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Antibacterial activity of *Andrographis Paniculata* L.

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ABSTRACT

Objectives

Ethanollic extract and chloroformic extracts of *Andrographis Paniculata* leaves was investigated for antibacterial activities.

Methods

The well diffusion method was followed for antibacterial assay. Antibacterial activity of the leaves extract using well diffusion method according to Bauer et al., (1996). In this disc was prepared by using whatmann No.1 filter paper. Then, the filter paper disc of 5mm diameter were sterilized and soaked in the different concentration of plant extract.

Results

The antibacterial activity of *Andrographis Paniculata* was tested against 4 species of bacteria, viz, *Staphylococcus*, *Streptococcus*, *Bacillus pseudomonas* and *E.Coli*.

Conclusion

Aqueous ethanolic extract and chloroformic extracts of leaves was screened for their antibacterial potential. The zone of inhibition was seen to be largest when chloroform extract was used.

Keywords: *Andrographis Paniculata*, Antibacterial activity

INTRODUCTION

We all live in a world filled with microbes, from birth until death. Microorganism are in large part, responsible for determining the course of human history. They are carried by air currents from

the earth's surface to the upper atmosphere. The condition that favour the survival of growth of many microorganism is those under which people normally live, it is inevitable that we live among a multitude of microbes.^[1]

The use of antimicrobial agents for treating infectious disease has to greatly increased life expectance. Human disease caused by bacterial pathogens includes Tuberculosis, Whooping cough, Diphtheria, Cholera, Typhoid fever, Tetanus, Gonorrhoea, Syphilis and Pneumonia.

Many new biological pathogens have been recognised in the past twenty five years. Which are responsible for causing numerous diseases. [2]

Staphylococcus aureus produces disease by either production of toxin or direct invasion and destruction of tissue due to toxin activity. Example Staphylococcal scaled skin syndrome, toxic shock syndrome and staphylococcal food poisoning, whereas other disease involving proliferation of the organism with abscess formation and tissue destruction. [3]

Streptococci are gram- positive bacteria that characteristically form pair of chain during growth. They are widely associated with normal human flora others are associated with important human disease attributable in part to infection *streptococci*.

Most *streptococci* that causes human infections are facultative anaerobes, although some are strict anaerobes. [4] The clinical symptoms associated with *Pseudomonas* are pulmonary infection, ear infection and other infection include infection of gastrointestinal tract, urinary tract, eye, central nerves system and musculo skeletal system. [5]

Bacillus cereus is a gram – positive bacteria and it is a rod shaped bacteria. It is common mesophilic saprophytic and is widely distributed in nature. It produces exoenzymes that hydrolyze starch and casein. It can cause type of food poisoning. It is highly pathogenic for human. [6]

Escherichia coli are referred to as colon bacillus. Because, it is the predominant facultative species of the large bowel. Its presence in a water supply usually indicated fecal contamination. *E.coli* is the head of the large bacterial family, Enterobacteriaceae, the enteric bacteria, which are facultative anaerobic gram- negative rods that live in the intestinal tracts of animals in health and disease.

Now – a – day's number of biologically active antibacterial and antifungal compounds is extracted from various plants. [7] Hence in our study, Microbial activity of *Andrographis paniculata* has been studied against *Staphylococci*, *streptococci*, *Pseudomonas*, *Bacillus* & *E. coli*.

The present study was undertaken to the antibacterial effect of *Andrographis Paniculata* against *Staphylococcus*, *Streptococcus*, *Bacillus pseudomonas* and *E.Coli*.

MATERIAL AND METHODS

The present study has been under taken to focus the effect of alcoholic extract of *Andrographis paniculata* on bacterial pathogen such as *staphylococcus*, *streptococcus*, *pseudomonas*, *bacillus* & *E. coli*.

