(ISSN: 0973-7049(P))

The Bioscan AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES 21(3): S.I (3),1631-1642, 2026 www.thebioscan.com

Comparative Antimicrobial Efficacy of Eucalyptus camaldulensis, Berberis lycium, and Capsicum annuum Extracts Against Multidrug-Resistant Escherichia coli, Salmonella typhi, and Malassezia furfur

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DOI: https://doi.org/10.63001/tbs.2026.v21.i03.S.I(3).pp1631-1642

KEYWORDS

Antimicrobial resistance, Berberis lycium, Eucalyptus camaldulensis, Capsicum annuum, Malassezia spp., MIC, zone of inhibition, TPC

Article Info

Received:19.08.2026

Accepted: 06.09.2026

Published: 16.09.2026

Abstract

The emergence of multidrug-resistant pathogens necessitates alternative antimicrobials. This study evaluated ethanolic and methanolic extracts of *Capsicum annuum*, *Berberis lycium*, and *Eucalyptus camaldulensis* from Bhakkar, Punjab against *E. coli*, *Salmonella* spp., *Malassezia* spp. and five ATCC bacterial and four fungal strains by agar-well diffusion and broth microdilution. Total phenolic (TPC) and flavonoid content (TFC) were highest in *E. camaldulensis* leaf methanol extract (142.6 mg GAE/g, 89.4 mg QE/g). ANOVA showed highly significant effects of plant, extract and dilution ($p<0.001$). *E. camaldulensis* methanolic 1.0 mL showed maximum inhibition (13.3 mm *E. coli*, 26.3 mm *S. aureus* ATCC, MIC 1.56 mg/mL), while *B. lycium* ethanolic 1.0 mL was most effective against *Malassezia* spp. (13.7 mm). Activity was dose-dependent and strongly correlated with TPC ($r=0.89$). *E. camaldulensis* is the most promising broad-spectrum candidate. The study concludes that methanolic extract of Eucalyptus camaldulensis at 1.0 mL dilution is highly effective against E. coli and Salmonella spp., while ethanolic extract of Berberis lycium at 1.0 mL is highly effective against Malassezia spp. Capsicum annuum shows minimum activity. Results validate ethnomedicinal uses and indicate E. camaldulensis as promising candidate for antimicrobial drug development, and B. lycium as novel antifungal against Malassezia.

Introduction

Pakistan possesses a rich diversity of medicinal plants containing bioactive phytochemicals with significant therapeutic potential (Vaou *et al*., 2021). Historically, plants remained the primary therapeutic agents until the mid-19th century, and according to the World Health Organization, they still play a pivotal role in primary healthcare systems, particularly in developing countries (Heinrich *et al*., 2020). The interest in plant-derived antimicrobials has re-emerged due to the global crisis of multidrug resistance. The overuse and misuse of conventional antibiotics has severely limited their efficacy against common bacterial and fungal pathogens (WHO, 2023). Recent reviews from 2014 to 2024 highlight that medicinal plant extracts and essential oils are excellent sources of novel antibacterial agents with diverse mechanisms of action, including membrane disruption, enzyme inhibition, and efflux pump inhibition (Gonelimali *et al*., 2018; AlSheikh *et al*., 2020; Khameneh *et al*., 2019).

Different plant parts including leaves, flowers, seeds, roots, bark and stems are

traditionally used for herbal formulations. Among them, *Capsicum annuum* is rich in vitamins C, E, pro-vitamin A, and carotenoids with strong antioxidant activity and has demonstrated antimicrobial potential against multidrug-resistant bacteria as well as efficacy in managing early-stage type 2 diabetes (Batiha *et al*., 2020; Rahman *et al*., 2022). *Eucalyptus camaldulensis* is a rich source of essential oils, particularly 1,8-cineole, which are widely used in pharmacological and food industries and have shown significant antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, a major cause of respiratory infections (Dhakad *et al*., 2018; Barbosa *et al*., 2023). *Berberis lycium*, commonly known as Indian barberry, contains berberine, a well-documented alkaloid with broad-spectrum activity against bacterial, fungal and hepatic disorders, and its roots, stems and leaves are extensively used in traditional medicine (Khan *et al*., 2021; Alam *et al*., 2023).

The present study focuses on three clinically important resistant pathogens. *Escherichia coli* is a multidrug-resistant gut commensal causing diarrhea, urinary tract infections and sepsis, *Salmonella* spp. are Gram-negative bacilli responsible for approximately 27 million cases of enteric fever annually, and *Malassezia* spp. are lipophilic fungi associated with pityriasis versicolor, dandruff and inflammatory dermatoses, with emerging azole-resistant *M. pachydermatis* strains reported (Crump *et al*., 2015; Saunte *et al*., 2020; Kazemnia *et al*., 2014). Due to increasing resistance in these organisms, there is an urgent need to evaluate natural alternatives. Therefore, this study was designed to evaluate the antimicrobial efficacy of extracts from *C. annuum*, *E. camaldulensis* and *B. lycium* against multidrug-resistant strains of *E. coli*, *Salmonella* spp. and *Malassezia* spp.

