WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 14, 817-829. Research Article ISSN 2277– 7105

PHYTOCHEMICAL ANALYSIS AND STUDY OF ANTI-OXIDANT POTENTIAL OF A WILD PLANT: *NICOTIANA PLUMBAGINIFOLIA* Viv. FROM CHANDIGARH

Nishtha*[1], Richa[2] and Anju Rao[3]

Department of Botany, Panjab University, Chandigarh, 160014.

Article Received on
11 Sept. 2017,

Revised on 03 Oct. 2017,
Accepted on 24 Oct. 2017

DOI: 10.20959/wjpr201714-9984

***Corresponding Author**
Nishtha
Department of Botany,
Panjab University,
Chandigarh, 160014.

ABSTRACT

Nicotiana plumbaginifolia Viv. also known as wild tobacco is an erect annual or perennial weedy plant with spreading radical and slender leafy branches and belongs to the family Solanaceae. The plant is of great significant importance in terms of its uses as it acts as an effective antispasmodic, diuretic and expectorant. It is used to treat human ailments like in treating swelling, rheumatic fever, it acts as analgesic and possess larvicidal and anti-bacterial properties. Decoction and infusions of the roots and leaves are used to treat syphilis ulceration in men. Present study was undertaken for the qualitative and quantitative analysis of active phytochemical constituents present in the leaves of this plant. Qualitative tests reveal the presence of alkaloids, flavonoids, glycosides and tannins in aqueous, ethanolic and methanolic leaf extracts. Quantitative studies detected the presence of nicotine and nornicotine as major alkaloids by HPLC in the methanolic leaf extracts. The anti-oxidant studies were estimated by DPPH assay and H_2O_2 radical scavenging activity showing the highest % inhibition of 86.54% and 88.13% as compared to the reference standard respectively. The total phenolic and flavonoid contents were also calculated along with reference standard and calculated as mg equivalents per g of the sample. (mg/g).

KEYWORDS: Phytochemical, Flavonoids, Antioxidant and HPLC.

INTRODUCTION

Plants are wide source of medicines used since ages. Due to medicinal properties, they are valuable source of potent and powerful drugs. (Shrivastva *et al*, 1996). Plants and plant based products are being used for prevention and treating human ailments especially in developing

countries. Among the large diversity of plants, only a small number of plants are for their medicinal purposes and there is indeed the need for exploration and investigation of plants for pharmacological purposes. The medicinal value of the plants is due to the presence of active chemical substances in any part of the plant (stem, root, bark, fruit, leaf etc.) which are termed as secondary metabolites or phytochemicals such as alkaloids, saponins, steroids, tannins, flavonoids etc. These exhibit definite physiological activity on human body. (Software, 1993) by dealing and controlling a large number of diseases that cause severe problems to mankind. Phytochemical investigations are the tools which evaluate the active components in the plants that can be further explored in the production of useful plant based medicines. Plants not only contain the metabolites but show an effective antioxidant property wherein these molecules are capable of preventing the oxidation of free radicals. (Satyanarayana *et al*, 2016). The family Solanaceae is the third most important plant taxon in terms of economy and is useful as the source of large amounts of bioactive compounds. *Nicotiana plumbaginifolia* belonging to this family has been selected for studying qualitative and quantitative analysis of phytochemicals and anti-oxidant potential.

Nicotiana plumbaginifolia also known as wild tobacco, is a weed herb which is annual having hairy stem and found to be originated from Mexico and West Indies. It is mostly found along roadsides in damp areas attaining a height of 60 cm with spreading radical and slender leafy branches. It has great medicinal importance because it is used in treating several human ailments like rheumatic and relieves pain caused by swelling. It is highly antispasmodic and expectorant and the dried leaves are used to treat travelling sickness and nausea. (Singh *et al*, 2010). Studies have confirmed that the syphilis ulceration is treated by drawing the infusion of roots or inner leaves in the nostrils. (Dhar, *et al*, 1968). Leaves are found effective larvicide against female vector. (Singh *et al*, 2015).The present study is undertaken to analyze the active phytochemicals, anti-oxidant potential and estimation of total phenolic and flavonoid content in the leaves of this plant.

MATERIALS AND METHODS

Plant collection and identification: Fresh plant samples were collected from two localities. Identification of species was done by comparing with authenticated herbarium specimens, (PAN, CHANDIGARH) and later confirmed with the help of diagnostic keys and morphological descriptions given in various floras. The useful parts such as leaves, stems etc. were separated and preserved for study.

