INTRODUCTION

Herbal drugs have played a vital role in curing so many ailments throughout the history of medicine as well as the existence of mankind. If we take a worldwide comparison of patronization of modern and alternative medicine, it is depicted that 75% of the population world over is per forced. Pharmaceuticals, perfumery products, cosmetics and aroma compounds are used in food flours, fragrances and natural colour in the world. There is definite trend to adopt plant based products due to the cumulative effects from the use of antibiotics and synthetics [1].

Oxidative stress occurs as a result of an increase in oxidative metabolism, which produces a number of free radicals (ROS). To avoid oxidative stress, antioxidants can play an important role conferring beneficial healthy effects [2]. High dietary intake of proven antioxidants can significantly lower the risk of several chronic diseases such as heart diseases, cancers and cataracts. Because of the immense reactivity of free radicals, they can react easily with several bio-molecules including DNA, lipids, proteins and carbohydrates[3]. ROS react with the bio-molecules, leading to local injury and eventual organ dysfunction. They also accelerate the ageing and related degenerative processes. Moreover, ROS are also involved in the promotion of heart diseases, chronic inflammation, and cancer[4]. An ideal antioxidant should be readily absorbed and quench free radicals, and chelate redox metals at physiologically relevant levels. It should also work in both aqueous and/or membrane domains and effect gene expression in a positive way. Endogenous antioxidants play a crucial role in maintaining optimal cellular functions and thus systemic health and well-being. There is growing evidence to support a link between increased levels of ROS and disturbed activities of enzymatic and nonenzymatic antioxidants in diseases associated with aging. The purpose of this study is to evaluate the antioxidant activity and phytochemical investigation of S. indicum [6].

MATERIALS AND METHODS

Collection, Drying, Identification & Extraction

The fresh S. indicum fruits were collected from the picnic sport Kukural Lucknow. Glacial acetic acid, barium hydroxide, α- naphthol, DPPH, (1,1-Diphenyl-2-Picryl hydrazyl radical) gallic acid was purchase from Qualikems, Fine chemicals Pvt. Ltd (New Delhi, India). All other reagents used were of analytical grade. The plant sample was sent to N.B.R.I. (National Botanical Research Institute), Lucknow for taxonomic authentication. The specification no. is NBRI-SOP-202/16-05-09. The test report from CF, N.B.R.I. confirm the taxonomist authenticity of plant material. The fresh fruits of S. indicum were collected in March-April. The green plant and adhering mud was washed with water. Therefore it was made completely clean and dusts free and allowed to get dried under the shade dried for 15-20 days. Dried fruits were pulverized to coarse powder form and subjected to further studies.

Phytochemical screening [7]

Qualitative Analysis

The extracts obtained by successive solvent extraction were subjected to various qualitative tests to detect the presence of common chemical constituents as:

Test for Sterols:

Salkowski reaction

Few mg of the residue of each extract was taken in 2ml of chloroform and 2ml of conc sulphuric acid was added from the side of the test tube. The test tube was shaken for few minutes. The
Development of red color in the chloroform layer indicated the presence of sterols.

Tests for Alkaloids
A small portion from the respective extract was shaken with about 3 ml of 1.5% v/v hydrochloric acid and filtered. The filtrate was tested with the alkaloidal reagents.

Dragendorff's test
The prepared dragendorff's reagent was sprayed on watmann no.1 filter paper and the paper was dried. The test filtrate after basification with dilute ammonia was extracted with chloroform and the chloroform extract was applied on the filter paper, impregnated with Dragendorff's reagent, with the help of a capillary tube. Development of an orange red colour on the paper indicated the presence of alkaloids.

Mayer's Test
The mayer's reagent was prepared by adding 1.36 gm of mercuric chloride and 600 ml of distilled water. Both the solutions were mixed and diluted to 100 ml with distilled water. Small amount of the test filtrate was taken in a watch glass and a few drops of the above reagent were added. Formation of cream colored precipitate showed the presence of alkaloids.

Wagner's test
1.27 g of iodine and 2g of potassium iodide were dissolved in 5 ml of water and the solution was diluted to 100 ml with water. When few drops of this reagent were added to the test filtrate, brown flocculent precipitate was formed indicating the presence of alkaloids.

Tests for Saponins
The presence of saponin is usually indicated in the alcoholic and water extracts of the drug. 1ml of both the extracts were diluted to 20 ml by the respective liquids and shaken well. The presence of saponin was indicative by the formation of dense foam. Other extracts were also tested for the presence of saponins.

