



ANTI-BACTERIAL ACTIVITY OF ETHANOLIC EXTRACT OF ANDROGRAPHIS PANICULATA

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ABSTRACT

This present was an attempt to study the antibacterial activity of Ethanolic Extract of *Andrographis Paniculata* the extracts of the roots were obtained by successive extraction with solvents according to increasing polarity. These extracts were subjected to photochemical screening and tests for antibacterial activity. Disc diffusion method was chosen to elucidate the antibacterial activity of the extracts, using penicillin as a reference standard. The activity was tested on *Bacillus cereus*, *Staphylococcus aureus*, *E. coli* and *Pseudomonas aeruginosa* variety of bacteria. All the extracts except the aqueous extract were found to have antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* cultures, among which the petroleum ether extract was seen to have maximum activity.

KEYWORDS: *Andrographis Paniculata*, *Bacillus cereus*, *Staphylococcus aureus*, *E. coli* and *Pseudomonas aeruginosa*.

INTRODUCTION

Indian medicine has come down to us in several strands, four of which stand out rather conspicuously. The first strand is composed of professional physicians, called *Vaidyas* or *Bhishaks* originally belonging to a class of people known as the *ambashtas*. The second consists of wondering pedlars, mostly of tribal origin, who collect herbs and drugs from forests and mountains and sell them in villages. The third group comprises temple priests, especially of the *Vaikhanasa* persuasion, who are also required to function as physicians. The fourth strands is illustrated by what may be called the 'Home Remedies' the medical wisdom and practices that were until recently current within the domestic set-up as "grandmother's prescription" all over the country.^[5]

Ayurveda consider human being as a whole. It believes that imbalance in the Dosha, Dhatus and Malas generated disease and the restoration of balance in these climates disease. The aim of treatment is not only to cure the disease but also to root out the cause so that it may not take place in future. The aim of treatment is also to improve the vitality and to strengthen the immune system.^[6] The identity of drug plant was based on the doctrine of signatures, viz. "Pashanbhed" meaning thereby the plant, which can split a stone or grow through rock. It is also indicative of dissolving or splitting of the stones in the bladder or kidney. The doctrine of signatures was not often recorded, but the knowledge was handed down from "guru" to discipline

or father to son where the Ayurveda profession was confined to the family.^[7]

A deeper study of ayurveda can suggest the activities, which can be tested by modern methodologist. Natural products are contemplated the standard of efficiency and safety as applicable to any modern drug should be applied and if found acceptable, the only they become real ayurvedic remedies, which can be any modern medical practitioner of any healing art. In these times of iatrogenic (drug induce) disease and drug price causing economic catastrophies in families and to the country, it is necessary to take resource to ayurvedic not merely herbal remedies for wider benefit of ailing humanity and living being the world.^[8]

Drug Research and Development Using Plants

Nature always has been a valuable source of drugs and despite the unprecedented opportunities afforded by medicinal chemistry continues to deliver lead compounds. "there are more things between heaven and earth..." Despite the progress of science during the past four centuries, Shakespeare's word did not lose their actuality. Knowledge about the etiology of disease is still limited, and for many life threatening illness no effective treatment exist.^[9] Natural products, including plants, animals and minerals have been the basis of treatment of human diseases. History of medicine dates back practically to the existence of human civilization. The current accepted modern medicine or allopathy has

gradually developed over the years by scientific and observational efforts of scientists. However, the basis of its development remains rooted in traditional medicine and therapies.

The history of medicine includes many ludicrous therapies. Nevertheless, ancient wisdom has been the basis of modern medicine and will remain as one important source of future medicine and therapeutics.^[10]

The search of new drugs of plant origin has yielded fruitful result in the past. Today it is possible to use molecular techniques for detection of genetic variability and tagging desired traits as well as culling out duplicates in accessions. The availability of high throughput screening has made the possibility of converting 'hits' into lead compounds in comparatively short time. Drug development from plant sources using gene/molecular techniques is becoming increasingly important.