A) Qualitative Analysis

1. Phytochemical study: Leaves were washed in a solution of 5% mercuric chloride for 5 minutes and then thoroughly washed with sterile distilled water in order to remove any dirt or filthy particles present on the surface and then were shade dried as well as oven dried and made into fine powder. The solvent extracts were evaporated to dryness in rotary evaporator in ethanol, methanol and distilled water. The dried residues thus obtained were stored in screw capped vials at -4°C. Qualitative phytochemical screening of plant extracts was carried out by using standard procedures. Specific qualitative tests were performed:

- **Test for alkaloids**

Mayer's reagent and Wagner's Reagent: The plant extract was warmed with 2% H_2SO_4 for two minutes, then it was filtered and few drops of reagents were added separately.

A) Mayer's reagent: A creamy white colored precipitate showed the presence of alkaloids.

B) Wagner's Reagent: A reddish-brown precipitate appears which also confirmed presence of alkaloids in the extract.

- **Test for cardiac glycosides (Keller-Killiani test):** To the solution of extract in glacial acetic acid solution, few drops of $FeCl_3$ and conc. H2SO4 were added and later observed for the reddish-brown coloration at the junction of two layers and bluish-green color in upper layer showed the presence of cardiac glycosides. (Siddiqui and Ali, 1997)

- **Test for Tannins:** The substance (extract) was mixed with basic lead acetate solution. Formation of white precipitate indicated the presence of tannins.

- **Test for Saponins:** Froth test: 1g of the sample was weighed into a conical flask in which 10ml of sterile distilled water was added and boiled for 5 min. The mixture was filtered and 2.5ml of the filtrate was added to 10ml of sterile distilled water in a test tube. The test tube was stopped and shaken vigorously for about 30 second. It was then allowed to stand for half an hour. Honeycomb froth indicated the presence of saponins.

- **Test for Flavonoids**

1. Ferric- Chloride test: Few drops of $FeCl_3$ solution were added to the extract which formed the black color indicated the presence of flavonoids.

2. Lead-acetate solution test: To the extract was added a few drops of lead acetate (10%) solution which resulted in the yellow precipitate thus indicating the presence of flavonoids.

• **Test for Steroids and Terpenoids:** 4mg of extract was treated with 0.5ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of H_2SO4 was added and res violet color was observed for terpenoids and green-bluish color for steroids. (Siddiqui and Ali, 1997).

B) Quantitative Analysis

2. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

The methanolic leaf extracts were analyzed using HPLC (Shimadzu, 2LC-10 ATVP pumps, SPD-10AVP, UV –visible detector, Rheodyne injector with 50 μL loop. The data was acquired and processed using Shimadzu LC- solution version 6.42 software. Phenomenex C18 column (250mm×4.6mm, I.D., 5μm) and 0.4% aqueous acetonitrile containing 0.1% (v/v) phosphoric acid buffered to pH 3.5 with triethylamine was used as mobile phase. The mobile phase was filtered through 0.22μm membrane filter and degassed by sonication for 10 mins. Injection volume was adjusted to 20μL and detection was made at 260nm.

3. TOTAL PHENOLIC CONTENT

Folin Ciocalteu reagent was used for analysis of total phenolic content (TPC) according to Chun *et al*. (2003) with little modifications. Plants extract was prepared in methanol with concentrations ranging from 50-100µg/ml. 10% Folin Ciocalteau Reagent was made and 2.5 ml was added in each concentration. 7.5% Na_2CO_3 was prepared and after 5 min 2.5 ml was added in each plant extract. Addition of distilled water was done to make the volume upto 5.5.ml.Solution was allowed to stand for 2 hours at room temperature. Then absorbance was taken spectrophotometrically at 765nm. Gallic acid was used as standard for the experiment TPC was expressed as mg gallic acid equivalents (GAE) per gram of sample (mg/g).

4. TOTAL FLAVONOID CONTENT

Total flavonoid content was measured by $AlCl_3$ colorimetric assay as proposed by (Chang et al, 2002) with slight modifications. 1mg/ml of plant extract was prepared in methanol and different concentrations were taken ranged from 50-250µg/ml. Sample was mixed with 0.1ml of 10% $AlCl_3$ and 0.1ml of Potassium acetate. Then 80% methanol was added and final volume was raised to 3ml. After shaking the mixture, the sample was incubated for 30 minutes at 37°C water bath. Then absorbance was measured at 420 nm. Quercetin was used

as standard for experiment.TFC was expressed as mg Quercetin equivalents per g of the sample. (mg/g).