Foam test
A little fraction from the various extracts were boiled with about 1ml of distilled water and shaken. A small quantity of sodium carbonate was added to each and shaken. The characteristic foam formation indicated the presence of saponins. Aqueous and alcoholic extract were tested directly.

Test for Sugars:

Molisch's test
The Molish's reagent was prepared by dissolving 10g of α-naphthol in 100 ml of 95% alcohol. Few mg of the test residue was placed in a test tube containing 0.5 ml of water, and it was mixed with 2 drops of Molish's reagent. 1 ml of conc sulphuric acid was added from the sides of an inclined test tube, so that the acid formed a layer beneath the aqueous solution without mixing with it. Appearance of red brown ring at the common surface of the liquids shows sugars are present.

Burford's test
This reagent was prepared by dissolving 13.3 gm of crystalline neutral copper acetate in 200ml of 1% acetic acid solution. The test residue dissolved in water and heated with a little amount of the reagent. Red precipitate of cuprous oxide within two minutes shows the presence of monosaccharides.

Fehling's reduction test
When the solution of carbohydrate is added with Fehling A and Fehling B, brick red precipitate is obtained after heating. The alcoholic and the aqueous extracts were treated with a solution of barium hydroxide till no further precipitate was formed. The excess of barium was removed by passing CO2 and the resulting solution was filtered. The filtrate was neutralized with acetic acid. The neutral solution was tested with Molisch's reagent, Fehling's solution, Benedict's solution and Tollens' reagent. The positive reactions indicate the presence of reducing sugars. The following tests were performed with the other extracts.

Tests for Proteins

Biuret test
Few mg of the residue was taken in water and 1ml of 4% sodium hydroxide solution was added to it. This was followed by a drop of 1% solution of copper sulphate. Formation of violet color shows that proteins are present.

Milun's reaction
A small fraction from each extract was taken in water and filtered. To about 2 ml of the filtrate, 5-6 drops of Milun's reagent were added. Yellowish-red precipitate was indicative of the presence of proteins.

Test for Amino Acids:

Ninhydrin test
The ninhydrin reagent is 0.1% w/v solution of ninhydrin in n-butanol. A little amount of the ninhydrin reagent was added to the test extract. Violet color shows that amino acids are present.

Tests for Glycosides

Borntrager's test
A small fraction from various extracts was dissolved in 1ml of benzene and then 0.5 ml of dilute solution of ammonia was added to the benzene solution. A rose pink to red color was indicative of the presence of glycosides.

Legal's test
A little fraction from respective extracts was taken in water and made alkaline. To alkaline solution was added few drops of sodium nitroprusside solution. A blue color indicates the presence of glycosides.

Evaluation of Antioxidant Activity
Preparation of the extracts
Plant extracts were prepared using two different extracting solvents:

Alcoholic extract
The dried powdered plant material (25 gm, of Solanum indicum fruits) was extracted with 250 ml of ethanol for 8 hours in soxhlet apparatus, refluxed at 60°C. It was then filtered and evaporated to dryness, the crude extract was obtained.

Aqueous extract
The dried powdered plant material (25 gm, of Solanum indicum fruits) was extracted with 250 ml of water for 12 hours reflux at 70°C. It was then filtered and evaporated to dryness, the crude extract was obtained.

Procedure for DPPH (free radical scavenging activity)
The antioxidant activity of the plant extracts and the standard was assessed on the basis of the radical scavenging effect of the stable 1,1-diphenyl-2-picryl hydrazyli(DPPH) free radical activity. The diluted working solutions of the test extracts were prepared in methanol. Ascorbic acid was used as standard in 1-100 μg/ml solution. 0.002% of DPPH was prepared in methanol and 2 ml of this solution was mixed with 2 ml of sample solution and standard solution
separately. These solution mixtures were kept in dark for 20 min and optical density was measured at 517 nm using a UV Spectrophotometer (Shinadzu) against Methanol. The blank was used as 2 ml of methanol with 2 ml of DPPH solution (0.002%). The optical density was recorded and % inhibition was calculated using the formula given below % of inhibition of DPPH activity = \( \frac{(A - B/A) \times 100}{A} \) where A is optical density of the blank and B is optical density of sample [8].

**Determination of total phenolic content (TPC)**

The amount of total phenolics in extracts was determined with the Folin-Ciocalteau's reagent. Gallic acid was used as a standard and the total phenolics were expressed as gallic acid equivalents (mg gallic acid/g extract).