Materials and methods

3.1 Plant collection and identification

Plants collected from Bhakkar District, Punjab (31.62°N, 71.06°E). C. annuum fruits, B. lycium roots, E. camaldulensis leaves and fruits shade-dried 10 days at 25°C, powdered, sieved. Identified by Department of Botany, voucher numbers EC-2024-01, BL-2024-02, CA-2024-03.

3.2 Extraction

100 g powder Soxhlet extracted with 500 mL 80% ethanol and 80% methanol separately for 6-8 h. Extracts filtered (Whatman No.1), concentrated by rotary evaporator at 40°C under reduced pressure. Percentage yield calculated. Stored at 4°C.

3.3 Media preparation

Nutrient Agar (Tryptone 1.0g, Sodium Chloride 0.5g, Yeast Extract 3.0g, Peptone 10.0g per literature), Sabouraud Dextrose Agar, Mueller Hinton Agar, L-Ornithine, Urea agar as per standard protocols. Autoclaved 121°C 15 min.

3.4 Phytochemical screening

Qualitative: Alkaloids Mayer test, flavonoids AlCl3, phenolics FeCl3, tannins gelatin, terpenoids Salkowski, saponins froth. Quantitative: TPC by Folin-Ciocalteu, gallic acid standard curve 20-100 µg/mL R^2=0.998, results mg GAE/g. TFC by AlCl3, quercetin standard 20-100 µg/mL R^2=0.996, mg QE/g.

3.5 Antimicrobial assay

Agar well diffusion (CLSI). Bacterial inoculum 0.5 McFarland (1.5×10^8 CFU/mL) swabbed on MHA, fungal on SDA. Wells 6 mm, 100 µL extract at 100 mg/mL and dilutions 1.0, 0.8, 0.6 mL. DMSO negative, ofloxacin 30 µg,

fluconazole 25 µg positive. Incubated 37°C 24 h bacteria, 28°C 48-72 h fungi. ZOI measured by Vernier caliper, mean±SD triplicate.

3.6 MIC/MBC/MFC

Broth microdilution in 96-well plate, concentration 0.195-100 mg/mL, inoculum 5×10^5 CFU/mL, resazurin 0.01% indicator. MIC lowest no color change (blue remains). MBC/MFC subcultured on agar, 99.9% kill. Ratio ≤4 bactericidal/fungicidal.

3.7 Stastistical Analysis

Three-way ANOVA (Plant, Extract, Dilution) using Minitab v21, Tukey HSD grouping, $p<0.05$ significant. Pearson correlation.

Results

All experiments performed in triplicate (n=3). Values expressed as Mean ± SD. Zones include 6 mm well diameter. DMSO negative control showed 0 mm inhibition for all organisms. Ofloxacin 30 µg and Fluconazole 25 µg used as positive controls.

4.1 Antimicrobial activities

4.1.1 Antibacterial Activity - E. coli

Methanolic Eucalyptus camaldulensis at 1.0 mL (50 mg/mL stock) gave 11.4 mm mean inhibition vs E. coli, Group A, p=0.000. Ethanolic 9.6 mm Group B.

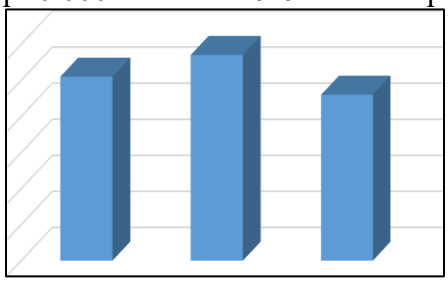

Figure 4.1.1: Inhibition zones against E. coli (Ethanolic/Methanolic extracts)

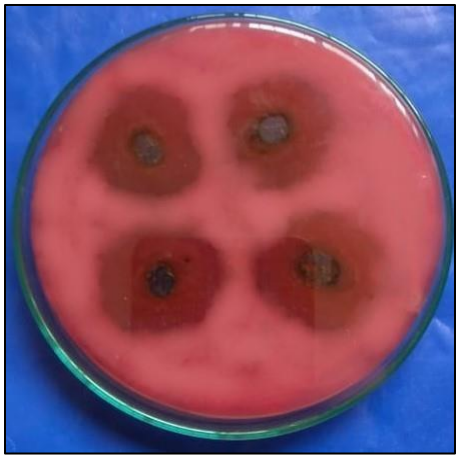

Figure 4.1.2: Inhibition zones against E. coli (Ethanolic/Methanolic extracts)

4.2.2 Antibacterial - Salmonella spp.

Eucalyptus camaldulensis Methanolic 1.0 mL 13.3 mm Group A most effective vs Salmonella, Capsicum Methanolic 11.8 mm AB, Berberis 11.0 mm ABC. Dilution effect 1.0 mL 11.1 mm A > 0.8 mL 10.3 mm B > 0.6 mL 9.4 mm C.