5. *IN VITRO* ANTI-OXIDANT ACTIVITY

- **DPPH radical scavenging activity**

The anti-oxidant activity was determined by 2, 2 -diphenyl-1-picrylhydrazyl (DPPH) assay (Harini *et al*, 2012) with some modifications. DPPH is a stable free radical and is widely used to assess the radical scavenging activity of anti-oxidant compounds. 0.1mM DPPH solution was made in methanol and was taken as negative control. Plant extract was prepared as 1mg/ml in methanol and different concentrations ranging from 50-250µg/ml were prepared and DPPH was added to them. The reaction mixture was performed in the dark condition and subsequently it was incubated in dark for 30 minutes and then absorbance was measured spectrophometrically at 517nm. The observations showed the change in the color of reaction mixtures which indicated the increase in the percentage of the free radical inhibition due to the radical scavenging activity. The DPPH radical scavenging activity (%) was calculated by the following equation: Ascorbic acid was taken as standard for this experiment.

Activity (%) = $(A_1\text{-}A_0)/ A_0) \times 100$

Where A_1 is the absorbance of the control and A_0 is the absorbance of test solution. For the performed experiment ascorbic acid was used as standard. The results are expressed as mean % antiradical activity.

- **Hydrogen peroxide radical activity**

The ability of the plant extracts to scavenge hydrogen peroxide was determined according to the method of (Ruch *et al* 1989). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Extracts ranging from 50-250µg/ml in distilled water were added to a hydrogen peroxide solution (0.6 mL, 40mM). Absorbance of hydrogen peroxide at 230 nm was determined 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide. Ascorbic acid was taken as blank. The percentage of hydrogen peroxide scavenging activity of the extract and standard was calculated

% Scavenged [H2O2] = [(AC – AS)/AC] x 100

Where AC is the absorbance of the control and AS is the absorbance of the extract.

RESULTS

1. PHYTOCHEMICAL STUDIES

Tab. 1: Qualitative analysis of secondary metabolites in different extracts of the plant.

Phytochemicals	Aqueous extract	Ethanolic extract	Methanolic extract
Alkaloids	+	+	+
Flavonoids	+	+	+
Glycosides	+	+	+
Tannins	+	+	+
Steroids	-	+	+
Terpenoids	-	+	+

+ indicates the presence and –indicates the absence of secondary metabolites

2. QUANTITATIVE ANALYSIS OF ALKALOIDS BY HPLC

In the present studies, it has been observed that peak area was found at 23794 with retention time 4.576. These peaks were observed by comparing with the reference standard which showed the retention time of 4.576. The peaks thus found were of nicotine and nornicotine which showed the presence of alkaloids.

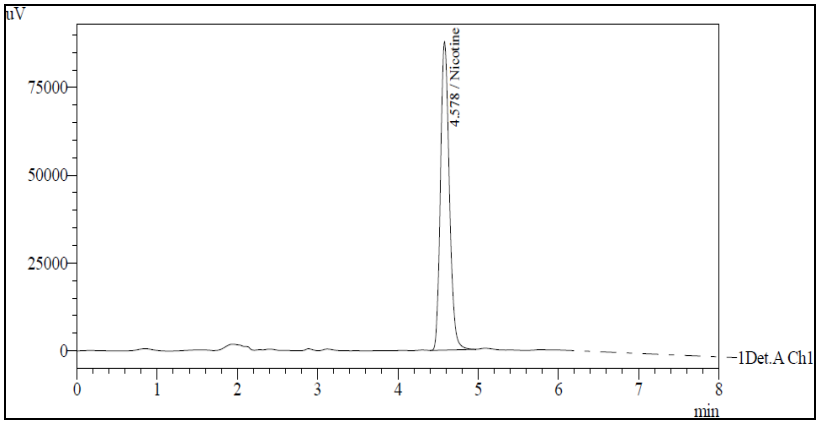

Fig. 1: HPLC Chromatogram of Standard (Nicotine).

