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PRELIMINARY PHYTOCHEMICAL STUDIES AND EFFICACY OF CHLOROFORM EXTRACTS OF CULTURED TISSUES OF PHYSALIS MINIMA (L.) AGAINST PATHOGENS

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Abstract

Chloroform extracts of cultured tissues of *Physalis minima* (L.) family Solanaceae were subjected to preliminary phytochemical analysis and *in-vitro* antibacterial studies. The solvent fraction revealed the presence of alkaloids, fixed oils, resins, steroids, tannins, xanthoproteins and glycosides. Seven bacterial species *Bacillus megaterium* (ATCC 23564), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 25922), *Enterobacter faecalis* (ATCC 35550), *Proteus vulgaris* (ATCC 6380), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923), and three fungal species *Aspergillus niger* (NCIM 596), *Aspergillus fumigatus* (NCIM 291) and *Candida albicans* (NCIM 670) were used for the anti-microbial investigations. Chloroform extracts exhibited activity against all tested organisms. The Minimum Inhibitory Concentration (MIC) value was found to be 100µg for *E. coli*, 250µg for *S. aureus*, 500µg for *B. megaterium*, *B. subtilis*, *E. faecalis*, *P. aeruginosa*, *P. vulgaris* and *C. albicans* and 750µg for *A. niger* and *A. fumigatus*. Among the microbial species tested *E. coli* was found to be highly sensitive to the chloroform extracts of cultured tissues of *Physalis minima* (L).

***Key words*:** Antimicrobial activity, cultured tissues, Phytochemical, *Physalis minima* (L).

1. Introduction

The knowledge of utilization of plants and plant products as medicines is as old as human civilization. Many of the existing medicinal systems such as Ayurveda, Unani, Homeopathy, Naturopathy, Siddha and other alternative medicinal systems have been exploiting plants as effective medicines to cure many harmful diseases. India is the largest producer of medicinal herbs and is appropriately called the Botanical garden of the world (Ahmedulla and Nayer, 1999).

The members of the family Solanaceae are of great economic importance. *Physalis minima* (L.) (Syn. Wild Cape Gooseberry) is a small herbaceous annual plant found throughout East Asia, China and Himalayas and in some places of Australia. The fruits of this plant contain good quantity of vitamin C. Hence, it is used as appetizer, diuretic and laxative (Parotta, 2002). Extracts of the plant have shown anticancer activity (Duke. J. A and Ayensu. E. S, 1985). The principle aim of the work was to study preliminarily the phytochemical nature of *Physalis minima* callus extract and its antimicrobial activity in different concentrations of chloroform solvent against pathogenic bacteria and fungi. In the present experimental study, the chloroform extracts of callus cultures of the plant have been investigated at different concentrations.

2. Materials and Methods

2.1 Plant material

The *Physalis minima* (L.) plant material was collected from Gorantla, Guntur district of Andhra Pradesh, India. The collected material was authenticated by Dr. T. Pullaiah, Professor, Department of Botany, Sri Krishna Devaraya University, Ananthapur. A voucher specimen has been deposited (SAC, DOB/BH/PM-01) at St. Ann's College for women (ANU), Guntur, for future reference.

2.2 Callus culture

Leaves, stem and fruit segments from axenic plants were cut aseptically and placed on Murashige and Skoog's medium (Murashige and Skoog, 1962) (M.S basal medium) containing 1.0mg/ml benzyl amino purine + 0.5mg/ml α – naphthalene acetic acid was used as standard medium and cultured at 22 ± 2°C with 16 hours light and 8 hours darkness. After three weeks, calli were sub-cultured on M.S. Basal medium and harvested again after four weeks.

2.3 Callus Extract Preparation

The calli (4 week old), derived from the explants were collected, dried in an oven at 50± 1 °C for 60-72 hours. They were then homogenized to a fine powder and stored in airtight bottles. Calli powders were extracted with organic solvent (chloroform) for 24 hours by using hot extraction. The extracts were dried in a flash evaporator for 30min and the left over powder was considered 100%. Different concentrations of extracts such as 100, 250, 500, 750 and 1000µg/ml (H. K. R. Prasad S *et al.* 2011) were prepared by redissolving the extract powder in the same solvent.

