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Phytochemical Screening and Study of Antibacterial Effect of Ethanolic Extract of Aerial Part of Euphorbia Nivulia

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Abstract*— Traditionally, medicinal herbs have been used to treat a wide range of infections. Also, bioactive chemicals with an appropriate therapeutic index have been isolated from medical plants to create novel medications. The objective of this research is to investigate the existence of antibacterial activity in crude extracts of Euphorbia Nivulia (EN) using standard inhibitory zone diameter and phytochemical screening. The leaves were used in this preliminary inquiry, and the crude extracts were screened using the standard Disc Diffusion Method (DDM) methodology against two strains of the bacteria species K. pneumonia MTCC 39 and S. pneumonia MTCC 39. The antibacterial activity is evaluated based on the presence or absence of inhibition zones and MIC values. The results evaluated the mean outcomes of the zone of inhibition and the antibacterial susceptibility along with the standard deviation. The herbal extract EN found to be more effective against K. pneumonia MTCC 39 and S. pneumonia MTCC 39 along the lines of antibacterial susceptibility. The results of this study serve as a foundation for future research on the antibacterial qualities of extracts from local medicinal plants.

Keywords: Euphorbia Nivulia, antibacterial, zone inhibition, ethanolic extract, disc diffusion method.

I. INTRODUCTION

Anti- Bacterial Study leads to the killing or suppressing the growth of bacteria which is caused due to Air borne or droplet, direct or indirect contact, vector or by vehicular. Natural products are now thought to be one of the main sources of novel medication compounds; they may also act as models for the development of emerging antibiotics [1]. The application of herbal plants and their unique extract, which has unexplored potential, could contribute to the development of bacterial growth inhibition.

The Plant *Euphorbia Nivulia* (EN) belongs to the family Euphorbiaceae. In Traditional system of medicine leaves, bark, stem, root and latex posses wound healing, cytotoxic activity, antimicrobial activity and insecticidal activity. The pharmacognostical evaluation of E. nivulia was conducted using the WHO recommendations on quality control for medicinal plant materials; phytochemical screening aids in identifying the main classes of active elements that are accountable for the activity [2].

Raghunath et al. [3] investigated that the Aqua-alcoholic extract of leaves of EN has antimicrobial activity. Chemically, it contains terpenes, glycoproteins, phytoelements and phytochemicals. Nineteen different bacterial strains and two fungal cultures were used for anti-microbial activity. The latex has wound healing property, and also reduced blood clotting and bleeding time and possess haemostatic effect. Yonus et.al [4] investigated and revealed that different concentration of chloroform, crude hexane, butanol and aqueous extract of EN had been tested for their insecticidal potential and phytotoxic against (dusky cotton bug) Oxycarenus hyalinipennis costa and (duck weed) Lemna minor. Also demonstrated that all extracts exhibited insecticidal activity, while EN chloroform extract displayed notable phytotoxicity.

Badgujar and Mahajan [5] measured the residual enzyme activity of aliquots of the enzyme by incubating them at various temperatures in order to evaluate the biochemical characteristics of the cysteine protease of EN. By topically applying the latex to the region of the healed wound thrice a day for 23 days, Badgujar et al. [6] investigated the wound-healing ability of EN latex. This was similar to soframycin in that it greatly increased the pace of wound contraction and the length of the epithelialization stage. The latex speeds up both bleeding and blood clotting time. Alkaloids, phenolics, tannins, cyanogenic glycosides, and saponins were among the phytochemicals identified.

Wesley et al. [7] investigated several extracts of EN for phytochemical content, antioxidant activity, and antidiabetic activity. The ethanolic extract of EN shows greater anti-oxidant activity than the ethyl acetate extract, according to the results of the DPPH experiment. The extract increases the alpha glycosidase activity; thus, the in vitro research indicates the anti-diabetics and anti-oxidant activity. Arivukkarasu et al. [8] investigated and revealed that the plant EN has anticancer activity. Using the HPTLC technique, the study identifies quercetin and apigenin elements in the methanol extract. It was discovered that the Ic 50 values of normal and cancer cells had percentages of cell inhibition concentrations of 135.5 μg/ml, 99.63 μg/ml and 118.1μg/ml, respectively. EN showed notable activity against MCF-7,

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NIH 3 T3 and HeLa cancer cells.

