Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Issue 3, 2013

Research Article

PHYTOCHEMICAL INVESTIGATION AND EVALUATION OF ANTIOXIDANT ACTIVITY OF FRUIT OF *SOLANUM INDICUM* LINN.

RIZWAN UL HASAN*[1], PRANAV PRABHAT[1], KAUSAR SHAFAAT[1], RIZWANA KHAN[2]

[1]Faculty of Pharmacy, S. Sinha College, Aurangabad, Bihar, India, [2]Institute of Pharmacy, Bundelkhand University, Jhansi, (U.P.) India.
Email: rizwanhasan786@gmail.com

Received: 28 Feb 2013, Revised and Accepted: 14 Apr 2013

ABSTRACTS

Objective: The objective of the present study is to investigate the phytochemical constituents and to evaluate the antioxidant potential of fruit of *Solanum indicum* Linn. In spite of the modern chemical analytical procedures available, only rarely do phytochemical investigations succeed in isolating and characterizing all secondary metabolites present in the plant extract.

Methods: The extracts obtained by successive solvent extraction were subjected to various qualitative tests to detect the presence of common chemical constituents as: Alkaloids, Carbohydrates, Glycosides, Phytosterols, Saponins, Tannin, Phenolic compounds and Proteins and free amino acids were separated by TLC. The antioxidant evaluation was performed by using three complementary in *vitro bio-assay*, DPPH (1,1-Diphenyl-2-Picryl hydrazyl radical) Scavenging assay, total phenolic content and β-Carotein linoliec acid.

Results: The spots with (Rf=0.46) confirms presence of solanine in chloroform extract. The Ethanolic extract showed (10.17 ± 0.6) Ic50 for DPPH assay where aqueous extract showed (21.83 ± 0.84). β - Carotene assay showed that the ethanolic extract (37.22 ± 1.3) possesses more antioxidant activity than water extract (29.07 ± 1.5) but both extract contain less antioxidant activity as compared to BHA (46.86 ± 0.1) as standard. The total phenolic content that was estimated for ethanolic & aqueous extract was (20.49), (36.11) respectively.

Conclusion: The present findings suggest that the crude aqueous and ethanolic extract of *Solanum indicum* fruits is a potential source of natural antioxidants. The ethanolic extract showed both, the highest antioxidant activity and phenolic contents than aqueous extract.

Keywords: Phytochemical, DPPH, TPC, β Carotene, Antioxidant, Solanine

INTRODUCTION

Herbal drugs have played a vital role in curing so many aliments 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 flavours, 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 Reactive Oxygen Species (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 cancers[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[5].

The plant *S. indicum* is much-branched perennial shrub, up to 1.8 m high, found mostly throughout warmer parts of India, Africa and Asia up to an elevation of 1500 m. Traditionally the plant roots are used as diaphoretic, diuretic, expectorant, and stimulant. The root is used against bronchitis, itches and for bodyaches. The leaves are placed in the cradles of infants to promote sleep. It is useful in cutaneous disorders. Their juice has been used for ringworm, gout and earache and when mixed with vinegar, is considered to be good for gargle and mouthwash. 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 *Solanum indicum* fruits were collected from the picnic sport Kukural Lucknow.Glacial acetic acid, barium hydroxide, α-nepthol,DPPH(1,1-Diphenyl-2-Picryl hydrazyl radical), galic 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-202/16-03-09. The test report from CIF, N.B.R.I. confirm the taxonomist authenticity of plant material. The fresh fruits of *Solanum 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:

Salkowaski 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 colour 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.

Dragondroff's test

The prepared dragondroffs 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 Dragondroff'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 coloured precipitate showed the presence of alkaloids.

Hager's reagent

A saturated aqueous solution of picric acid was employed for this test. When the test filtrate was treated with this reagent, an orange yellow precipitate was obtained, indicating 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 saponins is usually is 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. However, 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 Molisch'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 monosaccharide.

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 CO_2 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, Benedicts solution and Tollen's 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 colour shows that proteins are present.

Xanthoproteate test

Little amount of residue was taken in 2ml of water and 0.5ml of concentrated nitric acid was added to it. Appearance of yellow colour shows that proteins are present.

Millon'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 Millon'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 colour 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 colour 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 colour 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 hydrazyl(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 (Shimadzu) 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= (A – B/A) × 100 where A is optical density of the blank and B is optical density of the 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/linoleate model system. A solution of β-carotene was prepared by dissolving 2.0 mg of β-carotene in 10 ml of chloroform. One milliliter of this solution was then pipetted into a round-bottom flask. After chloroform was rotary evaporated at 40 °C under vacuum, 20 mg of purified linoleic acid, 200 mg of Tween 40 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].

$$AA = \left(1 - \frac{(A_\circ - A_t)}{(A^\circ_0 - A^\circ_t)}\right) \times 100$$

Where A_0 and A^0_0 are the absorbance values measured at initial time of the incubation for samples and control respectively, while A_t and A^0_t are the absorbance values measured in the samples or standards and control at t= 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 them 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 R_f value[12]. The R_f value indicates the position the position at which a substance was located in the chromatogram as shown in (fig. 1, 2, 3, 4).