In terms of modern research endeavor drug development from plants must necessarily imply a multi-disciplinary approach. Recent computerized studies of ethnomedical information, albeit in specialized areas of biological activity, have nevertheless confirmed that plants are a reservoir of chemical agents with therapeutic potential. What has been hitherto missed is collection of data gathered in the trial-and-error is a prolonged clinical trial. A multidisciplinary approach to drug development from medicinal plants used in traditional medicine was tried out in several technical assistance programmes by UNIDO. UNIDO technical assistance programme envisaged that if a successful candidate plant was identified, then the large-scale plant cultivation was to be implemented with FAO and the clinical assessment of the drug be conducted with WHO participation. Success was recorded in a number of instances and the programme introduced the concept of goal-oriented research.^[11]

The goals of using plants as source of therapeutic agents are

- a) To isolate bioactive compounds for direct use as drugs.
eg. Digoxin, digitoxin, morphine, reserpine, taxol, vinblastin, vincristine.
- b) To produce bioactive compound of novel or known structures as lead compounds for semi-synthesis to produce patentable entities of higher activity and / or lower toxicity. Eg. Metformin, nabilone, oxycodon, taxotere, teniposide, verapamil and amiodarone.
- c) To use agents as pharmacologic tools.
Eg. Lysergic acid diethylamide, yohimbine.
- d) To use the whole plant or part of it as a herbal remedy.
Eg. Cranberry, Echinacea, feverfew, garlic, ginkgo biloba.^[12]

Ethnobotanical approach to Drug Discovery

For pharmaceuticals ranging from digitalis to vincristine the ethnobotanical approach to drug discovery has proved successful. The advent of high-throughput, mechanism-based *in vitro* bioassays coupled with candidate plants derived from ethnopharmacological research has resulted in the discovery of new pharmaceuticals such as prostatin, a drug candidate for treatment of human immunodeficiency virus, as well as a variety of novel anti-inflammatory compounds.

Historically, ethnobotanical leads have resulted in three different types of drug discovery.

1. Unmodified natural plant products where ethnomedical use suggested clinical efficiency. Eg. Digitalis
2. Unmodified natural products of which the therapeutic efficiency was only remotely suggested by indigenous plant use.
Eg. vincristine.^[11]

The present study is aimed at Phytochemical screening and Anti-bacterial activity on roots of *Andrographis paniculata* belonging to the family Acanthaceae.

METHODOLOGY

Leaves of *Andrographis paniculata* were collected. They were dried, powdered and stored in an air tight container for further use.

EXTRACTION OF PLANTS

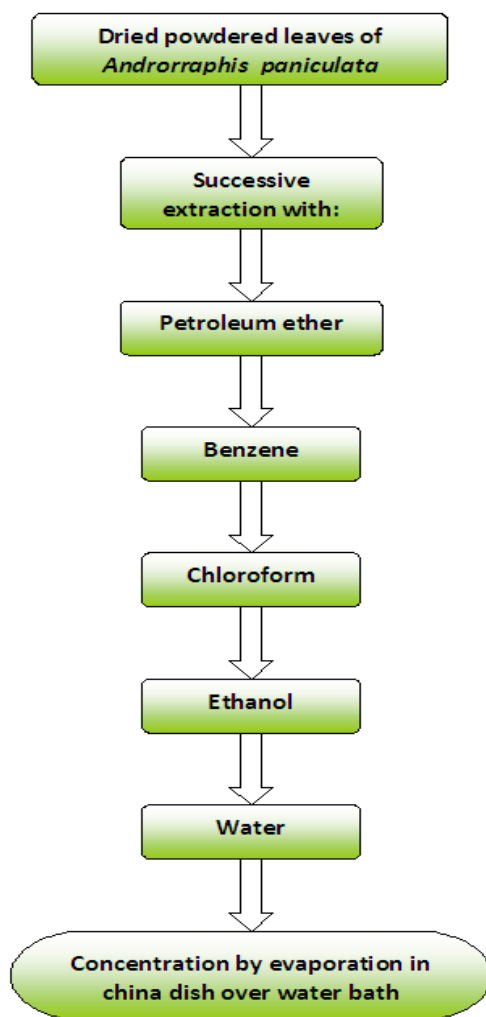
Successive solvent extraction

The method is based on the extraction of active constituents present in the crude drug using solvents ranging from non-polar to polar. The solvents used were Petroleum ether, benzene, chloroform, ethanol and water. The extraction was carried out in a Soxhlet apparatus. The apparatus included a Soxhlet tube in which the drug was placed, a round bottomed flask for collection of extract, a condenser for reflux and a heating mantle to supply heat. The extract collected in the round bottomed flask was evaporated in china dish.