When compared to the acute convulsant dose of Pentylenetetrazol, the ethanolic extract of EN was reported by Rehman et al. [9] to exhibit a concentration-dependent anti-convulsant activity. Jamalpoor et al. [10] investigated and revealed that the hydroalcoholic extract of EN possesses hypolipidemic activity. The leaves were extracted using 70%v/v hydroalcohol at 75-80 at the soxhlet apparatus. A rotary flash evaporator (50°) was used to concentrate the extract. A qualitative analysis was conducted on carbohydrates, alkaloids, glycosides, flavonoids, terpenes, phytosterols, saponins and proteins.

Using HPLC research, Younus et al. [11] examined the potential antidiabetic effects of EN and discovered that this plant includes polyphenols, flavonoids, quercetin, gallic acid, ferullic acid and benzoic acid. Studies are conducted in vivo and in vitro to evaluate its effects on a diabetic Wistar rat model. The experiment has been conducted and contrasted with glibenclamide, the control medication. At 1.0 mg/ml, EN's 70% hydroalcoholic extract showed 97.8% in vitro α-glucosidase inhibitory activity. Using stem extract of EN, Devi et al. [12] studied the manufacture and characterization of silver nanoparticles (AgNPs) and their anti-microbial impact on specific harmful bacteria. Eight bacterial strains and one fungus strain were screened for by antibacterial activity. With respect to Escherichia coli, AgNPs exhibited the highest level of inhibition (33.5±0.5) followed by Bacillus subtilis (29±1), Pseudomonas aeruginosa (30.5±0.5), Bacillus cereus (27±1), Salmonella typhimurium (28±1), Klebsiella pneumoniae (23.5±5.5) and Staphylococcus aureus (24.5±1.5), as well as one fungal strain Candida albicans (26 ±1). Many literatures has shown that the plant possesses antibacterial activity, yet there is no study has been conducted. Hence the present study of antibacterial activity will be useful and valuable for future studies.

In this paper, the experiments containing procedures with respect to EN herbal extract collection, antibiotic sensitivity testing were evaluated and multiple trials were conducted for fluency with the procedure. The study aims to investigate the effective treatment of ethanolic EN herbal extracts and streptomycin (an antibiotic) in treating K. pneumonia MTCC 39 and S. pneumonia MTCC 39 due to the development of drug resistance in pathology.

II. MATERIALS AND METHODS

2.1 Plant collection and selection

In the Sathuragiri hills of Tamil Nadu's Virudhunagar district, *Euphorbia nivulia* aerial parts were collected and verified by the Central Council for Research in Siddha and Ayurveda, Government of India. The plant parts were crushed, dried, and stored in an airtight container for later extraction.

2.2 Extraction of plant Material:

After thoroughly washing them in water to get rid of any foreign objects, the Euphorbia nivulia leaves were shade-dried at a humidity of 40–45%. The leaves were then processed via a roller grinder (No. 40) and sieved. After extracting the 150 gm powdered sample from the mixture using petroleum ether, it was extracted using Soxhlet apparatus for 72 hours at room temperature and 1 litre of 95% ethanol. The resultant samples were concentrated and filtered at a lower pressure in a rotary evaporator, yielding a thick brown paste that was kept at -20^{0}C until it was needed. It was found that the extraction yield was 11.87 % w/w. Figure 1 displays the extraction of EN was done by Soxhlet apparatus.

