ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL OF *PHLOMIS BRACTEOSA*

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Abstract: The essential oil was isolated from the aerial parts of *Phlomis bracteosa* Royle ex Benth., a hairy erect herb. The leaves and flowers of this species are used as carminative, stimulant and locally known as 'Ballikotu, Calba, Salba' in Turkish traditional medicine. The antimicrobial activity of *Phlomis bracteosa* was studied using well diffusion method. The activity was tested against human pathogenic bacteria and fungi at different concentrations (0.25 µg/ml, 0.125 µg/ml and 0.062 µg/ml) of the essential oil.

Keywords: *Phlomis bracteosa*; Lamiaceae; Antimicrobial activity.

INTRODUCTION

Essential oils are known to possess antimicrobial activity, which has been evaluated mainly in liquid medium. Several essential oils and their isolates have been found to exhibit strong antibacterial and antifungal activity. The essential oil are supposed to interfere with intermediary metabolism of microorganisms by changing the rate of an enzyme reaction influencing nutrient uptake from the medium affecting enzyme synthesis at nuclear or ribosomal level or changing membrane structures.

Phlomis bracteosa Royle ex Benth. of the family Lamiaceae is an erect hairy plant 20-80 cm, with heart-shaped toothed leaves, and pink-purple flowers crowded into a few large whorls and forming an interrupted spike. The whorls are 2.5-4 cm across, corolla 1.5-2 cm, the tube shorter than the calyx, upper lip larger hooded, very hairy and with a fringe of white hairs, the lower lip smaller, 3-lobed, calyx hairy, with five narrow awl-shaped teeth, much shorter than the calyx tube. The bracts linear-lanceolate, bristly-haired, without a spiny tip. The leaves are 5-10 cm, stalked, hairy. The plant is distributed in temperate region from Kashmir to Kumaun and Afghanistan to South West China from an elevation of 1200-4000 m[1,2].

Phlomis species are recorded as herbal drugs being used ethno pharmacologically, tonic and as stimulant[3]. The leaves and flowers of *Phlomis* species are used as carminative, stimulant and locally known as 'Ballikotu, Calba, Salba' in Turkish traditional medicine[4,5]. Several essential oil constituents of the *Phlomis* species have been reported from different parts of the world[6-16]. The major constituent of the essential oil of *Phlomis bracteosa* was reported as germacrene D (34.3%). Other minor constituents were sabinene (6.2%), germacrene D-4-ol (4.9%), linalool (4.8%) and α-bulnesene (3.5%)[17].

The essential oils of *Phlomis lanata, Phlomis fruticosa, Phlomis cretica, Phlomis samia, Phlomis ferruginea* and the ethanol extract of *Phlomis fruticosa* showed antimicrobial activities[15, 18-20], while the essential oil of *Phlomis linearis* has also been reported anti-angiogenic and anti-inflammatory activity[21]. The iridoids, like lamiide isolated from the dichloromethane and methanol extracts of *Phlomis crinita* showed the topical anti-inflammatory activity[22]. The compounds, luteolin 7-O-â-D-glucopyranoside and chrysoeriol 7-O-â-D-glucopyranoside were isolated from *Phlomis brunneogaleata* and have anti-malarial property[23]. *Phlomis grandiflora* used in folk remedy as gastroprotective crude drug and possess antiulcerogenic activity[24]. To the best of our knowledge this is the first report on the antimicrobial activity of the essential oil of *Phlomis bracteosa.*

MATERIALS AND METHODS

Plant Material

The aerial parts of *Phlomis bracteosa* were collected before flowering phase from the Pindari glacier area, district Bageswer of North-West Himalaya of Uttarakhand, India, at a height of 3200 m. The plant was identified by Prof. Y. P. S. Pangtey, Botany Department, Kumaun University, Nainital. The

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voucher specimen (No. 3318) was confirmed and deposited in the herbarium of the Botany Department, Forest Research Institute, Dehradun.

Isolation of Essential Oil

The aerial parts (1 kg) of *Phlomis bracteosa* were steam distilled for 3 hours using a copper still fitted with spiral glass condensers for 3 hours and extracted with *n*-hexane and dichloromethane. The organic phase was dried over anhydrous sodium sulphate and the solvent was recovered using a thin film rotary vacuum evaporator at 25°-30°C. The oil yield was 0.02% (v/w).

Antimicrobial Assay

Media Preparation

Bacterial Media

Nutrient agar (NA) was used for the screening of antibacterial activity of Gram-positive and Gram-negative bacteria. NA was weighed as per instructions provided by the manufacturer and dissolved in distilled water. After proper plugging, it was autoclaved at 120°C and 15 lbs for 20 minutes. Autoclaved nutrient agar when cooled at 45°C was poured into sterilized petri dishes containing nearly 20 ml agar medium under aseptic condition and kept undisturbed as such till solidify. After solidification, these petri plates were incubated at 37°C±1°C overnight for sterility testing.

Fungal Media

Preparations of potato dextrose agar (PDA), sabouraud's agar (SA) and yeast extract potato dextrose agar (YEPDA) media were used as per instruction provided by the manufacturer, for different fungal strains viz., *Micrococus canis, trichophyton rubrum* and *Candida albicans,* respectively. After proper plugging, the media were autoclaved at 120°C and 15 lbs for 20 minutes. Autoclaved PDA, SA and YEPD media when cooled at 45°C was poured into sterilized petri plates containing nearly 20 ml growth media under aseptic condition till solidified. After solidification these petri plates were incubated for sterility testing. The PDA plates incubated at 25°C± 1°C while SA and YEPD plates at 30°C± 1°C for ten days, seven days and 48 hours, respectively.