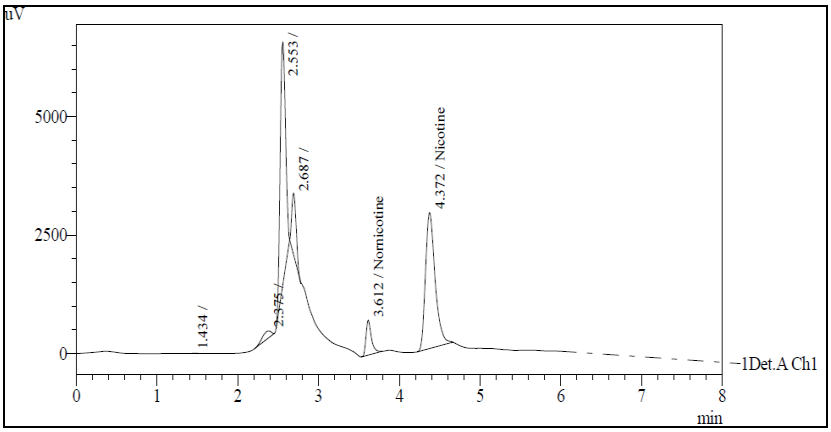

Fig. 2: HPLC Chromatogram of Leaf Extract of *Nicotiana plumbaginifolia.*

Tab. 2.

Sample ID	area	conc.(µg/ml)	DF	Nicotine present (µg/ml)
Nicotiana plumbaginifolia	23794	2.81101598	5.622032	5.622032

1. DPPH RADICAL SCAVENGING ACTIVITY

The ability of methanolic leaf extract of *Nicotiana plumbaginifolia* to scavenge DPPH radical was compared with ascorbic acid used as standard. It was observed that at the concentration of 250µg/ml, the percentage inhibition of plant extract was found to be 86.54% as compared to 80.12% of ascorbic acid at same concentration. The results of the present study at various concentrations are given in the table below along with fig 3.

Tab. 3: % inhibition of DPPH by methanolic leaf extract of the plant and ascorbic acid.

Concentrations (µg/ml)	%inhibition(ascorbic acid)	% inhibition (Plant extract)
50	36.97±0.40	41.25±0.25
100	52.94±0.47	54.09±0.44
150	56.64±0.63	58.87±1.00
200	65.96±0.47	67.73±1.2
250	80.12±0.96	86.54±0.42

Values represent mean±SEM of three replicates.

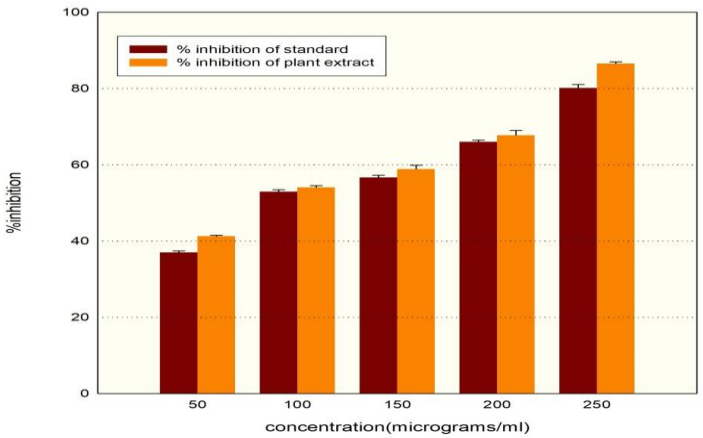

Fig. 3: Determination of free radical scavenging activity of methanolic leaf extracts showing difference in the %inhibition of DPPH of plant extract and standard (Ascorbic acid) at various concentrations.

Values are represented as mean±SEM of three values and bars represent standard errors at 5%level.

2. Hydrogen peroxide radical activity.

Tab. 4: % inhibition of H2O2 scavenging ability by methanolic leaf extract of the plant and ascorbic acid.

Concentrations (µg/ml)	%inhibition(ascorbic acid)	% inhibition (Plant extract)
50	36.97±0.75	71.87±0.76
100	52.94±0.54	76.90±0.55
150	56.64±0.40	81.36±0.81
200	66.63±0.37	85.00±0.23
250	80.12±0.50	88.13±0.43

Various values represent mean±SEM of three values.

