

IN VITRO RADICAL SCAVENGING ACTIVITY, ANTI MICROBIAL AND ANTI DIABETIC POTENTIAL OF SOLANUM BETACEUM

SRUTHI S¹, ANBARASI K²

Department of Chemistry, Nirmala College for Women, Coimbatore-18

ABSTRACT:- Medicinal and aromatic plants contain biologically active chemical substances such as Flavanoids, alkaloids, steroids, carotenoids, saponins, tannins, glycosides and other chemical compounds which have curative properties. The plant products are also an alternative source of oils, hydrocarbon and phytochemicals. The phytochemicals have important biological activities like antibacterial, antifungal, antiviral, antiscorbutic, anthelmintic, anti inflammatory and antipyretic. The present study focuses to phytochemical constituents, antifungal, antioxidant and antidiabetic activities of *Solanum betaceum* plant extract.

Key Words: alkaloids, antifungal, antioxidant, antidiabetic

1. INTRODUCTION

Plants provide the traditional medicine systems that have been in existence for thousands of years and continue to provide new medicinal treatments. Plants are being easily available to human beings, have been explored for the medicinal properties, various parts of the plants like leaves, bark, roots etc., [1-3]. The term phytochemistry is the study of phytochemicals, which are chemicals derived from plants. Studying phytochemistry strive to describe the structures of the large number of secondary metabolic compounds found in plants, the functions of these secondary metabolic compounds in human and plant biology and biosynthesis of these compounds. Plants have provided a basis of inspiration of novel drug compounds, as plant derived medicines have made large contributions to human health and well-being. Their role is twofold namely; they provide key chemical structure for the development of new antimicrobial drugs and also as a phyto medicine [4]. The pharmacological studies was already done by several researchers with various plants such as *Leucas aspera* [5], *Bellis perennis* [6], *Gomphrena globosa* L [7], *Amorphophallus campanulatus* [8], *Amorphophallus commutatus* [9].

2. EXPERIMENTAL SECTION

2.1 MATERIALS

2.1.1 PREPARATION OF PLANT EXTRACT

The plant materials were collected from the locality of Kerala (Palakad). The leaves were initially separated from plants body and rinsed with distilled water. Afterwards the samples were dried under shade of sunlight and then homogenized into fine powder using a mortar and were stored in air bottles and the powdered seeds was soaked in solvent ethyl acetate for 3 days. The collected portion was subjected to screening studies.

2.2 METHODS

2.2.1 PRELIMINARY PHYTOCHEMICAL SCREENING

The extract obtained from successive solvent extraction of *Solanum betaceum* were then subjected to various qualitative chemical tests to determine the presence of various phytoconstituents like alkaloids, carbohydrates, proteins/amino acids, glycosides, phenol, tannins, phytosterols, flavonoids, Saponins, lactones, coumarins, terpenes [10].

2.2.2 ANTI-FUNGAL ANALYSIS

The Anti fungal activity of the extract (SBL EA) were performed with *Aspergillus niger*, *Aspergillus flavus* were used as test organism by Agar diffusion method. The stock cultures of bacteria were revived by inoculating in broth media and grown at 37 degree Celsius for 18 hrs. The agar plates of the media were prepared and wells were made in the plate. Each plate was inoculated with 18 hold cultures (100µl, 10⁴cfu) and spread evenly on the plate. After 20 min, the wells were filled with of compound at different concentrations. All the plates were incubated at 37 degree celcius for 24 hours and the diameter of inhibition zone were noted and compared with standard Amphotericin.[11,12].

2.2.3 ANTI-OXIDANT ACTIVITY

The antioxidant activity of the sample was determined in different concentrations (5µg, 25µg and 50µg) of samples in Dimethyl sulfoxide (DMSO), were taken in a series of test tubes. The volume was adjusted to 500µl by adding Methanol. Five milliliters of a 0.1 mM methanolic solution of 1,1-diphenyl-2-picryl hydrazyl (DPPH; from Sigma –Aldrich, Bangalore) was added to these tubes and shaken vigorously. A control without the test compound, but with an equivalent amount of methanol was maintained. The tubes were allowed to stand at RT for 20 min. The absorbance of the samples was measured at 517 nm. Butylated Hydroxy Anisole (BHA) was used as reference standard.[13]