Plant material

The leaves of *Andrographis Paniculata* were collected from S.T.E.T Medicinal plant garden, Mannargudi, Thiruvavur District and authenticated by Botany Department of A.V.V.M. Sri Pushpam College, Poondi. After authentication the plant material were washed under running water.

Preparation of plant extract

Chloroform extract:

The *Andrographis paniculata* was dried and extracted with 1.0 litre of chloroform by continuous hot percolation, (59-61°C) until the completion of extraction. The extract was filtered and the solvent was removed by distillation under reduced pressure. A dark green coloured residue was obtained.

Methanol extract

The mark left after the chloroform extraction was dried and extracted with 100ml of methanol (59.5-61.5°C) by continuous hot percolation, until the extraction was completed. After the completion of extraction, the extraction was filtered and the solvent was removed by distillation under reduced pressure. A dark green coloured residue was obtained.

Preliminary phytochemical screening

Qualitative phytochemical examinations were carried out for all the extracts as per the standard methods.

Test for Steroids

0.5g of the various solvents extract fraction of each plant was mixed with 2ml of acetic anhydride followed by 2ml of sulphuric acid. The colour

changed from violet to blue (or) green in some samples indicates the presence of steroids.

Test for Phenols

1ml of various solvents extracts of sample, 2ml of distilled water, followed by a few drops of 10% aqueous ferric chloride solution were added formation of blue colour (or) green colour indicated the presence of phenols.

Test for Tannins

0.25g of various solvent extract was dissolved in 10ml of distilled water and filtered 1% aqueous iron chloride (FeCl₃) solution was added to the filtrate.

The appearance of intense green, purple, blue or blank colour indicated the presence of tannins in the test sample.

Test for Flavonoids

One ml of extract, 5-10 drops of diluted HCL taken in a test tube. Kept in boiling water both for few minutes. Mejanya colour indicates the presence of Flavonoids.

Test for Carbohydrates

To 2ml Benedict's reagent 5 drops of extract was added. It was boiled for 5 minutes and cooled. Formation of reddish yellow or green colour precipitated showed the presence of carbohydrates.

Test for Alkaloids

2ml of extract was taken in a test tube and few ml of Wagner's reagent to be added. Formation of reddish brown colour precipitated indicates the presence of alkaloids.

Test for Glycosides

2ml of extract, 1ml of Chloroform taken in a test tube. Added 10% Ammonium solution. Pink colour not formed absence of Glycosides.

Test for Saponin

About 1ml of the extract was diluted to 5ml of water was and the tube was shaken vigorously. Formation of 1cm layer of foam indicates the presence of saponin.

Test for Terpenoids

One ml of extract with 2ml Chloroform and concentrated sulphuric acid added. Red brown colour shows the presence of Terpenoids.

Test for Anthraquinones

5ml of the extract solution was hydrolysed with diluted concentrated H₂SO₄ extracted with benzene. 1ml of diluted ammonia was added to it. Rose pink colouration suggested the positive response for Anthraquinones.

Test for Proteins: (Pitrowski Test)

2ml of the extract, 2 drops of 0.05 % CuSO₄ and 2ml of 10% NaOH was added. Appearance of violet/purple colour is considered as a positive test for Proteins.

Test for Anthocyanidins

5 ml of the extract, 5 ml of methanolic HCL was added. The Formation of red or purple colour considered as a positive test for Anthocyanidins.

Test for reducing sugars

5 ml of the extract, 5ml of Benedict's reagent was added. The test tube is incubated in boiling water bath for 10 – 30 minutes. The development of an orange red precipitate indicates presence of Reducing Sugars.

Antibacterial activity

The antibacterial activity was carried out against *Staphylococcus*, *Streptococcus*, *Bacillus pseudomonas* and *E.Coli*. The well diffusion method was followed for antibacterial assay. Antibacterial activity of the leaves extract using well diffusion method according to Bauer et al., [8]. In this disc was prepared by using whatmann No.1 filter paper. Then, the filter paper disc of 5mm diameter were sterilized and soaked in the different concentration of plant extract.