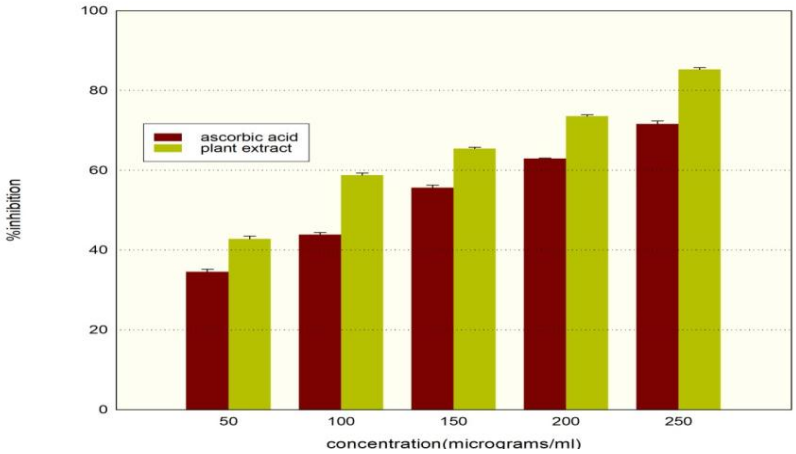

Fig. 4: Determination of %inhibition of H_2O by methanolic leaf extract and positive control ascorbic acid at various concentrations. Values are represented as Mean±SEM of three values and bars represent standard errors at 5%level.

3. TOTAL PHENOLIC CONTENT

The phenolic content of the extract is expressed in terms of gram Gallic acid equivalent obtained from the standard curve equation: y=0.0088x + 0.0328 R^2= 0.999. The values are expressed as mg GAE/g of extract. The values are based on the chemical reducing power which is in relation to an equivalent reducing the capacity of Gallic acid.(Mc Donald *et al.*, 2001).

Concentration of extract of the plant	Total phenolic content (mg GAE/g) of leaf extract	Total phenolic content (mg GAE/g) of root extract
50	56.89±0.13*	31.63±0.14*
100	75.67±0.14*	43.09±0.07*
150	86.02±0.10*	55.29±1.02*
200	104.07±0.17*	67.52±0.12*
250	111.03±0.15*	73.89±0.06

*Each value is the average of three analyses±SEM.

The values of tpc present in the leaf extract of the plant at various concentrations are obtained from the regression equation of standard used.

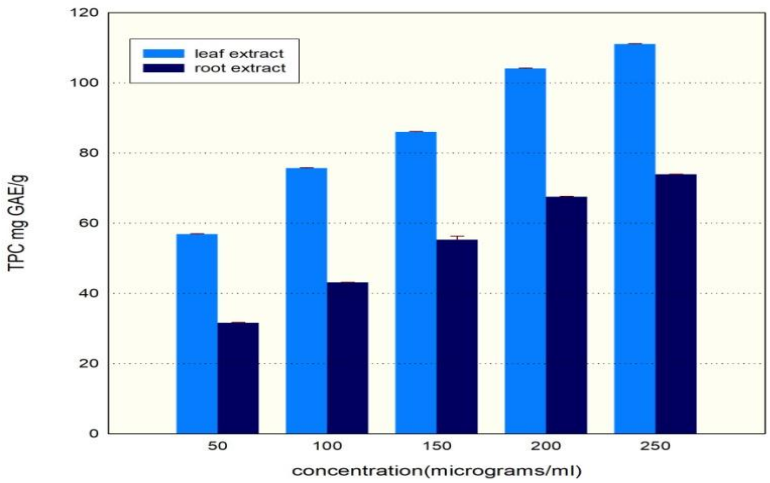

Fig. 5: Determination of total phenolic content of methanolic leaf and root extract of the plant represented as mg equivalent of gallic acid where each value is presented as mean±SEM (n=3) and the bars represent errors at 5% level.

4. TOTAL FLAVNOID CONTENT

Total flavonoid content is expressed as mg of quercetin/g of the extract and calculated using the standard curve equation: y=0.2439x – 5.1781 R^2 =0.9489. The total flavonoid content at different concentrations is calculated and given in table as well as represented in figure 4.

Concentration of extract of the plant(µg/ml)	Total flavonoid content (mg equivalent quercetin/g) of leaf extract	Total flavonoid content(mg equivalent quercetin/g) of root extract
50	08.34±0.10*	07.16±0.04*
100	17.79±0.04*	15.44±0.03*
150	20.78±0.11*	18.89±0.07*
200	34.78±0.05*	25.80±0.10*
250	35.89±0.60*	28.67±0.05*

*Values are expressed as mean±SEM of three replicates.

