

ANTIFUNGAL ACTIVITY AND PRELIMINARY PHYTOCHEMICAL STUDIES OF LEAF EXTRACT OF *SOLANUM NIGRUM* LINN

SWETA PRAKASH*¹ AND ASHOK K. JAIN²

¹R.V.S.K.V.V, Department of Plant Breeding, College of Agriculture, Gwalior (M.P.), ²School of Studies in Botany, Jiwaji University, Gwalior, India.
Email: sweta.shrivastava28@gmail.com

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ABSTRACT

Solanum nigrum Linn. is extensively used in Indian traditional and folk medicines to cure various skin ailments. The present study aims to evaluate the possibility for the presence of novel bio-active compounds against fungal pathogens. To determine antifungal activity, aqueous and crude extracts from leaves of *S. nigrum* Linn. was used against *A. niger*, *A. flavus*, *C. albicans* by dry weight method. Extracts prepared using crude solvents exhibited higher antifungal activity as compared to their corresponding aqueous extracts. No good activity was observed in the aqueous extract. The pathogen inhibiting activity was found to be dose dependent.

The preliminary phytochemical screening of the leaves revealed the presence of Alkaloids, Flavonols, Flavones, Flavanols, Saponins and Steroids.

Keywords: Skin ailments, Antifungal, Phytochemical, Dry weight method

INTRODUCTION

Plant derived drugs even today remain important resource especially in developing countries, to combat serious diseases. Approximately 62–80% of the world's population still relies on traditional medicines for the treatment of common illness (WHO, 2002; Zhang, 2004). Extracts of many plants are highly efficient against parasitic as well as microbial infections. It is estimated that around 70,000 plant species, from lichens to tall trees, have been used at one time or another for medicinal purposes (Purohit & Vyas, 2004). In fact, plants produce a diverse range of bioactive molecules making them a rich source of different types of medicines. Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times (Farombi, 2003).

The antimicrobial activity have been screened because of their great medicinal relevance with the recent years, infections have increased to a great extent and resistant against antibiotics, becomes an ever increasing therapeutic problem (Austin *et al.*, 1999). Natural products of higher plants may give a new source of antimicrobial agents. There are many research groups that are now engaged in medicinal plants research (Samy *et al.*, 1998; Hamil *et al.*, 2003; Motsei *et al.*, 2003).

The medicinal plants are the plants whose parts (leaves, seeds, stems, roots, fruits etc.), extracts, infusions, decoctions, powders are used in the treatment of different diseases of humans, plants and

animals (Nostro *et al.*, 2000). The medicinal plants occupy a significant place in modern medicine as a raw material for some important drugs, although synthetic drugs and antibiotics brought about a revolution in controlling different diseases. But these synthetic drugs are out of reach of millions of people. Those who live in remote places depend on traditional healers, whom they know and trust. The judicious use of medicinal herbs can even cure deadly diseases that have long defied synthetic drugs (Bhattacharjee, 2001).

In the present study a focus has been placed on phytochemical constituent and evaluation of antimicrobial activity of crude and aqueous extracts from the leaves of *S. nigrum* are bioassay against *A. flavus*, *A. niger* and *C. albicans*.

Solanum nigrum Linn. (Solanaceae) is commonly known as 'Black nightshade'. The plant has been extensively used in traditional medicine in India and other parts of world to cure liver disorders, chronic skin ailments (psoriasis and ringworm), inflammatory conditions, painful periods, fevers, diarrhoea, eye diseases, hydrophobia etc (Kirtikar and Basu, 1935).

In the literature very few works has been carried out in this plant. Thus it was thought worthwhile to explore this plant for antibacterial activity. Phytochemicals obtained from medicinal plants are the sole remedy to this emerging problem. Therefore, an attempt has been made to study the antifungal activity of the extracts of *Solanum nigrum* Linn. on certain pathogenic microorganisms.



Solanum nigrum Linn

MATERIALS AND METHODS

Ethnobotanical survey

Ethnobotanical survey was made around Gwalior and Sheopur districts in 'Sahariya' tribe dominated localities. Emphasis was laid on medicinal plants and parts used for the treatment of skin diseases.