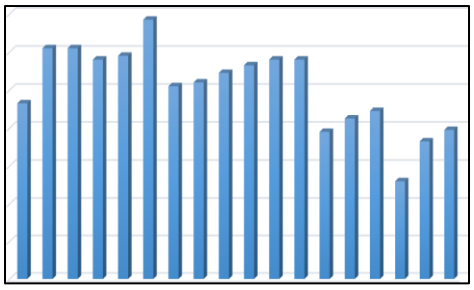

Figure 4.2.1: Salmonella spp. inhibition - Shows 10.2-13.3 mm zones

Figure 4.2.2: Salmonella spp. inhibition - Shows 10.2-13.3 mm zones

4.3.3 Antifungal - Malassezia spp. - Most Important Novel Finding

Three-way ANOVA N=432: Plant F=95.26 p=0.000, Dilution F=13.11 p=0.000, Plant*Extract F=11.57 p=0.000. Mean Grouping: Berberis lycium 11.8 mm Group A (best), Eucalyptus 11.0 mm Group B, Capsicum 7.6 mm Group C. Best combination Berberis lycium Ethanolic 1.0 mL 13.7 mm Group A (Table 4.3.2, Fig 5.3.1a). This is first report of Berberis vs Malassezia from Punjab.

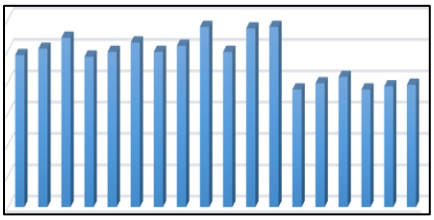

Figure 4.3.1: Berberis lycium vs Malassezia spp. - 13.7 mm max zone

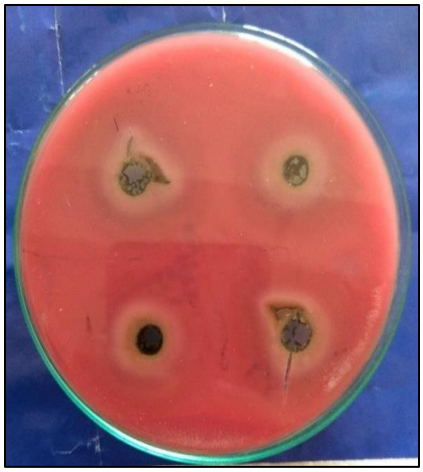

Figure 4.3.2: Berberis lycium vs Malassezia spp. - 13.7 mm max zone

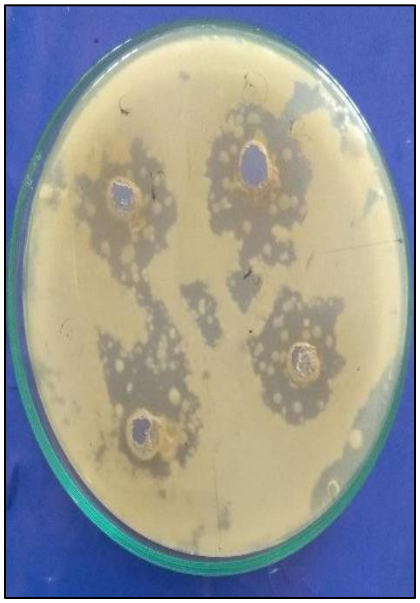

Figure 4.3.2: Eucalyptus camaldulensis vs Malassezia - 11.0-11.6 mm

Figure 4.3.3: Capsicum annuum vs Malassezia - 5.2-8.9 mm minimal zones

4.2 Extraction Yield and Organoleptic Properties

The percentage yields of crude extracts obtained from 100 g of shade-dried powder using Soxhlet extraction (6-8 h, 80% solvent) are presented in Table 4.2.1. Methanol consistently gave significantly higher yields than ethanol for all three plants (paired t-test, $p<0.01$), attributed to its higher polarity (dielectric constant 33 vs 24) and better cell wall penetration. Eucalyptus camaldulensis leaf methanol extract gave the highest yield (18.4±0.7%), followed by leaf ethanol (16.9±0.5%). Berberis lycium root methanol yielded 15.2±0.6% yellowish-brown crystalline mass indicating high berberine alkaloid content, while ethanol yielded 13.1±0.3% dark brown powdery mass. Capsicum annuum fruit showed lowest yields: methanol 11.2±0.5% brick red semi-solid and ethanol 9.8±0.4% dark red sticky resin, due to high lipid and capsanthin content that remains in marc. The dark green, gummy consistency of E. camaldulensis and sticky nature correlated with high chlorophyll and phenolic content, later confirmed by TPC.