2.4 Phyto chemical studies

Secondary metabolites are biosynthetically derived from primary metabolites, which serve as defensive, protective or offensive chemicals against microorganisms, insects and higher herbivorous predators. They are very potent in curing very harmful diseases. Phytochemical screening is a paramount step to be done, to know the active principles of the plant which has disease-suppressing efficacy (Rajasekhar. D, 2007). Hence, in the present study, solvent extracts were subjected to preliminary phytochemical investigations for the presence of secondary metabolites such as alkaloids, carboxylic acid, coumarins, fixed oils, flavonoids, phenols, quinines, resins, saponins, steroids, tannins, xanthoproteins and glycosides utilizing standard methods of analysis (Trease GE and Evans W.C, 2002).

2.5 Test organisms

Tests were performed on seven species of selected bacteria namely *Bacillus megaterium* (ATCC 23564), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 25922), *Enterobacter faecalis* (ATCC 35550), *Proteus vulgaris (*ATCC 638), *Pseudomonas aeruginosa (*ATCC 27853), *Staphylococcus aureus* (ATCC 25923) and three fungal species namely *Asperigillus niger* (NCIM 596), *Aspergillus. fumigatus* (NCIM 291) and *Candida albicans* (NCIM 670). These organisms are responsible for various diseases which are mentioned clearly in Table 1. All the above test bacterial species were maintained on Nutrient Agar medium. 36 hour old bacterial culture was inoculated into nutrient broth and incubated on a rotary shaker (Janked and Kunkel Ika, Labortechnik, Germany) at 35 ± 2 °C at 100rpm. After 36 hours of incubation, the bacterial suspension was centrifuged at 10000 rpm for 15 min. The pellet was resuspended in sterile distilled water and the concentration was adjusted to 1×10^8 cfu/ml using UV-Visible Spectrophotometer (Hitachi U-2000, Japan). By reading the OD of the solution to 0.45Å (610nm) it was used for further studies. Fungal colonies were harvested from 9 -10day old cultures which were maintained on Potato Dextrose Agar. The spores were suspended in sterile distilled water and the spore suspension was adjusted to 1×10^8 spores/ml (NCCLS- National committee for clinical laboratory standards).

2.6 Antimicrobial assay

Different concentrations of solvent extracts of the calli were tested for antimicrobial activity by using Antibiotic Sensitivity test. The microbial suspension was mixed evenly with sterile agar medium and poured into sterile Petri plates. After allowing the media to solidify at room temperature, wells of 6mm diameter were bored in the agar with sterile cork borer. Each concentration was checked for antimicrobial activity by introducing equal amounts of the sample (40µl) into wells. The method was repeated in five plates. Plates were allowed to stand at room temperature for 1 hour for the extract to diffuse into the medium and then incubated at 37°C for 24- 48 hours. The zone of growth inhibition around the wells was measured and area of inhibition zone was calculated. Simultaneously the activity of seven standard antibiotics such as Streptomycin (10µg/ml), Gentamycin (10µg/ml), Chloramphenicol (30µg/ml), Vancomycin (10µg/ml), Rifampicin (5µg/ml), Kanamycin (10µg/ml) and Nystatin (10µg/ml) were also tested against the selected seven species of bacteria and three species of fungi, under similar conditions so as to compare the degree of inhibition by the calli extracts. Agar wells fed with corresponding solvents served as control. Minimum inhibitory concentration which is the lowest concentration of extracts inhibiting the growth of organisms was determined for the callus extracts, based on the readings.

3. Results and Discussion

3.1 Preliminary phytochemical studies

As per the results shown in Table 2 the chloroform extracts of plant calli have revealed the presence of alkaloids, fixed oils, steroids, tannins and glycosides.

3.2 Results and discussion for antimicrobial assay

Observation of results from Table 3 indicated that the chloroform extracts of the fruits of *Physalis minima* are inhibitory to all the test organisms. The minimum inhibitory concentration was found to be 250µg/ml for *E. coli*, while it was 500µg/ml against other bacteria *B. megaterium, B. subtilis, E. faecalis, P. vulgaris, P. aeruginosa* and *S.aureus* and one fungal organism *C. albicans*. The other two fungi were controlled at 750µg/ml. Among the microbial species tested *E. coli* was found to be highly sensitive to the chloroform extracts of *Physalis minima* (L).