Selection of plants

Information on uses of plants against skin diseases such as eczema, scabies, ringworm, cuts, wounds, rashes, itching etc. was recorded from Sahariya tribe. *Solanum nigrum* was for the experimental work :-

Preparation of plant extracts

The following methods were used for preparation of plant extracts:-

(i) Aqueous extract

25 gm of fresh leaves were boiled in 100 ml of water for one hour, thereafter the extract was filtered and 50 ml was maintained by adding the required quantity of water. The extract was used to test fungus at the concentration of 1%, 2% and 3%.

(ii) Crude extract

25 gm of fresh leaves were chopped and grounded in a pestle and mortar with 50ml of water after crushing, the extract was kept overnight and then filtered. After filtration 15ml was maintained by adding required quantity of water. The concentration of this extract is taken to be 100% (1:1 weight/ volume basis). These crushed extracts were used at 1%, 2% and 3% concentrations.

Test organisms:

The selected microorganisms were obtained from Cancer Hospital and Research Institute, Gwalior and Birla Hospital, Gwalior. The extract was tested on the following three fungi: *Aspergillus niger*, *Aspergillus flavus* and *Candida albicans*.

Phytochemical studies (Qualitative chemical analysis)

The plant leaves were air dried in laboratory and ground into uniform powder and stored in container. These plant parts were subjected to qualitative chemical screening for the identification of the various classes of active chemical constituents using standard prescribed methods (Amarsingham *et al.* 1964; Gibbs, 1974; Dan *et al.*, 1978; Harborne, 1984; Trease and Evans, 1987; Faraz *et al.*, 2003; Edeoga *et al.*, 2005).

(i) Test for Alkaloids

5 gm of dried powder was kept in 50ml of 10% ethanol for 48 hours at room temperature with occasional shaking. The extract was filtered and distilled water vacuum. The dried concentrated extract was acidified with 25ml of 0.1 NH_2SO_4 . The acid extract was centrifuged and the clear supernatant was tested with Mayer's, Wagner's and Dragendorff's reagent.

(ii) Test for Anthraquinones

The powder was boiled with sulphuric acid for one hour, cooled and filtered. To the filtrate was added Chloroform. The mixture was vigorously shaken and allowed to stand, organic layer gets separated. Ammonia was added to organic layer slowly. Development of red, pink, or violet colour indicates the presence of Anthraquinones. This is to detect glycosides.

(iii) Test for Cardiac glycosides (Cardenolides)

Fresh tissue was extracted with rectified spirit. To the extract 10% solution of Sodium Hydroxide and 0.3% solution of Nitroprusside were added. Appearance of transient pinkish red colouration indicates the presence of Cardenolides.

(iv) Test for Flavonoids

Different tests were carried out for different types of flavonoids. Tests were carried with dry sample.

a) Flavonoids (Shinoda test)

To the extract, a piece of Magnesium ribbon and Hydrochloric acid were added. Purple, red, pink or orange colour developed, which confirm flavonoids.

b) Flavanonols

If deep colour developed with Shinoda test, then instead of Magnesium ribbon, Zinc powder was added with Hydrochloric acid. Deep magenta colour developed which confirmed flavanonols.

c) Flavonols

To the extract a pinch of Boric acid and few drops of Acetic acid were added. Bright yellow colour with green fluorescence indicated flavonols.

d) Flavones and Flavanols

Firstly extract was dissolved in Sulphuric acid to give yellow solution and the flavanones produced lively orange to crimson colours. To the extract few drops of Sulphuric acid were added and colour was noted. This further confirmed the presence of Flavones, Flavanols and Flavanones.

e) Test for Simple Phenolics

Plant powder was extracted with aqueous ethanol overnight. To the extract 1-2 drops of 1% aqueous Ferric chloride was added. Development of specific colours was indicative of the presence of Phenol.

f) Test for Saponin

About 2g of the powdered sample (shoot and rhizome) was boiled in 20ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth.

g) Test for Steroids

2ml of acetic anhydride was added to 0.5 gm ethanolic extract of each sample with 2ml H_2SO_4 . The colour changes from violet to blue or green in some samples indicating the presence of steroids.