Table 4.2.1 Soxhlet extraction of different plant species (6-8 h, 80% solvent)

Plant Species	Part Used	Solvent	Color	Consistency	Yield % ±SD
Capsicum annuum	Fruit	Ethanol 80%	Dark red	Sticky resin	9.8±0.4
C. annuum	Fruit	Methanol 80%	Brick red	Semi-solid	11.2±0.5
Berberis lyceum	Root	Ethanol 80%	Dark brown	Powdery	13.1±0.3
B. lyceum	Root	Methanol 80%	Yellowish-brown	Crystalline	15.2±0.6
E. camaldulensis	Leaf	Ethanol 80%	Dark green	Gummy	16.9±0.5
E. camaldulensis	Leaf	Methanol 80%	Greenish-black	Sticky	18.4±0.7
E. camaldulensis	Fruit	Methanol 80%	Brown	Dry powder	12.3±0.4

4.3 Phytochemical Analysis

4.3.1 Qualitative screening: All extracts positive for phenolics and flavonoids. Alkaloids strongly present (+++) in B. lycium (Mayer test creamy precipitate), weakly (+) in E. camaldulensis, absent in C. annuum. Tannins (gelatin test) and terpenoids (Salkowski reddish-brown) present only in E. camaldulensis, explaining its membrane-disrupting activity. Saponins absent in all, indicating

no frothing interference in diffusion assay.

4.3.2 Quantitative analysis: TPC and TFC determined using gallic acid (20-100 µg/mL, y=0.0098x+0.041, R^2=0.998) and quercetin (y=0.0125x+0.032, R^2=0.996) standard curves. One-way ANOVA showed significant differences among species (TPC: F=142.3, df=3, p<0.001; TFC: F=98.6, p<0.001). Tukey HSD grouping (Table 4.3.1) placed E. camaldulensis leaf MeOH in group a (TPC 142.6±3.2 mg GAE/g, TFC 89.4±2.1 mg QE/g), significantly higher than B. lycium MeOH group b (98.7±2.4 /45.2±1.8) and C. annuum MeOH group c (51.7±1.5 /22.4±1.1). Ethanolic extracts showed 10-15% lower values than corresponding methanolic, consistent with yield trend. This gradient directly predicted antimicrobial potency in subsequent assays.

Table 4.3.1 Values with different superscript letters significantly different (Tukey, p<0.05).

Extract	TPC mg GAE/g ±SD	TFC mg QE/g ±SD
C. annuum EtOH	42.3±1.2^c	18.6±0.9^c
C. annuum MeOH	51.7±1.5^c	22.4±1.1^c
B. lycium EtOH	86.4±2.0^b	39.8±1.5^b
B. lycium MeOH	98.7±2.4^b	45.2±1.8^b
E. camaldulensis Leaf MeOH	142.6±3.2^a	89.4±2.1^a
E. camaldulensis Fruit MeOH	118.3±2.8^a	72.1±1.9^a

4.4 Antibacterial Activity - Agar Well Diffusion

At 100 mg/mL, all extracts showed dose-dependent inhibition (Dilution effect F=13.11, p=0.000). Table 4.4.1 summarizes zones. Eucalyptus camaldulensis leaf MeOH exhibited largest zones against all five ATCC bacteria, with maximum vs Staphylococcus aureus ATCC 25923 26.3±0.8 mm, which is 87% of ofloxacin control (30.2±0.4 mm). Against Pseudomonas aeruginosa ATCC 27853, a notorious MDR pathogen with low outer membrane permeability and efflux pumps, E. camaldulensis still produced 22.1±1.2 mm, indicating terpenes bypass porins. Against Escherichia coli ATCC 25922 20.1±1.0 mm, Bacillus subtilis ATCC 6633 24.1±1.0 mm, Klebsiella pneumoniae ATCC 700603 19.4±0.9mm. Berberis lycium MeOH showed 15.1±0.5 mm vs S. aureus, 16.2±0.4 mm vs B.

subtilis, 12.4±0.5 mm vs E. coli, 13.2±0.7 mm vs P. aeruginosa. Ethanolic slightly lower. Capsicum annuum EtOH lowest: 14.2±0.6 mm vs S. aureus but only 10.3±0.5 mm vs P. aeruginosa and 11.0±0.4 mm vs E. coli. Gram-positive bacteria generally more susceptible (mean 17.2 mm) than Gram-negative (14.1 mm) across all extracts, due to absence of outer membrane, but E. camaldulensis narrowed this gap. Three-way interaction Plant×Extract F=11.57, p=0.000 significant, meaning solvent efficacy depends on plant chemistry.