Figure 1. Soxhlet Extraction of EN

2.3 Phytochemical Screening:

A phytochemical screening of *Euphorbia nivulia* can help determine the active constituents responsible for its activity. The screening can also help identify the fresh and dried samples of the aerial parts. The plant *Euphorbia nivulia* are rich in varieties of active constituents such as terpenoids, proteins, amino acid, steroids, flavonoids, phenols, tannins, anthraquinones, saponins which are responsible for antibacterial activity.

2.4 In vitro Antibacterial Activity by Disc diffusion method

In order to evaluate whether plant extracts possessed antibacterial properties, the disc diffusion method for antimicrobial susceptibility testing was conducted in accordance with Bauer et al. [13] standard procedure. Using a sterile swab, a bacterium culture that has been calibrated to the 0.5McF Arland standard was used to uniformly mow Muller Hinton agar plates. After 15 minutes of drying, the plates were used for the sensitivity test. The Mueller-Hinton agar surface was covered with the discs that had been impregnated with a variety of plant extracts. Six discs make up each test plate. Four treated discs, one negative control, and one positive control—a typical commercial antibiotic

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disc. The standard antibiotic discs were loaded with 1mg in 10ul for each disc (+C- Streptomycin 1mg/disc) for K. pneumonia MTCC 39 and S. pneumonia MTCC 39, respectively. DMSO (100% concentration) served as the negative control. Each plate included four treated discs spaced roughly equally apart in addition to the controls. Afterwards, the plate was incubated for 18 to 24 hours at 37°C, depending on the type of bacteria utilised in the experiment. The plates were checked for an inhibitory zone after the incubation period. The inhibitory zone was then measured and noted using calipers. To guarantee reliability, the tests were conducted three times.

III. EXPERIMENTAL RESULTS

3.1 Phytochemical Screening

To perform phytochemical screening of EEEN, the powdered flower is extracted with like 70% ethanol and subjected to qualitative chemical analysis to test the presence of alkaloid, glycoside, terpenoids, proteins, amino acid, carbohydrate, steroids, flavonoids, phenols, tannins, quinones and saponins. The highest number of phytochemicals in the ethanolic extract were identified. The ethanolic extract of *Euphorbia nivulia* may show highest inhibition zone activity against bacteria. Qualitative chemical analysis of phytoconstituents of extracts of Euphorbia nivulia was shown in Table 1

Table 1. Qualitative chemical analysis of phytoconstituents of extracts of *Euphorbia nivulia*

S. No.	TEST	EEEN	S. No.	TEST	EEEN
1	Alkaloid	-	7	Steroids	+
2	Glycoside	-	8	Phenols	+
3	Terpenoids	+	9	Tannins	+
4	Proteins	+	10	Quinones	-
5	Amino acid	+	11	Flavonoid	+
6	Carbohydrate	-	12	Saponins	+

3.2 In vitro Antibacterial Activity

Using the approach of a disc diffusion method, the antibacterial properties of five materials with various chemical compositions—NC, +C, LA-I, LA-II, and LA-III—were tested against the two recommended clinical bacterial cultures: K. pneumonia MTCC 39 and S. pneumonia MTCC 39. The samples were loaded with 1mg in 10ul for each disc (+C- Streptomycin 1mg/disc).

The results of the disc diffusion test (diffusion method) showed that, for the four materials +C, LA-I, LA-II, and LA-III, there was increasing antibacterial activity against both Gram-positive and Gram-negative bacteria, while the sample NC showed no antibacterial activity as it did in the control disc (see Figure 2).