Antifungal assay

The toxicity of the aqueous and crude plant extract was determined against the pathogen following the Mycelial weight loss method. In this method 30ml Sabouraud dextrose agar media was added to a sterilized in conical flask of 150ml capacity. In each conical flask 1ml. plant extract was added at three different concentrations i.e. 1%, 2% and 3% w/v.

These different sets were prepared with different plant extract. From the pure fungus already grown on Sabouraud broth medium small discs of 6mm. diameter was cut with the help of sterile cork borer and transferred aseptically in the centre of the conical flask, containing the liquid medium and plant extracts of known concentration. The prepared flask was incubated for 4-7 days under standard growth condition i.e. adequate moisture and temperature. Similar preparation of checks (control) was also kept where the culture disc were grown under similar conditions but without plant's extract in Sabouraud broth medium for some period.

After seven days of incubation, the content of different flasks including control was filtered separately through weighed Whatmann filter paper no.1. The filter paper with fungal biomass was dried at $110 \pm \text{C}$ for 5-6 hours in oven. The dried filter paper with fungal biomass obtained from the above procedure was weighed by chemical balance. The loss in weight is due to plant extract was calculated by the formula given below:-

$$\text{Weight Loss} = W_2 - W_1 \text{ (Fergus, 1969)}$$

Where,

W_2 = Weight of over dried filter paper along with treated mass after filtration.

W1 = Weight of over dried filter paper along with untreated mass after filtration.

Percentage weight loss inhibition in growth was calculated with following formula:

$$\frac{\text{Weight of mycelium in control} - \text{Weight of mycelium in extract}}{\text{Weight of mycelium in control}} \times 100$$

The above test was repeated twice with two replicates

RESULTS AND DISCUSSION

Ayurveda remains one of the most ancient and yet living traditions practiced widely in India. Medicinal herbs consist of

wide variety of chemical compounds nearly 80% of the world population depends upon traditional system of health care. Survey had revealed that 50% of the top prescription drugs in the world are based on natural products. Hence this present study was conducted to study the *in vitro* antimicrobial activity of medicinal plant used by Indian peoples to show that the therapeutic properties of *Solanum nigrum* Linn. used in traditional medicine coincide with laboratory findings.

The leaf powder of *Solanum nigrum* revealed the presence of flavonols, flavones, flavanols, saponin, steroids and suspected alkaloids, but shows negative results for cardiac glycosides, terpenoids and saponins were discussed in the table -1

Table 1: Phytochemical profile of *S nigrum* Linn. Leaves extract

S.No.	Phytochemical	Observation	Remark
1.	Alkaloids	Brown precipitate	Alkaloids present
2.	Anthraquinone	Brown colour	Anthraquinone absent
3.	Cardenolides	Pale green colour	Cardenolides absent
4.	Flavonoids	Olive green colour	Flavonoids absent
5.	Flavononols	Dark grey colour	Flavononols absent
6.	Flavonols	Yellow colour	Flavonols present
7.	Flavones & Flavanols	Bright yellow colour	Flavones, Flavanols present
8.	Flavonones	Olive green colour	Flavonones absent
9.	Iridoides	Pale yellow colour	Iridoides absent
10.	Phenolics	Pale yellow colour	Phenolics absent
11.	Saponin	Persistent frothing	Saponin present
12.	Steroids	Bright green colour	Steroids present

Table 2: Dry mycelial weight loss (%) of fungi with different concentrations in *S. nigrum* Linn. leaf extracts

S.No.	Pathogens	Extract	Concentration			Mean (%)	* G. Mean
			1%	2%	3%		
1.	<i>A.flavus</i>	Aqueous	6.39	8.52	19.15	11.35	(0.42)
		Crude	72.31	85.39	95.38	64.30	(0.97)
2.	<i>A.niger</i>	Aqueous	14.29	42.86	71.43	42.84	(0.46)
		Crude	3.71	49.39	75.31	42.80	(0.55)
3.	<i>C.albicans</i>	Aqueous	20.00	24.00	26.00	23.33	(0.40)
		Crude	5.48	28.77	28.77	21.00	(0.60)

