Vol 4 | Issue 2 | 2013 | 46-53.

e - ISSN 2249-7544
Print ISSN 2229-7464

INTERNATIONAL JOURNAL
OF
PHYTOPHARMACY RESEARCH
www.phytopharmacyresearch.com

QUALITATIVE ASSESSMENT OF PHYTOCHEMICALS AND ANTIOXIDANT POTENTIAL OF *SOLANUM NIGRUM* LINN. WITH THIN LAYER CHROMATOGRAPHY

Ashwani Kumar, Priya Chaudhary, RK, Deshwal, Varun Kumar, Somiya Sagwal

Department of Bioscience, Shri Ram College, Muzaffarnagar Uttar Pradesh-251002, India.

ABSTRACT

Solanum nigrum is also called 'Black Nightshade' this plant is used in the world for the treatment of various ailments. In Ayurveda *Solanum nigrum* is indicated for various disorders. For the pharmacological discovery of novel drug plants, the essential information's regarding the chemical constituents are generally provided by the qualitative phytochemical screening of plant extracts. While proteins, ascorbic acid and reducing sugar could not be detected in the extracts. Saponins were uniformly found in this case. In the present state of affairs, TLC profiling of all the plant extract in different solvent system indicated the presence of diverse type of phytochemicals in these plant. Different RF values of the compound also reflect an idea about their polarity. This information will help in selection of appropriate solvent system for further separation of compound from these plant extracts. DPPH radicals are widely used in evaluation of antioxidant activity. When DPPH radical is scavenged, the colour of the reaction mixture changes from purple to yellow with decreasing of absorbance at 517 nm. Results showed that, the scavenging activity against DPPH radicals of methanolic extracts of *Solanum nigrum* was found, while ascorbic acid was used as standard. All these data and concepts are in need to re-research on the present scientific tools. It can really contribute to medical and pharmaceutical practices. There are still many more activities waiting for screening the drug.

Keywords: *Solanum nigrum*, Phytochemical, Antioxidant, TLC.

INTRODUCTION

Nature has provided a complete store-house of remedies to cure all aliments of mankind [1]. This is where, nature provides us drugs in the form of herbs, plants and an alga's to cure the incurable diseases without any toxic effect [2]. There is a growing interest in herbal remedies because of their effectiveness, minimal side effects in clinical experience and relatively low cost. Herbal drugs or their extracts are prescribed widely, even when their biological active compounds are unknown [3]. Epilepsy (sometimes referred to as a seizure disorder) is a common chronic neurological condition that is characterized by recurrent unprovoked epileptic seizures. These seizures are transient signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain. It affects approximately 50 million people worldwide [4]. Inflammation is a disorder involving localized increases in the number of leukocytes and a variety of complex mediator molecules. Prostaglandins are ubiquitous substances that indicate and modulate cell and tissue responses involved in inflammation. Their biosynthesis has also been implicated in the pathophysiology of cardiovascular diseases, cancer, colonic adenomas and Alzheimer's disease [5]. Because of increasing side effects of available synthetic drugs for epilepsy and inflammation, there is need to focus on the scientific exploration of herbal drugs having fewer side effects. *Solanum nigrum* Linn. (Solanacea) is a thorny shrub widely distributed in Sikkim, Uttar Pradesh, Southern India and Sri Lanka in moist places. This plant is well known in English and Tamil system as 'Black night shade' and 'Kakamachi', respectively [6]. This research was aimed to investigating the possible anticonvulsant and anti-inflammatory activities of *Solanum nigrum* in order to support or refute the claims by traditional herbalists in India. *Solanum nigrum* (European Black Nightshade or locally just "black nightshade", Duscle, Garden Nightshade, Hound's Berry, Petty Morel, Wonder Berry, Small-fruited black nightshade or popolo) is a species in the *Solanum* genus, native to Eurasia and introduced in the Americas, Australasia and South Africa. Parts of this plant can be highly toxic to livestock and humans, and it's

Corresponding Author: **Dr. Ashwani Kumar Email:-** ashwani_biotech@yahoo.co.in

considered a weed. Nonetheless, ripe berries and cooked leaves are used as food in some locales; and plant parts are used as a traditional medicine. There is a tendency in literature to incorrectly refer to many of the black nightshade species as *'Solanum nigrum'* [7].

