

Efficacy of the bioactive compounds of medicinal importance in the leaves of *Lantana camara* L. and *Andrographis paniculata* (Burm. f.) Wall. ex Nees.

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Abstract: The present study includes the phytochemical detection and antimicrobial activity of the leaves of *Lantana camara* L. and *Andrographis paniculata* (Burm. f.) Wall. ex Nees. Phytochemical screening of the leaf extract of leaves shade dried indicated the presence of flavonoids, terpenoids, tannins, alkaloids, steroids and glycosides. The antibacterial activity of ethanolic and aqueous extracts of leaves of *Lantana camara* and *Andrographis paniculata* were evaluated against the human pathogenic bacteria like *Staphylococcus aureus* and *Pseudomonas aeruginosa* by well diffusion method. Among the extracts analysed ethanolic extracts showed promising results whereas aqueous extracts did not report any zone of inhibition. The ethanol leaf extract (200µg/ml) showed maximum inhibition against *Pseudomonas aeruginosa* (16mm) and *Staphylococcus aureus* (16mm). Phytochemical tests carried out showed that the antibacterial activity of plant *Lantana camara* and *Andrographis paniculata* leaves was due to the presence of phytochemical compounds present in it.

Keywords: *Lantana camara*, *Andrographis paniculata*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*.

I. Introduction

Drugs based on plant origin are being used worldwide which are either extracted and used directly from plants or in a processed form [1]. The chemicals belong to plant derived compounds called phytochemicals such as the metabolites of primary and secondary origin. The secondary metabolites are much of interest because of their antimicrobial, antibiotic, insecticidal and hormonal properties [2]. Medicinal plants contain physiologically active principles that over the years have been exploited in traditional medicine for the treatment of various ailments (3). *Andrographis paniculata* contains diterpenes, lactones and flavonoids and flavonoids mainly exist in the root, but have also been isolated from the leaves. Aerial parts contain alkanes, ketones, and aldehydes and the bitter principles in the leaves were due to presence of the lactone andrographolide named kalmegin. Four lactones Chuaxinlian A (deoxyandrographolide), B (andrographolide), C (neoandrographolide) and D (14-deoxy-11, 12-didehydroandrographolide) were isolated from the aerial parts. [4]. The leaf and stem extracts showed the presence of glycosides, flavonoids, gums, steroids, terpenoids, tannins, saponins and phenolic compounds (5). The ethanol, acetone, methanol, petroleum ether and chloroform extracts of Kalmegh were screened for the presence of secondary metabolites (6). Traditionally *Lantana camara* is considered antiseptic, antispasmodic, carminative and diaphoretic. Anti-inflammatory, antipyretic and analgesic properties of extracts of *Lantana camara* leaves has been reported [7]. *Lantana camara* is used in herbal medicine for the treatment of skin itches, as an antiseptic for wounds, and externally for leprosy and scabies. Major natural products investigated in *Lantana camara* belong to the group of triterpenoids, flavonoids and other compounds. In herbal medicine, infusions of the leaves and other plant parts are used as anti-inflammatory, tonic and expectorant [8]. The present study aims to investigate the phytochemicals and antimicrobial potential of leaves extract of the commonly used medicinal plants human pathogenic bacteria like *Staphylococcus aureus* and *Pseudomonas aeruginosa* by well diffusion method.

II. Materials and Methods

Collection of raw materials and preparation of extracts

The leaves of *Andrographis paniculata* and *Lantana camara* were collected locally and authenticated. Shade dried leaves were used for the study. The leaves were cleaned and were shade dried for one week and pulverized to coarse powder. For the preparation of the ethanolic, methanolic and aqueous extract, 1g of the dried ground plant material was weighed and extract was prepared by refluxing with 10ml of the solvent. The extract was filtered and collected. The extract was then wrapped with aluminium foil to prevent evaporation. Extracts of leaves were stored in air tight containers at 4⁰ C for various procedures.

Phytochemical Screening of extracts

Ethanol methanol and aqueous extracts were used for preliminary phytochemical screening using standard procedures[9].

Determination of Anti bacterial activity

Preparation of Culture Medium

Nutrient agar medium

1000ml of distilled water was boiled in a round bottom flask and 5gm of NaCl, 20gm agar,10gm meat extract and peptone were added by continuous stirring. The mouth of the flask was wrapped with aluminium foil and tied tightly. The medium was sterilized at a pressure of 15 lbs and 121⁰C for 15 minutes in an autoclave. The pH of the Nutrient agar medium was adjusted to 7.4 (at room temperature after gelling).

Microorganisms used

The bacterial strains were procured from the Cambrit Biosolution, Ernakulam were used for the present study. They include *Staphylococcus aureus* (Gram positive) and *Pseudomonas aeruginosa* (Gram negative). The bacterial strains were maintained in Muller Hinton Agar (MHA, pH 7.2) at 37±1⁰C .The stock culture slants were maintained at 4⁰C.