Procedure

10ml of sterilized agar medium for *Staphylococcus*, *Streptococcus*, *Bacillus pseudomonas* and *E.Coli*. were poured into the each sterile petridish. After solidification, the sterile cotton swab dipped into the culture or broth of *Staphylococcus*, *Streptococcus*, *Bacillus pseudomonas* and *E.Coli*. The entire agar surface of each plate was inoculated with this swab first in a horizontal direction and then in a vertical direction, which ensure the distribution of organism over the agar surface. The filter paper disc soaked in leaves extracts was placed on the surface of the bacteria seeded agar plate and then the plate was

incubated at 37 hours. The antibacterial activity was recorded by measuring the width of the clear zone around each disc.

Sensitivity test for antibiotics

Commercially available antibiotics discs viz, Ampicillin 10 ug, ciprofloxacin (CL) 5 g were used. In this study for the entire test microorganism and considered as standard controls. The sensitivity patterns were recorded and the readings were interpreted according to the critical diameter given by National committee for clinical laboratory standards.

RESULT AND DISCUSSION

The objective of the present study is to prove the antibacterial activity of chloroform and methanolic extracts of *Andrographis paniculata* on the pathogenic bacteria *staphylococcus*, *streptococcus*, and *Bacillus*, *Pseudomonas* and *E. coli*.

Investigation were carried out to study the effect of the chloroform and methanolic extracts on pathogens were observed by well diffusion method.

The inhibition developed by the chloroform and methanolic extracts were observed by measuring the width of the zone (in mm).

Phytochemical screening

Alcoholic extracts of *Andrographis paniculata* was subjected to evaluate the phytochemical constituents. The result was presented in Table (1). The alcoholic extract of *Andrographis paniculata* showed higher amount of carbohydrate alkaloids fixed oils, fats, flavonoids, protein free amino acids, tannins, phenols were recorded. Small amount of phytosterol and saponins were present. Gums and Mucilage was absent.

Phytochemical screening revealed the present of flavonoids which could be responsible for its anti-inflammatory and oxidant activity. Flavonoids have been reported to exert multiple biological effects such as anti-inflammatory, anti-allergics, anti-viral and anti-cancer activities.^[9]

Alkaloids are important defence of the plant against pathogenic organism and herbivores or protoxins for insects which further modify the alkaloids and incorporated into own defence/ secretion.^[10]

Table -1: phytochemical analysis of ethanol and aqueous extract of *Andrographis Paniculata* leaves

S.NO	PHYTOCHEMICALS	AQUEOUS EXTRACT	ETHANOL EXTRACT
1.	Steroids	+	+
2.	Phenols	+	+
3.	Tannins	+	+
4.	Flavonoids	+++	+++
5.	Carbohydrate	+++	+++
6.	Alkaloids	+++	+++
7.	Glycosides	-	-
8.	Saponins	++	++
9.	Terpenoids	-	-
10.	Anthraquinones	-	-
11.	Protein	+++	+++
12.	Anthocyanidins	-	-
13.	Reducing sugars	-	-

(+++) Appreciable amount

(++) Moderate amount

(+) small amount

(++) Minor

(-) No phytochemical

Antibacterial study

Efficacy of whole plant of *Andrographis paniculata* against the pathogens has been performed by well diffusion method which is supported by the earlier works.^[11] The present work has done to compare the ethanolic & chloroformic extracts of *Andrographis paniculata* to prove the antibacterial effects against *staphylococcus*, *streptococcus*, *Bacillus Pseudomonas* and *E. coli* species.

Effects of *Andrographis paniculata* extract of *staphylococcus* species

Preliminary screening of *Andrographis paniculata* extract against *staphylococcus* under laboratory condition revealed that it was found to be inhibition of the bacterial growth by chloroform extract which is evident from table(2). The width inhibition Zone was found to be 32 mm. The other extract methanolic also forms inhibition zones of width 30 mm. A clear Zone is observed by using Antibiotics as control. The width of Zone was found to be 30 & 32 mm.