MATERIALS AND METHODS

To collect specimens of *Solanum nigrum* (SN) Black nightshade is an annual to short-lived perennial plant that has white or mauve flowers followed by berries that are first green, but change to black as they ripen. Mature plants can grow up to 75cm tall and plant collecting session is between April to June. Firstly plant is collected from the road side field. Now the sample shade dries than make powder with the help of the blender. 5g. of shade dried *Solanum nigrum* samples were ground at a high speed with blender and extracted in methanol with the soxhlet apparatus.

Prepration of methanolic extract with soxhlet appraturs

Weight 12 gm of powder (leaves) of *Solanum nigrum*. Took 120 ml methanol in round bottom distillation flask. Put the sample on whatman filter paper; make the thimble of filter paper placed in the Soxhlet assembly. Placed the assembly on heating mental at 60^0C. After 12 hrs, the extract was filtered through whatman no. 1 filter paper in a Buchner funnel. The solvent was evaporated in a rotary vaccume evaporator model then crude extracts were stored in amber glass vials in refregerator at 4^0C. Crude extracts were diluted with methanol for further investigation.

Preparation of water soluble plant extract

First of all take fresh healthy leaves of *Solanum nigrum* and dried shad then until they dried properly. After dried used blend then into the blender and from a thin powder. Now this powder is used for the experiments. Now plant extract is available in the from of powder. We take 25gm of powder and mix 25ml distilled water & kept it in beaker for boiling on the hot plate for 15 min also at 80-100^0C. After boiling we kept it for cooling. Now we weight the empty crucible after heating in oven to dry at 60^0C. Now we take filter paper for filtrated the boiled sample in crucible with the help of whatmann paper. After filtration the sample into the crucible we weight it & than kept it into the oven for totally evaporation at 60^0C. After evaporation the extract is remain which is used for the experiment.

Preliminary phytochemical screening

Phytochemical analysis of all the procedure of Indian Pharmacopoeia [8]. By this analysis the presence of several phytochemicals listed in **table: 2**. was tested for phytochemical analysis as follows:

Detection of Alkaloids

Dissolve 1.358 g of $Hgcl_2$ in 60 ml of water and pour into a solution of 5g of KI in 10 ml of H_2O, add distilled water to make the volume 100 ml. (White precipitate with most alkaloids in slightly acid solution). Dissolve 1g of picric acid in 100 ml of water. To one ml of the methanolic extract sample in a test tube was mixed with one ml of Hager's reagent/Wagner's reagent. The appearance of coloured precipitates indicated the presence of aikaloids [9].

Detection of Flavonoids

Dilute ammonia solution concentrated sulphuric acid. To 5ml of the dilute ammonia solution apportion of the aqueous extract was added, followed by addition of concentrated sulphuric acid. Appearance of yellow coloration indicated the presence of flavonoids [10].

Detection of Saponins

The extract was diluted with distilled H_2O and made up to 20 ml. The suspension was shaken in a graduated cylinder for 15 min. 2 cm layer of stable foam indicated the presence of saponins [11].

Detection of Terpenoids (Salkowski test)

Chloroform and concentrated H_2So_4 5 ml of aqueous extract was mixed with 2 ml of Chloroform and concentrated H_2So_4 to form a layer. A reddish brown coloration on the interface showed the presence of terpenoids [12].

Detection of Carbohydrates

Prepare reagent by dissolving o.5 g reagent grade naphthol in 10 ml of 95% ethanol. Store the reagent at room temperature. To one ml of the sample few drops of molish reagent were added. There after con. H_2So_4 was sided along the walls of the test tube. Appearance of purple ring at the interface indicated presence of carbohydrates[13].

Detection of Anthraquinones

25% ammonia solution was made with 75 ml of distilled water in 25 ml of dilute ammonia solution. 5 ml of the extract was dried and shaken with 3 ml petroleum ether. The filtrate was added to 2 ml of a 25% ammonia solution. The mixture was shaken. Presence of red coloration was taken as indication of the presence of anthraquinone[14].