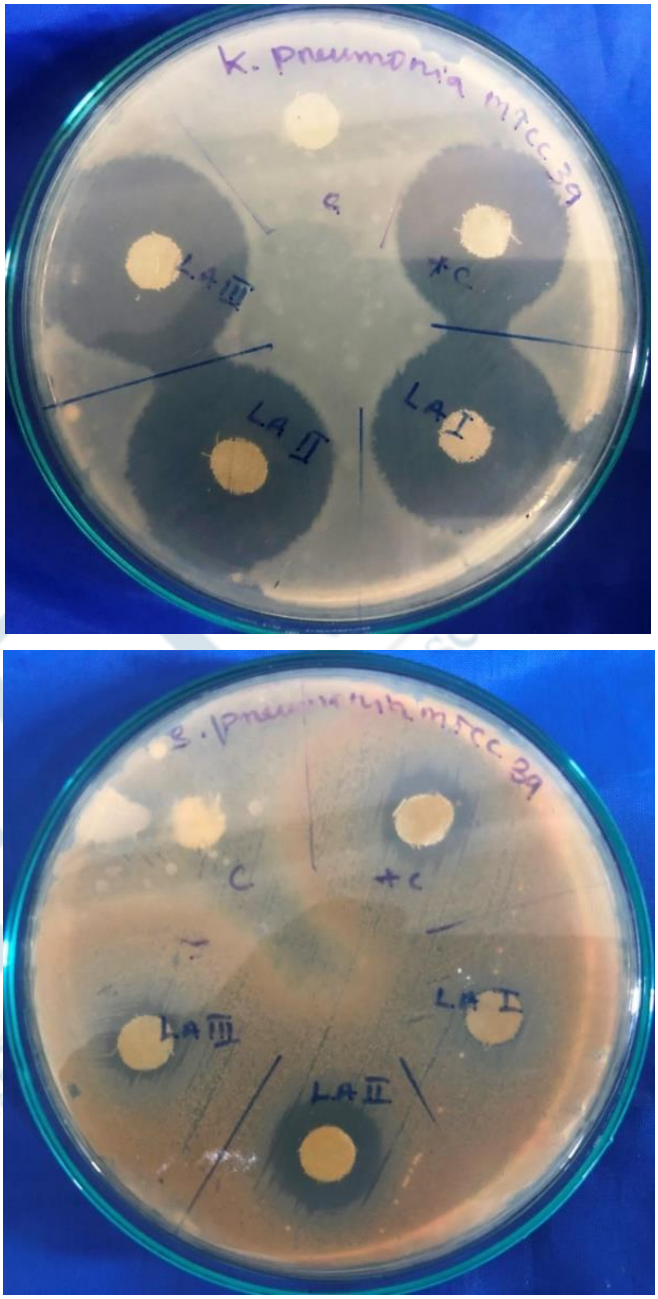

Figure 2. K. pneumonia MTCC 39 and S. pneumonia MTCC 39

Figure 3 and 4 depicts the inhibitor diameter of the *E. nivulia* leaf extract, against K. pneumonia MTCC 39 and S. pneumonia MTCC 39 after incubation for 24 hours post streaking and diffusion. Table 2 and 3 shows the area, perimeter and radius of K. pneumonia MTCC 39 and S. pneumonia MTCC 39 for the samples of NC, +C, LA-I, LA-II and LA-III.

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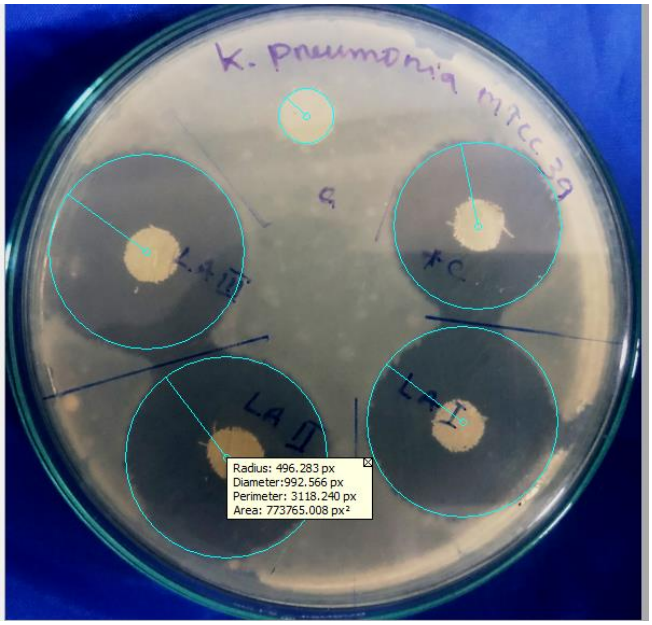