Antibacterial Activity of the Essential Oil

The essential oil of 0.25 µg/ml, 0.125 µg/ml and 0.062 µg/ml were prepared with some modification in 50% ethanol[25-27] and tested against human pathogens *Staphylococcus aureus* (Gram-positive), *Pseudomonas aeruginosa, Escherichia coli and Proteus vulgaris* ((Gram-negative) bacteria. It was demonstrated by well diffusion method.

Antifungal Activity of the Essential Oil

The essential oil of 0.25µg/ml, 0.12µg/ml and 0.062µg/ml were prepared with some modification in 50% ethanol[25-27] and tested against *Microsporum canis*, *Trichophyton rubrum* and *Candida albicans* fungi. It was demonstrated by well diffusion method.

Well Diffusion Method

Antibacterial and antifungal activities of the essential oil of *Phlomis bracteosa* were tested using well diffusion method[28]. The autoclaved media was poured in the sterilized petri plates. These plates were dried for a period of 20 minutes under aseptic condition before its use. Freshly grown cultures of the tested bacteria and fungi in their media were streaked over the plates using a platinum wire inoculation loop. On sterile media plates, well of 6.0 mm diameter were punched with the help of a sterile gel cutter. Wells were sealed with the molten media to prevent the escape of essential oil through bottom. In the well of separate Petri plates 15 µl of different concentrations (0.25µg/ml, 0.125µg/ml and 0.062µg/ml) of essential oil were delivered. The positive control were used *gentamicin* 1% (w/v) (Fulford (India) Limited, Hyderabad) and *fluconazole* 1% (w/v) (Lark Laboratories (India) Ltd., New Delhi) for antibacterial and antifungal activity, respectively. The plates were incubated at 37°C ± 1°C for 24 hours for Gram-positive and Gram-negative bacteria and 25°C ± 1°C, 30°C ± 1°C and 30°C ± 1°C for *Microsporum canis*, *Trichophyton rubrum* and *Candida albicans* fungi for ten days, seven days and 48 hours respectively. The plates were observed for the zone clearance around the wells. The zones of inhibition were calculated by measuring the diameter of the inhibition zone around the well in millimeter including the well diameter. The readings were taken in three different replicates and the average values were tabulated (Table 1).

Table 1: Antimicrobial activity of the essential oil of *Phlomis bracteosa* Royle ex Benth.

Microorganism / Bacteria	Zone of Inhibition (mm)			
Gram positive bacteria	Concentration (µg/ml) of essential oil [a]			Standard (µg/ml)[b]
	0.25	0.125	0.062	
Staphylococcus aureus (MTCC 737)	18	18	14	33
Gram negative bacteria				
Pseudomonas aeruginosa (MTCC414)	21	10	NA	28
Escherichia coli (MTCC 443)	18	14	12	31
Proteus vulgaris (MTCC 426)	20	16	15	34
Fungi				
Microsporum canis (MTCC 2820)	18	18	14	20
Trichophyton rubrum (MTCC 296)	16	16	14	20
Candida albicans (MTCC 183)	28	27	24	30

[a] Dilution of essential oil in 50 % ethanol, V/V: applied dose: 15 µl

[b] Standard: applied dose: 15 µl

Diameter of well = 6 mm

NA= No activity

RESULTS AND DISCUSSION

The present study was designed to evaluate the qualitative antimicrobial activity of the aerial parts of *Phlomis bracteosa* essential oil. The essential oil from this plant exhibited antimicrobial activity. The activity may be attributed to the presence of germacrene D, sabinene, α-bulnesene, germacrene D-4-ol, linalool, eugenol and isoeugenol or other miner constituents[17] were present in *Phlomis bracteosa* essential oil. The chemical components exert their toxic effects against the microorganisms through the disruption of bacteria or fungal membrane integrity[29-31]. The antimicrobial activity of the essential oil of *Phlomis bracteosa* showed significant activity against the tested microorganisms at three different concentrations (0.25 µg/ml, 0.125 µg/ml and 0.062 µg/ml). The results of antimicrobial activity of the essential oil using well diffusion assay are summarized in Table 1. The oil showed significant activity against tested Gram-positive and Gram-negative bacteria and *Trichophyton rubrum* was much susceptible to the essential oil, followed by *Microsporum canis* and *Candida albicans* are relatively less susceptible. The principal component of the essential oil of *Phlomis bracteosa* was germacrene D. The literature revealed that the germacrene D rich essential showed good antimicrobial and cytotoxicity activity. *In vitro* cytotoxicity activity of germacrand D was found cytotoxic against Human Hs 578T breast ductal carcinoma cells[32]. According to previous reports eugenol displayed potent activity against *Candida albicans* biofilms *in vitro* with low cytotoxicity and therefore has potential therapeutic implication for biofilm-associated candidal infections[33]. Another study also revealed the presence of eugenol in the essential oil of *Ocimum gratissimum* showed good antimicrobial activity[34].

CONCLUSION

In vitro antimicrobial activity of the essential oil of *Phlomis bracteosa* showed significant activity against tested human pathogens. Thus the oil of *Phlomis bracteosa* could be a source of germacrene D a good cytotoxic and antimicrobial constituent.

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