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Research Article

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Effect of Temperature and Light on Phytochemical Profiling and Antimicrobial Activity of *Andrographis paniculata*

B. Jaikumar, R. Sharmila*

PG-Research Department of Biotechnology and Bioinformatics, Bishop Heber College Trichy-17, Tamil Nadu, India

ABSTRACT

Andrographis paniculata (Acanthaceae) is an annual herb. It is found in Sri Lanka, and throughout the plains of India especially Tamilnadu, Maharashtra, Karnataka, and Orissa. Various medicinal properties like cholevetic, antidiarrheal, immunostimulant and anti-inflammatory have been attributed to this plant in the traditional system of Indian medicine. Further reported activities are hepatoprotective, antimalarial, anticancer, antihypertensive, antipyretic, antithrombotic and antidote for snake bites. The present study aimed to evaluate the anti-microbial activity for the isopropanol extract of *A. paniculata* against different bacterial strains such as *E. coli, K. pneumoniae, P. vulgaris, S. pneumoniae, P. aeruginosa, S. aureus* by determining inhibitory concentration and zone of inhibition. Minimum inhibitory concentration and zone of inhibition values and the high bioactive metabolites production was observed in different extracts of *A. paniculata* under different physical stress (Light and temperatures). The results revealed that, the isopropanol extract of *A. paniculata* is potent for inhibiting bacterial growth and various secondary metabolites production in dark condition at 37°C than other tested parameters.

Keywords: Antimicrobial activity, Phytochemical analysis, Minimum Inhibitory Concentration, *Andrographis paniculata.*

INTRODUCTION

In recent times, there have been increases in antibiotic resistant strains of clinically important pathogens, which have led to the emergence of new bacterial strains that are multi-resistant. [1] The non-availability and high cost of new generation antibiotics with limited effective span have resulted in increase in morbidity and mortality. [2] Therefore, there is a need to look for substances from other sources with proven antimicrobial activity. Consequently, this has led to the search for more effective antimicrobial agents among materials of plant origin, with the aim of discovering potentially useful active ingredients that can serve as source and template for the synthesis of new antimicrobial drugs. [3]

Medicinal plants contain many bio- active principles that over the years have been exploited in traditional medicine for the treatment of various ailments. [4] Secondary metabolite biosynthetic mechanism in plants produces a wide range of chemical compounds. They generally regarded as a nonessential process that produced as by products or plant waste. However, there is evidence that secondary metabolites such as alkaloids, phenolics and terpenoids may provide defence against infection. [5] Recent year, these secondary metabolites are attractive targets for the development of new pharmaceutical drugs, herbicides,

***Corresponding author: Mrs. R. Sharmila,**
PG-Research Department of Biotechnology and Bioinformatics, Bishop Heber College Trichy-17, Tamil Nadu, India;
E-mail: sharmilabiotech81@gmail.com
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cosmetics and pesticides. Many new and interesting bioactive metabolites such as antibiotics, antiviral, anticancer and antioxidant compounds having pharmaceutical value produced by plants. [6] *A.paniculata* is well known plant in Southeast Asia and it is used in traditional Siddha and Ayurvedic systems of medicine as well as in tribal medicine in India and some other countries for multiple clinical applications. [7] *A. paniculata* commonly known as "king of bitter". Andrographolide, the chief constituent extracted from the leaves of this plant, is bitter water-soluble lactones exhibiting various pharmacological activities. [8] Chemical profile of plants and accumulation level of a special metabolite in plant tissues can be influenced by several environmental factors such as temperature light quality and light intensity In this sense, determination of optimum temperatures and light intensities for chemical accumulation as well as plant growth and development is an important topic in obtaining the increased concentration of phytochemical. [9] There are few reports are available on effect of physical stress (temperature and light) on secondary metabolite production in *A. paniculata.* On the basis of this background, *in-vitro* antimicrobial activities and phytochemical profile of the extracts *A. paniculata* as affected by light and temperature were tested against clinically important pathogens.

MATERIAL AND METHODS

Sample Collection

The plant of *A. paniculata* was collected around Tiruchirappalli District, Tamilnadu. The plant material were cleaned with distilled water and shade dried at room temperature. The shade dried plant material was powdered by using electric blunder.

Preparation of Plant extracts

The plant leaf powder (500 g) of *A.paniculata* was extracted separately to exhaustion in a soxhelet apparatus using isopropanol. The extracts were filtered through a cotton plug followed by whatman no: 1 filter paper and stored in different physiological condition.