Figure 3. Diameter of the inhibition zone of EN against K. pneumonia MTCC 39

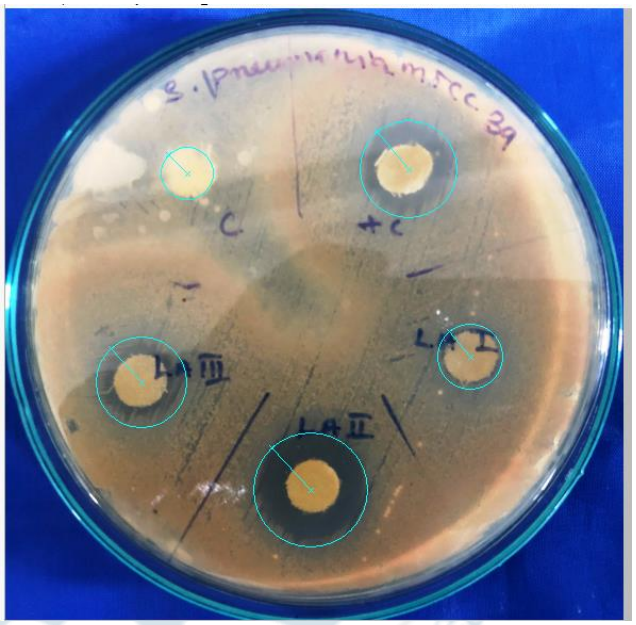

Figure 4. Diameter of the inhibition zone of EN against K. pneumonia MTCC 39

Table 2. K. pneumonia MTCC 39 measurement list

Sample	Area	Perimeter	Radius	Unit
NC	59728.3	866.353	137.884	px
+C	539298	2603.272	414.324	px
LA-I	693807	2952.735	469.942	px
LA-II	773765	3118.240	496.283	px
LA-III	731737	3032.372	482.617	px

Table 3. S. pneumonia MTCC 39 measurement list

Sample	Area	Perimeter	Radius	Unit
NC	56502.1	842.631	134.109	px
+C	191479	1551.193	246.880	px
LA-I	85101.9	1034.129	164.587	px
LA-II	258880	1803.661	287.062	px
LA-III	162617	1429.517	227.515	px

Table 4. Statistics of antibacterial activity by disc diffusion method

Outcome	Measure	n	Mean	SD	Min	Max
Zone of inhibition of EN	Area	11	328422	294548	56502.1	773765
	Perimeter	11	1827.31	931.0219	842.631	3118.240
	Radius	11	290.8261	148.1767	134.109	496.283

Table 4 depicts the average mean value, standard deviation (SD), minimum and maximum values evaluated for *E. Nivulia* leaf extract with respect to their corresponding zone of inhibition and the antibacterial susceptibility against K. pneumonia MTCC 39 and S. pneumonia MTCC 39. The images and the normalised width of the antibacterial activity are quantitative and qualitative results acquired with this technique, together with a good study of its reproducibility (mean ± standard deviation). To determine whether the mean values obtained from the diffusion and contact technique results analysis are statistically substantially different (p <0.01) when comparing different materials, a Digimizer 6 analysis is performed.

IV. CONCLUSION

In this paper, our findings are about new drug sources using the ethanolic extract of *Euphorbia Nivulia* to treat diseases infected by antibacterial-resistant bacteria using standard inhibitory zone diameter and phytochemical screening. The zone of inhibition and antibacterial susceptibility mean results were assessed, as well as the standard deviation, in the findings. According to antibacterial susceptibility, the herbal extract EN proved to be more effective against S. pneumonia MTCC 39 and K. pneumonia MTCC 39.

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