PROCEDURE

Plant extracts were prepared by successive Soxhlet extraction method. The plant materials (leaves) were powdered and extracted successively with different solvents (non-polar to polar). The solvents used were Petroleum ether, Benzene, Chloroform, Ethanol and Water. The plant extracts were filtered through Whatman No. 1 filter paper into beaker. The filtrates were dried until a constant dry weight of each extract was obtained. The residues were stored at 4°C for further use.

Flow chat of extraction procedure



PHOTOCHEMICAL SCREENING

Detection of Tannins

The extracts are dissolved in the respective solvents and subjected to the following tests.

- Test with 5% FeCl₃ solution:** Formation of a blue-black or greenish yellow colour indicates the presence of tannins.
- With Lead Acetate:** Formation of a white precipitate indicates the presence of tannins.
- With Acetic Acid:** Formation of red colour indicates the presence of tannins.
- With dilute Iodine:** Formation of a transient red colour indicates the presence of tannins.

Detection of Alkaloids

Small portions of extracts were dissolved in the respective solvents and subjected to the following tests:

- Dragendorff's test** (Potassium bismuth iodide solution): Extracts were treated with dragendorff's reagent. Formation of reddish-brown precipitate indicates the presence of alkaloids.
- Mayer's test** (Potassium mercuric iodide solution): Extracts were treated with Mayer's reagent. Formation of cream coloured precipitate indicates presence of alkaloids.

- Hager's test** (saturated solution of picric acid): Extracts were treated with Hager's reagent. Formation of yellow precipitate indicates presence of alkaloids.

- Wagner's test** (Iodine and Potassium iodide): Extracts were treated with Wagner's reagent. Formation of a reddish brown precipitate indicates the presence of alkaloids.

Detection of Glycosides

Extracts were dissolved in the respective solvents and subjected to the following tests.

Cardiac glycosides

- Baljet's test:** The extract is treated with sodium picrate solution. Formation of a yellow colour indicates the presence of cardiac glycosides.
- Legal's test** (for cardenolides): The extracts were treated with pyridine and 1ml of sodium nitroprusside. A pink to red colour indicates the presence of cardenolides.
- Kellar-killiani test:** The extract is treated with glacial acetic acid and 1 drop of FeCl₃ and concentrated sulfuric acid. Formation of a reddish

brown precipitate indicates the presence of cardiac glycosides.

- d) Liebermann's test** (for bufadienolides): Extracts were treated with 3ml acetic anhydride and heated. To it a few drops of concentrated sulfuric acid was added. Formation of a blue colour indicates the presence of bufadienolides.

Steroids

Salkowski test: Extract is treated 2ml chloroform and 2ml concentrated sulfuric acid. If the chloroform layer appears red and acid layer shows greenish yellow fluorescence, it indicates the presence of steroids.

- b) Liebermann-Burchard test:** The extract is treated with chloroform and 1-2ml acetic anhydride followed by 2 drops of concentrated sulfuric acid.

The appearance of an initial red colour which changes to blue then green indicates the presence of steroidal glycosides.

- a) Liebermann test:** The extract is treated with 3ml acetic anhydride and heated. After cooling, a few drops of concentrated sulfuric acid are added. A blue colour indicates the presence of steroids.

Saponin glycosides

Foam test: Water is added to the extract and shaken vigorously. Formation of foam indicates the presence of saponin glycosides.

Flavonoid glycosides

- a) Shinoda test:** The extract is treated with 5ml of 95% ethanol and a few drops of concentrated hydrochloric acid and 0.5gms of magnesium turnings. A pink colour indicates the presence of flavonoids.

b) Lead acetate test: The extract is treated with lead acetate. Formation of a yellow colour precipitate indicates the presence of flavonoids.

c) Sodium hydroxide test: The extract is treated with sodium hydroxide. A yellow colour which is decolorized by acids indicates the presence of flavonoids.

Anthraquinone glycosides

b) Borntrager's test: The extract is heated with dilute sulfuric acid and filtered. To the filtrate an equal volume of chloroform is added and shaken well. The organic layer is separated and to it ammonia is added. A pink to red colour in the ammoniacal layer indicates the presence of anthraquinone glycoside.

c) Modified Borntrager's test: Dilute FeCl_3 is added and heated and then the extract is heated with dilute sulfuric acid and filtered. To the filtrate an equal volume of chloroform is added and shaken well. The organic layer is separated and to it ammonia is added. A pink to red colour in the ammoniacal layer indicates the presence of anthraquinone glycoside.