Well Diffusion Method

The antibacterial activity of the leaf extracts was determined using agar well diffusion method by following the following procedure. Nutrient agar was inoculated with the given microorganisms *Staphylococcus aureus* and *Pseudomonas aeruginosa* by spreading the bacterial inoculums on the media. Wells were punched in the agar and filled with plant extracts. Control wells containing neat solvents (negative control) were also run parallel in the same plate. The plates were incubated at 37⁰C for 18 hours and the antibacterial activity was analysed by measuring the diameter of the zone of inhibition. The antibacterial potential of the different extracts was evaluated by comparing their zones of inhibition [10].

III. Results and Discussion

Andrographis paniculata commonly known as Kalmegh (King of Bitters) is an important plant species in Ayurvedic medicine[11]. Wounds dressed with *A. paniculata* extracts showed enhanced rate of wound healing in rats [12].The ethanol extracts of *Andrographis paniculata* leaf possess significant inhibitory effect against the tested organisms. The results of the investigation support the traditional claimed of this plant. Apart from this investigation, there are no reports of antibacterial, antifungal and phytoconstituents studies of *A. alata*. [13]. The results of phytochemical screening in the present investigation showed that the shade dried leaves of *Lantana camara* and *Andrographis paniculata* contained alkaloids, flavanoids, resins, bitter, tannins, cardiac glycosides, reducing sugar and triterpenes, volatile oils, saponins and steroids in the ethanol extracts. (Table 3.1).

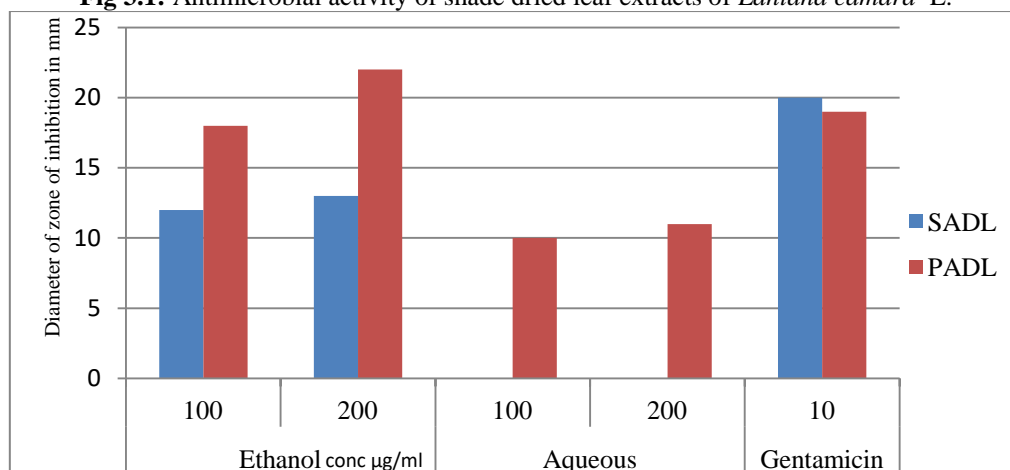
Table 3.1 Phytochemical screening of the shade dried leaves of *Lantana camara* and *Andrographis paniculata*

chemical constituent	<i>Andrographis paniculata</i>			<i>Lantana camara</i>		
	ethanol	methanol	aqueous	ethanol	methanol	aqueous
Alkaloids	+	+	-	+	+	-
Flavonoids	+	+	-	+	+	-
Resins	+	-	-	+	-	-
Bitter	+	-	-	+	-	-
Saponins	+	-	-	+	-	-
Tannins	+	-	-	+	-	-
Steroids	+	-	-	+	-	-
Cardiac glycosides	+	+	+	+	+	+
Reducing sugars	-	-	-	+	+	-
Volatile oils	-	-	-	-	-	-

Lantana camara has been studied extensively for their antibacterial properties. They possess antipyretic, antimicrobial, antimutagenic, antimicrobial, fungicidal, insecticidal and nematicidal properties[14]. The ethanolic extract of *Andrographis paniculata* of the leaves showed a zone of inhibition (17mm) against *Staphylococcus aureus* while the zone inhibition of 18mm was recorded against *Pseudomonas aeruginosa* at 100 µg/ml. The extract (200 µg/ml concentration) displayed same inhibition zone(21mm) against both *Staphylococcus aureus* and *Pseudomonas aeruginosa*. It can be suggested that leaves of *Andrographis paniculata* could be used as best antimicrobial drug against *Staphylococcus aureus* and *Pseudomonas aeruginosa* than the Gentamicin (20mm against *Staphylococcus aureus* 19mm against *Pseudomonas aeruginosa*) (Fig 3.1). However the aqueous extract showed no effect against the pathogenic bacteria. *Lantana camara* L and *Andrographis paniculata* Nees could