Detection of Tannins

10% of lead acetate solution: Add 1 gm of lead acetate in 10 ml of distilled H_2O and mix properly. To 1 ml of sample in a test tube, 10% of lead acetate solution was added mixed well. The presence of yellow precipitates indicated tannins. All above experiments were performed in triplicates [15].

Thin Layer Chromatography (TLC)
Instrumentation and Experimental Procedures

The phytochemical analysis of this plant revealed the presence of flavonoids, saponins, alkaloids, carbohydrates & tannins etc. this extract was further subjected to TLC to confirm the presence of major group like alkaloids, flavonoids, saponins, etc. in the extract .Individual substances separated out based on RF value. The solvent evaporated dried extracts were redissolved in

methanol. TLC performed on Merck Silica Gel 60 glass plate using different eluents analyzed the fraction obtained. The chromatograms were observed in UV/VIS before and after processing with spraying agent. The flavonoids and phytochemicals were identified by comparison to co-chromatographed standards and available literature data [16]. 42 g of silica gel was dissolved in 25 ml chloroform, 25 ml methanol. Prepared the TLC plates by spreading the gel on it. The silica gel TLC plates were marked by using pencil. Placed the TLC plate in an oven at 50-60 for 15-20 min to "activate it". Activation involves driving of water molecules that bond to the polar sites on the plate. The narrow end of capillary was placed into the extract. When extract rises into the capillary then touch the capillary on the silica plate very carefully. Allowed the solvent to completely evaporate from the spot. The TLC plate was placed very carefully in the developing bottle containing mobile phase solvent system. Left it for some time so that solvent front can move. Placed the slide in an oven at Temperature 50-60^{0}C to evaporate the solvent.

Sample detail : *Solanum nigrum* leaves.
Adsorbent : Merck Silica Gel 60 on glass plate

Solvent system and detecting agents for Thin Layer Chromatography- Seven solvents systems were applied to achieve the banding profile of *Solanum* extract, five solvent systems for phytochemical identification and other two solvent systems used for antioxidant compounds. These are as follows

Solvent system- 1
Solvent system : Ethyl acetate: methanol: water (10:1.35:1)
Detection : Iodine vapour gave pinkish red spot
Solvent front run upto : 10 cm

Solvent system- 2
Solvent system : Chloroform: Ethyl acetate: Formic acid (10:8:2)
Solvent run : 10 cm
Detection : Iodine vapour gave pinkish red spot

Solvent system- 3
Solvent system : Benzene: Ethanol: Amonia (18:2:0.2)
Solvent front run upto: 10 cm
Detection : Iodine vapour gave pinkish red spot

Solvent system- 4
Solvent system : Toluene: Ethyl acetate(5:7)
Solvent run : 10 cm
Detection : Iodine vapour gave pinkish red spot

Solvent system- 5
Solvent system : Choroform: Ethanol (8:2)
Solvent run : 10cm
Detection : Iodine vapour gave pinkish red spot

Solvent System- 6
Solvent system : Toluene: Ethyl acetate (5:7)
Solvent run : 10cm
Detection : Spraying with DPPH

Solvent System- 7
Solvent system : Chloroform: Ethanol (8:2)
Solvent run : 10cm
Detection : DPPH

TLC Analysis of the Fractions

For each extract, seven different solvent systems were used as developing systems. These were Ethyl acetate: methanol: water (10:1.35:1), Chloroform: Ethyl acetate: Formic acid (10:8:2), Benzene: Ethanol: Ammonia (18:2:0.2), Toluene: Ethyl acetate (5:7), Choroform: Ethanol (8:2). In first five cases, the spots were visualised by exposure of the plates to iodine vapour and last two Chloroform: Ethanol (8:2), Toluene: Ethyl acetate (5:7) plates spots were detected with spraying of methanolic solution of DPPH.

α, α – diphenyl – β – Pieryl- hydrazyl (DPPH) radical Scavening assay:

According to the adopted method, 4mg of DPPH was dissolved in methanol (50ml) to obtained a dilution of 80µg/ml [17].. Serial dilution were made with stock solution (10mg/ml)of the plant extracts to obtain concentration of 1-500mg/ml. Diluted solutions (2ml each) were mixed with DPPH (3ml) and allowed to stand for 30min for any reaction to occur. The UV absorbance was recorded at 517nm. The experiment was performed in duplicate and the average absorption was noted for each concentration [18].

