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A STUDY ON PHYTOCHEMICALS, ANTIOXIDANT, ANTIDIABETIC AND ANTIMICROBIAL ACTIVITY OF THE LEAVES OF *SOLANUM NIGRUM*

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ABSTRACT

Medicinal plants serve as a source of novel therapeutic agent due to the presence of diverse bioactive compounds like alkaloids, flavonoids, terpenoids, phenolic compounds, glycosides etc in plants. These phytocompounds are synthesized by primary or rather secondary metabolism of living organisms. They are widely used in the human therapy, veterinary, agriculture, scientific research, etc. The utilization of several medicinal plants as medicine lies in the fact that they contain various phytoconstituents of therapeutic value. Extraction and characterization of various active phytoconstituents from these green factories have given birth to some highly active profile drug. Most of the plants are found to be rich in one or more elements, thus providing a probable relation to the therapeutic action of the medicine. *Solanum nigrum* is commonly used as a food since early times, and the fruit was recorded as a famine food in 15th – century in China. In India, the berries are casually grown and eaten while the berries are referred to as '**fragrant tomato**'. The plant also has a long time history of medicinal usage, dating back to ancient Greece, being used for cancer and with Horehound and wine taken for dropsy. The present study aims at analyzing the Phytochemical, Antioxidant, Antidiabetic and Antimicrobial activity of the leaves of *Solanum nigrum.*

KEYWORDS: Medicinal plants, Phytocompounds, *Solanum nigrum,* Medicinal Usage and Cancer Treatment.

INTRODUCTION

Solanum nigrum is a common blackshade herb or short-lived perenial shrub found in many wooded areas as well as disturbed habitats (**Amir Muhammad Khan *et al*., 2011).** It reaches a height of 30 to 120cm (12 to 47 inches), leaves 4 to 7.5cm (1.6 to 3inch) long, 2 to 5cm (1 to 2inch) wide; ovate to heart shaped with wavy or large toothed edges; both surfaces hairy or hairless (**Sweta Prakash And Ashok K. Jain, 2011)**. The flowers have petals greenish to whitish, recurved when aged and surround prominent bright yellow anthers (**Ibraheem O.** and **Maimako R. F, 2014)**. The berry is mostly 6 to 8 mm (0.24 to 0.31 inch) in diameter, dull black or purple-black. In India, another strain is found with berries that turn red when ripe **(Dushyant Kumar Sharma *et al*., 2015).**

The ***Solanum nigrum*** is a strong sudorific, analgesic and sedative with powerful narcotic properties. It was also used topically as a treatment for herpes zoster. It is an important ingredient in traditional Indian medicines (**Muhammad *et al*., 2003).** Infusions are used in dysentery, stomach complaints and fever (**Santhosh Kumar S and Uma C, 2013)**. The juice of the plant is used on ulcers and other skin diseases (**Figure. 1**). The fruits are used as a tonic, laxative, appetite stimulant, and for treating asthma and tuberculosis (**ANM Mamun-or-Rashid *et al*., 2014).** The boiled extracts of leaves and berries are also used to alleviate liver – related ailments, including jaundice. The juice from its roots is used against asthma and whooping cough (**Akilan.C.A *et al*., 2014).** It is widely used in oriental medicine where it is considered to be antitumorigenic, antioxidant, anti – inflammatory, hepatoprotective, diuretic and antipyretic (**M. Pratheeba *et al*., 2014).** Chinese experiments confirm that the plant inhibits growth of cervical carcinoma in mice **(Priya G and Chellaram C.2014).**

Figure. 1: Whole plant of *Solanum nigrum.*

MATERIALS AND METHODOLOGY

Collection of Sample: The mature and the young leaves of *S. nigrum* were collected from Guduvanchery, Tamil Nadu. The fresh leaves of these species were collected, washed under running tap water to remove dust and other foreign matter and allowed to shade dry at room temperature for 10 – 15days for long term storage purpose. The shade dried leaves were then powdered using a mechanical grinder.

