinnamomum cassia* (bark) were collected from the local market and validated by the botanist and a voucher specimen of the plant was kept in quality control department for future reference. The scientific name of the plants and the plant parts used (Fig. 1) is mentioned below.

Fig 1: The name of the plants used in the study

Essential oil extraction
Each of the samples (100 g) were cleaned and subjected to hydro distillation for 4 hour using clevenger-type apparatus. The percentage yield of essential oil was calculated on dry weight basis using the equation:

% Yield = [volume of distilled oil collected (mL)/weight of herbal material taken (g)] × 100

HPTLC analysis
HPTLC analysis of essential oils was done by using precoated TLC plate (Merck-Germany) of silica gel $60F_{254.}$ For HPTLC fingerprinting essential oil was dissolved in hexane at 50µL/mL concentration and 10 µL of the sample was applied on the plate with 6mm 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 consisting of hexane-diethyl ether [8:2; v/v]. The plate was visualized in CAMAG UV cabinet under UV 254 nm and 366 nm. After chromatographic development, derivatization of the plate was done with 4-Anisaldehyde-sulphuric acid reagent. The scanning was done using Camag Scanner-III equipped with win CATS software version 1.4.3.

GC Analysis
The essential oils were analyzed by gas chromatograph (GC) to obtain the GC chromatogram for each sample. The GC was carried out on a Chemito GC-1000 model. A capillary column BP5 (30 m × 0.32 mm × 1.0 µm) was used. The carrier gas was helium and the injection port temperature was 260 °C. The temperature program of GC began at 80 °C and increased at the rate of 17 °C/min up to 250 °C. Volume of injection was 0.2 µl

Preliminary Phytochemical analysis
Preliminary phytochemical analysis was carried out through extensive TLC analysis for identification phytosterols, terpenpoids, alkaloids, and phenolics in the essential oil. The TLC plates were derivatized with various reagents such as Liebermann-Burchard, 4-Anisaldehyde-sulphuric acid, vanillin-sulphuric acid, Iodine, 10% alcoholic H_2SO_4, ferric chloride, and Dragendorff reagent for identification of various classes of compound [34-36].

TLC-Bioautography assay with DPPH:
The TLC bioautography is a simple and rapid chromatography based method for the separation and in-situ identification of the bioactive compounds on the TLC plates The reaction between 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) radical and the active compound is commonly used to determine its antioxidant activity. The characteristic reaction between DPPH and the active free-radical scavenging compound produces a pale yellow spots on the TLC plates indicating their antioxidant activity [18]. The plate were developed using Hexane-Diethyl ether (v/v; 8:2) as mobile phase and was sprayed with 0.2% methanolic solution of DPPH and then incubate in the dark till the colored zones are developed on purple background of the TLC-plate. Then the photograph of the TLC was recorded when the TLC showed maximum numbers of intense yellow-colored spots.

Antimicrobial activity
The reference strains used in the present study composed of gram-negative *Escherichia coli*-ATCC-8739™ (*E. coli*) gram-positive *Staphylococcus aureus*-ATCC® 6538™ (*S. aureus*), and fungus *Candida albicans*-ATCCC 10231 (*C. albicans*) and *Aspergillus brasiliensis*-ATCC 16404 (*A. brasiliensis*) were purchased from Hi Media. The bacteria and fungus culture was reactivated in trypton soya broth and then maintained in soyabein caseine digest agar and sabouraud dextrose emmons agar respectively. The subculture was prepared from the stock for testing the anti-microbial activities.

Antimicrobial Assay
The antibacterial and antifungal activity was assessed following the agar well-diffusion method. The bacterial and fungus inoculum was uniformly spread over the nutrient-agar plate using a sterile cotton swab. The holes of 6mm diameter were made on the agar plates and 100 µl of each of the essential oils were added into the resulting wells in triplicate to evaluate antibacterial activity. As a positive control for antimicrobial activity, ciprofloxacin antibiotic disc was used for bacteria *E. coli* and *S. aureus* and Itraconazole antibiotic disc was used for fungus *A. brasiliensis* and *C. albicans.* The plates were incubated for 48 h at 37 ℃. The antimicrobial activity was evaluated by measuring the inhibition zone diameter (mm) using a zone- scale. The results of zone of inhibition was compared with the activity of standards, ciprofloxacin and itraconazole.

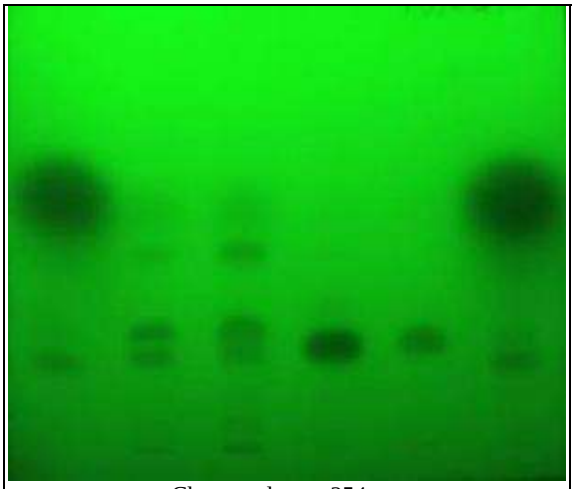
Results
Extraction of Essential Oil
The essential oil collected from the herbs were analyzed for different physicochemical parameters (Table 1). Among the oils the plant *Myristica fragrans*, *Illicium verum* were has highest yield followed by *Syzygium aromaticum*, *Cinnamomum cassia* and *Foeniculum vulgare* found to contain the least quantity of essential oils.