Free Radical scavenging activity was calculated using the following formula:

$$\text{Percentage radical scavenging activity} = (\text{control OA} - \text{sample OA}) / \text{control OA} \times 100$$

2.2.4 ANTI-DIABETIC ACTIVITY

The inhibition assay was performed using the chromogenic DNSA method The total assay mixture composed of 1400 µl of 0.05 M sodium phosphate buffer (pH 6.9), 50 µl of amylase (Diastase procured from Hi Media, Mumbai, Cat No. RM 638) and samples at concentration 20, 60 and 80 µl were incubated at 37°C for 10 min. After pre-incubation, 500 µl of 1% (w/v) starch solution in the above buffer was added to each tube and incubated at 37°C for 15 min. The reaction was terminated with 1.0 mL DNSA reagent, placed in boiling water bath for 5 min, cooled to room temperature and the absorbance measured at 540 nm. The control amylase represented 100% enzyme activity and did not contain any sample of analysis. To eliminate the absorbance produced by sample, appropriate extract controls with the extract in the reaction mixture in which the enzyme was added after adding DNS. Acarbose was used as a standard inhibitor and it was assayed at above mentioned test sample concentrations and activities were calculated according to the following formula

$$\text{Activity} = \frac{\text{Conc. of Maltose liberated} \times \text{ml of enzyme used}}{\text{Mol. wt of maltose} \times \text{incubation time (min)}} \times \text{dilution factor}$$

One unit of enzyme activity is defined as the amount of enzyme required to release one micromole of maltose from starch per min under the assay conditions.[14,15] The inhibitory/induction property shown by the sample was compared with that of control and expressed as percent induction/inhibition. This was calculated according to the following formula

$$\% \text{ inhibition/induction} = \frac{\text{Activity in presence of compound}}{\text{Control Activity}} \times 100$$

3. RESULTS AND DISCUSSION

3.1 Phytochemical Analysis

Preliminary phytochemical screening of (SBL EA) mainly revealed the presence of alkaloids, carbohydrates, proteins, glycosides, lactones, saponin, steroids, flavanoids and negative results were obtained for tannins, terpenes, anthocyanin, phenol and coumarin in ethyl acetate extract.

3.2 Antibacterial sensitivity

The Anti fungal activity of SBL EA at 20 µl concentration DIZ showed 4 mm against A.niger and 5mm against A. flavus , at 60 µl concentration DIZ showed 6 mm against A.niger and 14 mm against A.flavus and at higher concentration 80 µl showed DIZ against A.niger was 9 mm and A.flavus was 20 mm. The antifungal activity of standard drug Amphotericin shown in below table 2 and fig 4,5. Thus the plant extract shows moderate activity against both the microbial species.

Table 1: Anti-fungal Activity of SBL-EA

Organisms	20 μ l	60 μ l	80 μ l	MIC μ l
A.niger	4	6	9	20
A.flavus	5	14	20	20

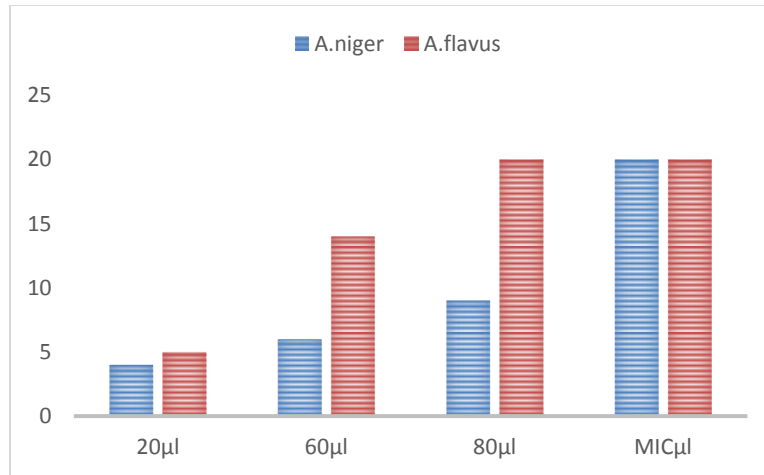


Fig:1 Anti-fungal Susceptibility Assay of ethyl acetate extract leaves of *Solanum betaceum*