Preparation of Extract: 10g of the powder was extracted with different organic solvents viz, Chloroform, ethanol, and water and placed on a mechanical shaker for overnight. The powdered samples were initially air dried and then extracted with solvents. The extracts were filtered through Whatman No.1 filter paper to remove all unextractable matter, including cellular materials and other constitutions that are insoluble in the solvent extraction. The entire extract was concentrated to crude extract and was collected in amber colored sample bottles and was stored.

Biochemical Assay for Phytochemical Screening

Qualitative Analysis: Preliminary Qualitative Phytochemical screening of various alcoholic and aqueous extracts of *S. nigrum* was carried out by following standard procedures to identify the secondary metabolites present in them.

Biochemical Assay for Phytochemical Screening

Quantitative Analysis: Quantitative analyzes of alkaloids, flavanoids, tannins, saponins, terpenoids, proteins, amino acids and cardiac glycosides were carried out for the plant leaves of *S. nigrum.*

Antimicrobial Assay of Plant Extracts

The ethanolic extracts of *S. nigrum* was individually tested against a panel of microorganisms, including four bacteria, *Escherichia coli, Bacillus subtilis, Staphylococcus aureus* and *Pseudomonas aeruginosa* and three pathogenic fungi, *Aspergillus niger, Candida albicans* and *Penicillium spp*. The pure bacterial and fungal strains were obtained commercially. Bacterial strains were cultured overnight at 37 °C in Muller Hinton Agar while fungal strains were cultured for three days at 28 °C in Sabouraud Dextrose Agar.

Besides this Antibacterial and Antifungal Activity, Minimum Inhibitory Concentration, Antioxidant Activity by DPPH Method and Antidiabetic Activity were carried out.

RESULTS AND DISCUSSION

Qualitative Phytochemical Screening

Out of the three extracts (ethanol, chloroform and aqueous) ethanolic extract demonstrated the maximum occurrence of phytoconstituents in *S. nigrum* (10/15) such as alkaloids, fixed oils, proteins, amino acids, terpenoids, carotenoids, glycosides, flavanoids and tannins. In the case of chloroform extract of *S. nigrum* alkaloids, phenols, tannins, carotenoids and fixed oils were only present. Whereas in aqueous extract of *S. nigrum*, it had alkaloids, fixed oils, proteins, amino acids, terpenoids, glycosides and flavanoids **(Table 1).** The presence and absence of the phytoconstituents depends on the solvent medium used for extraction and the physiological property of individual taxa.

Table 1: Phytochemical analysis conducted on different extracts of *S. nigrum.*

S. No	Qualitative Phytochemical Test	Ethanol extract	Chloroform extract	Water extract
01.	Alkaloids	+	+	+
02.	Carbohydrates	-	-	-
03.	Saponin	-	-	+
04.	Fixed Oils	+	+	-
05.	Protein	+	-	+
06.	Amino Acid	+	-	+
07.	Terpenoids	+	+	+
08.	Carotenoids	+	-	-
09.	Sterols	-	-	-
10.	Glycosides	+	-	+
11.	Flavanoids	+	-	+
12.	Phenols	-	+	+
13.	Tannins	+	+	-
14.	Quinones	-	-	-
15.	Anthroquinones	-	-	-

Quantitative Phytochemical Screening

The quantitative estimation of the phytochemicals which showed better results in all the three extracts were quantitatively analysed and their concentration was estimated using standard methods **(Table. 2)**. The quantitative estimation was carried out in the leaf powder of *S. nigrum* for phytochemicals like alkaloid, flavanoid, saponin, tannin, terpenoid, cardiac glycosides, proteins and amino acids. The estimation revealed that *S. nigrum* had 4.6% of alkaloid, 18.5% of flavanoid, 20% of glycosides, 4% of saponin, 2.5% of terpenoid, 41% of

protein, 11% of amino acid and 0.88mg GAE/g extract of tannin.