Table 1: Physicochemical parameters of the essential oils

*Essential Oils	Description	Refractive Index	Weight/ml	% Yield (v/w)
MFS-EO	Yellow color oil with characteristic spicy odour	1.4105	0.9103	5.11
MFM-EO	Light Yellow oil with with characteristic odour	1.3960	0.9106	7.50
IV-EO	Light Yellow oil with with characteristic odour	1.3845	0.9104	6.66
SA-EO	Light yellow oil with with characteristic odour	1.3765	1.0500	3.33
FV-EO	Light yellowish-green colored oil with characteristic odour	1.3865	0.9107	0.15
CZ-EO	Light yellow transparent liquid with slightly viscous nature and with characteristic odour	1.3880	1.0300	2.00

**Myristica fragrans* seed Essential oil-MFS-EO, *Myristica fragrans* - mace-MFM-EO, *Illicium verum*-IV-EO, *Syzygium aromaticum*-SA-EO, Foeniculum vulgare-FV-EO, Cinnamomum cassia-CZ-EO

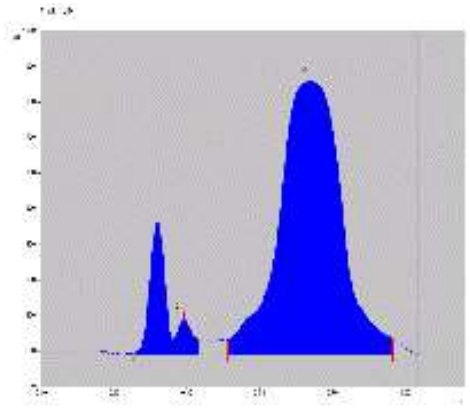
HPTLC analysis: In the present work suitable method was developed for simultaneous HPTLC fingerprint of the essential oil. HPTLC chromplate under UV 254 and derivatized plate are shown in the Figure-1. The TLC plates any oils did not show any prominent spots under 366 nm. The characteristic HPTLC fingerprint is shown in Table 2.



