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ANALYTICAL STUDY ON PHYTOCHEMICAL AND ANTIMICROBIAL SCREENING OF *SOLANUM NIGRUM IN VITRO*

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ABSTRACT

Solanum nigrum(Black Nightshade) is a medicinal plant member of the Solanaceae family of plants. This family comprises many genera, well known for their therapeutic properties. In addition to *S. nigrum*, this family includes fruits and vegetables such as potato (*Solanum tuberosum*), tomato, peppers, ornamental plants such as petunia, and other medicinal plants[1].The present work is focused on the phytochemical analysis and of aqueous, methanolic and hexane extracts of *Solanum nigrum.* And screening for there antimicrobial activity against *Staphylococcus aureus* and *Candida albicans* by well diffusion method measuring the zone of inhibition by turbidity method. This study assures that methanol is the best available solvent for phytochemical extraction and also it confirms the antimicrobial activity of the plant extract.

KEYWORDS: **Antimicrobial screening, *Candida albicans*, Phytochemical analysis, *Solanumnigrum*, *Staphylococcus aureus*.**

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INTRODUCTION

In the present scenario as most of the available antibiotics are running over the cost and also are not appropriate to use because of adulteration and several side effects. Blind dependence on synthetics seems to be over and people are returning to the naturals with the hope of safety. Nature has provided a complete store-house of remedies to cure almost all aliments of human[2].Recently, clinical use of indigenous drugs for the treatment of various diseases is on great demand. Herbs are staging a comeback and herbal craze is all over the globe. Therefore, novel natural antibiotics are encouraged as they being safe as well as are of relatively low cost. As *S. nigrum* has been extensively used traditionally to treat various ailments such as pain, inflammation and fever[3]. It possess many activities like antitumorgenic, antioxidant[4], anti-inflammatory[5],hepatoprotective[6], diuretic[7], antipyretic agent[6,7], antibacterial[8],mycoticinfection, cytotoxicity[9], anti-convulsant, and antiulcerogenic[10,11]. It is also used against sexually transmitted diseases[12].It has also been studied that the plant extracts have great potential as immunostimulant against microorganisms and that they can be used in the treatment of infectious diseases caused by microorganisms[13]. So, being a source of extreme medicinal usage it is significant to screen the phytochemical potential of the plant and to find out its effect on some common human disease causing pathogens. Therefore, This study was carried out on two widely distributed disease causing pathogens i.e. *Staphylococcus aureus* and *Candida albicans. Staphylococcus aureus* is one of the most commonly found pathogenic bacteria and is hard to eliminate from the human and animals environment. It is responsible for many nosocomial infections, besides being the main causative agent of food intoxication by virtue of its variety of enterotoxins[14]. Whereas, *Candida albicans* is an opportunistic fungal pathogen found as part of the normal microflora in the human digestive tract. It is just one of approximately 200 species in the genus Candida, but accounts for up to 75% of all candidal infections[15].

MATERIALS AND METHODS

Dried Plant was collected from Herbal garden of Himalaya Drug Company, Dehradun. The collected plant included leaves, roots, fruit, stem, seeds (panchang). Boiling hot water bath, Methane, Hexane and Distilled water.

(i) Chemicals

For phytochemical analysis chemicals were prepared according to the Indian Pharmacopoeia-"sixth edition".

- Dilute ammonia solution-10% w/w of ammonia i.e. 425ml ammonia solution in 1000ml.
- Ferric chloride solution-5% w/v of ferric chloride.
- KOH solution-10% w/v of KOH.
- NaOHSolution- 20% i.e. 20gm of NaOH in 1000ml of distilled water.
- Dilute sulphuric acid-10% w/w sulphuric acid i.e. 57ml of sulphuric acid and volume make up to 1000ml by distilled water.
- NaOH solution 40%- 40gm NaOH in 1000ml distilled water.
- HCl solution 1%- 1ml of hclin 999ml of distilled water.
- Sulphuric acid solution 50%-50ml of sulphuric acid in 950ml of distilled water
- Fehling's solution[Alkaline cupric tartarate solution]- Copper solution-34.66gm cupric sulphate in 500ml distilled water, Alkaline tartarate solution-176gm sodium potassium tartarate +77gm of NaOH volume made up to 500ml by distilled water. Both the solution were mixed when in use and freshly prepared Fehling's solution was used.

