

EVALUATION OF ANTIBACTERIAL ACTIVITY OF METHANOLIC EXTRACT OF SEEDS OF *PHYLA NODIFLORA* LINN.

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ABSTRACT

'Jalapippali' described in classical texts of Ayurveda is botanically identified as *Phyla nodiflora* Linn. (Syn. *Lippia nodiflora* Rich). In present study methanolic extract of seeds of *Phyla nodiflora* Linn. was screened for in-vitro antibacterial activity against gram positive and gram negative bacteria by cup- plate method. The methanolic extract of the seeds significantly inhibit the growth of bacteria as compared to the standard bactericide (streptomycin). The study reveals that the methanolic fraction of seeds of *Phyla nodiflora* Linn possesses significant antibacterial activity.

KEYWORDS Antibacterial activity, streptomycin, seeds of *Phyla nodiflora*, cup- plate method.

INTRODUCTION

Phyla nodiflora Linn. (family– Verbenaceae) Known as *Lippia nodiflora* Rich, Jalapippali. As per Ayurvedic literature the drug is shukrala, laghu, sheeta, katu, ruksha, kashya and agnivardhaka. *Phyla nodiflora* Linn. is an evergreen, creeping, much branched herb is distributed in India, Sri Lanka, Ceylon, Baluchistan, South and Central America and Tropical Africa. It is found throughout warmer parts of India ascending upto 900 m in the hills including Andhra Pradesh, Karnataka, Kerala, Maharashtra, some parts of Rajasthan, Tamilnadu, Uttar Pradesh and West Bengal. It is common in wet places, along bunds or irrigation channels, canal edges and river banks.^{1,2}

Phyla nodiflora Linn. is a creeping, prostrate, much branched perennial herb with branches spreading profusely and rooting at the nodes. Fruits are Capsular, 1.5 – 2 mm long, globosely – oblong, dry, splitting into two seeded plan convex glabrous pyrenes.³⁻⁵ In literature review it was found that the aerial parts are used as anodyne, antibacterial, diuretic, emmenagogue, parasiticide, refrigerant, febrifuge and cooling^{6, 7}. According to traditional uses and Unani system of medicine the plant is acrid, hot and dry; diuretic, maturant, useful in fevers and cold, astringent to bowels, stomachic, used in lack of bowel movements, pain in knee joints and in lithiasis⁸⁻¹¹. *Phyla nodiflora* contains flavonoids, sugars, sterol, an essential oil, resin, non-glucosidal bitter substance, tannin, large amount of potassium nitrate and other constituents¹². Several

workers have reported many pharmacological properties including antispasmodic¹³, hair afflictions¹⁴, anti-inflammatory, analgesic and antipyretic¹⁵, antibacterial¹⁶, anti *Helicobacter pylori* activity¹⁷, hypotensive activity¹⁸, antinociceptive¹⁹ and antifungal²⁰. Yet, antibacterial potential of methanol fraction of seeds of the plant was not proved; it was studied in the present research-work.

MATERIALS AND METHODS

Collection and identification of the plant material

Seeds of *Phyla nodiflora* Linn. were collected from wet places, along irrigation channels and canal edges of Kalsar village, near Dakor, Gujarat and its authentication was confirmed by Dr. A. S. Reddy, Prof. and Head of Botany Dept., Sardar Patel University, Vallabh Vidyanagar. A voucher specimen has been retained in the department of Pharmacognosy, B.Pharmacy College, Rampura, Kakanpur, Dist: Panchmahal, Gujarat, India for future reference.

Preparation of the extract

Phyla nodiflora Linn. seeds were collected, shade dried seeds were coarsely powdered with a mechanical grinder. Powder was passed through sieve No. 40 and stored in an airtight container for the extraction. 100gm of powdered drug was extracted with petroleum ether for 24 hours by Soxhlet apparatus. The marc left after petroleum ether extract were dried and then extracted with 95% methanol in Soxhlet apparatus for 72 hours. After completion of extraction, the extracts were filtered through Whatmann No. 1 filter paper. The filtrates were

concentrated to dryness in vacuum and stored in a dessicator.

Microorganisms used

The test microorganisms used for the antimicrobial activity were four bacterial species (two Gram positive and two Gram negative) – *Bacillus subtilis* MTCC (121), *Staphylococcus aureus* MTCC (96), *Pseudomonas aeruginosa* MTCC (429), *Escherichia coli* MTCC (443). These organisms were identified and procured from Institute of Microbial Technology (IMTECH-CSIR), Chandigarh, India. The stock cultures were maintained on nutrient agar medium at 4°C. The microorganisms were activated by inoculating a loopful of the strain in the nutrient broth (25ml).

Antimicrobial screening

Evaluation of antimicrobial activity was performed by Cup plate method^{21, 22}. Sterile Muller Hinton agar media was poured in sterile Petri plates under aseptic conditions. 0.1 ml of the test organisms were spread on agar plates. Cups were made at the size of 6 mm diameter, in the agar plates using the sterile borer. Streptomycin (10µg/disc) was served as reference standard. The disc (6 mm in diameter) was impregnated with 10 µl of each of 125 mg/ml (1.25 mg/disc), 250 mg/ml (2.5 mg/disc) and 500 mg/ml (5 mg/disc) methanolic extracts of seeds of the plant. The plates containing bacterial strains and standard were incubated at 37±0.5°C for 48 h depending on the incubation time required for a visible growth. The zone of inhibition (mm) was calculated by measuring the diameter of zone of bacterial growth around the cup. The average of three independent determinations was recorded^{23, 24, 25, 26}.