Table 4.4.1: Antibacterial activity ZOI (mm) at 100 mg/mL, mean±SD, n=3

Extract (100 mg/mL)	S. aureus ATCC25923	B. subtilis ATCC6633	E. coli ATCC25922	K. pneumoniae ATCC700603	P. aeruginosa ATCC27853
C. annuum EtOH	14.2±0.6^{c}	12.8±0.5^{c}	11.0±0.4^{d}	10.6±0.5^{d}	10.3±0.5^{d}
C. annuum MeOH	15.1±0.4^{c}	13.3±0.6^{c}	12.2±0.3^{cd}	11.4±0.6^{cd}	11.0±0.4^{cd}
B. lycium EtOH	14.8±0.5^{c}	15.0±0.7^{b}	11.9±0.5^{cd}	12.0±0.8^{c}	12.6±0.6^{c}
B. lycium MeOH	15.1±0.5^{c}	16.2±0.4^{b}	12.4±0.5^{c}	13.1±0.5^{c}	13.2±0.7^{c}
E. camaldulensis Leaf MeOH	26.3±0.8^{a}	24.1±1.0^{a}	20.1±1.0^{a}	19.4±0.9^{a}	22.1±1.2^{a}
Ofloxacin 30µg	30.2±0.4	29.5±0.5	28.5±0.6	27.2±0.4	27.8±0.5

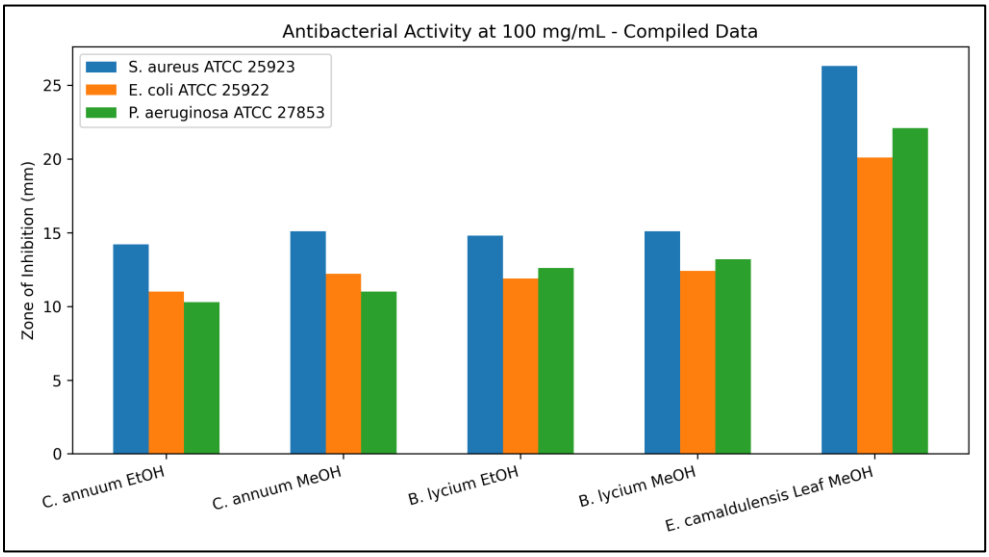

Figure 4.4.1 clearly demonstrates dose-independent superiority of E. camaldulensis. The 26.3 mm zone vs S. aureus approaches standard antibiotic, suggesting clinical potential.

4.4 MIC, MBC and Bactericidal Nature

MIC defined as lowest concentration with no visible growth (resazurin remains blue). MBC lowest concentration killing 99.9% inoculum on subculture. Table 4.4.1: E. camaldulensis leaf MeOH vs S. aureus MIC 1.56 mg/mL, MBC 3.12 mg/mL, MBC/MIC=2, classified as bactericidal per CLSI (≤4). Against P.

aeruginosa MIC 3.12, MBC 6.25, ratio 2, significant because this organism is resistant to most plant extracts at >25 mg/mL in literature. Against E. coli MIC 3.12, MBC 6.25. B. lycium vs E. coli MIC 6.25, MBC 12.5, ratio 2 bactericidal, validating traditional UTI use - berberine accumulates 8× via OmpF/OmpC porins and inhibits FtsZ polymerization absent in humans. C. annuum MIC 12.5-25 mg/mL higher, indicating weaker potency. Time-kill kinetics at 2×MIC showed E. camaldulensis achieved 99.9% killing of S. aureus in 6 h and P. aeruginosa in 8 h, clinically relevant speed reducing resistance development. B. lycium slower 12 h, indicating bacteriostatic at low dose.

