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Antimycotic activity of low polar petroleum ether and interpolar methanolic young leaf extracts of *Solanum nigrum* L.

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ABSTRACT

Antimycotic activity of Petroleum ether and 98% methanolic young leaf soxhlet extract of *Solanum nigrum* (Solanaceae) was evaluated against dermatophytic fungi namely, *Trichophyton rubrum, Trichophyton tonsurans, Trichophyton mentagrophytes, Microsporium gypseum, Candida albicans*, and bacteria like, *Staphylococcus aureus, Psudomonas aeruginosa, Bacillus subtilis*, *Escherichia coli.* The maximum activity was observed in interpolar methanolic extract when compared to low polar petroleum ether extract. The minimum inhibitory concentration, minimum fungicidal concentration and minimum bactericidal concentration were determined against all the test strains. This study provides a basis for the isolation and purification of anti-dermatophytic compounds from the young leaves of *S. nigrum.*

Key words: Antimycotic activity, 98% methanolic young leaf extract, *Solanum nigrum,* MIC, MFC, MBC.

Introduction

In recent years, plant secondary metabolites (phytochemicals) have been extensively investigated as a source of medical agents (Krishnaraju AV et al., 2005). Thus, it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections (Balandrin MF et al., 1985). The Greek physician, Galen (AD129-200) devised the first pharmacopoeia describing the appearance, properties and use of many plants. Approximately 20% of the plants in the world are found to have pharmacological or biological activities (Suffrendini JB et al., 2004). The plants having capacity to produce a large number of secondary metabolites (Evans JS 1986), which are divided into Chemotherapeutic, bacteriostatic, bactericidal and antimicrobial agent (Purohit SS, and Mathur SK 1999).

The chemical constituent of plants plays an important role in modern medicine after profiling against different biological activities. Antibiotics brought revolution to control pathogenic diseases and fungal infections. But, these synthetic drugs are out of reach to millions of people. People who live in remote places mostly depend on traditional healers

(Bhattacharjee SK 2001). About three quarters of world population are estimated to be dependent mainly on plants drugs (Jamil A et al., 2007). Medicinal plants possess a variety of phytochemical constituents, which cast a definite physiological action on human body. Some of these chemicals are toxic and may lead to death (Sheeba E 2010).

A large number of plants have been reported by researchers and practitioners of traditional medicine to be useful in the treatment of skin diseases (Miranda 1976, Berlin B et al., 1993). Fungi that infect the skin, nails, and hair, are generally called as ‘‘ringworm’’ or ‘‘tinea,’’ and are classified as dermatophytes. Three important genera that are closely related botanically are *Microsporum, Trichophyton*, and *Epidermophyton*. The genus *Microsporum* is the most frequent cause of ringworm of the scalp and may give rise to ringworm in other parts of the body. *Trichophyton* causes ringworm of the scalp, beard, and other areas of the skin and nails. *Epidermophyton* is largely responsible for ringworm of the skin, hand and feet and appears as interlacing threads in the skin, but does not invade the hair (Rippon JW 1982). *Candida* spp. have been reported to be commensal fungi commonly found in the gastrointestinal tract, mouth, and vagina; they become pathogenic only when natural defence mechanism fails (Grabue GE 1994).

The plant *Solanum nigrum* belongs to Family Solanaceae. The family consists of 90 genera and approximately 2000-3000 species. In this family, Solanum constitutes the largest and the most complex genus and it consists of more than 1500 species, many of which are also economically important throughout their cosmopolitan distribution. The generic name Solanum is considered to be derived from the Latin “Solamen” to refer to the quieting or sedative effects associated with many species (Edmonds JM and Chweya JA 1997). It is commonly known as, ‘Black nightshade’. *S. nigrum* elaborated a wide spectrum of medicinal properties such as anticancer, antioxidant (Al-Qirim T et al., 2008), neuroprotective(Jainu M and Devi CSS 2005), liver disorders, chronic skin ailments, inflammatory conditions, painful periods, fevers, diarrhoea, eye diseases, hydrophobia (Kritikar KR and Basu BD 1935), antimicrobial, and antipyretic properties. *S. nigrum* has been used as the important ingredient for herbal formulations in India, namely Liv. 52 mainly used for treating liver diseases (Ikeda T et al., 1992). *S. nigrum* seed extract exhibited selective antifungal activity, against *P. nicotianae* (Sashikumar JM et al., 2003).