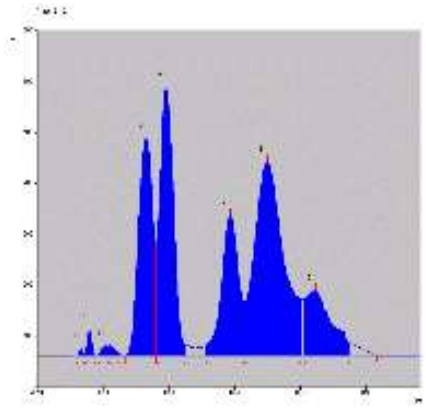
Chrome plate at 254 nm

Chromplate after derivatization in white light

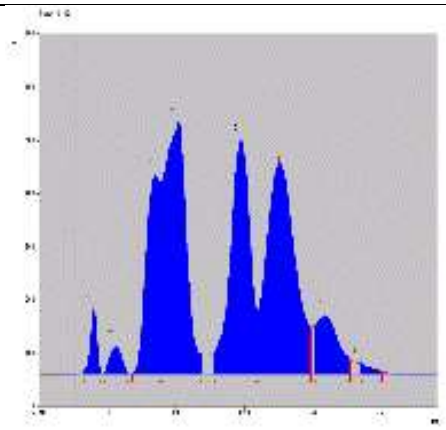
Densitogram at 254 nm



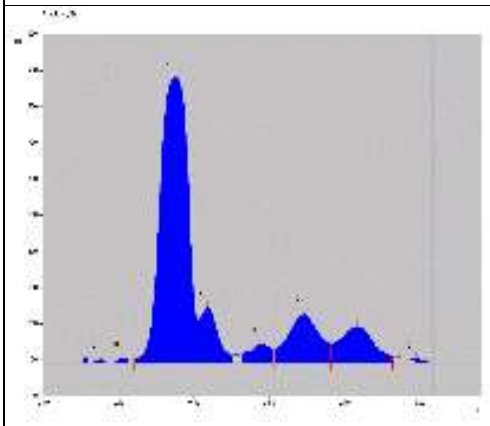
Illicium verum



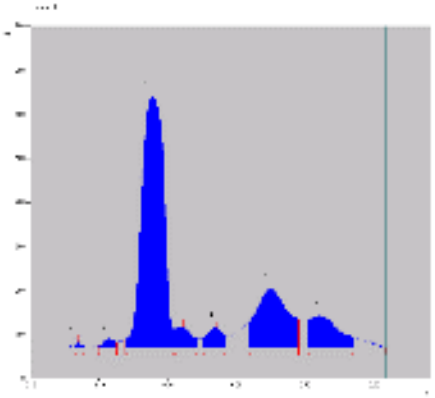
Myristica fragrans seed



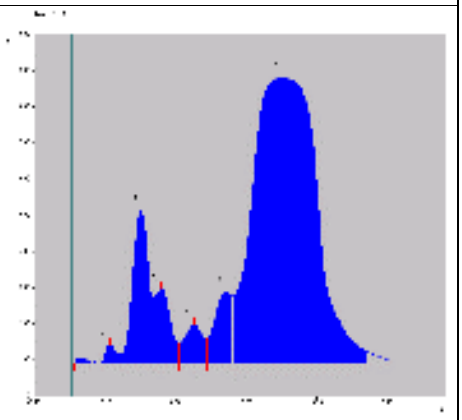
Myristica fragrans - rind



Cinnamomum cassia

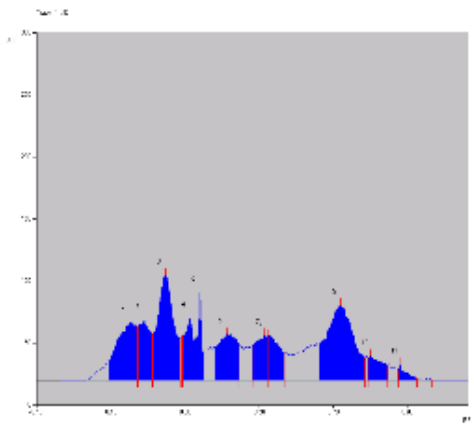


Syzygium aromaticum

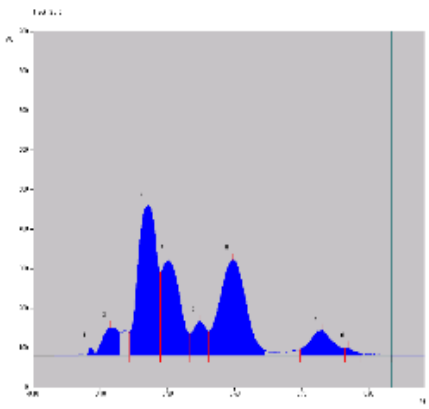


Foeniculum vulgare

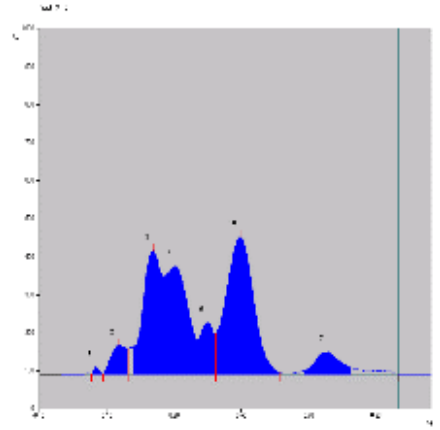
Densitogram at 520 nm after derivatization



Illicium verum



Myristica fragrans seed



Myristica fragrans - ***rind***

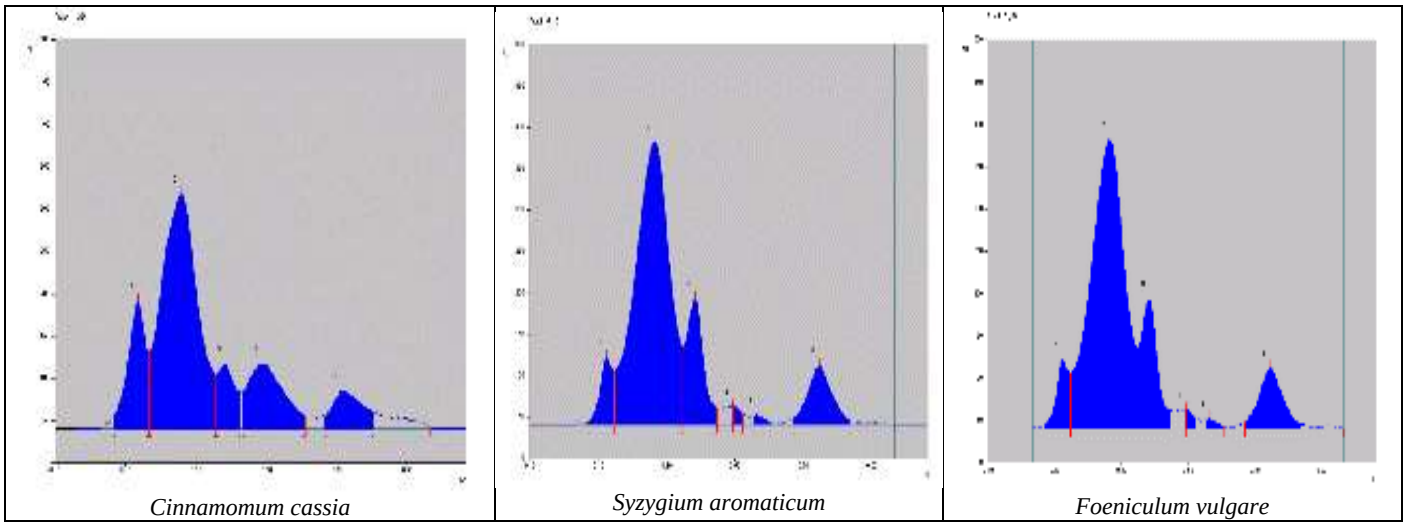


Fig 2: HPTLC chromatogram of Essential Oils

Track 1: *Illicium verum*, Track 2: *Myristica fragrans*-seed, Track 3: *Myristica fragrans*-mace, Track 4: *Cinnamomum cassia*, Track 5: *Syzygium aromaticum*, Track 6: *Foeniculum vulgare*.