(i) Extraction

50gm grounded powder of the plant was added in 3 beakers separately containing 500ml each of methanol, distilled water and hexane and kept for around 48 hours after covering the

mixture with Aluminium foil and the extract was then filtered using handmade filter paper.

(ii) Phytochemical analysis

- Tannins (phenolic compound)
  Ferric chloride test: Extract was treated with ferric chloride solution;appearance of blue colour indicates hydrolysable tannins and green color indicates presence of condensed tannins.
- Flavonoids
  Alkaline reagent test: To the test solution few drops of sodium hydroxide was added andalso few drops of dilute hydrochloric acid. Appearance of yellow color indicated presence of flavonoids.
- Saponins
  Froth formation test: 2ml extract was placed in water in a test tube. Shaken well, stable foam was formed.
- Steroids
  Salkowski test: Extract was treated with few drops of concentrated sulphuric acid, red colour at lower layer indicates presence of steroids.
- Triterpenoids
  Salkowski test: Extract was treated with few drops of concentrated sulphuric acid, formation of yellow coloured lower layer indicated presence of triterpenoids.
- Protein
  Xanthoproteic acid test: To the 5ml of test solution,1ml of concentrated nitric acid was added and boiled, yellow precipitate was formed, after cooling it,40% sodium hydroxide solution was added, orange colour was formed.
- Anthraquinone glycosides
  Borntrager's test: Test material was boiled with 1ml of dilute sulphuric acid in a test tube for 5minutes. It was filtered while hot. Filtrate was cooled and shaken with equal volume of dichloromethane or chloroform. The lower layer of dichloromethane or chloroform was separated and it was shaken with half of its volume of dilute ammonia. Appearance of rose pink to red colour in the ammonical layer indicated the presence of anthraquinone glycosides.
- Alkaloids
  1ml of 1% HCl was added to 3ml of the extract in a test tube. The mixture was then heated for 20mins, cooled and filtered. About 2 drops of Mayer's reagent to 1ml of the extract. A creamy precipitate indicated the presence of alkaloids.
- Glycosides
  10ml of 50% Sulphuric acid was added to 1ml of extract and the mixture heated in boiling water bath for about 15min. 10ml of Fehling's solution was then added and the mixture boiled. A brick red precipitate indicated presence of glycosides.

(iii) Materials for antimicrobial analysis

Nutrient agar, yeast potato dextrose agar, sterile 6mm borer, Laminar air flow hood, Micropipettes, Colony counter, metric scale for measuring zone of inhibition.

(iv) Antimicrobial screening

The extracts were tested for their effect on some human pathogenic microorganisms. Antimicrobial activity was tested by well diffusion method using 24hr cultures of *Staphylococcus aureus* and *Candida albicans.* Stock cultures were maintained at 4°C.For active microbial culture the cells were transferred from the stock culture to conical flask containing 100ml nutrient broth and yeast potato dextrose broth for *Staphylococcus aureus* and *Candida albicans* respectively .They were then incubated at 37°C for 24hrs (bacteria) and 25°C for 48hrs(fungi). For positive control, tablet of broad spectrum antibiotic ciprofloxacin was taken having a concentration of 500mcg/ml i.e. 685mg including excipients. Active pharmaceutical ingredients for 50mcg/ml calculated as follows: 685×50/500=68.5mg. Then, 68.5mg of the antibiotic was taken and dissolved in 100ml of distilled water in a volumetric flask. To further make the concentration 20mcg/ml, 4ml of the solution was added in distilled water to make up 100ml volume. 1ml of the incubated suspension was inoculated in 700ml of the freshly prepared

agar media at 48°C.The media was then poured in petriplates and left to solidify. Specific Wells were made with the help of sterile stainless steelborer (6mm). In each plate 50µl of different extracts of the plants were poured with the help of micropipette. Positive control of 20mcg/l of ciprofloxacin solution was also poured in a plate for comparative study. Simultaneous addition of each solvent separately in plates were done and regarded as negative control. The plates were then kept for 2hrs so that the solution can diffuse properly in the agar media. Petriplates were incubated at 37°C and 28°C for *Staphylococcus aureus* and *Candida albicans* respectively in upright position. Zone of inhibition was measured after 24hrs.