The authors have documented and published (Shivakumar Singh P and Vidyasagar GM 2013) antidermatophytic plants from Hyderabad Karnataka region, however, the pharmacological work reported on these plants is limited. Therefore the present work was carried out.

Materials and methods

Collection of plant material and extraction of plant material by soxhlet apparatus: The young leaves of *Solanum nigrum* were collected in sterile bags from different places of Gulbarga University and identified with the help of Voucher specimen (HGUG-890) deposited in the herbarium centre, Department of Botany, Gulbarga University, Karnataka, India. The collected plant materials were initially rinsed with distilled water to remove soil and other contaminants and shade dried on paper towel at 37 ± 2ºC in the laboratory for a week.

The dried samples were ground in a grinder. 25g of shade dried powder was weighed and extracted successively with petroleum ether and interpolar methanol in soxhlet extractor for 48h. The extracts were concentrated under reduced pressure and stored at 4^0C in airtight bottles for further use.

Test fungi and bacteria: Five fungal culture strains, *Trichophyton rubrum*, *Trichophyton tonsurans, Trichophyton mentagrophytes, Microsporum gypseum, Candida albicans* and five bacterial strains, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* obtained from M.R. medical college, Gulbarga, Karnataka, India were used in the present study.

Agar-well diffusion method (Magaldi S et al., 2004): 15 to 20 ml of potato dextrose agar medium was poured in the sterilized petri dishes and allowed to solidify. Fungal lawn was prepared using 5 days old culture strains. The fungal strains were suspended in a saline solution (0.85% NaCl) and adjusted to a turbidity of 0.5 Mac Farland standards (108 CFU/ml). 1 ml of fungal strain was spread over the medium using a sterilized glass spreader. Using flamed sterile borer, wells of 4 mm diameter were punctured in the culture medium and required concentrations of serially diluted extract (0.62, 1.25, 2.5, 5, 10, 20 and 40mg/ml) was added to the 20µl to each wells. The plates thus prepared were left for diffusion of extracts into media for one hour in the refrigerator and then incubated at 37°C. After incubation for 48h, the plates were observed for zone of inhibition. Diameter zone of inhibition was measured and expressed in millimetres. Dimethyl formamide (DMF) was used as a negative control. The experiments were conducted in triplicates. The same method was followed for testing antibacterial activity using nutrient agar medium incubated at 37°C for 18h.

Minimum Inhibitory Concentration (NCCLS 1997)

One ml of sterile liquid Sabouraud medium was added to 11 sterile capped tubes, 1 ml of each solvent extracts suspension was added to tube 1. The contents were mixed and 1 ml was transferred to tube 2. This serial dilution was repeated through to tube nine and 1 ml was discarded from tube 9. Fifty µl of inoculum was added to tubes 1-10 and the contents were mixed. Medium control (no inoculum and no drug) and inoculum control (no drug) tubes were prepared. The final concentrations of each plant solvent extracts ranged from 05mg/ml to 0.02 mg/ml. The tubes were incubated at 30° C for 96 h. The fungal growth in each tube was evaluated visually depending up on the turbidity in the tubes. MIC was defined as the drug concentration at which the turbidity of the medium was the same as the medium control.

10µl aliquot of cell suspension from the tube without observed growth of dermatophytes was inoculated on to Sabouraud dextrose agar, and Minimum fungicidal concentration (MFC), Minimum bactericidal concentration (MBC) of test compound was determined as the lowest concentration of the agent at which no colonies were seen after 4 days at 30°C. Triplicate sets were maintained for each experiment.

Statistical Analysis

All the experiments were conducted in triplicate unless stated otherwise and statistical analysis of the data was performed by analysis of variance (ANOVA), using STATISTICA 5.5 (Stat Soft Inc, Tulsa, Oklahoma, USA) software. A probability value of difference $p<0.05$ was considered to denote a statistically significance All data were presented as mean values ± standard deviation (SD).