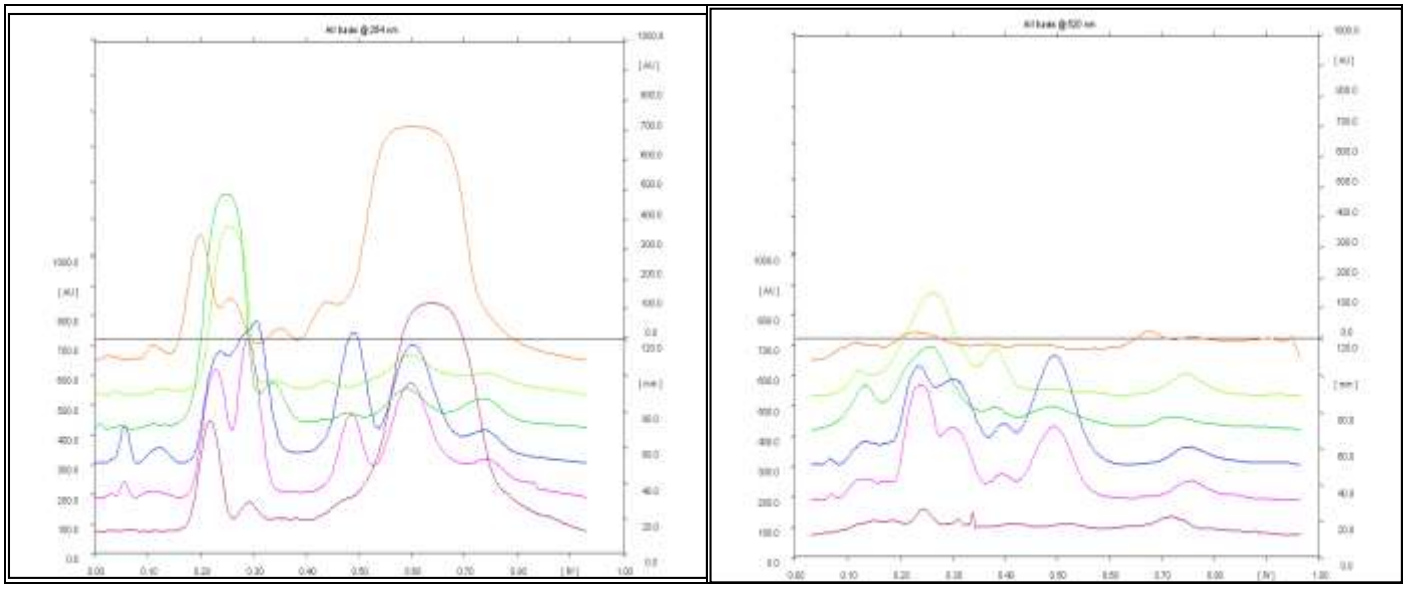


Fig 3: HPTLC Fingerprint of various essential oils

Table 2: HPTLC fingerprint of various essential Oils (R_f Value)

Plant name	R_f at 254 nm	R_f at 520 nm after derivatization
Illicium verum- essential oil	0.22, 0.29, 0.64	0.02, 0.11, 0.20, 0.26, 0.35, 0.44, 0.60
Myristica fragrans, seed-essential oil	0.03, 0.06, 0.10, 0.12, 0.23, 0.29, 0.48, 0.60, 0.75	0.08, 0.16, 0.28, 0.36, 0.42, 0.59, 0.83, 0.88
Myristica fragrans, mace-essential oil	0.06, 0.12, 0.24, 0.31, 0.49, 0.60, 0.74,0.84	0.09, 0.16, 0.27, 0.37, 0.42, 0.61, 0.82
Cinnamomum cassia -essential oil	0.01, 0.05, 0.11, 0.25, 0.34, 0.48, 0.59, 0.73, 0.89	0.16, 0.35, 0.42, 0.61, 0.80
Syzygium aromaticum-essential oil	0.04, 0.13, 0.25, 0.34, 0.44, 0.60, 0.75	0.14, 0.34, 0.45, 0.52, 0.61, 0.84
Foeniculum vulgare-essential oil	0.02, 0.11, 0.20, 0.26, 0.35, 0.44, 0.60	0.14, 0.33, 0.52, 0.72, 0.79, 0.92, 0.96

GC Analysis of the essential oils: GC analysis of the essential oils of the plants showed the presence of varied peaks at different percentiles at different Rt. The major peaks of the GC chromatogram were (Fig.4) *C. cassia*: R_t 1.68 min (18.51% area), 8.51 min (52.39 % area), 9.11 min (12.12 % area); *M. fragrance*, mace: 1.67 min (11.02 area %), 5.06 min (11.38 area %), 5.56 min (22.92 area %), 7.57 min (14.82 area %); *F. vulgare:* 1.67 min (32.06 % area), 6.71 min (6.23 % area), 7.72 min (19.24 % area), 8.53 (34.64 % area). *M. fragrance Seed*: Rt 5.50 (29.23 area %), 7.59 (15.13 area %), 10.49 (12.41 area %); *I. verum*: Rt 8.58 min (87.63 area %).

