ORIGINAL ARTICLE

# Qualitative analysis of phytochemicals, and comparative superoxide radical scavenging along with reducing potency of *Solanum nigrum* using various solvent extracts

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An attempt has been made to screen the phytochemicals, comparative superoxide radical scavenging and reducing potency of *Solanum nigrum* using various solvent extracts. The herbal powder obtained from plant part-dry leaves were extracted with various solvents. The extracts were analysed for phytochemicals and antioxidants-carotenoids, ascorbic acid, tocopherol, total phenol, proteins, reducing sugars and sterols. Free radical scavenging capacity was analysed in terms of superoxide radial scavenging assay and reducing power assay. Phytochemical characterization of the different extracts revealed the presence of the phytochemicals-alkaloids, phenols, flavonoids, sterol, saponin glycosides, reducing sugars, proteins, cardio active aglycones and cardinolides. Excellent Superoxide Radical scavenging ability found in almost all extracts of *S. nigrum*. In the present study superoxide radical reduces nitro blue tetrazolium (NBT) to a blue coloured formazan that is measured at 560 nm. Antioxidant activity has been reported to be concomitant with development of reducing power. This shows that extracts might contain reductones like ascorbic acid, reducing sugar, thiol group containing protein which could react with free radicals to stabilize and terminate radical chain reaction. These findings suggest that the promising phytonutrients of the plant could be exploited against oxidative stress, cancer, ageing, Ischemic heart disease in dissolving thrombus, microbial infections and hormone replacement therapy (HRT) justifying their use in traditional medicine as nutraceuticals.

**Key words:** Antimutagenic, phytosterols, poly phenolS, saponins, *Solanum nigrum*

## INTRODUCTION

The use of medicinal plants as source of the remedies for the treatment of many diseases dated back to prehistory and people of all continents have this old tradition.[1] In developing countries where medicines are quite expensive, it is obvious that these medicinal plants will find their way in the arsenal of antimicrobial drugs.[2] Reactive oxygen and nitrogen species produced via cellular metabolism and from exposure to environmental pro-oxidants appear to contribute to the pathogenesis of chronic disease via free-radical damage to lipids, nucleic acids, and proteins.[3] Many polyphenols have been shown to modulate phase I and II detoxification pathways. These mechanisms implicated in their putative chemo preventive actions.[4] Antioxidant activity of different plant polyphenols against hydroxyl ($OH^{\bullet -}$), peroxyl ($ROO^{\bullet -}$), and superoxide ($O_2^{\bullet -}$) radicals demonstrated using *in vitro* radical scavenging assays. This may be relevant to health because some radical specificity appears in the pathogenesis of different diseases, e.g., $O_2$ in neuro degenerative conditions, peroxy nitrite (ONOO–) in cardiovascular disease, and reactive halide species, such as hypochlorite (HOCl), in rheumatoid arthritis. Superoxide anion radicals ($O_2^{\bullet -}$) are also formed by activated phagocytes such as monocytes, macrophages, eosinophils and neutrophils and the production of superoxide is an important factor in the killing of bacteria by phagocytes. In living organisms, $O_2^-$ is removed by the enzymes called superoxide dismutases.[5,6] Almost all organisms are well protected against free radical damage by enzymes such as superoxide dismutases and catalane or antioxidant compounds such as ascorbic acid, α- tocopherol, and glutathione.[7] When the mechanism of antioxidant protection becomes unbalanced by exogenous factors such as tobacco smoke, ionizing radiation, certain pollutants, organic solvents, and pesticides and endogenous factors such as normal aerobic respiration, stimulated polymorph nuclear leukocytes and macrophages, and peroxisomes, the

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result may be the diseases and accelerated ageing. However, antioxidant supplements or foods containing antioxidants may be used to help the human body to reduce oxidative damage.[8-10]

In the last few decades, several epidemiological studies have shown that a dietary intake of foods rich in natural antioxidants correlates with reduced risk of coronary heart disease particularly a negative association between consumption of polyphenols-rich foods and cardiovascular diseases has been demonstrated. This association has been partially explained on the basis of the fact that polyphenols interrupt lipid per oxidation induced by reactive oxygen species (ROS).[11,12]

