International Journal of Botany Studies
ISSN: 2455-541X; Impact Factor: RJIF 5.12
Received: 16-05-2021, Accepted: 31-05-2021, Published: 15-06-2021
www.botanyjournals.com
Volume 6, Issue 3, 2021, Page No. 684-687

Antimicrobial activity of *Hyptis capitata* Jacq.-An ethnomedicinal plant of lamiaceae

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Abstract
Hyptis capitata Jacq. is commonly known as 'knob weed' is an exotic and slightly aromatic herb of Lamiaceae. The present study focuses on the primary phytochemical analysis and evaluation of the antimicrobial activity of methanolic leaf extract of *H. capitata* against bacterial strains such as *Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli* and fungal strains *viz*., *Candida albicans, Aspergillus niger* and *Penicillium chrysogenum* by agar well diffusion method. The methanolic leaf extract of *H. capitata* showed a maximum zone of inhibition of 12 ± 0.11 mm against *Staphylococcus aureus* ($P < 0.001$) followed by *Bacillus subtilis* and *Klebsiella pneumoniae* at the concentration of 160 µg. The methanolic leaf extract failed to display activity against the selected fungal strains. Moreover, the phytochemical screening revealed the presence of potential phytoconstituents which may account for the antibacterial activity of the promising medicinal herb, *Hyptis capitata*.

Keywords: *Hyptis capitata*, antibacterial, antifungal, phytoconstituents

Introduction
Although, 21st century witness incredible achievement in the development of science, technology and medicine, the control and prevent the sudden spreading of infectious diseases emerged as a major threat to global health security. World Health Organisation, 1998 reported that infectious diseases represent a serious problem to health and they are one of the main causes of morbidity and mortality. The majority of synthetic antibiotic drugs effectively control the growth and development of microorganisms and they are very much toxic at their optimum dosage level [1]. The commonly used antibiotics become less effective due to the toxic reactions and emergence of new drug-resistant microbes. The increasing number of pathogenic organisms and their resistance to antimicrobial drugs threatens the prevention of some infections. Consequently, some infections have become intensely difficult and in some cases nearly impossible to treat [2]. Natural products of traditionally important medicinal plants may serve as a new source of antimicrobial agents [3]. Samy and Ignacimuthu (2000) [4], informed that plant-based antimicrobials were effective in the treatment of several microbial infections and they possessed fewer side effects than synthetic antibiotic drugs.

The discovery of new phytodrugs with higher antimicrobial properties is the need of the day to protect human health. The plant *Hyptis capitata* is a slightly aromatic perennial herb commonly called knob weed belongs to Lamiaceae. The plant has some folk information on wound healing, anti-inflammatory, anti-cough and anti-spasmodic properties.

The traditional healers of tribal communities of the Philippines and Indonesia use *H.capitata* for the healing of wounds. The scientific validation of the antimicrobial activity of the herb is scanty. Hence, the present study was undertaken to evaluate the antibacterial and antifungal activity of the leaf of *H. capitata*.

Materials and Methods
Plant Collection and Extraction
Hyptis capitata was collected from the natural habitat of Thiruvananthapuram district, Kerala. The plant was identified and authenticated by the Department of Botany, University of Kerala and the voucher specimen (KUBH-6166) has been deposited in the herbarium. Fresh leaves were collected, washed thoroughly, shade dried and powdered. About 25 gm dried leaf powder was extracted in Soxhlet extractor using methanol for 48 hrs to 72 hrs. The extract was filtered through Whatman No.1 filter paper and evaporated to dryness at 55^0- 60^0C [5]. The solidified extract was stored in airtight container for further analyses.

Phytochemical Screening
The methanolic leaf extract of *H.capitata* was subjected to the qualitative analysis of phytochemicals to identify the presence or absence of different phytoconstituents [6].

Antimicrobial Assay
The bacterial strains such as *Bacillus subtilis* (MTCC-441) *Staphylococcus aureus* (MTCC-96) *Escherichia coli* (MTCC-452), *Klebsiella pneumoniae* (MTCC-109) and fungal strains such as *Candida albicans* (MTCC-183), *Aspergillus niger* (MTCC-281), *Penicillium chrysogenum* (MTCC-160) were employed for the present study. The microbial cultures were obtained from the Institute of Microbial Technology, Chandigarh, India. The selected strains are responsible for causing infections in the digestive tract (*Bacillus subtilis*), skin (*Staphylococcus aureus),* urinary tract (*Escherichia coli),* lungs and nose (*Klebsiella pneumonia),*genitourinary tract (*Candida albicans)* and lungs (*Aspergillus Niger).* The antimicrobial activity of methanolic leaf extract of *H.capitata* was determined by the agar well diffusion method [7].