Stock Solution prepared for DPPH radical scavenging was 1 mg/ml. dilution Prepared from Stock Solution for DPPH radical scavenging assay has been provide in table.

Stock Solution(ml)	Methanol added(ml)	Dilution(mg/ml)
1	9	0.1
2	8	0.2
3	7	0.3
4	6	0.4
5	5	0.5

Radical Scavenging Capacity was determined in each reaction, the sample solution were mixed with 3 ml of 0.25 mM DPPH. The mixture was shaken vigorously and allowed to reach. A Steady state at room temperature for 30 min, Determined by measuring the absorbance at 517 nm with spectrophotometer [19].The DPPH radical scavenging activity was calculated according to the following equation. % scavenging activity = $A_0 - A_1$. Where A0 is the absorbance of the control blank, without extract. A1 is the absorbance in the presence of the extract or standard sample.

RESULT & DISCUSSION

Results observed after performing various experiments were extreme good and indicated that *Solanum nigrum* has extreme scope as medicinal as well as antiaging components. The spectrophotometric analysis of *Solanum nigrum* crude extract represent various λ max, which indicates that *Solanum nigrum* extract have a pool of phytochemicals, which may have different type of

medicinal activities. Phytochemical characteristics verified with various test results given below. The preliminary phytochemical analysis indicates that Alkaloids**,** flavonoids, saponins, tannins, carbohydrates were present. But terpenoids and anthraquinones are not present, and show these secondary metabolites localized in leaf, collected in the plant.

Water extract- Initially water based extraction was done, 5 gm *Solanum nigrum* leave powder was extracted with 100ml of distilled water the boiled on hotplate for 30 min. then filtered with filter paper. Filtrate was transferred in crucible and evaporated and final weight was calculated-
Weight of empty crucible = 52.61
Weight of crucible with extract = 52.89
Weight of water soluble extract =52.89 – 52.61 = 0.28%

Methanolic Extract: Initially methanol based extract was done powder 11.24 gm of *Solanum nigrum* leaves powder extract with 300ml methanol with the help of soxhlet apparatus than extract is filtered with filter paper. Filtrate was transferred in crucible and evaporated and final weight was calculated-
Methanol = 200ml, Powder = 11.24gm
Weight of dry empty crucible = 113.24
Crucible with sample evaporated = 115.80, Total = 115.8-113.24= 2.56%.

Firstly two types of extracts were made, which was observed and found that 2.56% methanolic extract was sticky in nature and dark black in colour, aqueous extract characterstics was same as methanolic in nature while the yield was 0.28% in aqueous phase of *Solanum nigrum.*

TLC Profile of *Solanum nigrum* extracts in different solvent system is used on the slides than spry bands are developed on the slides. Solvent system used Choroform: Ethyl Acetate: formic acid (10:8:2), Benzene: Ethanol: Ammonia (18:2:0.2) Spraying with Methanolic DPPH.

Anti-oxidant compound were identified by Direct Bioautographic analysis. The methanolic extracts of *Solanum nigrum* were dissolved in respective solvent and chromatographed on precoated silica gel plates. The samples were loaded on plates as bands. The plates were developed in selected solvent systems. The plates were dried in air flow for 3hrs then sprayed with 0.008% solution of DPPH in methanol using TLC sprayer. Plates were placed in dark for 20min. for any reaction to be happened. Anti-oxidant compounds were identified as white spots on dark background. The RF value of these spots was calculated. The separation of antioxidant in the extract of *Solanum nigrum* in solvent system of Choroform: Ethanol (8:2) maximum 2 spots having RF value as 0.89 and 0.85, While Solvent System; Toluene: EA (5:7) have minimum spots RF value is 0.25 these chromatograms indicates the presence of antioxidant components in *Solanum nigrum.*

In this process plant extract of *Solanum nigrum* in concentration (µg/ml) in dilution of sample, Standarded (Sn), *Solanum nigrum* Methanolic extract (SNME), of % inhibition of scavenging effect of DPPH assay.