Results and discussion

The preliminary phytochemical analysis of pet ether and interpolar methanolic young leaf extracts of *S. nigrum* was carried out by adopting standard methods (Horborne JB 1998).The results represented in table-1 reveals the presence of phytochemicals such as, alkaloids, flavonoids, phenols, triterpenoids, glycosides, steroids and tainns. The interpolar methanolic extract shows strong positive response to flavonoids and phenols, moderate response to saponins and steroids, whereas petroleum ether extract showed strong positive response to alkaloids and moderate response to phenol, flavonoids, triterpenoids and glycosides.

The antimycotic studies was carried out against five fungal and five bacterial strains to determine the effect of petroleum ether and 98% methanolic young leaf extracts of *S. nigrum.* The interpolar methanolic extract exhibited an effective antidermatophytic activity as compared to low polar petroleum ether extract. The maximum antidermatophytic activity was observed against *T. rubrum* (16.66±0.57mm), followed by *M. gypseum* (16.00±0.00mm) *T.* mentagrophytes (12.66±1.52mm)*, C. albicans (*12.33±0.57mm) and *T. tonsurans* (11.66±1.52). Maximum antibacterial activity of 21.00±0.00 mm was observed in *S. aureus* followed by *B. subtilis* (18.66±1.15 mm), *E coli* (17.00±0.00 mm) and *P. aeruginosa (*17.33±1.15 mm). The zone of inhibition was found to be concentration dependent.

Ketoconazole and streptomycin sulphate used as positive controls at conc. 2 mg/ml against fungi and bacteria, showed 24.00±0.00 to 26.33±1.15 mm and 26.00±0.00 to 29.33±0.57 mm inhibition zone, respectively. The negative control (DMF) was showed no inhibition against all the tested fungal and bacterial strains.

The MIC value recorded against *Tt, Ca* and *Pa* was 0.15 mg^{-1}, similarly, the MFC value against *Tt, Ca* was 0.3 mg^{-1}. While 0.3 mg^{-1} MBC value recorded against all the tested bacteria (Figure 1, 2 & 3).

Table-3 shows the presence of flavonoids, phenols and tannins in methanolic extract. The earlier reports on phytochemical screening of this species showed positive response to alkaloid, flavonols, flavones, flavanols, saponin, flavonoids and steroids tests(Amir M and Kumar S 2004). Some of the alkaloids like, soladunalinidine, solasonine and solamargine have been isolated from leaf of *Solanum* species (Juneja D et al., 2007). However, in present studies the Alkaloid tests were weak in colours and the interpolar methanolic extract showed positive to cardiac glycosides. One of the reports from north India (Sweta Prakash and Ashok K. Jain, 2011) showed negative results for cardiac glycosides in aqueous extract. These types of variations may be due to change in climatic and soil conditions. The 98% methanolic young leaf extract showed effective antimycotic activity and found to have strong positive response to Flavonoids, Phenols and Tannins tests. These compounds may be responsible for antimicrobial activity as reported by Tsuchiya et al., in flavonoids (Tsuchiya H et al., 1996), Mason and Wasserman in phenolics and polyphenols (Mason TL et al., 1987), Ya et al., (1988) in tannins (Scortichini M et al., 1991), Scortichini and Pia Rossi (1996) in terpenoids,

Goren in sesquiterpenes (Goren N et al., 1996), Barnabas CG, Nagarajan S in alkaloids, glycosides, steroids (Barnabas CG, Nagarajan S 1988). The present study reveals an effective antimicrobial activity in interpolar methanolic extract. Whereas the previous reports showed that the methanolic extracts are weak against food borne and pathogenic organisms (Muhammad Zubaır et al., 2011). These observations may be attributed to the nature of biological active components whose activity can be increased in the successive extraction of interpolar methanol (solvent) extracts.