Detection of Carbohydrates

Extracts were dissolved individually and filtered. The filtrates were used to test for the presence of carbohydrates.

- a) Molisch test:** Filtrate is treated with 2 drops of molisch reagent (alcoholic α -naphthol solution) in a test tube and 2ml of concentrated sulfuric acid was carefully added along the sides of the test tube. Formation of a violet ring at the junction of two layers indicates the presence of carbohydrates.
- b) Benedict's test:** Filtrate was treated with Benedict's reagent and heated on a water bath. Formation of red precipitate indicates the presence of reducing sugars.
- c) Fehling's test:** Filtrates were heated with equal quantities of Fehling's A and B solutions. Formation of a brick red precipitate indicates the presence of reducing sugars.

Detection of Proteins

The extracts were dissolved in the respective solvents and subjected to the following tests.

- a) Biuret's test:** The extract is treated with 4% sodium hydroxide and a few drops of 1% Copper sulfate solution. Formation of a violet to pink colour indicates the presence of proteins.
- b) Million's test:** The extract is treated with 5ml million's reagent. Formation of a white precipitate which turns red on warming indicates the presence of proteins.

Antibacterial Activity of Extracts

The extracts obtained by successive extraction were subjected to tests for antibacterial activity.

Nutrient Media

Beef extract: 10.0gms
Peptone: 10.0gms
Sodium chloride: 5.0gms
Agar: 12.0gms
Distilled water: 1000ml

Generally, the antibacterial activity of a compound is expressed in terms of its ability to inhibit the growth of bacteria. The inhibition can be measured by either dilution method or by diffusion method. The serial dilution method is more useful in determining the antibacterial activity quantitatively. This method is not of much use in detection tests and also for evaluation of a large number of samples.

The method adopted for this investigation was the disc-diffusion method as it has the advantage of showing both inhibition and control growth (outside the zone of inhibition) of fastidious organisms on the same plate.

Preliminary Antibacterial Screening

The activity of all five extracts and isolated compounds were evaluated by disc diffusion method. This method is based on diffusion of antibacterial component from disc to the surrounding inoculated nutrient agar medium, so that the growth of microorganisms is inhibited as circular

zone around the disc. One gram positive and four gram negative organisms were Selected viz. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Proteus mirabilis* and *Klebsiella pneumoniae* for the present study.

Organism used

Standard cultures *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Proteus mirabilis* and *Klebsiella pneumoniae* were obtained from Microbiology department of Deccan school of pharmacy, Hyderabad. Staining techniques and biochemical reactions identified the microorganisms. The organisms were maintained by sub-culturing at regular intervals in nutrient agar medium.

Preparation of inoculum

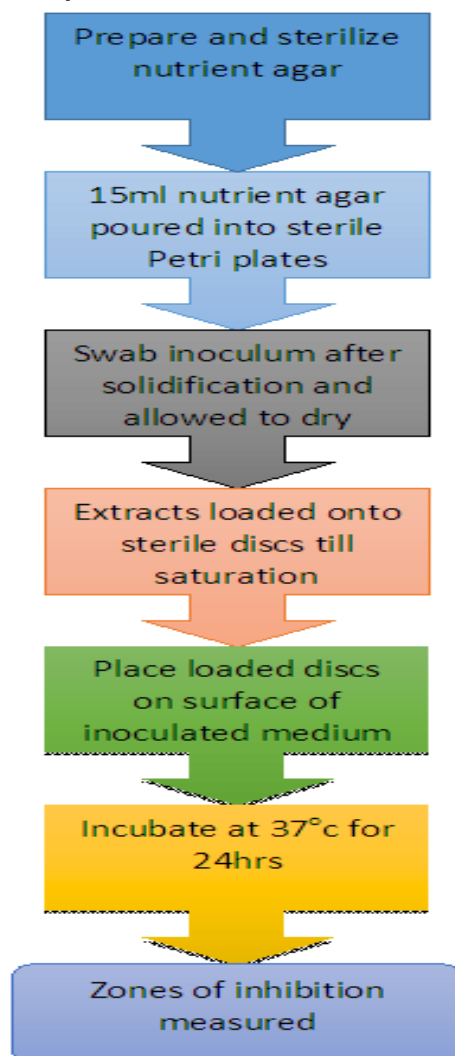
Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of Nutrient agar broth for bacteria

and incubated without agitation for 24 hrs at 37°C. The cultures were diluted with fresh Nutrient agar.

