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Phytochemical Investigation of the Plant *Solanum nigrum* L. used in Traditional Medicine from the Local Area of Bhopal

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Abstract: ***Solanum nigrum L. (Kaambal) (Kashmiri) has been traditionally used to treat pathological ailments like fever, ulcers, bacterial infections, fungal infections, jaundice and liver disorders (Creasy et al., 1981; Capizzi et al., 2003; and Bomkazi et al., 1981). The most important constituents from Solanum nigrum (i.e., vincristine, irinotecan, camptothecines) and microorganisms (i.e., doxorubicin, dactinomicines, mitomycin and bleomycin) (Grever, 2001) . The history of Solanum nigrum L. dates back to ancient China and the Mediterranean region as a highly popular laxative drug and a general tonic (Dashputre et al., 2010). It is used as purgative and astringent tonic; its stimulating effect combined with apparent properties renders it especially useful in tonic dyspepsia (Chintana et al., 2012). Powdered roots are sprinkled over ulcer for healing. Leaf and berries are eaten either raw or boiled, sprinkled with salt and pepper. Some workers have worked out antitumor activity of Solanum nigrum L. (Anindyajati et al., 2010) but very little is known about the mechanisms involved.***

Keywords: Phytochemical screening, Laxative, Antitumor, *Solanum nigrum* L.

1. Introduction

Medicinal plants are considered as a rich resources of ingrediants which can be used in drug development. Apart from the medicinal uses, herbs are also used in natural dye, pest control, food, perfume, tea and so on. plants have been used for medicinal purposes long before prehistoric period. Among ancient civilization India has been known to be rich repository of medicinal plants. According to WHO around 21,000 plant species have the potential for being used as medicinal plants. Treatment with medicinal plants is considered very safe as there is no side effects. These remedies are in sync with nature, which is the biggest advantage. The golden fact is that, use of herbal treatments is independent of any age groups and the sexes.

Owing to the significance in the above context, the phytochemical investigation of plant is the need of the hour inorder to discover and develop novel therapeutic agents with improved efficacy. Thus the present study deals with the screening based on phytochemical tests of the medicinal plant solanum nigram for identifying its chemical constituents.

2. Material and Methods:

Collection and Extraction of plant material

In the present investigation the whole plant of *Solanum nigrum* L. was collected from the local surrounding at Bhopal district of (M.P) during the months of October-November, 2012. A voucher specimen was submitted in the herbarium at the P.G. Department, Unique College, Bhopal, M.P, India, where it was authenticated by Dr. Jagrati Tripathi, Professor and head department of biotechnology and a herbarium number 280 was assigned to it. The specimen was kept in the herbarium of the said department for future references.

Preparation of plant extract

The plant *Solanum nigrum* L. was collected and washed thoroughly under running tap water and then rinsed in distilled water and allowed to dry for some time. Then the plant was shade dried without any contamination for about 3 to 4 weeks. The powder was extracted according to (Rashmi *et al.*, 2010). The dried plant was powdered (coarse) and subjected to Soxhlet appratus (Figure 2) using petroleum ether, ethyl acetate and chloroform respectively. Almost all the chlorophyll and lipid is deposited on the side of the flask and was removed carefully. The extraction was done with each solvent until the supernatant in the Soxhlet became transparent for 36 hours. Every time before taking the solvents of higher polarity to remove the traces of previous solvents, exhausted marc was completely dried. All the extracts were filtered, dried and weighed.

Phytochemical tests

Phytochemical screening of the extracts was carried out according to the standard procedures (Trease and Evans., 1989 and Kokate *et al.*, 2006). All extracts were subjected to preliminary phytochemical screening to identify the various phyto-constituents present in them i.e. alkaloids, terpenoids, glycosides, steroids, triterpenoids, flavonoids, carbohydrates, saponins and tannins.

Test for carbohydrates

1) Molish Test

Treat the test solution with few drops of alcoholic alpha-napthol. Add 0.2ml of conc. sulphuric acid slowly through

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the sides of the test tube, a purple to violet color ring appears at the junction.