Conclusion

The antimycotic activity of 98% methanolic young leaf extract of *S. nigrum* may be attributed to the phytochemical constituents like phenol, flavonoids and tannins present in crude extract. The purified components may have even more potency with respect to inhibition of microbes. The work carried out was a basic approach to find out the antimycotic activity in *S. nigrum*. Further study on the purification of individual groups of bioactive interpolar-components can reveal the exact potential of the plant to inhibit skin pathogenic microbes.

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Table 1- Antimycotic activity of petroleum ether and interpolar methanolic young leaf extracts of *Solanum nigrum*.

Selected medicinal plants	Concent-rations mg/ml	Test organisms & inhibition of zones in mm								
		Fungal strains					Bacterial strains			
		Tr	*Tt*	*Tm*	*Mg*	*Ca*	*Sa*	*Pa*	*Bs*	*Ec*
***S. nigrum* L.**										
A	40	06.00±1.00	04.66±0.57	04.33±0.57	06.33±0.57	05.00±1.00	06.33±1.15	05.33±0.57	05.33± 0.57	05.66± 0.57
	20	04.00±00	NA	NA	04.33±0.57	NA	05.00±0.0.00	04.00±0.00	NA	04.00± 1.00
	10	NA	NA	NA	NA	NA	04.66±0.57	NA	NA	NA
		NA	NA	NA	NA	NA	NA	NA	NA	NA
	5	NA	NA	NA	NA	NA	NA	NA	NA	NA
	2.5	NA	NA	NA	NA	NA	NA	NA	NA	NA
	1.25	NA	NA	NA	NA	NA	NA	NA	NA	NA
	0.62	NA	NA	NA	NA	NA	NA	NA	NA	NA
B	40	16.66± 0.57	11.66± 1.52	12.66± 1.52	16.00± 0.00	12.33± 0.57	21.00± 0.00	17.33± 1.15	18.66± 1.15	17.00± 0.00
	20	14.66± 1.52	10.33± 0.57	11.66± 0.57	15.33± 1.15	11.33± 1.15	16.66± 1.52	16.00± 0.00	16.66± 0.57	15.33± 0.57
	10	11.33± 0.57	08.33± 0.57	09.00± 0.00	13.33± 0.57	08.33± 0.57	14.66± 1.15	15.33± 0.57	14.00± 0.00	13.33± 1.15
	5	09.00± 0.00	06.00± 0.00	08.33± 0.57	11.33± 1.15	07.33± 0.57	13.00± 0.00	13.33± 1.15	13.33± 0.57	12.00± 0.00
	2.5	08.33± 0.57	05.33± 0.57	06.33± 1.15	09.66± 0.57	05.33± 1.15	12.33± 0.57	12.66± 0.57	08.00± 0.00	10.33± 1.15
	1.25	06.33± 1.15	NA	05.00± 0.00	07.00± 0.00	04.33± 0.57	10.66± 1.15	11.00± 0.00	06.33± 0.57	09.00± 0.00
	0.62	NA	NA	NA	05.66± 1.15	NA	08.66± 1.52	10.33± 1.15	05.00± 0.00	08.66± 0.57
K	02	24.00± 0.57	26.33± 1.15	26.00± 1.00	24.66± 1.52	24.00± 0.00	-	-	-	-
S	02	-	-	-	-	-	29.33± 0.57	28.33± 1.15	26.00± 0.00	26.33± 1.15

A- Petroleum ether extract, B- 98% methanolic extract, *Tr - Trichophyton rubrum, Tt – Trichophyton tonsurans, Tm- Trichophyton mentagrophytes, Mg- Microsporium gypseum, Ca - Candida albicans, Sa- Staphylococcus aureus, P a- Psudomonas aeruginosa, Bs- Bacillus subtilis*, *Ec- Escherichia coli, NA-Not Active, K-Ketoconazole, S-Streptomycin Sulphate.*

Table 2- Preliminary Phytochemical screening for secondary metabolites in *Solanum nigrum* young leaf extracts.