Anti-oxidant effect for *Solanum nigrum* Methanolic extract (SNME) was evaluated on the basis of its ability to inhibit free radical (DPPH. Reduction in absorbance by different concentration of test sample and ascorbic acid was recorded, result are compiled in (Table 4) Fig 8. The IC50 value of standard ascorbic acid was 2.04mg/ml. In *Solanum nigrum* the IC50 value was obtained 6.89mg/ml in DPPH assay.

Table 1. Nature and Percentage yield of extracts of *Solanum nigrum*

Sr. no.	Name of the extract	Nature	Colour	% Yield (w/w)
1	Methanolic	Shade	Green	2.56
2	Water	Shade	Green	0.28

Details of the Qualitative Phytochemicals Tests

Table 2. Phytochemical consitutentes of *Solanum nigrum* Alkaloids and Flavonoids are as fallows:

S.No.	Phytochemical Name	Reach or reagentes test	Observation	Test Result
1	**Alkaloids**	Mayer's reagent Hagar's reagent	Colour Precipitates	++
2	**Flavonoids**	Dilute ammonia solution, concentrated sulphuric acid	Yellow colour	+++

Table 3. Phytochemical consitutentes of *solanum nigrum* Saponins and Terpenoids are as fallows:

S.No.	Phytochemical Name	Reach or reagentes test	Observation	Test Result
1	**Saponins**	Vigrous shaking of plant extract	2 cm layer of stable foam indicated the presence of saponins	+++
2	**Terpenoids**	Chloroform and concentrated H_2So_4	**No Colour**	–

Table 4. Phytochemical consitutentes of *solanum nigrum* Carbohydrate are as fallows:

S.No	Phytochemical Name	Reach or reagentes test	Observation	Test Result
1	**Carbohydrate**	Molish reagent	Appearance of purple ring at the interface indicated presence of carbohydrates.	+++

Table 5. Phytochemical consitutentes of *solanum nigrum* Tannin are as fallows:

S.No.	Phytochemical Name	Reach or reagentes test	Observation	Test Result
1	**Tannin**	10% of lead acetate solution: Add 1 gm of lead acetate in 10 ml of distilled H2O and mix properly.	The presence of yellow precipitates indicated tannins.	+++

(Abesent = - , Persent = +), (- Low Concentration), (++ Medium Concentration), (+++ High Concentration)

Table 6. Thin layer chromatography of *Solanum nigrum* extracts showing experimental conditions and RF values of sample constituents

S.No.	*Solanum nigrum*	Solvent system	Identification reagents/Detection	Rf values
1	Alcoholic water	Ethyl acetate: methanol:water (10:1.35:1)	Iodine vapours	0.89 0.38
2	Alcoholic water	Chloroform: Ethyl acetate:Formic acid(10:8:2)	Iodine vapours	0.85 0.53
3	Alcoholic water	Benzene: Ethanol:Ammonia (18:2:0.2)	Iodine vapours	0.53 0.32
4	Alcoholic water	Toluene: Ethyl acetate(5:7)	Iodine vapours	0.79 0.32
5	Alcoholic water	Chloroform:Ethanol(8:2)	Iodine vapours	0.35 0. 75

Table 7. TLC analysis for antioxidant compounds in *Solanum nigrum*

1	Methanolic Water	Toluene:Ethyl acetate(5:7)	DPPH	0.55 0.25
2	Methanolic Water	Chloroform: Ethanol(8:2)	DPPH	0.75 0.35

Table 8. % inhibition of scavenging effect of DPPH assay of *Solanum nigrum*

S.No.	Dilution of Sample Concentration(µg/ml)	Standard (Sn)	*S. nigrum* Methanolic extract (SNME)
1	0	0	0
2	100	42.20	29.64
3	200	51.17	34.26
4	300	60.87	41.22
5	400	69.92	49.83
6	500	80.58	63.05

Figure 1. A digramatic repersentation of whole plant, flowers and fruits of *Solanum nigrum.*

Qualitative Phytochemials Analysis of *Solanum nigrum*

Figure 2. Alkaloids Test of *Solanum nigrum*; green colour precipitates were present after reaction. While in Flavanoids colour changed from light yellow to dark yellow after reaction.