Antibacterial susceptibility test

The disc diffusion method was used to screen the antibacterial activity. In vitro antibacterial activity was screened by using nutrient agar media obtained from Himedia (Mumbai). The nutrient agar plates were prepared by pouring 15 ml of molten media into sterile Petri plates. The plates were allowed to solidify for 5 minutes and 0.1 % inoculum suspension was swabbed uniformly and the inoculum was allowed to dry for 5 minutes. The different extracts and isolated compounds were loaded on 3mm sterile disc till saturation. The loaded disc was placed on the surface of medium and the compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37°C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. These studies were performed in triplicate by using standard drugs (10 units/disc Ampicillins and ciprofloxacin ; for bacteria).

Flow chart for antibacterial susceptibility test



RESULTS

Fresh leaves of the plant *Andrographis paniculata* were collected and dried.

PERCENTAGE YIELD OF EXTRACTS

EXTRACT	COLOUR OF EXTRACT	% YIELD (W/W)
Petroleum ether	Bottle green	5.3%
Benzene	Brown	5.2%
Chloroform	Dark Green	4.9%
Ethanol	Green	4.6%
Aqueous	Brown	6.6%

PHYTOCHEMICAL SCREENING

Chemical constituent	Test	P. E. Ext.	Ben. Ext	Chl. Ext	EtOH. Ext.	Aqu. Ext
Tannins	Ferric chloride test	+	+	-	+	-
	Lead acetate test	+	+	-	+	-
	Acetic acid sol.	-	-	-	+	-
	Dil. Iodine sol.	+	-	-	+	-
Alkaloids	Mayer's test	+	-	+	-	-
	Dragendroff's test	+	-	+	-	-
	Hager's test	+	-	+	-	-
	Wagner's test	+	-	+	-	-
Glycoside						
A. Cardiac glycosides	Baljet's test	+	-	-	-	-
	Legal's test	+	-	+	-	-
	Keller-killiani test	+	-	-	-	-
	Liebermann's test	+	-	+	-	-
B. Steroids	Salkowski test	+	-	+	-	-
	Liebermann-burchard test	+	-	+	-	-
	Liebermann's test	+	-	+	-	-
C. Saponins	Foam test	+	+	+	+	+
D. Flavonoids	Schinoda test	-	-	-	-	+
	Lead acetate test	-	-	-	-	+
	NaOH test	-	-	-	-	+
E. Anthraquinones	Borntrager's test	-	-	-	-	-
	Modified-borntrager's test	+	-	-	-	-
Carbohydrates	Molisch test	+	+	+	+	+
	Fehling's test	-	-	+	+	+
	Benedict's test	-	-	+	+	+
Proteins	Biuret's test	-	-	-	-	-
	Millon's test	-	-	-	-	-

Anti Bacterial Activity

The Extracts were subjected to antibacterial activity using different micro organisms.

Zone of inhibition of different extracts

Organisms	Zone of inhibition of extracts		STD
	EtOH.E	Aq.E	
1. <i>P.aeruginosa</i>	4.5mm	2.3mm	5 mm
2. <i>P.mirabilis</i>	3mm	2.8mm	5mm
3. <i>P.vulgaris</i>	4mm	3mm	5 mm
4. <i>K.pneumoniae</i>	3mm	2mm	5mm
5. <i>S.aureus</i>	3.2mm	1.8mm	5mm

NI = No Inhibition

EtOH. E = Ethanolic extract

Aq.E = Aqueous extract

STD =Standard (Ciprofloxacin 5 units/disc)

Antibacterial activity of different extracts



P.aeruginosa

**P.mirabilis****P.vulgaris****K.pneumoniae****S.aureus****EtOH**-Ethanol extract**Aq.E** – Aqueous extract**Std**-Standard ciprofloxacin**CONCLUSION**

The present project is an attempt to study the extraction, phytochemical screening and antibacterial properties of leaves of the plant *Andrographis paniculata*. The dried leaves were powdered and extracted successively with different solvents based on polarity (petroleum ether, Benzene, chloroform, ethanol and water). The concentrated extracts were subjected to phytochemical screening. All the extracts were tested for presence of antibacterial activity. Among all extracts, ethanolic extracts exhibited antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *proteus mirabilis*, and *klebsiella pneumoniae* cultures. The maximum activity was shown by ethanolic extract in *staphylococcus aureus* and *Pseudomonas aeruginosa*.

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