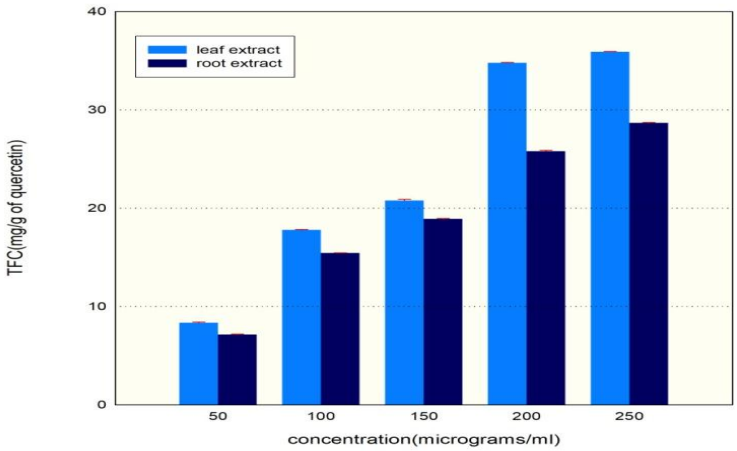

Fig. 6: Determination of flavonoid content of leaf and root methanolic extract represented as mg equivalent of quercetin. Here each value represents as mean±SEM (n=3) and bars represent standard errors at 5% level.

DISCUSSIONS

The experimental results reveal that the qualtitative analysis of the leaves show the presence of various phytochemicals in the aqueous, ethanolic and methanolic extracts. The tannins, steroids and terpenoids are absent only in aqueous extracts and all constituents are present in ethanolic and methanolic extracts. The quantitative analysis through HPLC reveal the presence of alkaloids in the methanolic leaf extract namely nicotine and nornicotine (Francois *et al* 1992) with retention time of 4372 and 3612 respectively as compared with the reference standard along with the peak area of 23794. The total yield of nicotine present is 5.622 µg/ml.

In-vitro antioxidant activity is assayed through DPPH radical scavenging activity and H_2O_2 radical activity which showed that the plant possess good anti-oxidant property with maximum % inhibition of 86.40% as compared to the reference standard of ascorbic acid in where the value came out be of 80.12% in case of DPPH radical scavenging activity. (Jain *et a*l 2016). DPPH termed as one of the powerful free radical has the ability of evaluating the electron donating capacity of the antioxidants. (Duan *et al*, 2006). In H_2O_2 radical activity, the maximum % inhibition is 88.13% as compared to reference standard ascorbic acid which showed maximum inhibition of 80.12% at the concentration of 250µg/ml. H_2O_2 inhibits oxidation of (SH) groups and can cross the cell membrane where in it can react with Fe^{2+} and Cu^{2+} ions forming hydroxyl radicals.(Miller *et al*; 1993).

Phenolics have essential role of scavenging free radical by the hydroxyl groups and screening of antioxidant activity can be conducted on this basis. (Bravo, 1998).With an increase in the total phenolic content, the antioxidant capacity of the extracts also increases. (Kurian *et al*., 2017). The TPC of leaf and root extracts was measured where the leaf extract exhibited the value of 111.03mg/g of phenolic content as compared to root extract showing 73.89mg/g of total phenolic content. Flavonoids also play essential role by suppressing the formation of reactive oxygen, scavenging of reactive species and can upgrade and protect the antioxidant defence system. (Agati *et al*, 2012). The TFC of the leaf and root extracts was measured where with increased concentration, the leaf extract showed the value of 35.89mg/g as compared to that of root extract which exhibited the value of 28.67mg/g.

CONCLUSIONS

The preliminary phytochemical analysis reveal that plant possess many secondary metabolites. The HPLC chromatogram showed the presence of alkaloids like nicotine and nornicotine which show many medicinal properties such as they are anti-bacterial, expectorant, vermifuge, anti-insecticidal, anti-inflammatory and many more.(Singh *et al* 2010). So this plant is a good source of medicine. The study of anti-oxidant activity and total phenolic and flavonoid content suggests that the plant is an important source of natural antioxidant, which can be useful as a protective agent against oxidative stress. So this plant serves as a good source of novel phytomedicine and further exploration of its parts can make it to play a beneficial role in pharmacognosy.