Test organisms

Bacterial isolates used in this study were (*E. coli, K. pneumoniae, P. vulgaris, S. pneumoniae, P. aeruginosa, S. aureus.)* collected from the Government Hospital, Tiruchirapalli, Tamilnadu and they were maintained on Mueller-Hinton Agar medium. Twenty-four hour old pure cultures were prepared for use each time.

Culture media and Inoculums preparation

Nutrient broth (NA) (HIMEDIA, 1993) was used as media for culturing of bacterial strains. A loop full of microbial cultures was inoculated in the nutrient broth stored in room temperature for 24 hours.

Phytochemical Analysis

Photochemical test were done to find the presence of the bioactive chemicals constituents such as alkaloids, flavonoids, terpenoids, carbohydrate, cardiac glycosides, phenols, phlobatannis, saponins, sterols, tennis, quinines, oxalate, reducing sugar, amino acids, anthraquinones, triterpenoids, leucoantholyanin, coumurins, fatty acids, diterpenes, physterols, protein, lactones, anthrocyanins, xanthoprotein, carboxylicacid, rasin, vitamin-c, starch, anthracenoxids, and catechin compounds and by the following procedure. [10-13]

Antimicrobial Activity

Disc Diffusion Method

In vitro antimicrobial was carried out by disc diffusion technique in whatman no;1 filter paper disc with 4mm diameter were impregnated with known amount test sample of the disc were loaded each with 10µl of the extract by the first applying 5µl with the pipette allowed to evaporate than applying another 5µl than drying again. The positive control contained a standard antibiotic disc sterile disc use as negative control. The impregnated disc along with control (streptomycin) was kept at the center of agar plates, seeded with test bacterial cultures. The discs were then placed individually using a sterile forceps in appropriate grids which were marked on the under surface of the plates Petri plates and kept for incubation at room temperature for 24 hours. After incubation plates were observed for zones of inhibition and recorded in millimeters.

Minimum Inhibitory Concentration and the Minimum Bactericidal Concentration

Minimum inhibitory concentration (MIC) was determined by the micro dilution method. A twofold serial dilution of the extract/fractions was prepared in Mueller Hinton Broth (MHB) and 100µl (approximately 1.5×10^8CFU/ml) of bacteria suspension was added. The samples were incubated for 24 h at 37°C. Resazurin solution (0.01%) was used as an indicator by color change visualization: any color changes from purple to pink were recorded as bacterial growth. The lowest concentration at which no color change occurred was taken as the MIC. Afterwards, cultures were seeded in MHA medium and incubated for 24h at 37°C to determine the minimum bactericidal concentration (MBC) which corresponds to the minimum concentration of extract/fractions that eliminated the bacteria. [14]

RESULTS AND DISCUSSION

Temperature and light are the major environmental factors affecting plant physiology, especially the photosynthesis and development. The physiological changes in plants in response to different stress factors may stimulate the secondary metabolite production for the restoration of the defensive systems. [15] In this study Antimicrobial activity of *A. paniculata* leaves extracts were assessed by using disc diffusion method against some bacterial strains are showed considerable effect (Table 1-3). Among the different physical parameters tested, the dark condition found to be a good result than light condition for its antimicrobial activity at 37°C. However the various tested organisms (*E. coli, K. pneumoniae, P. vulgaris, S. pneumoniae, P. aeruginosa,* and *S. aureus*); *P. aeruginosa* strongly

inhibited by the isopropanol extracts of *A. paniculata* under dark condition at 37°C when comparing with standard drug [streptomycin]. The increase in secondary metabolite concentrations of plants observed in the present study under moderate temperatures and dark conditions may be attributed to those possible physiological changes. It is also possible that biosynthesis of the secondary metabolites under stress conditions. [16] The extract was seen to be active against many opportunistic as well as pathogenic microorganisms like *E. coli* (Crohn's disease and ulcerative colitis), *B. subtilis* (food poisoning), *P. aeruginosa* (nosocomial infections) and *K. peumoniae* (urinary tract infections and pulmonary infections). Among the microorganisms used, *S. aureus* are potentially pathogenic. *S. aureus* in particular can cause a range of infections from minor skin infections to life threatening meningitis, toxic shock syndrome, endocarditis and septicemia. Some of the main components were seen to be present in both the extracts, but differed in their relative amounts, indicating their role in antimicrobial activity (Table 5). Hence the crude extract of *A. paniculata* in isopropanol can be used for further purification and preparation of new antimicrobials for the more resistant type of microorganisms. Bacterial infection is one of the most serious global health issues in 21st century.