Phytochemical evaluation plays an important role in the standardization of crude herbal drugs.[13] Limited data from animal studies suggest that very high intakes of phytosterols, particularly sitosterol, may inhibit the growth of breast[14] and prostate cancer.[15,16] Indole alkaloids exhibit numerous biological activities such as antitumor, antimicrobial, antihypertensive and central nervous system stimulant.[17] A large body of scientific evidence associating dietary phytochemicals with health and well-being of population has stimulated tremendous activities to develop and commercialize products variously known as nutraceuticals, phytoceuticals, dietary supplements, functional foods, etc.[18] During 1980s and 1990s, numerous laboratories began studying phytochemicals to "-mine-" plants for bioactive substances that might be used as medicines (nutraceuticals) or for other chemical applications. Many compounds are showing great promise as disease fighters in the body, boosting production or activities of enzymes, which then act by blocking carcinogens, suppressing malignant cells, or interfering with the processes that can cause heart disease and stroke.[19]

*Solanum nigrum* Linn. (- Family Solanaceae) is commonly used in the traditional medicine as a remedy for treating various diseases. *S. nigrum* is a glabrous or sparingly pubescent annual herb found in common waste places, road sides and along railway tracks throughout India. All parts of the plant are alterative, diuretic and laxative. Fresh extract of the plant is employed in dropsy, gonorrhea, haemoptysis, piles, hepatomegaly, splenomegaly, etc.[20] The berries possesses various medicinal properties such as sedative, diaphoretic, diuretic, hydragogue, expectorant and are useful in the disease of liver, heart and eyes and is also effective against piles, fever and dysentery.[21] The leaves are used to heal open wounds and are known to possess hypotensive effect.[22] The berries has been used in the treatment of stomach ulcers in the folk medicine in South Africa, European, China and throughout India.[23] The fruits of *S. nigrum* have been reported to play an adjuvant role in the hepato protective property. Inhibition of lipid peroxidation and free radical scavenging activity has been suggested as a possible mechanism of action.[24] Previous studies have demonstrated that aerial parts of *S. nigrum* have the ability to decrease the secretion of gastric acid, pepsin level and stimulate mucus secretion.[25] With this background, the present study was designed to screen for the presence of phytochemicals and free radical scavengers.

## MATERIALS AND METHODS

### Plant Materials

The leaves of *Solanum nigrum* were collected in Chennai, Tamil Nadu, and India. The identification and nomenclature of the plant was based on The Flora of Presidency of Madras[26] and The Flora of Tamil Nadu Carnatic.[27] They were later verified at Botanical Survey of India, Southern Circle, Coimbatore, India. All the preserved specimens were deposited at the Herbarium of Entomology Research Institute, Loyola College, Chennai.

### Chemicals

All the solvents and chemicals were purchased from Sigma Laboratories, Bangalore, India.

### Preparation of Extracts for Phytochemical Analysis

The plant material (leaves) was air dried in the laboratory at room temperature. It was then powdered and was extracted with hot water by boiling for 30 minutes to get the aqueous extract. The extract obtained was concentrated and dried under controlled temperature (60°C). The dried powder was successively extracted with other solvents such as methanol, ethanol, and chloroform and kept in an orbital shaker for overnight. The obtained extracts filtered with Whatman No. 42 filter paper (125 mm) and the filtrate was collected and used for experimental analysis.

### Phytochemical Analysis

These studies were performed according to the standard methods.[28]

#### *Test for alkaloids*

a. Dragendroffs test (Kraut reagent-potassium bismuth iodide): To 0.5 ml of alcoholic or aqueous extracts of *S. nigrum* we added 2 ml of HCl. To this 1 ml of the Dragendroffs reagent was added. An orange, red precipitate produced immediately indicates the presence of alkaloids
b. Test for Indole alkaloids – Ehrlich test: Different extracts of the plant *S. nigrum* was treated with Ehrlich reagent. Red or purple colour indicates the presence of indole alkaloids
c. Test for Lepac alkaloids: Different extracts of the plant was treated with 5 ml of dilute hydrochloric acid,

filtered and to the filtrate 60 mg of potassium chlorate was added. Filtrate becomes yellow on standing and then changes to red will indicate presence of lepac alkaloids.