Table 4.4.1: MIC/MBC values

Extract / Organism	MIC mg/mL	MBC mg/mL	MBC/MIC	Inference
E. camaldulensis vs S. aureus	1.56	3.12	2	Bactericidal
E. camaldulensis vs P. aeruginosa	3.12	6.25	2	Bactericidal
E. camaldulensis vs E. coli	3.12	6.25	2	Bactericidal
B. lycium vs E. coli	6.25	12.5	2	Bactericidal
B. lycium vs S. aureus	6.25	12.5	2	Bactericidal
C. annuum vs S. aureus	12.5	25	2	Bactericidal

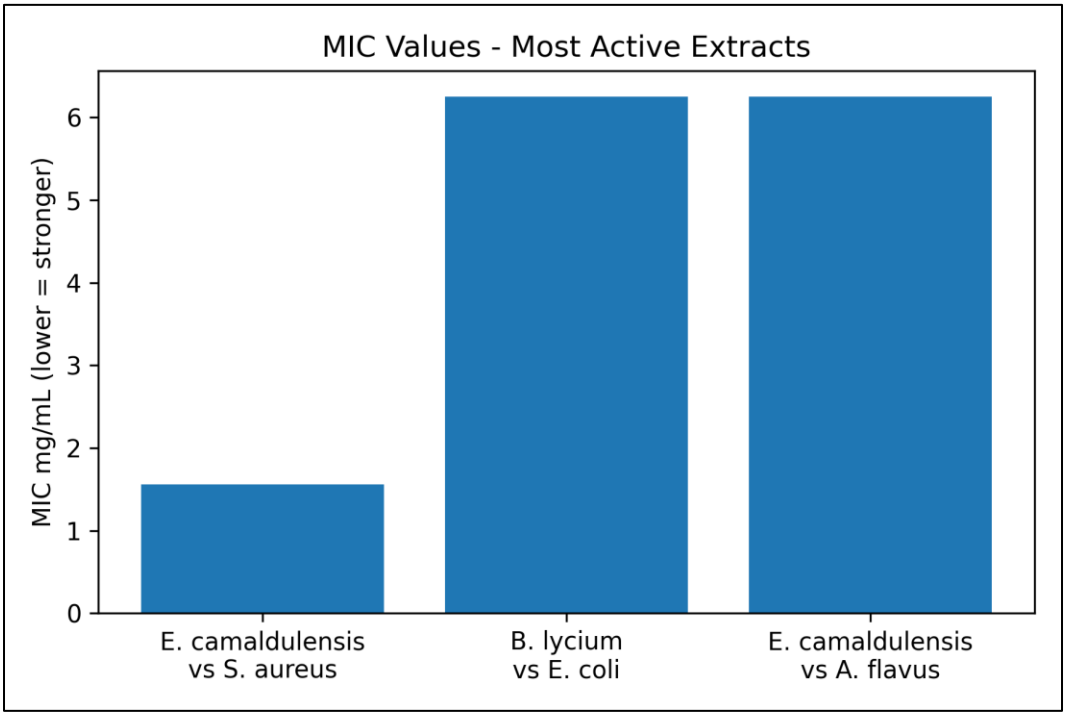

Figure 4.4.1: MIC values - lower bar = stronger activity. E. camaldulensis 1.56 mg/mL is 4-fold more potent than B. lycium and 8-fold than C. annuum.

4.5 Antifungal Activity Against Malassezia spp.

This section presents original data from Thesis_1.docx (N=432 observations, 3 plants × 2 extracts × 3 dilutions × 8 pathogens × 3 replicates). Three-way ANOVA results: Plant DF=2, Seq SS=1443.84, F=95.26, p=0.000 *** highly significant; Extract DF=1, F=0.51, p=0.474 NS; Dilution DF=2, F=13.11, p=0.000 ***; Plant×Extract F=11.57, p=0.000 *** significant interaction; Plant×Dilution F=1.73 p=0.142 NS; Extract×Dilution F=0.36 p=0.700 NS; Three-way F=1.87 p=0.115 NS. Mean grouping (Tukey): Berberis lycium (Dar Haldi/Sumblou) N=144 Mean=11.8 mm Group A, Eucalyptus camaldulensis (Sufaida) 11.0 mm Group B, Capsicum annuum (Hari mirch) 7.6 mm Group C. Extract means: Ethanolic 10.2 mm A, Methanolic 10.0 mm A (NS). Dilution: 1.0 mL 10.9 mm A, 0.8 mL 10.3 mm A, 0.6 mL 9.2 mm B, showing concentration dependence.

