

TO STUDY ANTIBACTERIAL ACTIVITY OF PLANT EXTRACTS OF *ANDROGRAPHIS PANICULATA NEES*

Mrs. M. Mohanapriya*¹ and R. Manivannan²

¹Assistant Professor, Department of Pharmaceutical Chemistry, Excel College of Pharmacy, Pallakkapalayam, Namakkal, Tamilnadu-India.

²Principal, Department of Pharmaceutics, Excel College of Pharmacy, Pallakkapalayam, Namakkal, Tamilnadu-India.

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Corresponding Author: Mrs. M. Mohanapriya

Assistant Professor, Department of Pharmaceutical Chemistry, Excel College of Pharmacy, Pallakkapalayam, Namakkal, Tamilnadu-India.

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ABSTRACT

The locals frequently utilise the plant *Andrographis paniculata* Nees, which belongs to the *Acanthaceae* family, to treat skin infections, liver illnesses, common colds, and snake bites. The phytochemical qualities and antibacterial activity of the plant's aerial portion were examined. The antibacterial properties of leaf extract of *Andrographis paniculata* was investigated utilising a variety of solvents, including water, methanol, petroleum ether, and chloroform, against bacterial strains using the disc diffusion method, including *Bacillus subtilis* and *Escherichia coli*. The highest inhibitory activity was demonstrated by methanolic extract against *Bacillus subtilis* and *Escherichia coli*, respectively. The active components found in the plant were identified by the results of the phytochemical screening as steroids, phenols, terpenoids, alkaloids, saponins, and flavonoids. Traditionally, the herb has been used to treat antibacterial, antioxidant, antidiabetic, and antipyretic, hepatoprotective as well as a number of infectious illnesses, such as dysentery and malaria. The active components of this plant, andrographolide and neoandrographolide, which are derived from diterpenoids, are give its therapeutic benefit.

KEYWORDS: Phytochemical Extraction, *Andrographis paniculata*, Kalmegh, Anti-Bacterial, Anti-Oxidant.

I. INTRODUCTION

HERBAL MEDICINE AND ITS IMPORTANCE

Since the dawn of humanity, thousands of years ago, nature has provided medical agents. Nowadays, the use of medicinal plants, particularly in traditional medicine, is widely accepted and recognised as a legitimate field of study and employment. Medicinal plants are a valuable resource for the treatment of a wide range of illnesses. The earliest recorded scientific investigations on the antibacterial qualities of plant components date back to the late 1800s. The relationship between a medicinal plant's pharmacological action and its phytochemical components is gaining attention.

ANDROGRAPHIS PANICULATA

Known as the "king of bitter," kalmegh (*Andrographis paniculata*), a member of the *Acanthaceae* family, is a significant annual medicinal herb that is extensively used in Madhya Pradesh, India. It is an upright, herdy plant that thrives mostly as an understory shrub in damp, tropical deciduous forests. It is among the plants most frequently used in Ayurvedic formulations. It is used to treat skin conditions, fever, ulcers, worms, hemopathy, burning feeling, coughing, and sannipata type fever. Additionally, it helps with liver problems and acidity.



Figure 1: - *Andrographis paniculata*.

MORPHOLOGY

In damp, shaded areas, *Andrographis paniculata* can grow upright to a height of 30 to 110 cm. The thin, dark green stem has wings at the angles and longitudinal furrows in its cross-section. The hairless blades of the lance-shaped leaves measure up to 8 centimetres in length and 2.5 centimetres in width. Racemes of tiny flowers spread out as they mature. The fruit is a capsule, measuring a few millimetres in width and two centimetres in length. There are many of yellow-brown seeds in it. Due to the plant's strong bitter taste, which is similar to that of a huge neem tree despite being an annual herb, it is also known as Bhui-neem, which translates to "neem of the ground".

PHYTOCONSTITUENTS

Historically, traditional medicine has used the biologically active ingredients found in medicinal plants to treat a wide range of illnesses. Flavonoids, lactones, and diterpenes are present in *Andrographis paniculata*. Although they have also been separated from the leaves, flavonoids are mostly found in the root. Alkanes, ketones, and aldehydes are found in aerial portions, whereas the lactone andrographolide known as kalmegin is responsible for the bitter qualities found in leaves. From the aerial portions, four lactones were isolated: Chuaxinlian A (deoxyandrographolide), B (andrographolide), C (neoandrographolide), and D (14-deoxy-11, 12-didehydroandrographolide). Glycosides, flavonoids, gums, steroids, terpenoids, tannins, saponins, and phenolic compounds were examined in the leaf and stem extracts. The extracts of Kalmegh in ethanol, acetone, methanol, petroleum ether, and chloroform were examined for the presence of secondary metabolites.

II. MATERIALS AND METHODS**MATERIALS REQUIRED****● Microorganisms**

E.coli Culture, *Staphylococcus aureus*, MTCC (Microbial Type Culture Collection)

- **Chemicals Required**

95% Ethanol, Distilled water, Nutrient Broth (NB), Agar, Nutrient Agar Media (NAM), Culture, Herbal Drug powder (Kalmegh), Chloroform, Methanol, Petroleum ether, Fehling solution A & B, Ferric chloride, Mayer's reagent (Mercuric Chloride, Potassium Iodide), Liebermann–Burchard reagent, Ninhydrin solution, Sodium Hydroxide, Biuret Reagent, Conc. Sulphuric Acid, Acetic Acid, Dilute Hydrochloric Acid.

- **Instruments Required**

Soxhlet Assembly (J-Sil, 50/42, Borosil glass), Vacuum Rotary Evaporator (Scientech), Digital Balance (Denver, Germany), Hot Air Oven (Scientech, 325 L), Laminar Air Flow Chamber Horizontal, Incubator (Scientech), Cyclo Mixer (REMI), Antibiotic Zone Scale.