Table 2: Quantitative Estimation of Phytochemicals in *S. Nigrum*.

S. No	Phytochemicals	*S. nigrum* % in 10g
01.	Alkaloid	4.6
02.	Flavanoid	10.9
03.	Cardiac Glycosides	20
04.	Terpenoids	2.5
05.	Saponins	4
06.	Protein	41
07.	Amino Acid	11
08.	Tannin	0.88*

*Unit - mg GAE/g extract.

Antimicrobial Activity

The Antimicrobial activity of the ethanolic extracts and different fractions from the leaves of *S. nigrum* against a panel of food-borne and pathogenic microorganisms were assessed. The results from the zone of inhibition method, followed by measurement of minimum inhibitory concentration (MIC), indicated that ethanolic extracts of concentrations 50, 100, 150 and 200µL showed good activity against the test organisms **(Table 3)**. In case of Antibacterial activity, the leaf extract was checked for its efficiency against four bacterial species among which two were gram positive - *Bacillus subtilis* , *Staphylococcus aureus* and two were gram negative - *Escherichia coli*, and *Pseudomonas aeruginosa* . The extracts showed least action towards *Pseudomonas aeruginosa* (10 – 16mm) and for others the zones were very prominent (10 – 26mm) inhibiting the bacterial growth **(Figures 3 & 4)**.

Table. 3: Antibacterial Activity by Zone Inhibition Method.

S. No	Test Organisms	Zone of Inhibition (mm)			
		50µL	100µL	150µL	200µL
01.	*Bacillus subtilis*	14	17	18	20
02.	*Escherichia coli*	8	15	20	23
03.	*Pseudomonas aeruginosa*	10	12	14	16
04.	*Staphylococcus aureus*	12	14	18	24

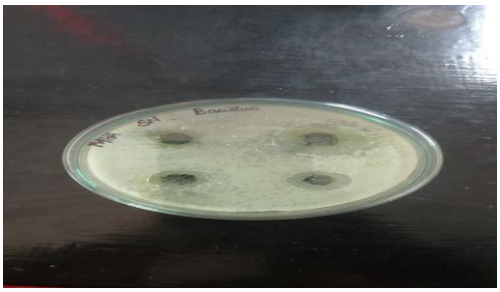

Figure 3: Antibacterial Activity against *B. subtilis.*

Figure 4: Antibacterial Activity against *E. coli.*

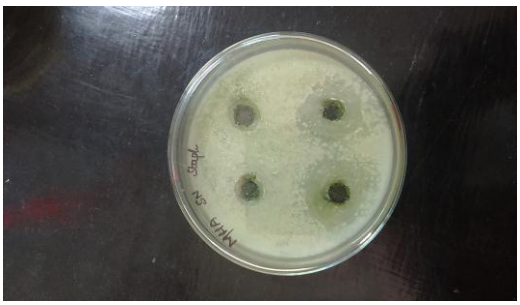

Figure 5: Antibacterial Activity against *S. aureus.*

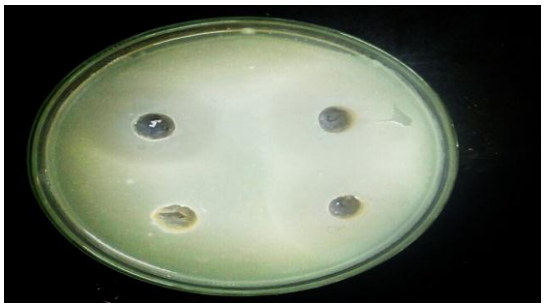

Figure 6: Antibacterial Activity against *Pseudomonas aeruginosa.*

The Antifungal activity of the ethanolic extracts and different fractions from the leaves of *S. nigrum* against three species of fungi - *Aspergillus niger*, *Candida albicans* and *Penicillium spp* was carried out. The extract showed good results by visible zones inhibiting fungal growth **(Table 4 and Figures 7 - 9).**

Table. 4: Antifungal Activity by Zone Inhibition Method.