Effects of *Andrographis paniculata* extract of *streptococcus* species

Streptococcus is gram – positive spherical bacteria and cause urinary tract infection, streptococcal, sorethroat, streptococca;, phoderma. Acute glomerulonephritis and rheumatic fever.^[4]

Preliminary screening of *Andrographis paniculata* extract against *staphylococcus* under laboratory condition revealed that it was found to be inhibition of the bacterial growth by chloroform extract which is evident from table(2).

The width of inhibition zone was found to be 32mm. The other extract methanolic which is evident from table (3). The width of inhibition zone was found to be 30 mm. A clear Zone is observed by using antibiotics as control. The width of zone was found to be 30 & 32mm.

Effects of *Andrographis paniculata* extract of *pseudomonas* species

Preliminary screening of *Andrographis paniculata* extract against *pseudomonas* under laboratory condition revealed that it was found to be inhibition of the bacterial growth by chloroform extract which is evident from table (2). The width of inhibition zone was found to be 24mm. The other

methanolic extract which is evident from table (3). The width of the inhibition zone was found to be 23mm. A clear zone is observed by using Antibiotics as control. The width of the zone was found to be 30 to 32mm.

Effect of *Andrographis paniculata* extract on *E. coli* species

Preliminary screening of *Andrographis paniculata* extract against *E. coli* under laboratory condition revealed that it was found to be inhibition of the bacterial growth by chloroform extract which is evident from table (2). The width of inhibition zone was found to be 20 mm. The other metabolic extract which is evident which from table (3). The width of the inhibition zone was found to be 18mm. A clear zone is observed by using Antibiotics as control. The width of the zone was found to be 30 & 32 mm.

Andrographis pediculate is effective at reducing the prevalence and intensity of cold and sinusitis and shortening the duration of the symptoms. Preliminary research has indicated that *Andrographis paniculata* may also be useful in cardiovascular disease and in preventing liver toxicity^[12]. The ability of *Andrographis paniculata* to lower fever has been demonstrated independently in several laboratories. Rat studies done in china have shown that neoandropholide, and dehydroandropholide can lower the fever produced by different fever including such as bacterial endotoxins (toxins chemically released from bacteria), pneumococcus, hemolytic streptococcus, typhoid, paratyphoid.^[13]

Extracts of *Andrographis paniculata* have been shown to have significant effects against the diarrhea associated with *E. coli* bacterial infection.^[14]

The *Andrographis paniculata* components, andropholide and neoandropholide showed similar activity to loperamide (Imodium) the most common antidiarrheal drug. In another study, *Andrographis paniculata* was used to treat 1, 611 cases of bacterial dysentery and 955 cases of diarrhea with overall effectiveness of 91.3%.^[13]

The above discussion clearly indicated the response of *Andrographis paniculata* against some pathogenic bacterial population and its phytochemical were well documented.

Table 2: Antibacterial effect of Chloroform extract of *Andrographis Paniculata*

S.No	Species Name	Zone of inhibition (in mm)
1.	<i>Staphylococcus</i> ,	32
2.	<i>Streptococcus</i>	28
3.	<i>Bacillus pseudomonas</i>	24
4.	<i>E.Coli.</i>	20
5.	Control	30

Table 2: Antibacterial effect of methanolic extract of *Andrographis Paniculata*

S.No	Species Name	Zone of inhibition (in mm)
1.	<i>Staphylococcus</i> ,	30
2.	<i>Streptococcus</i>	26
3.	<i>Bacillus pseudomonas</i>	24
4.	<i>E.Coli.</i>	20
5.	Control	32

CONCLUSION

Thus the presents study reveals that the selected plant extract have potential bacterial effect against the selected bacterial species and the efficiency was found to be varied according to the nature of solvent system.

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