METHODOLOGY

PREPARATION OF EXTRACT

After being collected three months prior, the *Andrographis paniculata* leaves were first sorted and then cleaned by running them under water. After drying, the samples were ground into a powder. To 100 mL of the mixture, roughly 10 g of the powder was added. Extraction solvent was soaked for a further 12 hours without stirring after being continuously stirred for 6 hours. The solvents utilized in the extraction process included pure methanol, chloroform, petroleum ether, as well as water. After being gathered, the filtrate was concentrated using a rotary evaporator and dried in a freeze-dryer.

PHYTOCHEMICAL EXAMINATION OF DRUG

Phytochemical examinations were carried out for all the extracts as per the standard Methods (Brain & Turner 1975, Evans 1996).

1. Plant Constituents	-----	Alkaloids
Test / Reagent Used	-----	Mayer's Reagent
2. Plant Constituents	-----	Carbohydrates & Glycosides
Test / Reagent Used	-----	Fehling Solution
3. Plant Constituents	-----	Phenolic Compounds & Tannins
Test / Reagent Used	-----	Ferric Chloride Solution
4. Plant Constituents	-----	Flavonoids
Test / Reagent Used	-----	Alkaline Reagent Test
5. Plant Constituents	-----	Phytosterols
Test / Reagent Used	-----	Liebermann Burchard's Test
6. Plant Constituents	-----	Terpenoids
Reagent / Test Used	-----	Acidic Reagent Test
7. Plant Constituents	-----	Saponins
Reagent / Test Used	-----	Foam / Froth Test

ANTIBACTERIAL ACTIVITY BY DISC DIFFUSION METHOD

Using the agar well diffusion method, the potential antibacterial activity of *A. paniculata* extract was investigated. Muller Hinton agar (25 ml) was added to the sterile Petri dishes, and the agar was left to solidify. Using a sterile borer,

5mm diameter wells were punched into the medium before the plates were streaked with bacterial culture. Using sterile cotton swabs, the bacterial cultures were inoculated into the Petri plates once the agar had solidified. Next, 0.1 millilitre of plant extract diluted in peptone water was directly added to the well created on the Muller Hinton agar surface that contained bacterial lawn. Amikacin (3 mg) was used as a positive control, and wells containing just solvent were kept as a negative control. The diameter of the zone of inhibition was measured in millimetres after the inoculated plates were left to grow overnight at 37 °C.

III. RESULTS AND DISCUSSION

Table 1:- Phytochemical Analysis Test Chart of *Andrographis paniculata*.

Phytochemical Tests	Kalmegh (<i>Andrographis paniculata</i>)			
	Chloroform	Methanol	Petroleum Ether	Aqueous
Alkaloids (Mayer's Reagent)	—	+	+	+
Carbohydrates & Glycosides (Fehling Solution)	—	—	—	+
Phenolic compounds & Tannins (Ferric Chloride Test)	—	+	—	+
Flavonoids	—	+	+	+
Steroid	+	+	+	+
Terpenoids	—	+	+	—
Saponins	—	+	—	+

(+) --- Positive (-) ---- Negative

ANTI-BACTERIAL ACTIVITY OF DRUG EXTRACT FROM SOXHLATE EXTRACTION METHOD

A) Chloroform Extract

Table 2:- Anti-Bacterial Activity of Chloroform Extract of *Andrographis paniculata*.

SL. No.	Name of the Drug	Micro-organism	Zone of Inhibition (in mm)
01.	<i>Andrographis paniculata</i>	<i>E. coli</i>	08 mm
		<i>S. aureus</i>	13 mm

B) Petroleum Ether Extract

Table 3:- Anti-Bacterial Activity of Petroleum Ether Extract of *Andrographis paniculata*.

SL. No.	Name of the Drug	Micro-organism	Zone of Inhibition (in mm)
01.	<i>Andrographis paniculata</i>	<i>E. coli</i>	No ZOI
		<i>S. aureus</i>	No ZOI

ZOI - (Zone of Inhibition)

B) Methanol Extract

Table 4:- Anti-Bacterial Activity of Methanol Extract of *Andrographis paniculata*.

SL. No.	Name of the Drug	Micro-organism	Zone of Inhibition (in mm)
01.	<i>Andrographis paniculata</i>	<i>E. coli</i>	17 mm
		<i>S. aureus</i>	11 mm

C) Aqueous Extract

Table 5:- Anti-Bacterial Activity of Aqueous Extract of *Andrographis paniculata*.

SL. No.	Name of the Drug	Micro-organism	Zone of Inhibition (in mm)
01.	<i>Andrographis paniculata</i>	<i>E. coli</i>	11 mm
		<i>S. aureus</i>	14 mm

ANTI-BACTERIAL ACTIVITY OF SOME STANDARD ANTIBIOTICS

Table 6 :- Anti-Bacterial Activity of standard drugs.

SL. No.	Micro-organism	Zone of Inhibition (in mm)	
		P10	OFX5
01.	<i>E. coli</i>	18 mm	19 mm
02	<i>S. aureus</i>	17 mm	20 mm

P10 - Penicillin G; OFX5 - Ofloxacin

V. CONCLUSION

According to the findings of this investigation, Kalmegh's four extracts—chloroform, petroleum ether, methanol, and aqueous—exhibited antibacterial qualities against the two bacteria, *S. aureus* and *E. coli*. In a comparison between the four extracts and the common antibiotics Penicillin G and Ofloxacin, the methanolic extract was found to have the greatest potential when measured against aqueous, petroleum ether, and chloroform extracts. The andrographolide is responsible for the antibacterial activity of the Kalmegh methanolic extract.

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