Table 1: Zones of inhibition by Isopropanol extracts of *A. paniculata* in light condition at different temperature by Disc diffusion method

S. No	Bacterial Starins	Extract Con. µL	Temperature in °C		
			4°C	37°C	70°C
1	*E. coli*	20	1.0	3.7	0.3
2	*S. aureus*	20	0.5	3.5	0.1
3	*K. pneumoniae*	20	1.1	2	0.9
4	*P. vulgaris*	20	1.7	3	0.6
5	*S. pneumoniae*	20	1.5	2.6	1.0
6	*P. aeurginosa*	20	1.1	2.1	1.1

Table 2: Zones of inhibition by Isopropanol extracts of *A. paniculata* in dark condition at different temperature by Disc diffusion method

S. No	Bacterial Starins	Extract Con. µl	Temperature in °C		
			4°C	37°C	70°C
1	*E. coli*	20	3	5	1.3
2	*S. aureus*	20	2	5.2	0.5
3	*K. pneumoniae*	20	3.7	5.9	1.7
4	*P. vulgaris*	20	2.5	6.2	1.1
5	*S. pneumoniae*	20	2.6	6.8	2.1
6	*P. aeurginosa*	20	2.6	7.8	1.0

Table 3: Zones of inhibition by Isopropanol extracts of *A. paniculata* in light & dark condition by Disc diffusion method

S. No	Bacterial Strains	Extract Con. mL	Light condition at 37°C	Dark condition at 37°C	Streptomycin
1	*E. coli*	10	3.7	5	0.6
2	*S. aureus*	10	3.5	5.2	0.9
3	*K. pneumoniae*	10	2	5.9	0.8
4	*P. vulgaris*	10	3	6.2	0.1
5	*S. pneumoniae*	10	2.6	6.8	0.9
6	*P. aeurginosa*	10	2.1	7.8	0.7

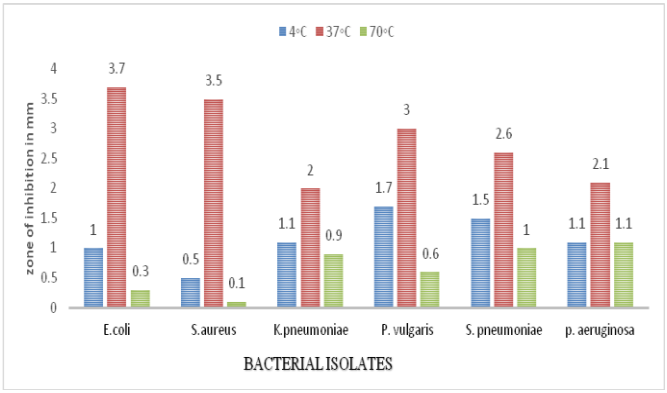

Fig. 1: Zones of inhibition (mm) by Isopropanol extract of *A. paniculata* in light condition at different temperature

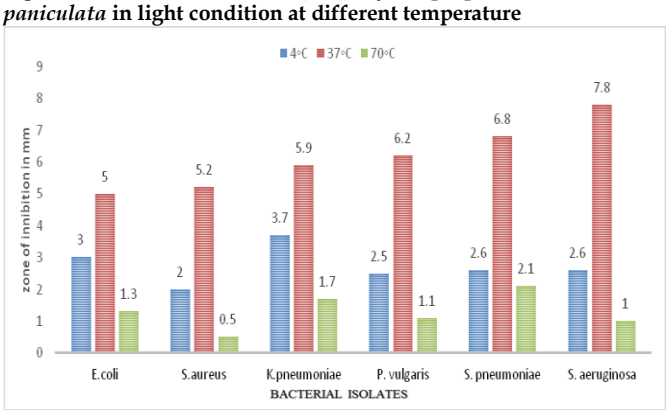

Fig. 2: Zones of inhibition (mm) by Isopropanol extract of *A. paniculata* in dark condition at different temperature