STATISTICAL ANALYSIS

The values are represented as mean ± standard error of mean (SEM) for triplicate set of experiments. P< 0.05 is considered statistically significant (ANNOVA, Dunnett's t-test).

RESULTS AND DISCUSSION

Antibacterial activity was done for methanolic extract of seeds of *Phyla nodiflora* Linn. During antibacterial study the methanolic extract of seeds showed concentration dependent increase in zone of inhibition against Gram +ve and Gram -ve bacteria by cup-plate method. The results of antibacterial activity are shown in Table 1, Figure 1

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TABLE 1: ANTIBACTERIAL ACTIVITY OF METHANOLIC EXTRACT OF SEEDS OF *PHYLA NODIFLORA* LINN.

Sample	Concentration	Mean \pm SEM of diameter of zone of inhibition (in mm)			
		Gram positive bacteria		Gram negative bacteria	
		<i>B. subtilis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P.aeruginosa</i>
Methanolic extract (a)	1.25 mg/disc	6.5 \pm 0.12	7.2 \pm 0.24	6.9 \pm 0.12	7.5 \pm 0.18
Methanolic extract (b)	2.5 mg/disc	16.3 \pm 0.26	17.0 \pm 0.23	16.8 \pm 0.19	17.4 \pm 0.15
Methanolic extract (c)	5 mg/disc	20.0 \pm 0.38	19.3 \pm 0.33	20.6 \pm 0.17	20.8 \pm 0.14
Streptomycin (d)	10 μ g/disc	24.7 \pm 0.11	23.7 \pm 0.18	25.2 \pm 0.18	25.6 \pm 0.20

Values are expressed as Mean \pm S.E.M of triplicate measurements. A value of P< 0.05 was considered statistically significant (By one way ANNOVA followed by Dunnett's t-test)

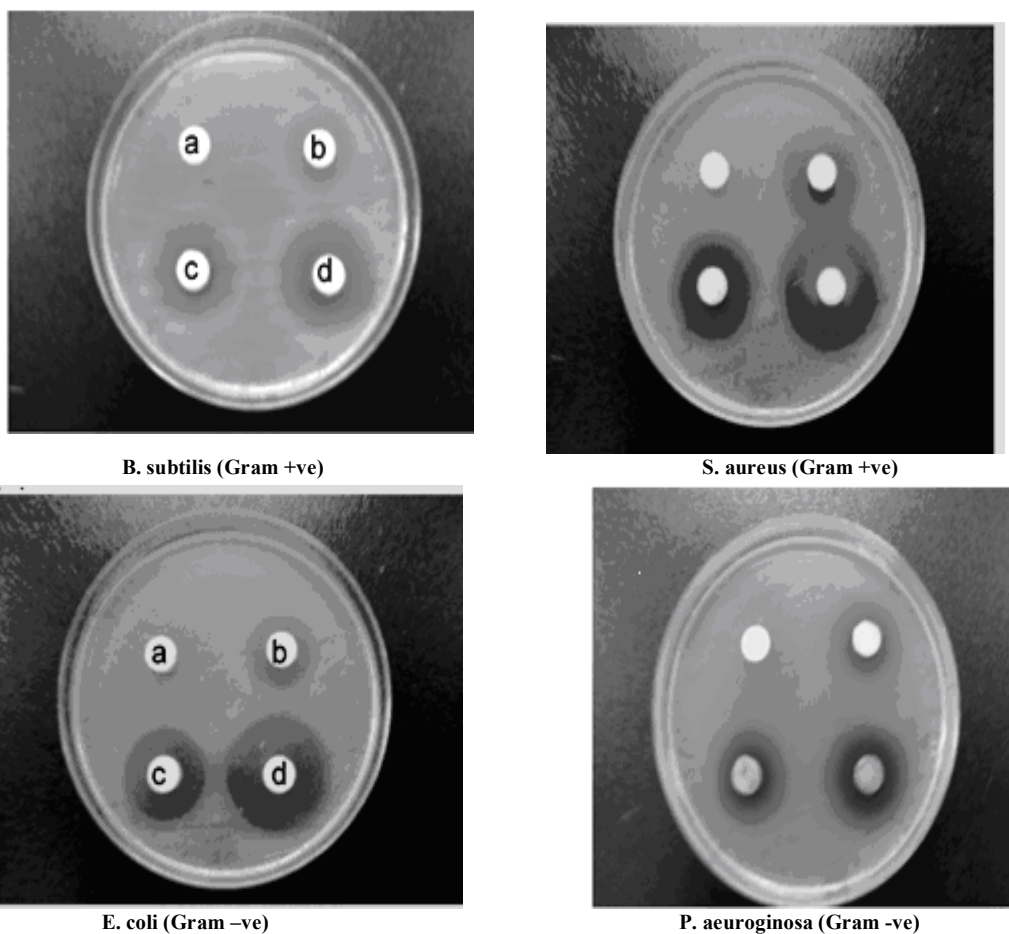


FIGURE 1: ANTIMICROBIAL ACTIVITY OF METHANOLIC EXTRACT OF SEEDS OF *PHYLA NODIFLORA* LINN. WITH GRAM POSITIVE BACTERIA AND GRAM NEGATIVE BACTERIA.

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