Mueller-Hinton agar (15-20 mL) was autoclaved, poured in glass petri plates and allowed to solidify. Standardized

inoculum of the selected test bacterial and fungal strains was uniformly spread on the surface of these plates using a sterile cotton swab. Four wells with a diameter of 8 mm (20 mm apart from one another) were punched aseptically with a sterile Cork borer in each plate. The different concentrations of extract (80 and 160 µL) from 2 mg/mL stock were added to two of the wells and one well with Gentamycin (80µg/well) as positive and one with the solvent used in extraction as the negative control for the antibacterial assay. The agar plates were incubated under at 37°C for 24 hrs. After incubation, a clear zone was observed. Inhibition of the bacterial growth was measured in mm. The desired concentrations of extract (20 and 40 µL) from 100mg/mL were added to two of the wells and clotrimazole 10µg/well as positive and solvent used in extraction as a negative control for the antifungal assay. The plates were incubated at room temperature for 48hrs, after which they were examined for inhibition zones. Inhibition of the fungal growth was measured in mm.

Statistical Analysis

The experiments were done in triplicate to calculate mean and standard deviation by Graph Pad Instat DTCG. Analysis of Variance was done according to Tukey- Kramer Multiple Comparison Test.

Results and Discussion

Phytochemical Analysis

The qualitative phytochemical screening of methanolic leaf extract of *H.capitata* showed the presence of therapeutically and pharmacologically important phytoconstituents such as alkaloids, coumarins, flavonoids, glycosides, iridoids, phenols, quinones. steroids, saponins, tannins and terpenoids (Table-1).

Table 1: Qualitative Phytochemical Analysis of Methanolic Leaf Extract of *Hyptis capitata* Jacq.

Phytochemicals	Methanolic Leaf Extract
Alkaloids	+
Coumarins	+
Flavonoids	+
Glycosides	+
Iridioids	-
Phenols	+
Quinones	+
Steroids	+
Saponins	+
Tannins	+
Terpenoids	+

+ = Present;
_= Absent.

Antibacterial Activity

The methanolic leaf extract of *H.capitata* was subjected to the antibacterial activity against Gram +ve (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram -ve strains (*Escherichia coli* and *Klebsiella pneumoniae*) by agar well diffusion method and the results depicted in Table-2 & Fig.1. Methanolic leaf extract of *H.capitata* showed significant ($P < 0.001$) antibacterial activity against *Staphylococcus aureus* with a maximum zone of inhibition 12 ± 0.11 mm and recorded an activity index as 0.4 followed by *Bacillus subtilis* (11 ± 0.05mm, 0.35) and *Klebsiella pneumoniae* (11 ± 0.05mm, 0.36) at the concentration of 160 µg.

The methanolic extract at a concentration of 80 µg failed to impart any activity against the selected bacterial strains. However, the methanolic leaf extract of *H. capitata* at the higher concentration (160 µg) showed antibacterial effect against the selected Gram +ve and Gram -ve strains which remained in unison with the study reports and inferred that the antimicrobial activities have a direct relation to the increasing extract concentration [8].

Table 2: Antibacterial Activity of Methanolic Leaf Extract of *Hyptis capitata* Jacq.