Secondary metabolites	Name of the test	PE	ME
Alkaloids	Mayers test	++	-
	Dragendoff's test	+	-
	Wagner's test	-	-
	Hot water test	-	++
Phenol	Ferric chloride test	-	+
	Ellagic acid test	+	++
	Ferric chloride test	-	++
Flavonoids	Lead acetate test	-	++
	Shinoda test	+	++
	Zinc/HCl test	+	+
Tannins	Gelatin test	-	+
	Salkowski's test	-	-
Triterpenoids	Libermann-Burchard test	+	-
	Salkowski's test	-	-
Steroids	Libermann-Burchard test	-	-+
Saponins	Foam test	-	+
	Keller-Killiani test	-	-
Glycosides	Conc. H_2So_4 test	-	+
	Molisch's test	-	+
	Glycoside test	+	-

PE: Petroleum ether extract, B- Interpolar methanolic extract + Present, + + strongly present, -+ moderately present, - absent.

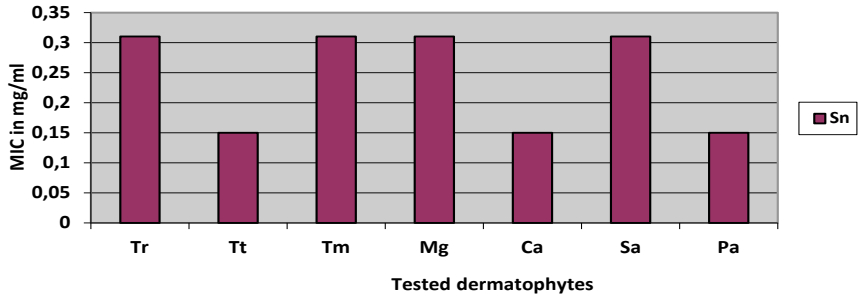


Figure 1- Minimum Inhibitory Concentrations (mg/ml) of young leaf inter polar extract of *S. nigrum.*

Sn- Solanum nigrum ,Tr - Trichophyton rubrum, Mg- Microsporium gypseum, Ca - Candida albicans, Tt -Trichophyton tonsurans, Tm -Trichophyton mentagrophytes, Sa- Staphylococcus aureus, Bs- Bacillus subtilis, *Ec- Escherichia coli*, *Pa- Pseudomonas aeruginosa.*

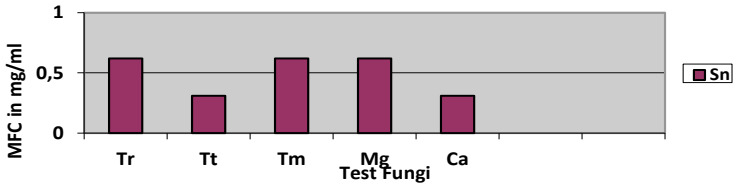
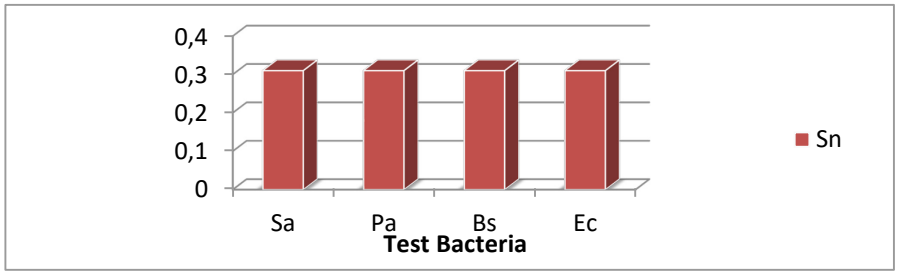


Figure 2-Minimum Fungicidal Concentration (mg/ml) of young leaf inter polar extract of *S. nigrum.*

Sn- Solanum nigrum, Tr - Trichophyton rubrum, Mg- Microsporum gypseum, Ca - Candida albicans, Tt -Trichophyton tonsurans, Tm -Trichophyton mentagrophytes.



Sn- Solanum nigrum, Sa- Staphylococcus aureus, Bs- Bacillus subtilis, *Ec- Escherichia coli*, *Pa-Pseudomonas aeruginosa.*

Figure 3- Minimum Bactericidal Concentration (mg/ml) of young leaf inter polar extract of *S. nigrum.*

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