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 with purified water. All the extracts thus obtained were concentrated 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 (Salkowaski reaction), alkaloids (Dragendroff, Mayer, Hager and Wagner), saponins (foam test), sugars (Molish, Fehling, Burford test), proteins(Biuret, Xanthoproteate, Millon and Xanthoprotein test), 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, steroids saponin and sugar. Ethanolic and water extract contains alkaloids, glycoside, flavonoids, steroids, sugar and tannin.

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

Phytoconstituents	Name of Test/Reagent	Different Extracts			
		Water	Ethanol	Chloroform	Ether
Steroids	Salkowaski	+	+	+	+
Alkaloids	Dragendroff	+	+	+	-
	Mayer	+	+	+	-
	Hager	+	+	+	-
	Wagner	+	+	+	-
Saponin	Foam	+	-	+	+
Sugar	Molish	+	+	-	-
	Fehling	-	+	+	-
	Burford	-	-	-	-
Proteins	Biuret	-	-	-	-
	Xanthoproteate	-	-	-	+
	Millon	-	-	-	-
	Xanthoprotein	-	-	-	-
Amino acids	Ninhydrin	-	-	-	-
	Benedict	+	-	-	-
Glycosides	Borntager's	+	+	-	-
	Legal	-	-	-	-

+ 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 dragendroff'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 2: TLC in Solvent System: Acetic acid: Ethanol (1:3)

Sample	Spots	Rf value
Chloroform extract	1	0.46
	2	0.59
Ethanol extract	1	0.47

Detecting agent: Dragendroff's reagent

Fig. 1: TLC of Chloroform extract of S. indicum by using solvent system of Acetic acid: Ethanol (1:3) and Detecting agent: Dragendroff's reagent.

Fig. 2: TLC of Ethanol extract of S. indicum by using solvent system of Acetic acid: Ethanol (1:3) and Detecting agent: Dragendroff's reagent.

***In-vitro* antioxidant activity**

DPPH free radical scavenging activity

In DPPH scavenging method ascorbic acid was used as standard and IC_{50} 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) IC_{50} 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 (IC_{50} 10.17) had high DPPH radical scavenging activity than the aqueous extract (IC_{50} 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 IC_{50}, the amount of antioxidant necessary to decrease the initial concentration of DPPH by 50%. This means that the lower is the IC_{50} value of the sample, the higher is its antioxidant activity [13].

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

Concentration	% inhibition			IC_{50}	Mean±SD
1.25	40.69	37.68	33.91		
3.1	55.86	50.44	46.97	2.5	3.0±0.4
6.1	73.55	65.48	66.76		
12.5	73.97	71.93	71.02	3	
25	79.95	78.11	80.15		
50	84.58	85.43	85.01	3.5	

Value are mean ± S.D.; n=3

Table 4: Antioxidant activity of Ethanolic Extract *Solanum indicum* fruits

Concentration	% inhibition			IC_{50}	Mean±SD
1.87	3.58	7.95	10.07		
3.95	16.72	18.15	22.83	11	10.17±0.6
7.81	39.70	44.04	40.92		
15.62	61.39	68.64	70.72	9.5	
31.25	70.05	76.91	72.96		
62.5	73.63	81.53	80.46	10	

Value are mean ± S.D.; n=3

Table 5: Antioxidant activity of aquous extract *Solanum indicum* fruits

Concentration	% inhibition			IC_{50}	Mean±SD
1.87	32.80	31.18	15.78		
3.95	34.79	33.10	27.05	21.5	21.83 ± 0.84
7.81	39.98	33.22	30.68		
15.62	43.89	41.61	40.51	23	
31.25	60.29	57.31	62.03		
62.5	81.91	77.88	71.19	21	

Value are mean ± S.D.; n=3

Determination of total phenolic content

In total phenolic content Folin-Ciocalteu's method gallic acid was used as standard. Phenolic compounds are widely distributed in plants[15]. and have gained much attention, due to their antioxidant activities and free radical-scavenging abilities,which potentially have beneficial implications for human health[16].The ethanolic and water extracts obtained from the samples seeds were evaluated for the presence of phenolic compounds. The samples were evaluated using the Folin–Ciocalteu assay, which was suggested as a fast and reliable method to quantify phenolics in foods[17]. The Total Phenolic (TPC) content of ethanolic and aqueous extracts is 20.49 and 36.11 mg gallic acid equivalent/g of extract, respectively. The total phenolic content (TPC) was determined in comparison with standard gallic acid and the results expressed in terms of mg GAE/g. The highest TPC values were obtained for the aqueous extract than the ethanolic extract.