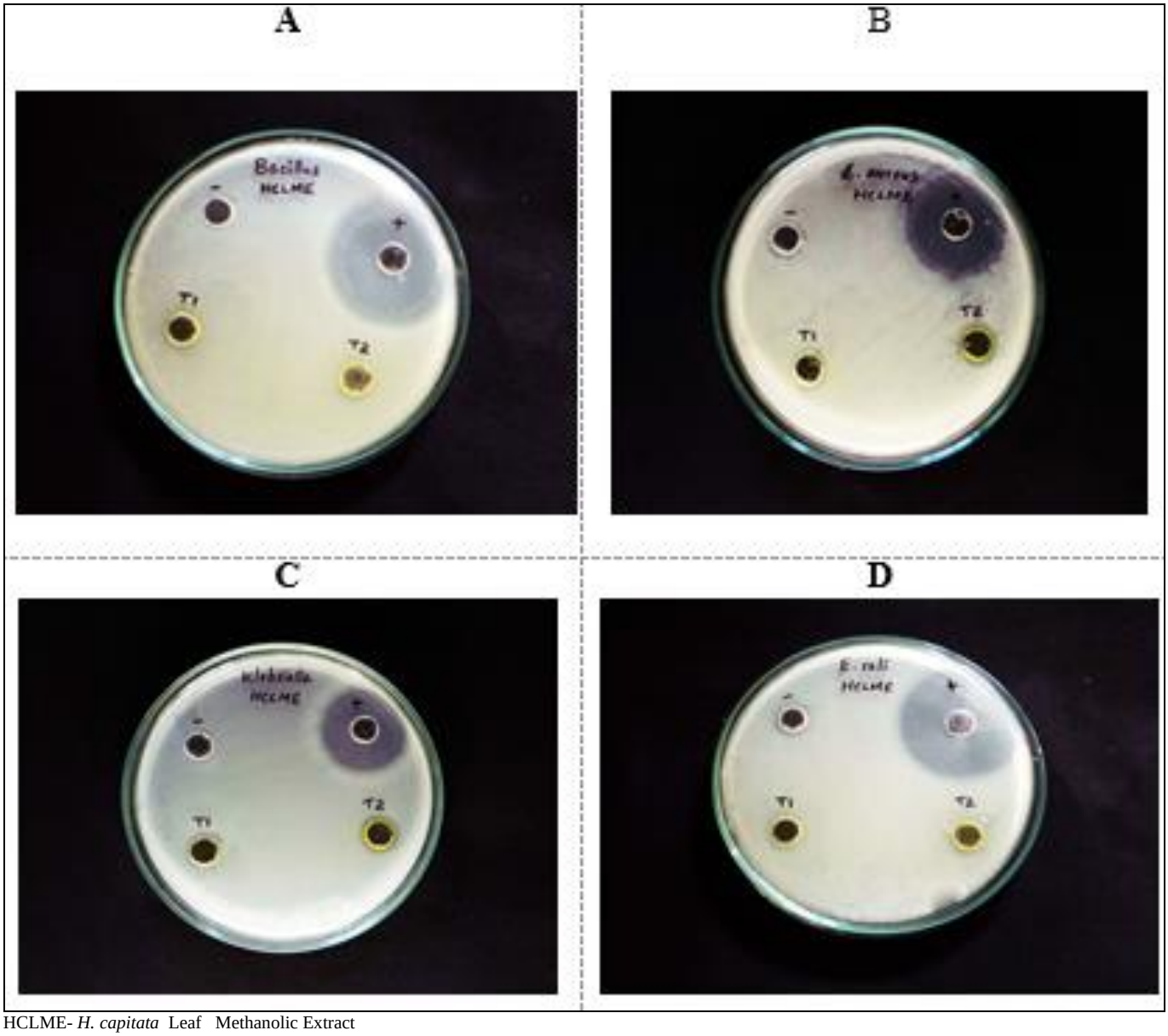
No:	Bacterial strains	Zone of inhibition (mm)			Activity index
		Methanolic Leaf Extract		Gentamycin	
		80 µg	160 µg	80 µg	
1	*Bacillus subtilis*	---	11 ± 0.05***	32 ± 0.05***	0.35
2	*Staphylococcus aureus*	---	12 ± 0.11***	30 ± 0.10***	0.4
3	*Klebsiella pneumoniae*	----	11 ± 0.05***	30 ± 0.12***	0.36
4	*Escherechia coli*	----	----	32 ± 0.05***	---

Each value represents the mean ± SD of triplicates and the superscript represents the level of significance comparing to control value (control-no activity) *** significant at $P < 0.001$ (according to Tukey-Kramer Multiple Comparison test)

The positive control Gentamycin showed very strong inhibition while the negative control failed to exhibit an inhibition zone against the tested organisms. The methanolic extract displayed less antibacterial activity comparable to standard Gentamycin. The variation in antibacterial sensitivity shown by the methanolic leaf extract against the test bacterial strains may be due to the permeability of the phytocompounds and resistance mechanisms displayed by microorganisms. The methanolic extract of *H.capitata* was found to be sensitive to human pathogenic Gram-positive *Staphylococcus aureus* and insensitive to the Gram-negative *E.coli* which supported the findings that the plant extracts are usually more active against Gram-positive than Gram-negative bacteria [9]. Studies reported that the Gram-positive bacteria have an outer peptidoglycan layer which makes the cell wall more permeable to antimicrobial chemicals than the outer lipopolysaccharide layer of Gram-negative bacteria. The higher complexity in the cell wall of Gram-negative bacteria makes it less susceptible to antimicrobial chemical substances than Gram-positive bacteria [10].

The phytochemical analysis of methanolic leaf extract of *H. capitata* also ascertained the presence of secondary

metabolites like alkaloids, flavonoids, quinones, phenols, tannins and terpenes which are with well-known antimicrobial effects [11]. These phytoconstiuents synergistically may be bacteriostatic against the tested strains of bacteria, which resulted in the formation of a zone of inhibition.



HCLME- *H. capitata* Leaf Methanolic Extract
- ve control – Methanol, + ve control –Gentamycin
T_1 - 80µl, T_2 – 160 µl
A- *Bacillus subtilis;* B – *Staphylococcus aureus;* C- *Klebisella pneumoniae*
D- *Escherechia coli*

Fig 1: Antibacterial Activity of Methanolic Leaf Extract of *Hyptis capitata* Jacq.