2) Fehling's Test:
Treat the 1ml of aqueous extract with1ml of Fehlings A and 1ml of Fehlings B solutions in a test tube and heated in a water bath for 10 minutes. The formation of red precipitate indicated the presence of reducing sugar.

3) Bareford's Test
1ml of extract and Barefords reagent were mixed in a test tube and heated in a water bath for 5-10 minutes. The solution appeared green, yellow or red depending on the amount of reducing sugar present in the test solution which indicated the presence of reducing sugar.

4) Benedict's Test
Equal volume of Benedict's reagent and extract were mixed in a test tube and heated in the water bath for 5-10 minutes. Solution appears green, yellow or red depending on the amount of reducing sugar present in the test solution which indicated the presence of reducing sugar.

Test for Alkaloids

1) Mayer's Test
Crude extract was mixed with Mayer's reagent (Potassium mercuric iodide solution) cream color ppt. was formed showing the presence of alkaloids

2) Hager's Test
To the 2-3 ml of filtrate, Hager's reagent was added. Yellow precipitate was formed showing the presence of alkaloids

3) Wagner's Test
To 1-2 ml of filtrate, few drops of Wagner's reagent were added in a test tube. The formation of reddish brown precipitate indicated the presence of alkaloids.

4) Dragendroff's Test
To 1-2 ml of filtrate, few drops of Dragendroff's reagent were added in a test tube. Formation of red precipitate indicated the presence of alkaloids.

5) Wagner's Test
To 1-2 ml of filtrate, few drops of Wagner's reagent were added in a test tube. Formation of reddish brown precipitate indicated the presence of alkaloids.

Test for Terpenoids

1) Salkowski Test
To 2 ml of extract, 2 ml of chloroform and 2 ml of conc. H_2SO_4 were added. The solution was shaken well. A reddish brown coloration of the interference indicated the presence of terpenoids.

2) Libermann Burchard's Test
The extract was treated with chloroform. To this solution few drops of acetic anhydride was added, boiled and cooled . Concentrated sulphuric acid was added through the sides of the test tube. The formation of brown ring at the junction of two layers, if upper layer turned green indicate presence of steroids and formation of deep red colour indicated presence of triterpenoids.

Test for Flavonoids:
1) Lead Acetate Test:
The extract was treated with few drops of lead acetate solution. The formation of yellow precipitate may indicated the presence of flavonoids.

2) Alkaline Reagent Test:
The extract was treated with few drops of sodium hydroxide separately in a test tube. The formation of intense yellow colour, which becomes colourless on addition of few drops of dilute acid, indicate presence of flavonoids.

3) Shinoda Test
To the extract, 5 ml (95%) of ethanol was added. The mixture was treated with few fragments of magnesium turning, followed by drop wise addition of concentrated hydrochloric acid. Formation of pink color indicated presence of flavonoids.

Test for Triterpenes:

To the extract, chloroform and conc. H_2SO_4 was added. The appearance of red color indicated the presence of triterpenes. Test for Tannins and Phenolic compounds.

1) $FeCl_3$ Solution Test
On addition of 5% $FeCl_3$ solution to the crude extract, deep blue black color appeared, indicated the presence of tannins.

2) Lead Acetate Test
Some amount of extract was dissolved in distilled water. To this solution few drops of lead acetate solution was added. The formation of white precipitate indicated presence of phenolic compounds.

3) Gelatin Test
Some quantity of extract was dissolve in distilled water. To this solution 2 ml of 1% gelatin solution containing 10% sodium chloride was added. The development of white precipitate indicated presence of phenolic compounds.

4) Dilute Iodine Solution Test
To 2-3 ml of extract, few drops of dilute iodine solution were added. Formation of transient red color indicated presence of phenolic compounds.

Test for Saponins

1) Froth Test
About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously. Persistent froth indicated the presence of saponins.

Test for Protein and Amino acids.
1) Ninhydrin Test
To the 3ml of crude sample, 3 drops 5% ninhydrin was mixed and heated for 10min in boiling water bath. Purple or bluish color indicated presence of amino acids

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2) Biuret's Test
The extract was treated with 1ml of 10% sodium hydroxide solution in a test tube and heated. A drop of 0.7% copper sulphate solution was added to the above mixture. The formation of violet or pink colour indicated the presence of proteins.