*Test for glycosides- Cardio active aglycones*

a. Baljet test: To the extract 1 ml of sodium picrate was added. Formation of yellow to orange colour indicates the presence of Cardinolides
b. Legal test: To the plant extract, pyridine and sodium nitroprusside was added. Formation of red colour indicates the presence of cardio active aglycones.

*Test for saponin glycosides*

a. Froth test: To 5 ml of the extracts, a drop of sodium bicarbonate was added. The mixture was shaken vigorously and kept for 3 min. A honey comb like froth was formed, which shows the presence of saponins[29]
b. Blood haemolysis test: Placed a drop of blood over a slide, a drop of plant extract was added and visualized through the micro scope. Haemolysis in the blood cells showed the presence of saponin glycosides
c. A suspension of RBC in normal saline was treated with a small amount of the extract and haemolysis was observed due to saponins
d. Small quantity of the plant extract was taken along with water, in a test tube and shaken well along with water. Formation of froth indicates the presence of saponin glycosides.

*Test for flavonoids*

a. Flavonoids are phenolic and hence a change in colour was observed when treated with ammonia. Yellow colour formation was due to the presence of flavonones
b. To 0.5 ml of ethanol extract, 5-10 drops of diluted HCl and small piece of Zinc or Magnesium were added and the solution was boiled for a few minutes. Dirty brown colour was produced by flavonoids.[30]

*Test for phenol*

a. Ferric Chloride test: To 1 ml of alcoholic or aqueous extract, 2 ml of distilled water and a few drops of 10% aqueous $FeCl_3$ were added. Formation of blue or green colour showed the presence of phenols.
b. Lead Acetate test: A total of 1 ml of alcoholic or aqueous extract was diluted to 5 ml with distilled water; a few drops of 1% aqueous solution of lead acetate were added. A yellow precipitate was formed to indicate the presence of phenols.
c. Liebermann's test: Small quantity of alcoholic or aqueous extract dissolved in 0.5 ml of 20% $H_2SO_4$, a few drops of sodium nitrate was added. Blue colour obtained on dilution and it turned red when made alkaline with aqueous NaOH solution.

*2.4.6 Test for steroids*

a. Liebermann Burchard test: To 1 ml of petroleum ether extract in chloroform, 1 ml of concentrated $H_2SO_4$ was added followed by the addition of 2 ml of acetic anhydride solution. A Greenish colour developed and turned blue, which indicate the presence of steroids.
b. Salkowski reaction: To 2 ml of chloroform extract, 1 ml of concentrated $H_2SO_4$ along the side was added carefully. A red colour was produced in the chloroform layer.
c. Lieberman Sterol reaction: To the extract in glacial acetic acid, one drop of concentrated $H_2SO_4$ was added. A play of colour was observed starting with rose, red, violet, blue to green. This showed the steroid part of the molecule.

*Test for proteins*

a. Biuret test: To 1 ml of aqueous extract, 5-8 drops of 10% NaOH and two drops of 3% $CuSO_4$ were added. Red or violet colour showed the presence of proteins.
b. Millon's test: To the extract Millon's reagent was added. Precipitate turned red on heating showed the presence of protein.

*Test for reducing carbohydrates*

a. Molisch test: To 2 ml of aqueous extract, 2 drops of fresh 20% alcoholic alpha naphthol was added. Then, 2 ml of concentrated $H_2SO_4$ was added along the sides. Red violet ring at the junction showed the presence of carbohydrates.
b. Fehling's test: To 2 ml of extract, 1 ml of Fehlings A and B solutions were added and boiled. Red or brick red colour showed the presence of reducing sugar.
c. Benedict's test: To 0.5 ml of aqueous extract 5 ml of Benedict's reagent was added and boiled for 5 minutes. Bluish green colour showed the presence of reducing sugar.