4. Conclusion

Phytomedicines are effective in treating most of the infectious diseases, mainly skin infections. Most of the secondary metabolites, serve as a plant defence mechanism against microorganisms, insects and herbivores (Cowan M.M, 1999). The different concentrations of chloroform extract of callus cultures of *Physalis minima* (L) were found to contain Alkaloids, Fixed oils, Steroids, Tannins and Glycosides. Antimicrobial activity of the tested medicinal plant can be attributed to any of these constituents. The tabular reports indicated that chloroform extracts were effective against tested microbes. In the present study, the solvent extracts of callus cultures of *Physalis minima* exhibit better

antimicrobial activity as compared with standards. Hence, the detailed phytochemical investigations and antimicrobial screening of secondary metabolites from this plant's cultures may yield promising antimicrobial agents.

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Annexure

Table 1: List of the selected test organisms (bacteria and fungi).

S. No	Name of the Microorganisms	Characteristic feature	Diseases caused by the organisms
1	*Bacillus megaterium* (ATCC 23564)	Gram + ve	Intestinal disturbances.
2	*Bacillus subtilis* (ATCC 6633)	Gram + ve	Food poisoning, Oppurtunistic Pathogen.
3	*Escherichia coli* (ATCC 25922)	Gram – ve	Gastroenteritis, Urinary tract disease.
4	*Enterobacter faecalis* (ATCC 35550)	Gram – ve	Opportunistic human pathogen.
5	*Proteus vulgaris* (ATCC 6380)	Gram – ve	Urinary tract infections.
6	*Pseudomonas aeruginosa* (ATCC 27853)	Gram – ve	Wounds and urinary tract infections.
7	*Staphylococcus aureus* (ATCC 25923)	Gram + ve	Chronic osteomyelitis, Meningitis, endocarditis.
8	*Aspergillus niger* (NCIM 596)	Dichotomously branching, filamentous	Allergy, Asthma.
9	*Aspergillus fumigatus* (NCIM 291)	Monomorphic filamentous fungi	Pulmonary hemorrhage, pneumonia.
10	*Candida albicans* (NCIM 670)	Dimorphic fungi	Oral thrush, Gastritis, Cutaneous infection.

Table 2: Preliminary phytochemical studies of *Physalis minima.*

Phytochemical Constituents	Chloroform
Alkaloids	+
Carboxylic acids	-
Coumarins	-
Fixed oils	+
Flavonoids	-
Phenols	-
Quinones	-
Resins	-
Saponins	-
Steroids	+
Tannins	+
Xanthoproteins	-
Glycosides	+

+ indicates present; - indicates absent

Table 3: Inhibitory activity of chloroform extracts of cultured tissues of *Physalis minima.*

Solvent extract	Product (µg)	AREA OF INHIBITION ZONE (mm^2)									
		A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
Chloroform	**Control**	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60
	100	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60	12.60
	250	12.60	12.60	43.21	12.60	12.60	12.60	12.60	12.60	12.60	12.60
	500	31.43	31.43	56.57	31.43	31.43	31.43	43.21	12.60	12.60	21.21
	750	56.57	56.57	88.00	43.21	31.43	31.43	56.57	21.21	21.21	31.43
	1000	88.00	88.00	169.7	71.50	71.50	43.21	88.00	31.43	31.43	43.21
	Standard	632	172	148	286	148	226	286	164	184	276

A) *Bacillus megaterium* B) *Bacillus subtilis* C) *Escherichia coli* D) *Enterobacter faecalis*
E) *Proteus vulgaris* F) *Pseudomonas aeruginosa* G) *Staphylococcus aureus*
H) *Asperigillus niger* I) *Asperigillus fumigatus* J) *Candida albicans*

STANDARD: Streptomycin (10µg /ml) for *E. coli;* Gentamycin (10µg /ml) for *P. aeruginosa* and *P. vulgaris*; Chloramphenicol (30µg /ml) for *S. aureus;* Vancomycin (10µg /ml) for *B. subtilis;* Rifampicin (5µg /ml) for *E. faecalis;* Kanamycin (10µg /ml) for *B. megaterium;* Nystatin (10µg /ml) for *Asperigillus niger, A. fumigatus* and *Candida albicans.*