Figure 3. Saponins Test of *Solanum nigrum*. After vigorous shaking a thick layer of foames was present with high amount of saponins.

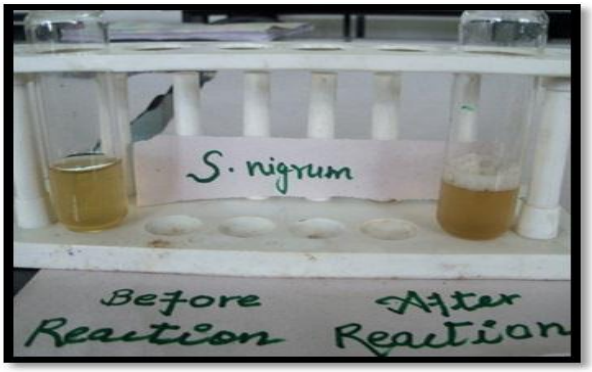


Figure 4. Carbohydrate Test of *Solanum nigrum* colour has been changed after reaction, Appearance of purple ring at the interface indicated presence of carbohydrates.

Figure 5. TLC fingerprints of *Solanum nigrum* with different solvent systems in A,B,C,D & E

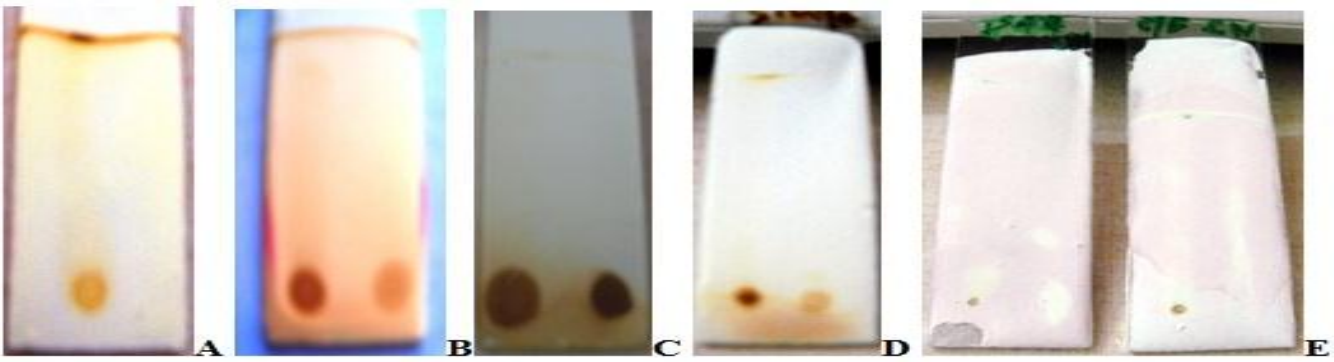


Figure 6. TLC Comparison of *Solanum nigrum* with other plant extracts; PE-EA (9:1) process used iodine vapour spry on the slides than bands present on the slides).