*Mean value of replicates; C. D. (at 5%) = 0.031, S. E. (m) \pm = 0.010 (Aqueous); C. D. (at 5%) = 0.088, S. E. (m) \pm = 0.030 (Crude)

S. nigrum exhibited good effect on the growth of *A. flavus* (64.30%). In some other studies *A. niger* was resistant (42.84), while *A. flavus* was susceptible to crude extracts (Jamil et al., 2007). Earlier studies research showed that *S. nigrum* showed higher potential of antimicrobial activity against all fungal forms (Ikeda et al. 2000; Qureshi et al. 1997; Katsura et al. 2001). *S. nigrum* was also found to be effective as it inhibited mycelial weight. *C. albicans* showed minimum inhibitory results in the aqueous (1%) and crude extract

(3%). Balakrishna et al. (2000) worked on antifungal activities of *S. trilobatum*, concluded that the higher concentration of the extract exhibits better activity.

Phytochemical screening of this species indicates the presence of alkaloids, flavonols, flavones, flavanols, saponin, flavonoids and steroids (Amir and Kumar, 2004). Alkaloids such as soladunalinidine, solasonine and solamargine have been isolated from leaf of *Solanum* species (Juneja et al., 2007).

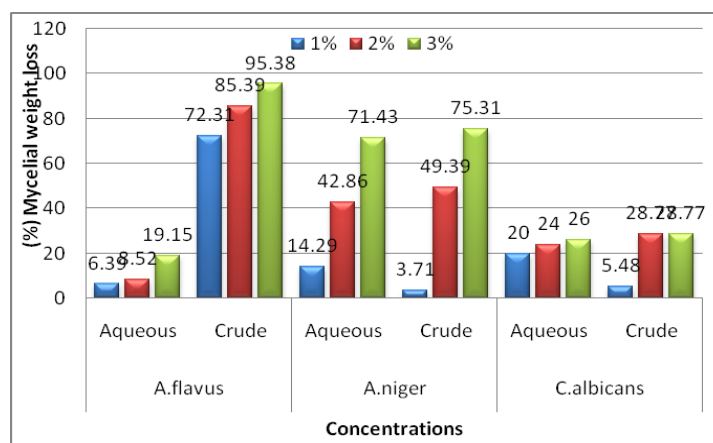


Fig. 1: Dry mycelial weight loss (%) of fungi with different concentrations of *S. nigrum* Linn. leaf extracts

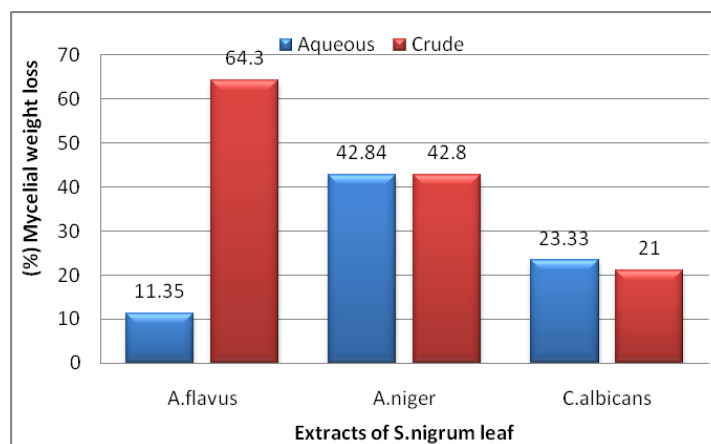


Fig. 2: Dry mycelial weight loss (Mean %) of fungi in *S. nigrum* Linn. leaf extracts *Solanum nigrum* Linn.

The present study *S. nigrum* showed maximum inhibition activity against *A. flavus* and *A. niger*. *S. nigrum* was recorded significant antifungal activity against *A. flavus* and *A. niger* tested. *A. flavus* recorded high susceptibility to crude extract of *S. nigrum*. Crude extract was found to be highly effective than aqueous extracts. *S. nigrum* did not show any inhibition effect on *C. albicans*. It is hoped that this study would lead to the establishment of some compounds that could be used to formulate new and more potent antifungal drugs of natural origin.

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