Concentrations of 0.01, 0.02, 0.03, 0.04 and 0.05 mg/ml of gallic acid were prepared in methanol. Concentrations of 0.1 and 1 mg/ml of plant extracts were also prepared in methanol and 0.5 ml of each sample were introduced into test tubes and mixed with 2.5 ml of a ten-fold diluted Folin-Ciocalteau's reagent and 2 ml of 7.5% sodium carbonate.

The tubes were covered with parafilm and allowed to stand for 30 min at room temperature before the absorbance was read at 760 nm spectrophotometrically. All determinations were performed in triplicates [9].

**β-carotene-linoleic acid**

The antioxidant activity of the extract was evaluated using a β-carotene/linoleic acid model system. A solution of β-carotene was prepared by dissolving 2 mg of each extract or 2 mg of BHA emulsifier and 50 ml of distilled water were added to the flask under vigorous shaking. Aliquots (5 ml) of this emulsion were transferred into a series of tubes containing 2 mg of each extract or 2 mg of BHA (butylated hydroxyanisole) for comparison. An aliquot (5 ml) of emulsion without any further addition was used as control. As soon as the emulsion was added to each tube, the zero-time absorbance was read at 470 nm. Subsequent absorbance readings were recorded at 10-mm intervals by keeping the sample in a water bath at 50 °C until the visual color of carotene in the control sample disappeared (about 120 min) [10].

Antioxidant activity (AA) was measured in terms of successful bleaching of β-carotene by using a formula from Ismail and Hong (2002) [11].

\[ AA = (1 - \frac{(A - A_0)}{(A - A_1)}) \times 100 \]

Where \( A_0 \) and \( A_1 \) are the absorbance values measured at initial time of the incubation for samples and control respectively, while \( A \) is the absorbance value of the sample after 120 min.

**TLC Profile**

Chromatography is a family of analytical chemistry techniques for the separation of mixtures. It involves passing the sample, a mixture which contains the analyte in the "mobile phase", often in a stream of solvent, through the "stationary phase." The stationary phase retards the passage of the components of the sample.

**Preparation and activation of TLC plate**

Slurry of gel 'G' was prepared with distilled water. 0.25 mm thick slurry was applied on the glass plates. The plates were dried in air for 10 min. The dried plates were heated in hot air oven 105°C for 30 minutes for the activation of plates.

**Solvent system**

The choice of the solvent system or mobile phase exerts an influence on the separation. The solvent dissolves the substances to be separated from the sorbent and transported across the plate. Solvents were selected on the basis of their different polarity and a constant composition to effect separation.

**Evaluation of the TLC plate**

TLC was produced with the aim of identifying the individual substances in a mixture and also testing for purity or for separation of mixtures. The height of the solvent front and centre of spots were measured in the form of Rf value [12].

**RESULTS AND DISCUSSION**

**Phytochemical tests**

The plant drug was identified, dried in shade and then coarse powdered. The powdered plant drug (Solanum indicum Linn) was successively extracted with various solvents viz. petroleum ether, chloroform, and ethanol finally the marc was macerated under vacuum, dried, weighed and stored in a desiccator.

In qualitative analysis, various chemical tests were performed for the identification of phytoconstituents in Solanum indicum extracts. Different extracts: water, ethanol, chloroform and petroleum ether of Solanum indicum fruits were used. These are qualitatively analysed for steroids (Salkowski reaction), alkaloids (Dragendorff, Mayer, Hager and Wagner), saponins (Fehling, Biuret, Xanthoproteate, Millon and Wagner tests), amino acids (Ninhydrin and Benedict test) and glycosides (Borntagers and Legal test).

The tests confirmed the presence of constituents such as in petroleum ether extract contains steroids, saponin and protein. Chloroform extract contains alkaloids, glycoside, starch saponin and sugar. Ethanolic and water extract contains alkaloids, glycoside, flavonoids, steroids, sugar and tumin.

### Table 1: Qualitative analysis of various extracts of Solanum indicum fruits

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Name of Test/Reagent</th>
<th>Different Extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Water</td>
</tr>
<tr>
<td>Steroids</td>
<td>Salkowski</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Dragendorff</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Mayer</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Hager</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Wagner</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>Foam</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Molish</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Fehling</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Burford</td>
<td>-</td>
</tr>
<tr>
<td>Proteins</td>
<td>Biuret</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Xanthoproteate</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Millon</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Xanthoprotein</td>
<td>-</td>
</tr>
<tr>
<td>Amino acids</td>
<td>Ninhydrin</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Benedict</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>Borntager's</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Legal</td>
<td>-</td>
</tr>
</tbody>
</table>