S. No	Test Organisms	Zone of Inhibition (mm)			
		50µL	100µL	150µL	200µL
01.	*Aspergillus niger*	10	12	15	17
02.	*Candida albicans*	10	12	14	16
03.	*Penicillium spp*	14	16	18	20

*SN-*S. nigrum.*

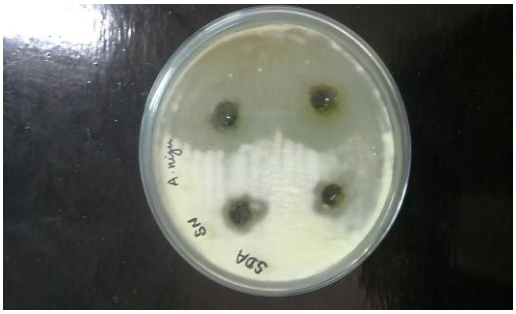

Figure 7: Antifungal activity against *A. niger*.

Figure 8: Antifungal activity against *C. albicans*.

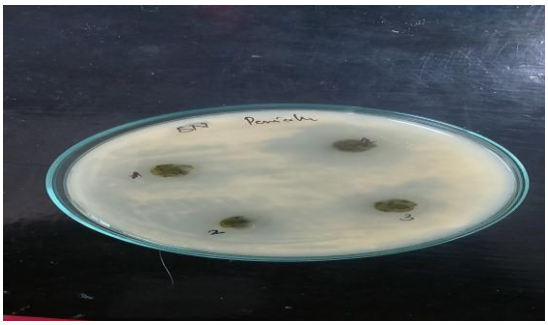

Figure 9: Antifungal activity against *Penicillium spp*.

Minimum Inhibitory Concentration

The MIC in this study against test organisms ranged between 0.4 and 1.5mg/ml for bacteria and while for fungi. MIC ranged between 0.8 and 2mg/ml. Antimicrobial agents with low activity against an organism had a high MIC while a highly active antimicrobial agent gave a low MIC. The results of the present study support the traditional use of the *S. nigrum* as a green medicine.

Antioxidant Activity

The redox properties of antioxidants play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides. In doing so, the antioxidants themselves become oxidised. This urges the constant need of antioxidants to replenish them. The antioxidant properties of *S. nigrum* and *S. trilobatum* are evaluated by DPPH assay. The aqueous extract was taken in different concentrations varying between 10 and 60µgmL and results showed that the antioxidant activity, the percentage of inhibition was 60% when analysed **(Table 5 and Figures 10 & 11).**

Table. 5: Antioxidant Activity using DPPH assay.

S. No.	Concentration(µg/ml)	% of Inhibition
		Solanum nigrum
1	10	14.85
2	20	20.35
3	30	21.79
4	40	25.29
5	50	33.28
6	60	59.9

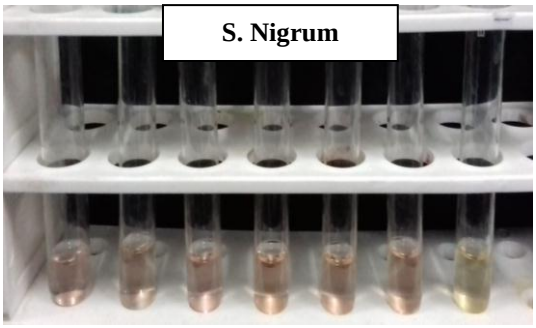

Figure 10: Antioxidant activity by DPPH assay.