β-carotene-linoleic acid test

In β-carotene-linoleic acid method Butylated hydroxyl anisole (BHA) was used as standard. β-Carotene assay showed ethanolic extract (37.22 ± 1.3) possess more antioxidant activity than water extract (29.07 ± 1.5) but both extract contain less antioxidant activity as compared to BHA (46.86 ± 0.1) as standard. The total phenolic content were estimated for ethanolic & aquous extract (20.49), (36.11) respectively as shown in Table 7. The above results signify that ethanolic extract has potent antioxidant activity. The crude plant drug was used to evaluate antioxidant potential and was found that ethanolic and water extract showed good antioxidant activity.

Table 6: Total Phenol Content of *Solanum indicum* fruits

Compound	Concentration	Abs.(mean)	SD	TPC
Gallic acid	2.5	0.0900	0.01	
	5	0.1121	0.01	
	10	0.2001	0.02	
	20	0.3352	0.03	
	40	0.5192	0.03	
Ethanolic Ext.	1000	0.3652	0.01	20.49
Aqueous Ext.	1000	0.6167	0.01	36.11

Table 7: Comparative antioxidant activity of *Solanum indicum* fruits by using β-carotene-linoleic acid method

Samples	Antioxidant activity			Mean±SD
Standard(BHA)	46.86	47.07	46.66	46.86±0.16
Aqueous extract	27.19	30.91	28.98	29.07±1.51
Ethanolic extract	38.7	37.6	35.36	37.22±1.38

Value are mean ± S.D.; n=3

CONCLUSION

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.

ACKNOWLEDGEMENT

The authors of this paper are very much thankful to the Faculty of Pharmacy, S. Sinha College, Aurangabad, Bihar for providing the facilities for conducting the research work. Authors are also thankful to N.B.R.I. (National Botanical Research Institute), Lucknow for taxonomic authentication of *Solanum indicum* fruits.

REFERENCES

1. Jain S.K. Medicinal plants. 1st ed. New Delhi: India National Book Trust. 1968.
2. Vaya J, Aviram M. Nutritional antioxidants: mechanism of action, analysis of activities and medical applications, Current Medicinal Chemistry. 2001: 1, 99-117

3. Halliwell B. Oxygen radicals, nitric oxide and human inflammatory joint disease. Ann. Rheam. Dis. 1995; 54:505-510.
4. Ivanova E, Ivanov B. Mechanisms of the extracellular antioxidant defend. Experimental pathology and parasitology. 2000; 4:49-59.
5. Haliwell B, Gutteridge JB. The antioxidants of human extra cellular fluids. Biochemistry Biophysics. 1990; 280:1-8.
6. The Ayurvedic pharmacopoeia of india. part1, volume 2, first edition. Ministry of health and family welfare, Government of india. The controller of publications, civil lines, Delhi. 1999; 27-28.
7. Singh S, Hussain A, Singh D. Phytochemical screening and determination of quinazoline alkaloid in *adhatoda vasica.* Int. J. Pharm. Sci. Rev. Res. 2012; 14(2):115-118.
8. Khalaf NA, Shakya AK Othman, AA Agbar ZE and Farah, H. Antioxidant activity of some common plants. 2008; 51-55.
9. Li F, Sun S, Wang J & Wang D. Chromatography of medicinal plants and Chinese traditional medicines. Biomedical Chromatography. 1998; 12:78-85.
10. Panovska TK, Kulevanova S, Stefova M. Invitro antioxidant activity of some *Teucrium* species (Lamiaceae). Acta Pham. 2005; 55: 207-214.
11. Ismail A, Hong TS. Antioxidant Activity of selected commercial seaweeds. Mal J Nutr. 2002; 8(2):167-177.
12. Mukherjee PK. Quality Control of herbal drugs. 1st ed. (Delhi) Birla horizon publication; 2002.
13. Khatri DK, Juvekar P, Juvekar AR. Phytochemical Investigation And *In Vitro* Antioxidant Activities *Indigofera Cordifolia* Seed Extracts. International Journal Of Pharmacy And Pharmaceutical Sciences. 2013; 5, Suppl 2:71-75.
14. Saptarini NM, Wardati Y, Juliawati R. Antioxidant Activity of Extract and Fraction of Yellow Passion Fruit (*Passiflora Flavicarpa*) Leaves. International Journal of Pharmacy and Pharmaceutical Sciences. 2013; 5:194-196.
15. Li BB, Smith B, & Hossain MM. Extraction of phenolics from citrus peels I. Solvent extraction method. Separation and Purification Technology. 2006; 48:182–188.
16. Govindarajan R, Singh DP, & Rawat AKS. High-performance liquid chromatographic method for the quantification of phenolics in 'Chyavanprash' a potent ayurvedic drug. Journal of Pharmaceutical and Biomedical Analysis. 2007; 43: 527–532.
17. Prior RL, Wu X, & Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in food and dietary supplements. Journal of Agricultural and Food Chemistry. 2005; 53:4290–4302.