RESULTS AND DISCUSSION

(i) Phytochemical screening

Several chemical tests were carried out for all the extracts to find out the phytochemical potential of *Solanum nigrum*. Out of all the tested extracts, methanolic extract showed to be the best solvent for extraction of phytochemicals from *Solanum nigrum*. As, there were more number of phytochemicals extracted in methanol than any other solvent used. Methanolic extract showed the presence of tannins, flavonoids, steroids, triterpenoids, proteins and alkaloids. Whereas in hexane extract only tannins, triterpenoids and proteins were present and in aqueous extract tannin, saponin and steroid were present.

Table 1
Phytochemical composition of methanolic extract

Phytochemical	Tests	Observation	Result
Tannins	Ferric chloride	Green colour	Tannins present
Flavonoids	Alkaline reagent test	Intense yellow colour that turned colourless on addition of acid	Flavonoids present
Saponins	Froth formation test	No stable froth formed	Saponins absent
Steroids	Salkowski test	No colour change	Steroids absent
Triterpenoids	Salkowski test	Yellow colour in lower layer	Triterpenoids present
Proteins	Xanthoproteic acid test	Orange colour	Proteins present
Alkaloids		Creamy white ppt.	Alkaloids present
Anthraquinone glycosides	Borntrager's test	No colour change	Absent
Glycosides		No colour change	Glycosides absent

Table 2
Phytochemical composition in hexane extract

Phytochemical	Tests	Observation	Result
Tannins	Ferric chloride	Green colour	Tannins present
Flavaonoids	Alkaline reagent test	No colour change	Flavonoids absent
Saponins	Froth formation test	No stable froth formed	Saponins absent
Steroids	Salkowski test	No colour change	Steroids absent
Triterpenoids	Salkowski test	Yellow colour in lower layer	Triterpenoids present
Proteins	Xanthoproteic acid test	Orange colour	Proteins present
Alkaloids		No colour change	Absent
Anthraquinone Glycosides	Borntrager's test	No colour change	Absent
Glycosides		No colour change	Glycosides absent

Table 3
Phytochemical composition in aqueous extract

Phytochemical	Tests	Observation	Result
Tannins	Ferric chloride	Green colour	Tannins present
Flavonoids	Alkaline reagent test	No colour change	Flavonoids absent
Saponins	Froth formation test	Stable froth formed	Saponin present
Steroids	Salkowski test	No colour change	Steroids absent
Triterpenoids	Salkowski test	Yellow colour in lower layer	Triterpenoids present
Proteins	Xanthoproteic acid test	Orange colour	Proteins present
Alkaloids		No colour change	Absent
Anthraquinone Glycosides	Borntrager's test	No colour change	Absent
Glycosides		No colour change	Glycosides absent

(ii) Antimicrobial screening

Antimicrobial activity on *Solanumnigrum* was performed with methanolic, aqueous, hexane and methanolic+hexane extract against *Staphylococcus aureus* and *Candida albicans*. Against *Staphylococcus aureus* zone of inhibition in methanolic extract was 12mm, in methanolic+hexane extract was 15mm, and no activity in aqueous and hexane extract. Ciprofloxacinwas taken as a positive control at a concentration of 20mcg/ml with zone of inhibition of 22mm, and no activity in negative control that were the solvents i.e. Hexane, methanol and distilled water. Against *Candida albicans* zone of inhibition in methanolic extraxt is 14mm,in methanolic+hexane extract is 15mm, in aqueous is 16mm and no activity in hexane extract. Positive control was taken ciprofloxacin at a concentration of 20mcg/ml with zone of inhibition of 23mm, and no activity in negative control that were the solvents i.e. Hexane, methanol and distilled water. Based on the result of this study it was concluded that methanolic extract *Solanum nigrum* was found to have the best antimicrobial activity out of all the extracts used in the study.

Table 4
Antimicrobial analysis of Solanum nigrum extracts against common pathogens

Extract	Zone of inhibition[mm]	
	Staphylococcus aureus	*Candida albicans*
Methanolic	12	14
Hexane	0	0
Aqueous	0	16
Methanolic+hexane	15	15
Ciprofloxacin[20mcg/ml]	22	23

CONCLUSION

From this preliminary study, it has been concluded that *Solanum nigrum* is a source of essential phytochemicals that has diverse importance and benefits, the best solvent that can be employed for the phytochemical extraction is methanol, as well as it can be an effective antimicrobial plant that can be used as a folk medicine and will be a good source for finding new antimicrobial agents in order to treat and control different human infections. The active ingredients, responsible for antimicrobial activity is yet to be determined, this can be carried out with the help of HPLC and other related techniques in future.

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