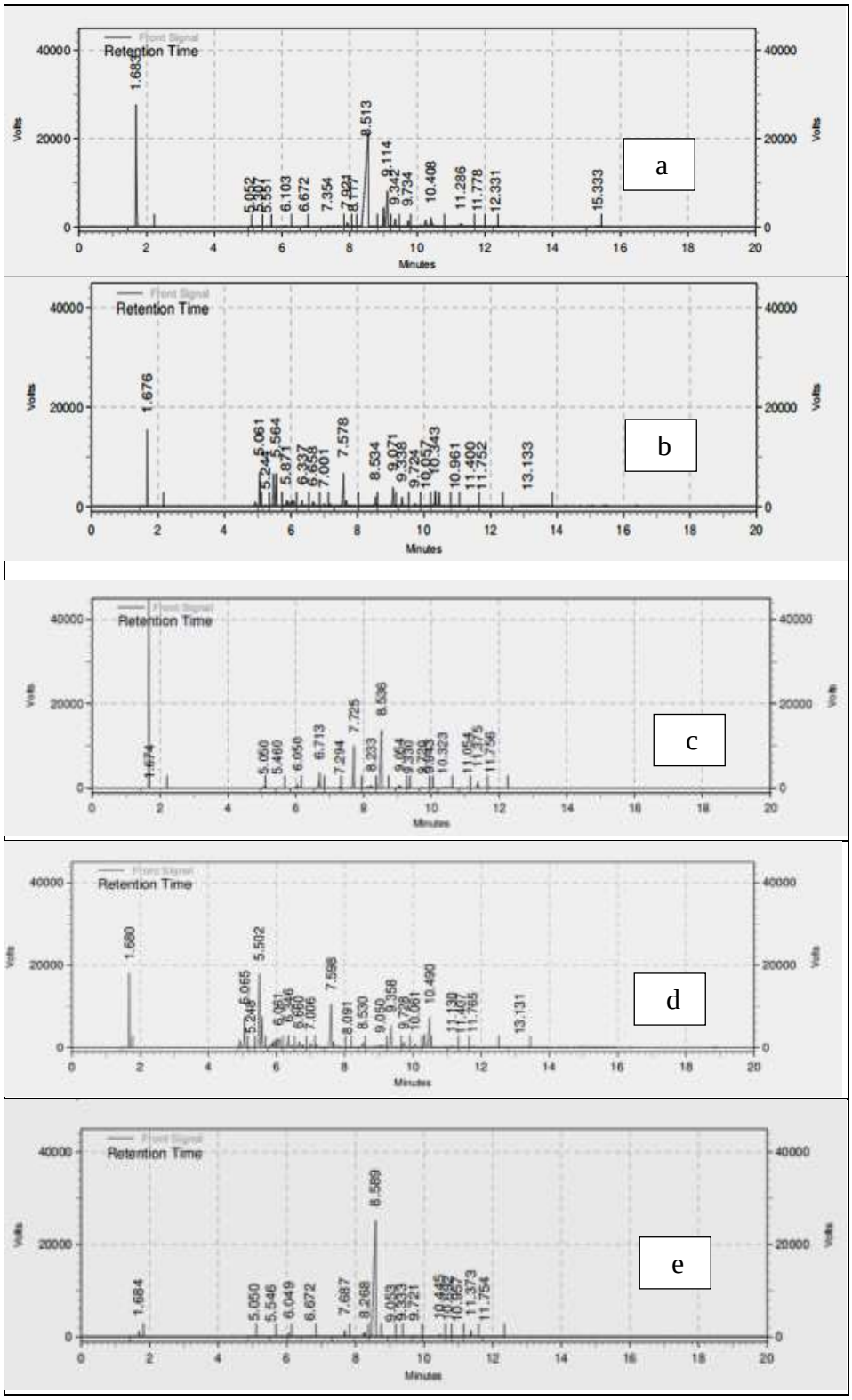
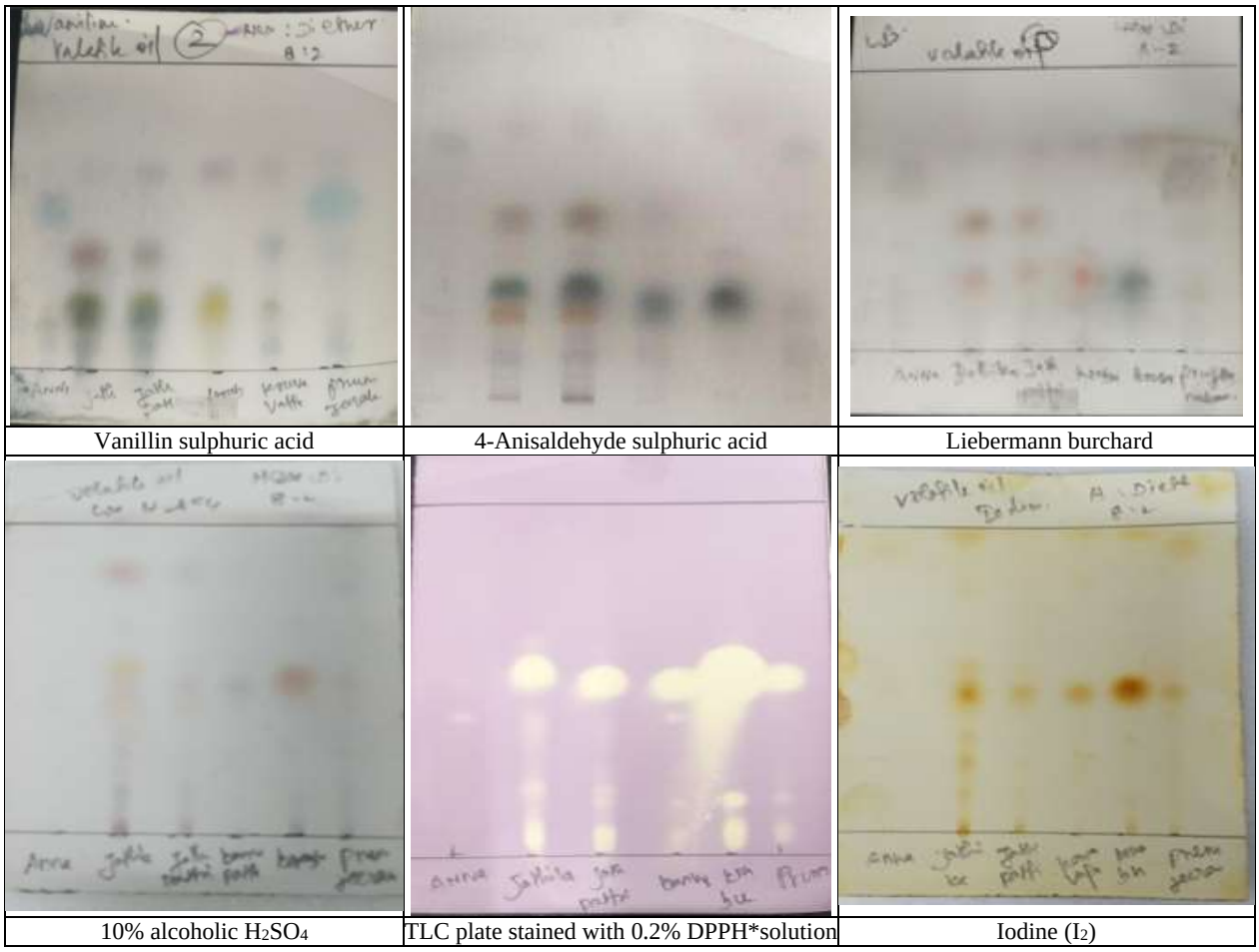