Fig: 2 SBL EA- A.niger

Fig: 3 SBL EA- A.flavus

Table 2: Anti-fungal Activity of std. Amphotericin

Organisms	25 μ g	50 μ g	100 μ g	250 μ g	500 μ g	1000 μ g	MIC μ g
A.niger	0	0	2	3	5	25	100
A.flavus	0	0	0	0	7	10	500



Fig: 4 *A.flavus* with Std. Amphotericin Fig: 5 *A.niger* with Std. Amphotericin

3.3. Anti-oxidant activity

DPPH anti-oxidant assay of SBL EA showed the activity of 19.29 %, 28.29 % and 31.18 % at three different concentrations (10 μ l, 50 μ l, 100 μ l).The leaf extract of *Solanum betaceum* was compared with reference standard butylated hydroxyl anisole with same concentrations are shown in table 3 fig 6.

Table 3: DPPH Scavenging assay of SBL EA

Concentrations	% free radical scavenging	
	SBL EA	BHS
10 μ l	19.29	54.18
50 μ l	28.29	70.10
100 μ l	31.18	91.64

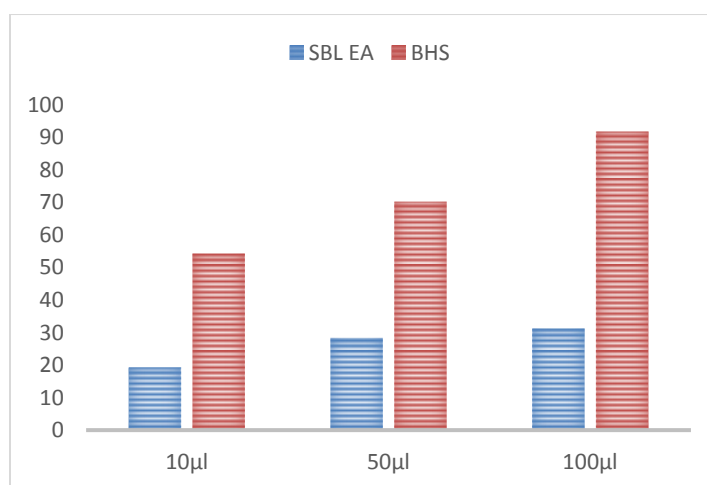


Fig: 6 Percentage Free radical scavenging activity of samples in comparison with BHA

3.4. Anti-diabetic activity

The leaves of *Solanum betaceum* in ethyl acetate extract (SBL EA) was tested for Anti-diabetic activity using amylase inhibition method at 20, 60 & 80 μ l concentration both showed very low percentage control. The result obtained are shown in table: 4 and fig: 7.

Table : 4 Anti- diabetic activity of SBL-EA

Concentrations	SBL-EA
20µl	0.05495268
60µl	0.058907607
80µl	0.055669655

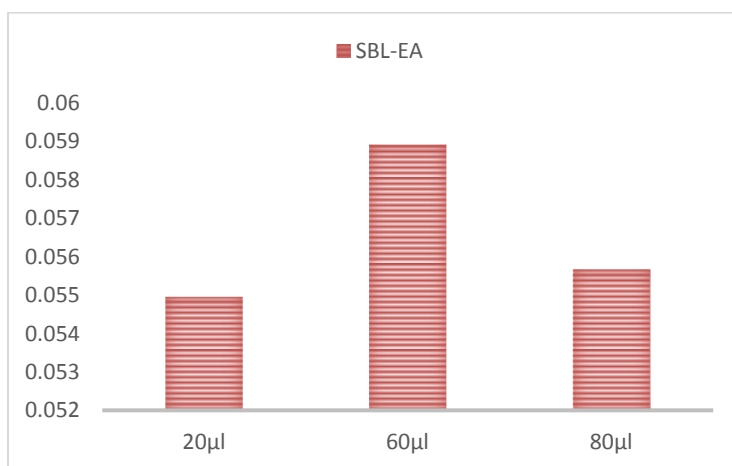


Fig: 7 Anti-diabetic assay of leaves of *Solanum betaceum* in ethyl acetate extract at 20,60&80µl concentrations.

4. CONCLUSION

From the above discussion it states that the phytochemical screening and pharmacological evaluation of the plant extract states that the plant extract possesses medicinally important secondary metabolites and these phytochemicals are may be responsible for the following activities like antifungal, antidiabetic and antioxidant.

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