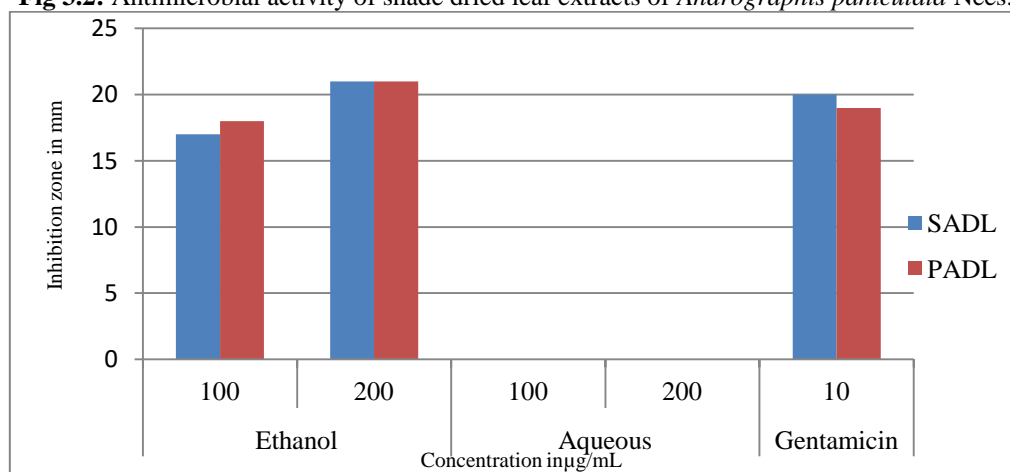
be used as a substitute for Gentamicin. Antioxidant activity was more in ethanol extract, which showed Inhibitory Concentration (IC₅₀) values of 0.5 mg/ml, 0.1 mg/ml and 0.9 mg/ml for DPPH scavenging activity, lipid peroxidation inhibitory activity and superoxide scavenging activity respectively. The result indicates that ethanolic leaf extract of *A. paniculata* shows potent antibacterial, antifungal and antioxidant activities [15]. Therefore, antibacterial activities of *L. camara* leaf and flower extracts might be due to the presence of some of these chemical constituents particularly lantadenes and thevetoside in the extracts [16]. The presence of phenolics, anthocyanins and proanthocyanidins in *L. camara* leaves which could also be responsible for the antibacterial properties of the *L. camara* have been documented [17]. *L. camara* leaves extract was active against *P. vulgaris* and *V. cholerae* (MIC 128 µg/mL for both strains); in addition the root extract was effective against *P. vulgaris* and *P. aeruginosa* (64 and 128 µg/mL, respectively). The leaves and roots *L. montevidensis* extracts were active against *P. vulgaris* and *P. aeruginosa* (MIC 8 µg/mL) and two strains of *E. coli* (MIC 16 µg/mL for the multiresistant strain) [18]. The ethanolic extract of *Andrographis paniculata* Nees leaf showed 17mm inhibition zone against *Staphylococcus aureus* and 18mm inhibition zone against *Pseudomonas aeruginosa* at 100 µg/ml whereas the extract displayed same inhibition zone (21mm) against both *Staphylococcus aureus* and *Pseudomonas aeruginosa* at 200 µg/ml (Fig 3.1). This proved that leaves of *Andrographis paniculata* can be used as best antimicrobial drug against *Staphylococcus aureus* and *Pseudomonas aeruginosa* than the Gentamicin (20mm against *Staphylococcus aureus* 19mm against *Pseudomonas aeruginosa*). The aqueous extract showed no effect against the pathogenic bacteria. The result obtained after experiment showed that *Lantana camara* and *Andrographis paniculata* can be used as a substitute for Gentamicin.

Fig 3.1: Antimicrobial activity of shade dried leaf extracts of *Lantana camara* L.



SADL - *Staphylococcus aureus* on shade dried leaves extract
 PADL - *Pseudomonas aeruginosa* on shade dried leaves extract

Fig 3.2: Antimicrobial activity of shade dried leaf extracts of *Andrographis paniculata* Nees.



SADL - *Staphylococcus aureus* on shade dried leaves extract
 PADL - *Pseudomonas aeruginosa* on shade dried leaves extract

IV. Conclusion

It was observed in the present study the plant contains potential antibacterial components that may be of useful for evolution of pharmaceutical for the therapy of ailments. Some of the characteristics of *P. aeruginosa* is its low antibiotic susceptibility, which is attributable to a concerted action of multidrug efflux pumps with chromosomally encoded antibiotic resistance genes (e.g., *mexAB*, *mexXY*, etc.) and the low permeability of the bacterial cellular envelopes. To overcome this barrier the use of plant extracts, as well as other alternative forms of medical treatments should open new avenues the patients. In the present study *Lantana camara* L. and *Andrographis paniculata* Nees. showed anti bacterial properties against gram positive and gramnegative bacteria can be used as a substitute for the antibiotic Gentamicin.

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