ACKNOWLEDGEMENT

The authors are thankful to UGC-BSR, Delhi for providing grant to carry the research work and to the Department of Botany, Panjab University, Chandigarh for providing necessary facilities to carry out the research work.

REFERENCES

1. Lambert J, Srivastava J and Vietmeyer N. Medicinal plants: An expanding role in development. World Bank technical paper., 1996; 320.
2. Sathyanarayana N, Sunitha P, Suresh C, Subramaniam S and Rathinam X. In vitro anti oxidant, HPLC profiling of polyphenolic compounds, AAs and FTIR spectrum of Malaysian origin Solanum torvum fruit. Indian J Pharm Edu, 2016; 50(2): s11-s20.

3. Chang CC, Yang MH, Wen HM and Chern JC. Estimation of total flavonoid content in Propolis by two complementary colorimetric methods. J Food Drug Anal, 2002; 10: 178-182.
4. Ruch RJ, Chang SJ. and Klaunig JE. Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis, 1989; 10: 1003-1008.
5. Harini R, Sindhu S, Sagadevan E and Arumugam P.2012 Characterization of in vitro antioxidant potential of Azadirachta indica and Abutilon indicum by different assay methods. J Pharm Res., 2012; 5: 3227-3231.
6. Siddiqui, AA and Ali M. Practical Pharmaceutical Chemistry. 1st ed., New Delhi; CBS Publishers and Distributors, 1997; 126-131.
7. Singh KP, Daboriya V, Kumar S, and Singh S. Antibacterial Activity and Phytochemcial Investigations On Nicotiana Plumbaginifolia Viv.(Wild Tobacco). Rom.J.Biol.- Plant Biol., 2010; 55(2): 135-142.
8. Dhar LM, Dhar MM, Dhawan BN, Ray C (1968). Screening of Indian plants for biological activity. Part I. Indian. J. Exp. Biol., 1968; 6: 232-247.
9. Software A. Medicinal plants and traditional medicines in Africa: Ibudan Nigerua; Spectrum book LTD, 1993; 289.
10. Chun OK, Kim DO and Lee CY. Superoxide radical scavenging activity of the major polyphenols in fresh plums. J Agric and Food Chem, 2003; 51: 8067-8072.
11. Singh A, Bhattacharya K, and Chandra G. Efficacy of Nicotiana plumbaginifolia (Solanaceae) leaf extracts as larvicide against malarial vector Anopheles stephensi liston 1901. Int J Pharm Bio Sci., 2015; 6(1): 860-868.
12. Jain N and Bhagia N. A Comparative study of total phenolic, total flavonoid and antioxidant assays of aqueous and DMSO extract from leaves of Origanum vulgare. World J Pharm Pharm Sci., 2016; 5(1): 726-737.
13. Franoise M, Marc Andre F and Jacquin-Dubreuil A. A high performance liquid chromatographic procedure for the analysis of tobacco alkaloids-Application to the evaluation of tobaaco alkaloids in plants and cell suspension culture. Phytochem Anal, 1992; 3: 65-68.
14. Duan XJ, Zhang WW, Li XM, Wang BG. Evaluation of antioxidants property of extract and fraction obtained from a red alga, Polysiphonia urceolata, Food Chem., 2006; 95: 37-43.

15. Miller MJ, Sadowska KH, Chotinaruemol S, Kakkis JL and Clark DA. Amelioration of chronic ileitis by nitric oxide synthase inhibition, J Pharm Ther., 1993; 264: 11-16.
16. Kurian A, Thiripuranathar G and Paranagama PA. Determination of total phenolic content and antioxidant activity of Borassus flabeliffer Linn. Fruit pulp collected from several parts of Sri Lanka. Int J Pharm Sci Res., 2017; 8(6): 2701-2705.
17. Bravo L, Polyphenols: chemistry, dietary sources, metabolism and nutritional significance. Nutr. Rev., 1998; 56: 317–333.
18. Agati, G., Azzarello, E., Pollastri, S., Tattini, M, Flavonoids as antioxidants in plants: location and functional significance. Plant Sci., 2012; 196: 67-76.
19. Mc Donald S, Prenzler PD, Antolovich M, Robards K. Phenolic content and antioxidant activity of olive extracts. Food Chem., 2001; 73: 74-84.