3) Million's Test
3 ml of extract was mixed with 5 ml of Million's reagent. White precipitate formed which on heating turned to brick red, indicated the presence of proteins.

Test for glycosides

1) Legal's Test
1ml of test solution was dissolved in pyridine.1ml of sodium nitropruside solution was added and made alkaline using 10% sodium hydroxide solution. The formation of pink to blood red colour indicated the presence of glycosides.

2) Keller Killani Test
To 2 ml of test solution, 3ml of glacial acetic acid and 1 drop of 5% ferric chloride were added in a test tube. 0.5 ml of concentrated sulphuric acid by the side of the test tube was added. The formation of blue colour in the acetic acid layer indicated the presence of cardiac glycosides.

3) Borntrager's Test
To 3 ml of test solution, dilute sulphuric acid was added, boiled for 5 minutes and filtered. To the cold filtrate, equal volume of benzene or chloroform was added and shake it welled. The organic solvent layer was separated and ammonia was added to it. Formation of pink to red color in ammonical layer indicated presence of anthraquinone glycosides.

3. Results

Table 5: Phytochemical investigation of various crude extracts of *Solanum nigrum* L.

S.No	Tests	Observation for extracts		
		Petrolem Ether	Ethyl acetate	Chloroform
1.	Test for Terpenoids			
	Salkowski's Test	+	_	+
2.	Test for Steroids			
	Salkowski's Test	+	_	+
	Libermann Burchard Test	+	_	+
3.	Test for Flavonoids			
	Shinoda test	_	_	+
	Lead acetate test	_	+	_
	Alkaline Reagent Test	+	_	_
4.	Test for Alkaloids			
	Wagner's test	+	+	+
	Mayer's Test	+	+	+
	Dragendroff's Test	+	_	+
5.	Test for Saponin's			
	Froth Test	+	+	+
6.	Tannin and Phenolic compounds			
	Lead acetate test	+	+	_
	Ferric chloride test	_	_	_
	Dilute Iodine Solution Test	+	+	_
	Gelatin Test	+	_	+
7.	Test for carbohydrates			
	Molish test	+	+	_
	Fehling's Test	+	+	_
	Benedict's Test	+	+	_
	Barfoed's Test	+	+	_
8.	Test for Protein's			
	Biuret's test	-	-	+
	Million's Test	+	-	-
	Ninhydrin Test	+	+	+
9.	Test for Amino acids			
	Biuret's test	+	_	+

4. Discussion

Phytochemical investigation revealed that petroleum ether extract was rich in terpenes, steroids, saponins, proteins, chloroform extract was rich in terpenes, alkaloids, saponins, phenols, proteins and ethyl acetate extract was rich in alkaloids, saponins, phenols and proteins. From the above phytochemical investigation it was observed that *Solanum nigrum* L. contains many steroidal glycosides 8, steroidal alkaloids, steroidal oligoglycosides, including solamargine, solasonine, solavilline, solasdamine, and solanine, steroidal saponins and glycoprotein, many polyphenolic compounds such as gallic acid, protocatechuic acid, catechin, caffeic acid, epicatechin, rutin, and naringenin, which possess strong antioxidant and anticancer activity (Xu Zhou *et al.*, 2006; Dai *et al.*, 2010; Lalit *et al.*, 1935; Desai *et al*., 2008). Besides these *Solanum nigrum* L. some proteins, carbohydrates, coumarins and phytosterols crude polysaccharides, polyphenols, gentisic acid, luteolin, apigenin, kaempferol, anthocyanidin have also been reported (Guilford *et al*., 2008); Soobrattee *et al*., 2006).

5. Conclusion

This study revealed that the whole plant of *solanum nigrum* L. have a potential source of useful drugs due to the presence of phytochemicals and can be used for the treatment of various diseases. The selected plant *solanum nigrum* L. is the source of the secondary metabolites i.e., alkaloids, terpenoids, phenols, carbohydrates, saponins, aminoacides.Taking great concern of the useful benefits of the plant it can be advocated as a safe, highly important medicinal plant for general mankind.

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