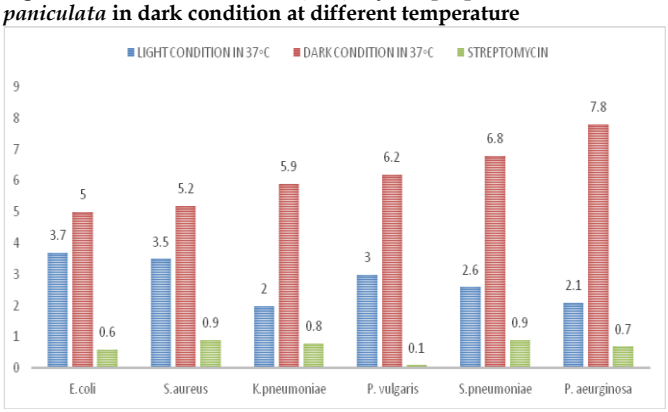

Fig. 3: Zones of inhibition (mm) by Isopropanol extract of *A. paniculata* in light & dark condition compare with Streptomycin.

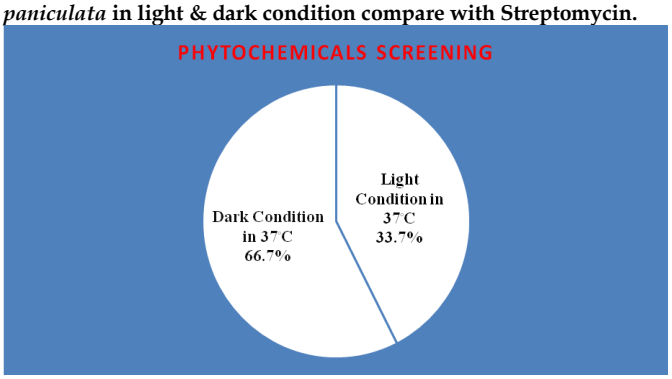

Fig. 4: Phytochemical screening of Isopropanol extract of *A. paniculata*

The emerging bacterial resistance to antibiotics is a major health problem and therefore, it is critical to develop new antibiotics with novel mechanisms of action to overcome these problems. Plants have traditionally provided a source of hope for novel drug compounds as plant herbal mixtures have made large

contributions to human health and wellbeing. The use of plant extracts with known antimicrobial properties can be of great significance of therapeutic treatments. The present study explicitly exhibited the antimicrobial effect of isopropanol extracts of *A. paniculata* against various bacterial strains.

Table 4: Minimal inhibitory concentration of Isopropanol extract of *A. paniculata* (in µg)

S. No	Bacterial Starins	Light condition at 37°C	Dark condition at 37°C
1	*E. coli*	500	250
2	*S. aureus*	500	125
3	*P. vulgaris*	500	500
4	*K. pneumoniae*	500	250
5	*S. pneumonia*	500	250
6	*P. aeurginosa*	250	16.125

Table 5: Phytochemical screening of Isopropanol extract of *A. paniculata*

S. No	Phytochemicals	Light condition in 37°C	Dark condition in 37°C
1	Alkaloids	+	+
2	Carbo hydrate	+	+
3	Cardiacglycosides	+	+
4	Flavonoids	+	+
5	Phenols	+	-
6	Phlobatannis	-	+
7	Saponins	+	+
8	Sterols	-	+
9	Tannis	+	+
10	Terpenoids	-	-
11	Quinines	+	-
12	Oxalate	-	+
13	Reducing sugar	+	+
14	Aminoacids	-	+
15	Anthraquinones	-	-
16	Triterpenoids	-	+
17	Leucoantholyanin	-	+
18	Coumurins	+	-
19	Emodins	-	+
20	Fattyacids	+	-
21	Diterpenes	-	-
22	Physterol	-	-
23	Protein	+	+
24	Lactones	-	+
25	Anthrocyanins	-	-
26	Xanthoprotein	-	-
27	Carboxylicacid	-	+
28	Rasin	-	+
29	Vitamin.c	-	-
30	Starch	+	+
31	Anthracenoxids	-	-
32	Catechin	-	+

The antimicrobial result were also comparable to that of the antibiotic (streptomycin) used as a standard reference. The result also indicated that isopropanol was a suitable organic solvent for extraction of active principles responsible for antimicrobial activity of *A. paniculata*. The inhibitory effect of the extracts justified the medicinal use of *A. paniculata* in the treatment of various diseases by medical practitioners and our results also suggested that temperature and light are important environmental factors to optimize the phytochemical production in extracts of *A. paniculata* under dark condition. These factors can significantly increase the phytochemical profile of it. Further study is mandatory to find out the active compounds of medicinal value.

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