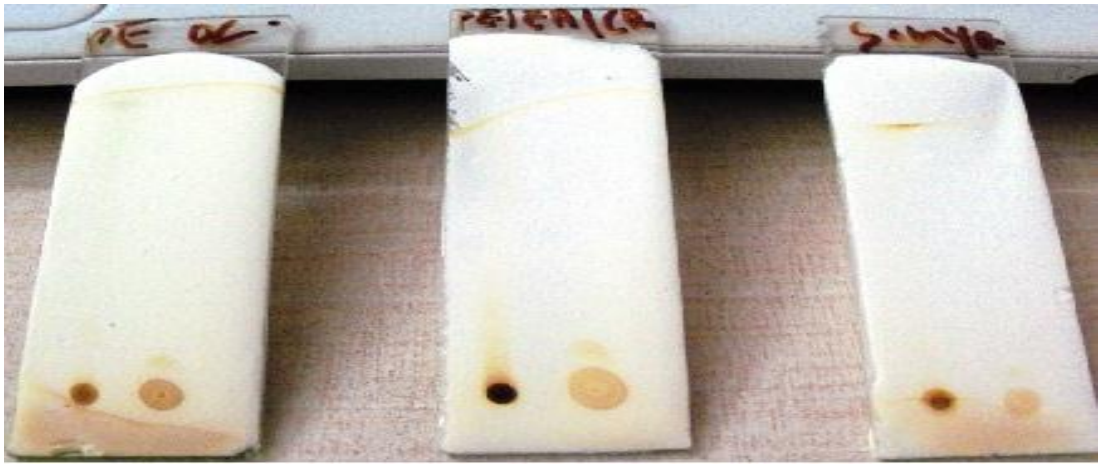


Figure 7. TLC based Chromatograms of *Solanum nigrum* having antioxidants with DPPH

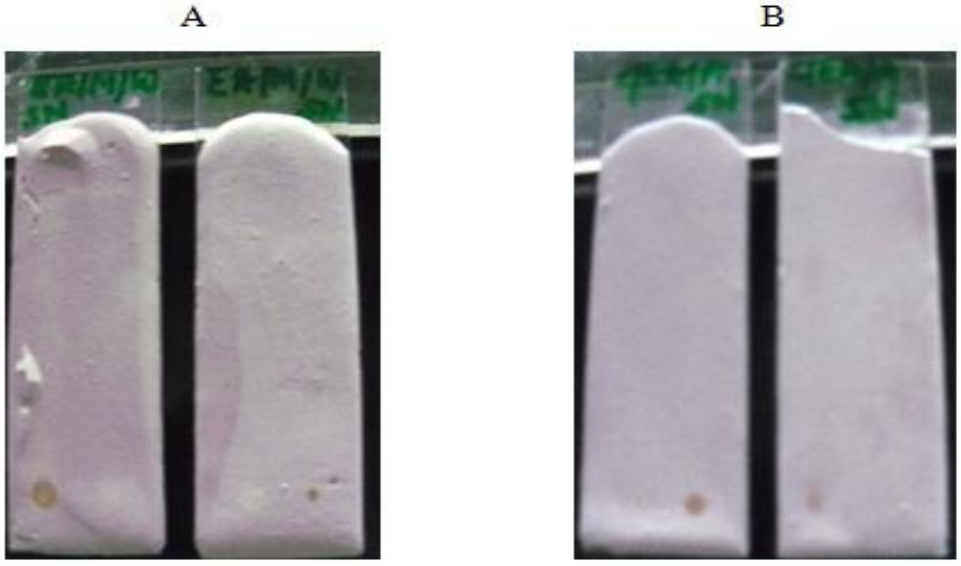
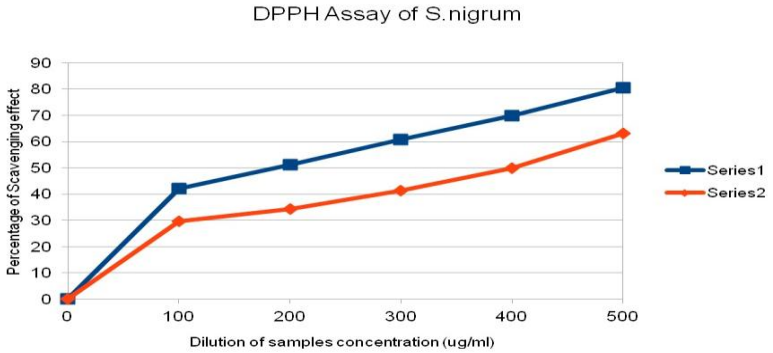


Figure 8. DPPH Radical Scavenging Antioxidant Assay Of *Solanum nigrum*; colour change due to increased concentration of Methanolic extracts (Red to Yellow)

Figure 9. Concentration and % inhibition of Scavenging effect of DPPH assay.



CONCLUSION

Hence there is a great scope to identify suitable techniques to exploit *Solanum nigrum* for different purpose. While scientists are advocating a change in food use behaviour, this can be materialized only when an interrogated, sustainable approach is a matter of policy and implemented accordingly. This is extremely disquieting that *Solanum nigrum* is still considered as weed and people continue to be in the grip of perpetual backwardness despite plenty of research findings due to lack of awareness. However, the government agency is not unaware of the scenario that the scientific investigations has brought into focus in this species, but it has chosen to remain silent for long and the species is still recognised as weed.