Antifungal Activity

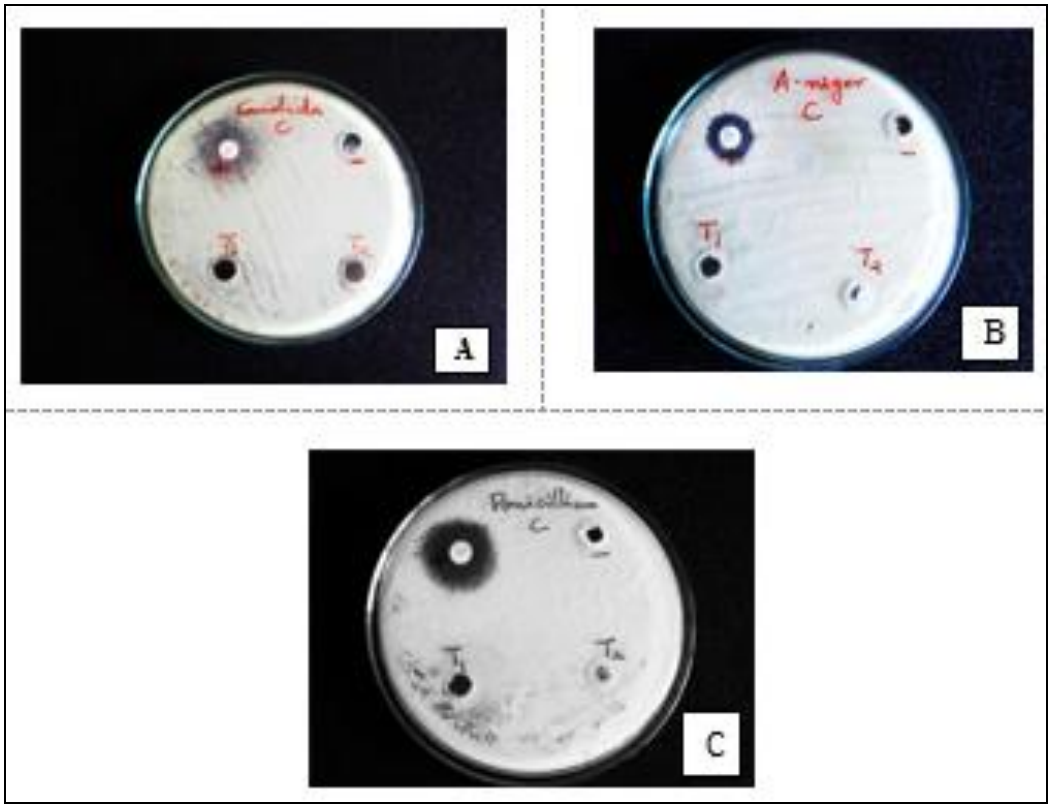
The effect of the methanolic leaf extract of *H.capitata* against test fungal strains such as *Candida albicans*, *Aspergillus niger* and *Penicillium chrysogenum* is depicted in Table-3 and Fig.2.The methanolic leaf extract of *H.capitata* failed to display an inhibition zone against the tested clinical and plant pathogenic fungal species. Methanol used as the negative control did not show an inhibition zone, whereas the positive control, Clotrimazole exhibited an inhibition zone in the range of 13 to 22 mm. The low antifungal activity may be due to the presence of a lesser amount of antifungal constituents in the extract.

The present study agreed with the study report that the antifungal activity of plant extracts against *Candida albicans* is not common. [12]

Table 3: Antifungal Activity of Methanolic Leaf Extract of *Hyptis capitata* Jacq.

Sl: No:	Fungal strains	Zone of inhibition (mm)		
		20 µg	40 µg	Clotrimazole
1	*Candida albicans*	---	----	21 ± 0.10
2	*Aspergillus niger*	---	----	13 ± 0.10
3	*Penicillium chrysogenum*	----	----	22 ± 0.10

Each value represents the mean ± SD of triplicates



C - Methanolic Leaf Extract of *H. capitata*
- ve Control - Methanol, + ve control - Clotrimazole,
T_1 – 20 µl, T_2 – 40 µl
A - *Candida albicans*; B- *Aspergillus niger*; C- *Penicillium chrysogenum*

Fig 2: Antifungal Activity of Methanolic Leaf Extract of *Hyptis capitata* Jacq.

Conclusion

Medicinal plants play an important role in health management. The present study revealed that the methanolic leaf extract of *H.capitata* was found significantly sensitive and bacteriostatic towards *Staphylococcus aureus*, *Bacillus subtilis* and *Klebsiella pneumoniae* while failed to display antifungal activity. The antibacterial activity may be due to the various phytoconstituents especially phenols, flavonoids and tannins. The present investigation proved the bacteriostatic or bactericidal efficacy of ethnomedicinally important herb *H. capitata* which supports the use of the herb by traditional healers for wound healing. However, further research is recommended for the isolation and identification of lead molecules involved in bioactivity.

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