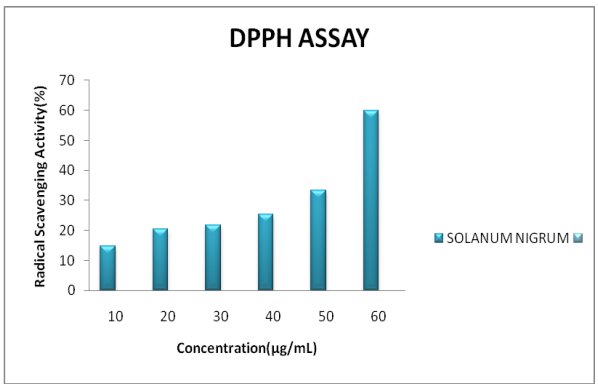

Figure 11: Graph showing Antioxidant activity.

Anti-Diabetic Activity

Diabetes being a metabolic disorder occurs when the blood glucose is too high which the main source of energy. Insulin hormone plays a vital role in aiding glucose from the food to reach the cells to be used as energy. When the body does not make insulin or is produced in inadequate amount then glucose stays in the blood causing hyperglycemia. Hence, to avoid or prevent this condition, these plant products may be used. When the extracts of these plants were analysed for anti-diabetic activity *S. nigrum*, it showed 17 – 61% activity **(Table 6 & Figures 12 - 13).**

Table. 6: Anti-diabetic activity - % of Inhibition.

S. No	Test Samples	Concentration (µg/mL)			
		100	200	300	400
1.	*S. nigrum*	17%	18%	36%	61%

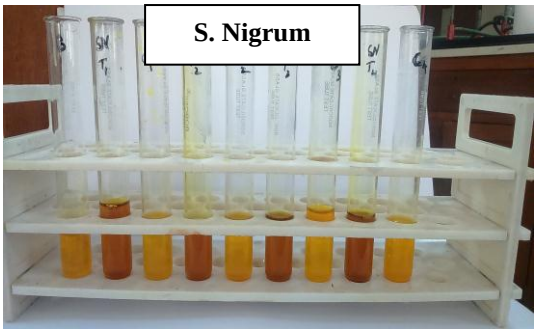

Figure 12: Anti-diabetic activity.

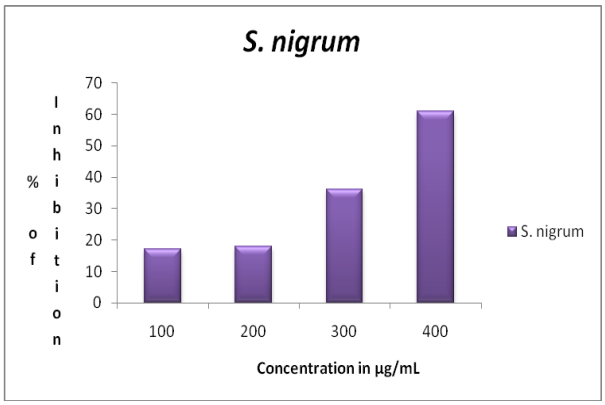

Figure 13: Graph showing Anti – Diabetic Activity.

SUMMARY AND CONCLUSION

This study was completely worked on the plant *S. nigrum* to evaluate the activities in terms of Antimicrobial, Antioxidant and Antidiabetic. The physio-chemical evaluation of drugs is an important parameter in detecting adulteration or improper handling of drugs. It is important to assess the quality of the plant material to suggest it as drug for application. So, the plant extracts containing alkaloids, flavanoids, phenolic, proteins, amino acid, sterols, fixed oil, carotenoid, carbohydrate, terpenoids, tannin, cardiac glycosides, saponin quinones and anthroquinones were all analysed quanlitavely. Among which the quantitative tests were carried out for alkaloids, flavanoids, tannins, terpenoids, saponins, proteins, cardiac glycosides and amino acid. These compounds are considered as potent bioactive compounds that could be used for therapeutic purpose or which are precursors for the synthesis of useful drugs as these compounds possess antimicrobial, antiviral, antidiarrhoeal, anticancer, antihelmintic property. Thus, from the study carried out it is very clear that all the above mentioned work were found to be dose dependent. Hence, to develop a therapeutic drug it is important to find out its exact concentration or dose in which a particular disease can be treated.

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