However, considering all the results, it can be concluded *Solanum nigrum* possess antioxidant activity, among the reactive oxygen species, the hydroxyl radical is the most reactive and induces severe damage to adjacent biomolecules and *Solanum nigrum* is a good scavenger of superoxide radical and DPPH radical. *Solanum nigrum* also has total good amount of flavonoid content. This property of *Solanum nigrum* could possibly be related to its higher flavonoid content. The present study concluded the presence of antioxidants in the *Solanum nigrum* provides useful information of *Solanum nigrum* on pharmacological activities and potential applications of such compounds as natural antioxidants in different food/pharmaceutical products. Further studies are being carried out on the other species of *solanum* of different habitats in order to provide complete data of the antioxidant activity and characterization of the principal antioxidant agents, which can be used to treat various oxidative stress-related diseases in plants.

REFRENCES

1. Kokate CK, Purohit AP, Gokhale SB. Textbook of pharmacognosy, Nirali prakasan Pune. 18, 2002, 1-4.
2. Trease GE, Evans MC. Text book of Pharmacognosy. 12th edition. Balliere, Tindall London, 1983, 343-383.
3. Gupta RK, Kesari AN, Murthy PS, Chandra R, Tandon V, Watal G. Hypoglycemic and antidiabetic effect of ethanolic extract of leaves of *Annona squamosa L.* in experimental animals. *J. Ethnopharmacol*, 99(1), 2005, 75-81.
4. Fisher R, Van Emde Boas W, Blume W, Elger C, Genton P, Lee P, Engel J. Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). *Epilepsia*, 46, 2005, 470-472.
5. Gupta M, Mazumder UK, Gomathi P, Thamilselvan V. Antiinflammatory evaluation of leaves of *Plumeria acuminate. BMC Complementary and alternative medicine*, 6, 2006, 1472- 6882.
6. Nadkarni KM, Indian Materia Medica. Bombay, Popular Prakasan Vol. 1,3rd ed, 1976, 1153-1154.
7. Mohy-ud-dint A, Khan Z, Ahmad M, Kashmiri M.A. Chemotaxonomic value of alkaloids in *Solanum nigrum* complex. *Pakistan Journal of Botany*, 42(1), 2010, 653-660.
8. Indian Pharmacopoeia - Third edition, followed by its addenda in 1989, 1991and 1985.
9. Kokate CK. Plant constituents in Practical Pharmacognosy.2001, 107- 110.
10. Mace,Gorbach SL, Anaerobic bacteriology for clinical laboraties. *pharmacognosy*, 23, 1963, 89-91.
11. Rajasekariah GR, Parab PB, Chandrashekar R, Deshpande L, Subrahmanyam D. Pattern of Wuchereria bancrofti microfilaraemia in young and adolescent school children in Bassein, India, an endemic area for Lymphatic filariasis. *Ann. Trop. Med. Parastiol*, 85(6), 1991, 663-665.
12. Evans WC. An index of medicinal plant. A Text book of Pharmacognosy, 14, 1997, 7(5), 12-14.
13. Mabry TJ, Markham KR, Thomas MB. The systematic identification of flavonoids. *Springer – Verlag, Berlin-Heiderbelrg New York*, 1970, 1-102.
14. Takao T, Kitatani F, Watanabe N, Yagi A, Sakata KA. Simple screening method for antioxidant and isolation of several antioxidant produced by marine bacteria from fish and shell-fish. *Biosci. Biotechnol. Biochem*, 58, 1994, 1780-1783.
15. Békro JAM, Konan KM, Djié Bi MG. Phytocompounds of the Extracts of Four Medicinal Plants of Côte D'ivoire and Assessment of their Potential Antioxidantly Thin Layer Chromatography. *European Journal of Scientific Research*, 24(2), 2008, 219-228.
16. Esmaeili MA, Sonboli A, Noushabadi MA. Antioxidant and protective properties of six Tanacetum species against hydrogen peroxide induced oxidative stress in K562 cell line. A comparative study. *Food chem.* 121, 2010, 148-155.