Fig 4: GC chromatogram of various essentials Oils: (a) *C. cassia, (b) M. fragrance*-mace, *(C) F. vulgare, (d) M. fragrance*-seed *(e) I. verum*

Preliminary phytochemical screening

The essential oils were analyzed for identification of phytosterols, terpenoids, alkaloids and phenolics etc. Derivatization of TLC with vanillin sulphuric acid, 4-Anisaldehyde sulphuric acid, Liebermann Burchard and 10% alcoholic H_2SO_4 displayed various colours like green, violet, blue, pink and grey colored zones indicating presence of terpenoids and phenolic functional groups. On exposure to Iodine vapors, the TLC plates showed dark brown colored spots inferring the presence of unsaturated hydrocarbons in the phytochemicals present in the essential oils. The TLC plates on treatment with DPPH reagent and followed by incubation showed purple color of DPPH turned into yellow colored zones indicating the presence of antioxidant compounds in essential oils. This is due to the reduction of DPPH radical to stable DPPH molecules due to the antioxidant molecules in the essential oils [19]. The derivatized TLC plates are shown in Fig.6 and the TLC pattern is summarized in Table 4.



Vanillin sulphuric acid	4-Anisaldehyde sulphuric acid	Liebermann burchard
10% alcoholic H_2SO_4	TLC plate stained with 0.2% DPPH*solution	Iodine (I_2)

Fig 6: The TLC derivatization with various derivatizing reagents

Track 1: *Illicium verum*, Track 2: *Myristica fragrans*-seed, Track 3: *Myristica fragrans*-rind, Track 4: *Cinnamomum cassia*, Track 5: *Syzygium aromaticum*, Track 6: *Foeniculum vulgare*.

Table 4: Spray reagent and color zone of various secondary metabolite using TLC derivatization for essential oils

Sample	Color of the spot with reagent in visible light					
	Vanillin sulphuric acid	**4-Anisaldehyde sulphuric acid**	**Liebermann burchard**	**10% alcoholic H_2SO_4**	**Iodine exposure**	**DPPH**
MFF-EO	Green, violet	Green and violet	Pink	Light grey spots	Dark brown	Light Yellow zones
MFR-EO	Green, violet	Green and violet	Pink	grey spots	Dark brown	Yellow color zones
IV-EO	Light blue	Blue	Light grey	grey spots	Dark brown	Yellow color zones
SA-EO	Yellow and pink	Green color	Deep red color	Red colored spots	Dark brown	Yellow color zones
FV-EO	Light blue	Dark blue color	Light grey	Light grey spots	Dark brown	Yellow color zones
CC-EO	Grey, pink	Light-grey color spots	Dark green color	Light grey spots	Dark brown	Light yellow zones

Antibacterial and anti-fungal activity of essential oil

Out of all the oils from *M. fragrance* mace and *C. cassia* bark displayed over all better anti-microbial activity with clear and larger zone of inhibition against all four. Essential oils of *S. aromaticum* showed potent anti-fungal activity against *A. brasiliensis*. The essential oils of *C. cassia* and *M. fragrance*-rind showed better anti-bacterial activity against two pathogenic bacteria *E. coli* and *S. aureus*. The essential oil of F. vulgare showed a very moderate antimicrobial activity against *S. aureus* and *A. brasiliensis.* The antibacterial activity of the essential oils is mentioned in Table 5 and Figure 6.

Table 5: Antibacterial activity of various essential oils

S. No	Name of the essential oil	*E. coli*	*S. aureus*	*A. brasiliensis*	*C. albicans*
1	*Illicium verum*-fruit	14	12	10	20
2	*Myristica fragrance-aril (Mace)*	20	38	34	14
3	*Myristica fragrans* seeds	16	18	16	22
4	*Cinnamomum cassia*	22	32	36	26
5	*Syzygium aromaticum*	12	18	30	12
6	*Foeniculum vulgare*	Nil	20	16	Nil
7	*Ciprofloxacin*	32	38	-	-
10	*Itraconazole-IT-30cg*	-	-	32	36

A. brasiliensis

S. aureus

C. albicans

M. fragrance-aril (Mace)

M. fragrance seeds

A. brasiliensis

E. coli

C. albicans

C. cassia

S. aureus

A. brasiliensis

--

C. cassia

S. aromaticum

Fig 6: Few Prominent anti-biogram with clear ZOI against the tested pathogens

Discussion:

Out of all the *Myristica fragrans* mace and seed followed by *Illicium verum* yield maximum essential oils. The HPTLC analysis showed a unique fingerprint for each oil and derivatization of TLC with various derivatizing reagents displayed distinguished pattern of colored fingerprints that can be used as a diagnostic feature for all the oils. The plant *Myristica fragrans* Houtt. (nutmeg) belongs to the Myristicaceae family. The principal phytoconstituents reported from its essential oils consist of monoterpene hydrocarbons like sabinene, pinene, camphene p-cymene, phellandrene, terpinene, limonene, myrcene, and their oxygenated derivatives like linalool, geraniol, and terpineol, aromatic ethers such as myristicin, elemicin, safrole, and eugenol and sesquiterpenes such as germacrene D and β-bergamotene [20-21]. The plant *Illicium verum*, commonly known as star anise is an aromatic spice belonging to the family Illiciaceae. Numerous phytochemicals have been reported from *I. verum*, including monoterpenids, sesquiterpenes, phenylpropanoids, lignans, and flavonoids.

The compound *trans*-anethole constitutes major component (75-90%) of its essential oil [22-24]. *Syzygium aromaticum* commonly known as clove belongs to the family of Myrtaceae. The major constituents in the clove essential oil is eugenol (80.19%) and other compounds reported are eugenyl acetate and caryophyllene [25-26]. The essential oil of *C. cassia* contains Trans-cinnamaldehyde as major compound with (69.1%) [27-28]. The plant *Foeniculum vulgare* commonly known as Fennel contains *m*ore than 85 constituents in its essentials. The major phytochemicals reported in the essential oil are *trans*-anethole, alpha-pinene, limonene and fenchone [29-31]. The observed antibacterial, antifungal and antioxidant activity exhibited by these essential oils may be due to the presence of diverse kinds of phytochemical constituents in these plants. The increasing incidence of antimicrobial resistance species has become a major public health concern worldwide. So several research studies are under progress for alternative and potent antimicrobial agents against emerging resistant species. The natural products can be promising drugs for fight against the microbial infection.

Conclusions

The results of the study demonstrates use of HPTLC and TLC finger print as easy, simple less expansive and quick approach for analysis of essential oil extracted from different plants. HPTLC could provide useful insights about the photochemical composition of essential oils and can be a routine method for preliminary analysis of the essential oil. In the present study each essential oil displayed a unique fingerprint pattern and spectrum of color upon derivatization with different TLC derivatizing reagents. The essential oils exhibited good antioxidant and potent free-radical scavenging activity by DPPH based TLC-Bioautography analysis. In antimicrobial study, the essential oils showed good in-vitro antibacterial and anti-fungal activities against the tested human pathogens. These results substantiates the wide application essential oils in treatment of microbial infections and as well as a natural food preservative in traditional uses. Nevertheless, further studies will be needed for the potential application of essential oils as promising alternatives for the treatment of microbial infections of bacterial and fungal origin.

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References

1. Dhifi W, Bellili S, Jazi S, Bahloul N, Mnif W. Essential Oils' Chemical Characterization and Investigation of Some Biological Activities: A Critical Review. Medicines (Basel). 2016 Dec;3(4):25.
2. Zuzarte M, Salgueiro L. Essential Oils Chemistry. In: de Sousa, D. (eds) Bioactive Essential Oils and Cancer. Springer, Cham; c2015.
3. Tongnuanchan P, Benjakul S. Essential Oils: Extraction, Bioactivities, and Their Uses for Food Preservation. Journal of Food Science. 2014;79:7. R1231-R1249..
4. Sharifi-Rad J, Sureda A, Tenore GC, Daglia M, Sharifi-Rad M, Valussi M, *et al*. Biological Activities of Essential Oils: From Plant Chemoecology to Traditional Healing Systems. Molecules. 2017;22(1):70.
5. Guruvaurappan P, Sahoo MR, Varier RR, Rajendran A, Harekrihshna M, Karuppasamy R. Phytochemical Profiling, HPTLC Fingerprint and Antibacterial, Anti-fungal, and Antioxidant properties of essential oils extracted from *Cumminum cyminum*, *Zingiber officinale*, *Trachyspermum ammi*, *Alpinia galanga*, *Cedrus deodara*, and *Elettaria cardamomum*. Asian Journal of Pharmaceutical and Clinical Research. 2022;15:50-55.
6. Stojanović NM, Mladenović MZ, Randjelović PJ, Radulović NS. The potential of lemon balm (*Melissa officinalis* L.) essential oil as an anti-anxiety agent–is the citronellal the activity carrier?. Journal of Ethnopharmacology. 2023 Oct 5;314:116661.
7. Cimino C, Maure OM, Musumeci T, Bonaccorso A, Drago F, Souto EMB. Essential Oils: Pharmaceutical Applications and Encapsulation Strategies into Lipid-Based Delivery Systems. Pharmaceutics. 2021;13(3):327.
8. Dubar M, Anne-Charlotte D, Petit J, Delcourt-Debruyne E, Blaizot A, Agossa K. How effective are essential oils and essential oil constituents in the management of intra-oral halitosis: A systematic review. Journal of Herbal Medicine. 2023;39:100663.
9. Dagli N, Dagli R, Mahmoud RS, Baroudi K. Essential oils, their therapeutic properties, and implication in dentistry: A review. J Int Soc Prev Community Dent. 2015;5(5):335–340.
10. Abourashed EA, El-Alfy AT. Chemical diversity and pharmacological significance of the secondary metabolites of nutmeg (*Myristica fragrans* Houtt.). Phytochem Rev. 2016;15(6):1035–1056.
11. Ibrahim MA, Cantrell CL, Jeliazkova EA, Astatkie T, Zheljazkov VD. Utilization of Nutmeg (*Myristica fragrans* Houtt.) Seed Hydrodistillation Time to Produce Essential Oil Fractions with Varied Compositions and Pharmacological Effects. Molecules. 2020;25(3):565.
12. Sharafan M, Jafernik K, Ekiert H, Kubica P, Kocjan R, Blicharska E, *et al*. *Illicium verum* (Star Anise) and Trans-Anethole as Valuable Raw Materials for Medicinal and Cosmetic Applications. Molecules. 2022;19;27(3):650.
13. Sung YY, Kim YS, Kim HK. *Illicium verum* extract inhibits TNF-α- and IFN-γ-induced expression of chemokines and cytokines in human keratinocytes. J Ethnopharmacol. 2012 Oct 31;144(1):182-9.
14. Haro-González JN, Castillo-Herrera GA, Martínez-Velázquez M, Espinosa-Andrews H. Clove Essential Oil (*Syzygium aromaticum* L. Myrtaceae): Extraction, Chemical Composition, Food Applications, and Essential Bioactivity for Human Health. Molecules. 2021; 22;26(21):6387.
15. Badgujar SB, Patel VV, Bandivdekar AH. *Foeniculum vulgare* Mill: A Review of Its Botany, Phytochemistry, Pharmacology, Contemporary Application, and Toxicology. Biomed Res Int. 2014;2014:842674.