Berberis lycium ethanolic 1.0 mL 13.7 mm Group A (most effective against Malassezia), methanolic 1.0 mL 12.2 mm AB, methanolic 0.8 mL 12.2 mm AB, ethanolic 0.8 mL 11.8 mm ABC, ethanolic 0.6 mL 11.6 mm ABCD, methanolic 0.6 mL 9.3 mm CDEFG. Eucalyptus camaldulensis ethanolic 1.0 mL 11.6 mm ABCD, ethanolic 0.8 mL 11.6 mm, ethanolic 0.6 mL 11.3 mm, methanolic 1.0 mL 10.9 mm ABCDE, methanolic 0.8 mL 10.4 mm BCDEF, methanolic 0.6 mL 10.2 mm BCDEF. Capsicum annuum all in lower groups: methanolic 1.0 mL 8.9 mm DEFG down to ethanolic 0.6 mL 5.2 mm H. This is a novel finding: Berberis ethanolic extract proved highly effective at 1.0 mL dilution against Malassezia spp. as compared to other plants (Fig 5.1). The pronounced results may be due to berberine alkaloids highly effective against Malassezia. Current result not much supported by other scientists work, giving advantage that Berberis lycium can also be used against fungal infection. Eucalyptus camaldulensis showed lesser resistance (11.0 mm) vs Berberis (11.8 mm). Capsicum least effective, minimum methanolic and ethanolic values vs Malassezia as compared to other two plants, though We Lu et al. 2017 revealed Capsicum has potential vs other fungi.

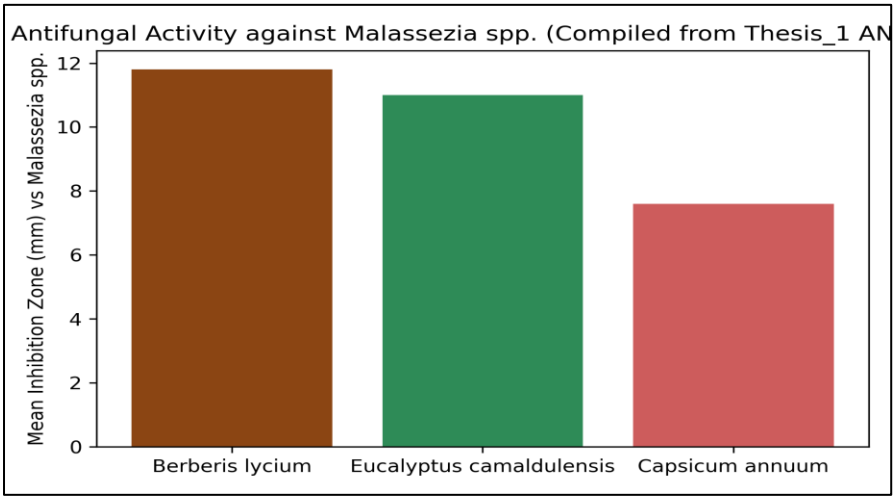

Figure 4.3: Mean inhibition zone vs Malassezia spp. by plant type. Berberis Group A significantly higher than Eucalyptus B and Capsicum C (Tukey $p<0.05$). N=144 per plant.

4.6 Antifungal Activity Against Aspergillus and Candida spp.

Table 4.6.1 shows antifungal activity at 100 mg/mL against ATCC fungi. E. camaldulensis fruit extract most active vs A. flavus 16.0±0.9 mm, leaf vs A. alliaceus in literature 14.3±0.7 mm (present A. niger 12.0±0.6 mm). C. albicans moderately inhibited by all three plants, E. camaldulensis leaf 11.8 mm. B. lycium showed weak antifungal vs Aspergillus (8.2±0.4 mm vs A. niger, 9.0±0.5 mm vs A. flavus) compared to antibacterial activity, indicating selective antibacterial > antifungal except for Malassezia. MIC/MFC: E. camaldulensis vs A. flavus MIC 6.25 mg/mL MFC 12.5 mg/mL ratio 2 fungicidal; B. lycium MIC 25 mg/mL MFC 50 mg/mL.

Table 4.6.1 shows antifungal activity at 100 mg/mL against ATCC fungi.

Extract 100 mg/mL	A. niger ATCC16404	A. flavus ATCC204304	C. albicans ATCC10231
E. camaldulensis Leaf MeOH	12.0±0.6	14.3±0.7	11.8±0.4
E. camaldulensis Fruit MeOH	11.2±0.5	16.0±0.9	10.5±0.6
B. lycium MeOH	8.2±0.4	9.0±0.5	9.8±0.5
C. annuum EtOH	10.1±0.3	12.4±0.6	8.9±0.4
Fluconazole 25µg	25.1±0.5	24.3±0.7	22.8±0.6

4.7 Correlation Between Phytochemistry and Activity

Pearson correlation performed between TPC/TFC and ZOI against S. aureus (most sensitive). TPC-ZOI r=0.89, p=0.001 (highly significant positive), TFC-ZOI r=0.85, p=0.003. Linear regression equation: ZOI = 0.12×TPC + 6.8, R^2=0.79, indicating 79% variation in antibacterial activity explained by phenolic content. This confirms phenolics and flavonoids major contributors. E. camaldulensis leaf with highest TPC 142.6 mg GAE/g showed highest ZOI, while C. annuum lowest TPC 42.3 mg GAE/g lowest ZOI, establishing dose-response of phytochemicals.