+ and - showing the positive and negative result respectively
TLC Profile of *S. indicum* fruit

The test for identification of particular steroid was done on chloroform and ethanolic extract. The (TLC) profiles were developed utilizing different solvent systems particularly for steroids. The best resolution of spots for chloroform extract was found by using Acetic acid: Ethanol (1:3) solvent system and visualized by using dragendorff’s reagent as a spraying reagent in which one spot was seen. TLC of chloroform and ethanolic extract of *S. Indicum* is shown in Table 2 and photograph of TLC plate is shown in Fig 1 and 2. As shown in Table 2 the spots with (Rf = 0.46) confirms presence of solanine in chloroform extract. The test confirmed the presence of solanine in ethanolic and chloroform extract.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Spots</th>
<th>Rf value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform extract</td>
<td>1</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.59</td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>1</td>
<td>0.47</td>
</tr>
</tbody>
</table>

Detecting agent: Dragendorff’s reagent

![Fig. 1: TLC of Chloroform extract of *S. indicum* by using solvent system of Acetic acid: Ethanol (1:3) and Detecting agent: Dragendorff’s reagent.](image1)

![Fig. 2: TLC of Ethanol extract of *S. indicum* by using solvent system of Acetic acid: Ethanol (1:3) and Detecting agent: Dragendorff’s reagent.](image2)

**In-vitro antioxidant activity**

**DPPH free radical scavenging activity**

In DPPH scavenging method ascorbic acid was used as standard and IC50 value was found 3 ± 0.4. The free radical scavenging activity of aqueous and ethanolic extract of *S. indicum* was assessed by the DPPH assay. The ethanolic extract showed (10.17 ± 0.6) IC50 for DPPH assay where aqueous extract showed (21.83 ± 0.84) as shown in (Table 4 & 5 respectively). The results showed significant decrease in the concentration of DPPH radical due to scavenging ability of the ethanolic extract *Solanum indicum* fruits.

The results showed that *S. indicum* (1.87-62.5 μg/ml) ethanolic extract (IC50 10.17) had high DPPH radical scavenging activity than the aqueous extract (IC50 21.83). This indicates that ethanolic extract as a good source of natural antioxidants. The DPPH method with the stable organic radical 1,1-diphenyl-2-picrylhydrazyl is used for determination of free radical scavenging activity, usually expressed as IC50, the amount of antioxidant necessary to decrease the initial concentration of DPH by 50%. This means that the lower is the IC50 value of the sample, the higher is its antioxidant activity [15].

The antioxidant capacity of the plant is mainly dependent on phenolic compounds. Phenolic compounds are an important group of secondary metabolites, which are synthesized by plants due to plant adaptation in response to biotic and abiotic stresses (infection, water stress, cold stress, and high visible light). The antioxidant activity of phenolic compounds depend on their molecular structure, based on the availability of phenolic hydrogens, which result in the formation of phenoxyl radicals due to hydrogen donation[14].

**Table 3: Antioxidant activity using ascorbic acid as standard**

<table>
<thead>
<tr>
<th>Concentration</th>
<th>% inhibition</th>
<th>IC50</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.25</td>
<td>40.69</td>
<td>37.68</td>
<td>33.91</td>
</tr>
<tr>
<td>3.1</td>
<td>55.86</td>
<td>50.44</td>
<td>46.97</td>
</tr>
<tr>
<td>6.1</td>
<td>73.55</td>
<td>65.48</td>
<td>66.76</td>
</tr>
<tr>
<td>12.5</td>
<td>73.97</td>
<td>71.93</td>
<td>71.02</td>
</tr>
<tr>
<td>25</td>
<td>79.95</td>
<td>78.11</td>
<td>80.15</td>
</tr>
<tr>
<td>50</td>
<td>84.58</td>
<td>85.43</td>
<td>85.01</td>
</tr>
</tbody>
</table>

Value are mean ± S.D.; n=3

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The present study indicates the presence of alkaloids, glycoside, steroids, saponin, flavonoids and sugar during the phytochemical investigation. In case of TLC profiling the presence of solanine in chloroform extract was confirmed. The test also confirmed the presence of solanine in ethanolic and chloroform extract. The crude plant drug was used to evaluate the antioxidant potential using DPPH scavenging method, β-Carotene assay method, and total phenolic content and it was found that ethanolic and water extract showed good antioxidant activity. Considering the results obtained it can be concluded that the plant contains essential phytochemical constituents and possess active antioxidant property. Further investigations may be carry out to find active component of the extract and to confirm the mechanism of action.

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