16. Zhang C, Fan L, Fan S, Wang J, Luo T, Tang Y, *et al*. *Cinnamomum cassia* Presl: A Review of Its Traditional Uses, Phytochemistry, Pharmacology and Toxicology. Molecules. 2019;24(19):3473.
17. Rao PV, Gan SH. Cinnamon: a multifaceted medicinal plant. Evid Based Complement Alternat Med. 2014;2014:642942.
18. Marston A. Thin-layer chromatography with biological detection in phytochemistry. Journal of Chromatography A. 2011;1218:2676–2683.
19. Akar Z, Kucuk M, Dogan D. A new colorimetric DPPH scavenging activity method with no need for a spectrophotometer applied on synthetic and natural antioxidants and medicinal herbs. Journal of enzyme inhibition and medicinal chemistry. 2017;32(1):640–647.
20. Matulyte I, Jekabsone A, Jankauskaite L, Zavistanaviciute P, Sakiene V, Bartkiene E, *et al*. The essential oil and hydrolats from Myristica fragrans seeds with magnesium aluminometasilicate as excipient: antioxidant, antibacterial, and anti-inflammatory activity. Foods. 2020 Jan 2;9(1):37.
21. Ashokkumar K, Simal-Gandara J, Murugan M, Dhanya MK, Pandian A. Nutmeg (*Myristica fragrans* Houtt.) essential oil: A review on its composition, biological, and pharmacological activities. Phytother Res. 2022;36(7):2839–2851.
22. Wang GW, Hu WT, Huang BK, Qin LP. Illicium verum: a review on its botany, traditional use, chemistry and pharmacology. Journal of Ethno Pharmacology. 2011 Jun 14;136(1):10-20.
23. Peng W, Lin Z, Wang L, Chang J, Fangliang G, Zhu X. Molecular characteristics of *Illicium verum* extractives to activate acquired immune response. Saudi J Biol Sci. 2016 May;23(3):348–352.
24. Sharafan M, Jafernik K, Ekiert H, Kubica P, Kocjan R, Blicharska E, *et al*. *Illicium verum* (Star Anise) and Trans-Anethole as Valuable Raw Materials for Medicinal and Cosmetic Applications. Molecules. 2022;27(3):650.
25. Selles SMA, Kouidri M, Belhamiti BT, Amrane AA. Chemical composition, *in vitro* antibacterial and antioxidant activities of *Syzygium aromaticum* essential oil. Food Measure. 2020;14(4):2352–2358.
26. Cortes-Rojas DF, de Souza CRF, Oliveira WP. Clove (*Syzygium aromaticum*): A precious spice. Asian Pac J Trop Biomed. 2014 Feb;4(2):90–96.
27. Chahbi A, Nassik A, El Amr H, Douaik A, El Haj El M, Boukharta M. Chemical Composition and Antimicrobial Activity of the Essential Oils of Two Aromatic Plants Cultivated in Morocco (*Cinnamomum cassia* and *Origanum compactum*). Journal of Chemistry; c2020. Article ID 1628710.
28. Huang DF, Xu JG, Liu JX, Zhang H, Hu QP. Chemical constituents, antibacterial activity and mechanism of action of the essential oil from *Cinnamomum cassia* bark against four food-related bacteria. Microbiology, 2014;83:357–365.
29. Miguel MG, Cruz C, Faleiro L, Simões MTF, Figueiredo AC, Barroso JG, *et al*. *Foeniculum vulgare* essential oils: chemical composition, antioxidant and antimicrobial activities. Nat Prod Commun. 2010;5(2):319-28.
30. Sharopov F, Valiev A, Satyal P, Gulmurodov I, Yusufi S, Setzer WN, *et al*. Cytotoxicity of the Essential Oil of Fennel (*Foeniculum vulgare*) from Tajikistan. Foods. 2017;6(9):73.
31. Badgujar SB, Patel VV, Bandivdekar AH. *Foeniculum vulgare* Mill: A Review of Its Botany, Phytochemistry, Pharmacology, Contemporary Application, and Toxicology. Biomed Res Int' c2014. p. 842674.