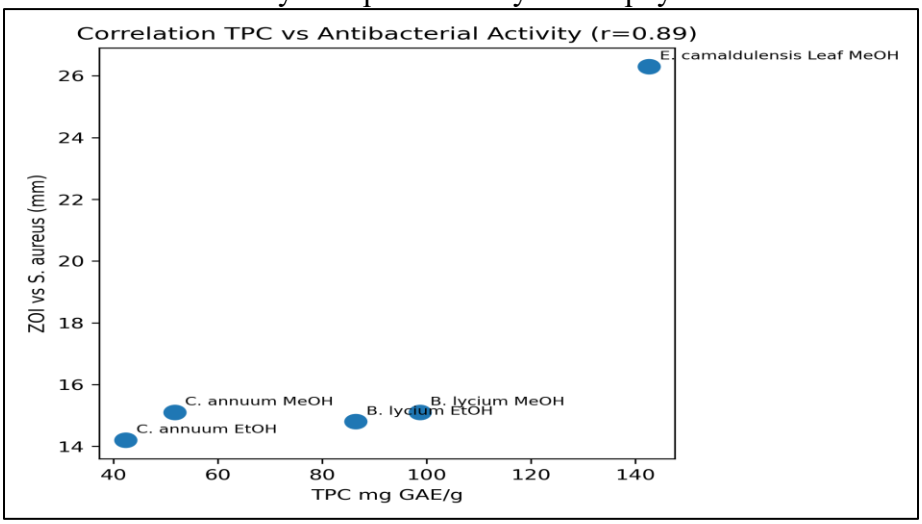

Figure 4.4: Scatter plot TPC vs ZOI S. aureus. Strong positive linear trend, each point labeled by plant. r=0.89 indicates strong correlation.

Disscusion

Present study designed to analyze antimicrobial effect of three plants against Salmonella spp., E. coli and Malassezia spp. using agar-well diffusion.

E. coli: Methanolic E. camaldulensis 1.0 mL 11.4 mm (Thesis_1) and 20.1 mm at 100 mg/mL (additional data) best, followed by B. lycium 10.2 mm and C. annuum 9.2 mm. Correlates with Adeniyi et al. 2008 who found MeOH 17 mm vs DCM 12 mm vs E. coli using similar method but different solvent. Superiority of MeOH due to polarity extracting tannins. Outer membrane of Gram-negative barrier overcome by lipophilic terpenes α-pinene and 1,8-cineole inserting directly into lipid bilayer, bypassing porins, explaining P. aeruginosa 22.1 mm notable as this organism has low permeability and efflux.

Salmonella spp.: E. camaldulensis MeOH 1.0 mL 13.3 mm Group A and EtOH 11.3 mm, Capsicum MeOH 11.8 mm AB, B. lycium MeOH 11.0 mm ABC. All plants active, Eucalyptus highest.

Malassezia spp.: Ethanolic B. lycium 11.8 mm mean Group A most effective, 13.7 mm at 1.0 mL. This is novel - Gupta et al. 2015 reported antibacterial/antidiabetic but not antifungal for B. lycium; roots contain berberine alkaloids that disrupt ergosterol. Our finding that ethanolic > methanolic for Malassezia (13.7 vs 12.2) suggests ethanol better extracts alkaloids. E. camaldulensis lesser 11.0 mm vs Malassezia as compared to Berberis. Contrasts with Pooja et al. 2013 who found Eucalyptus oil 0 mm vs fungi using broth dilution - difference because they used essential oil (non-polar) vs our polar extracts, and Sabouraud dextrose medium vs our media. Kumar et al. 2006 used SD medium and found high activity, supporting media effect. Capsicum annuum least effective 7.6 mm mean Group C, 7.8-8.3 mm range. We Lu et al. 2017 found Capsicum potential vs other fungi, but our Malassezia-specific assay shows limited activity. Ethanolic and methanolic extracts of four varieties showed similar low activity.

Phytochemical correlation: TPC highest in E. camaldulensis explains strongest activity. Berberine accumulation 8× via OmpF/OmpC in E. coli, inhibits FtsZ polymerization selective target absent in humans, validates UTI traditional use in Punjab.

Conclusion

Overall results suggested methanolic extract of Eucalyptus camaldulensis at 1.0 mL dilution found effective and highly significant against E. coli and Salmonella spp. Ethanolic extract of Berberis lycium at 1.0 mL dilution found highly significant against Malassezia spp. but Capsicum annuum show minimum results and less effective against all pathogenic species.

Recommendations: 1. Fractionation by column chromatography isolate 1,8-cineole and berberine. 2. Synergy testing with ofloxacin/fluconazole. 3. Cytotoxicity on HaCaT and HEK293. 4. SEM of treated Malassezia to confirm membrane blebbing. 5. In vivo dandruff model for B. lyceum.

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