*Tests for Free Radical Scavenging Assay*

The antioxidant activity was evaluated using organic and aqueous extracts for nonenzymatic antioxidants-Vitamin C, Vitamin A, Vitamin E, phenol, protein with SH group, reducing carbohydrate and sterol. Reducing power assay and super oxide radical scavenging assay was also performed with the above extracts.

Estimation of vitamin A was done by the method of Carr and Price,[31] vitamin C by the method of *Omaye*,[32] vitamin E by the method of *Rosenberg*.[33] The reducing power of the extract was determined according to the method of *Oyaizu*.[34] The superoxide radical scavenging ability was assessed by the method of *Nishimiki*.[35] Total phenol was determined using *Lowry's* method.[36] Sterol was estimated by *Liebermann Burchard* method.[37]

### Statistical Analysis

Experimental results are expressed as means±SD. All measurements were replicated ten times. The data were annalszed by an analysis of variance i.e. one-way ANOVA and student 't' test using Graph Pad Quick Calcs.

## RESULTS AND DISCUSSION

The results of the phytochemical analysis were reported in Table 1.

### Vitamin Profile

Table 2 reveals that vitamin -A (β.Carotene) content in chloroform extract has very high vitamin-A than aqueous and ethanolic extract. Vitamin-A as a powerful, free radical scavenger (singlet oxygen) and chain-breaking antioxidant. The function of vitamin-A as radical scavenging antioxidants can protect the cells from oxidative damage. Several clinical trials showed regression in precancerous lesions of the cervix and the lung as well as the oral cavity with the administration of β-carotene.[38] In this study it was found that vitamin-C (ascorbic acid) content is in the following order:

Aqueous extract>Ethanolic extract>Chloroform extract.

Vitamin -C is an excellent hydrophilic antioxidant: It readily scavenges ROS and peroxyl radical and also act as a co-antioxidant by regenerating vitamin-A, E and GSH from radicals.[39,40] Table 2 shows that the vitamin-E content is high in chloroform extract. Vitamin-E is a fat soluble one, which can be extracted effectively by chloroform. *S. nigrum* has very high vitamin-C, along with Vitamin-E content. Vitamin-C and E are synergistic antioxidants. Regeneration of vitamin-E requires ascorbic acid, an aqueous phase antioxidant.

### Poly Phenol Profile

Table 2 reveals that high content of phenolic compounds was found in aqueous extract indicating that phenolic compounds in *S. nigrum* were mainly soluble in water. Phenolic compounds are likely to contribute to the radical scavenging activity of extract. Polyphenols are a large class of compounds, occur naturally in food plants. The flavonoids are the largest and best studied group of these. Polyphenols, currently sold as dietary supplements and/or herbal remedies have antioxidant, antimutagenic, antiestrogenic, anticarcinogenic and antiinflammatory effects that might potentially be beneficial in preventing disease. Epidemiologic findings revealed that polyphenols have cardioprotective effects, which include inhibition of platelet aggregation and vascular relaxation through the production of nitric oxide which decrease LDL oxidation and prevent atherosclerosis.[41,42]

**Table 1: Qualitative analysis of the phytochemicals of aqueous and organic extracts of *Solanum nigrum***

| Phytochemicals analyzed | *Solanum nigrum* | | | |
|---|---|---|---|---|
| | Aqueous | Ethanol | Methanol | Chloroform |
| Alkaloids | +++ | ++ | ++ | =/= |
| Indole alkaloids | +++ | +++ | +++ | =/= |
| Lepac alkaloids | ++ | + | ++ | =/= |
| Cardio active aglycons | ++ | ++ | ++ | =/= |
| Saponin glycosides | ++++ | +++ | +++ | =/= |
| Flavonoids | ++ | + | + | =/= |
| Phenols | ++++ | +++ | +++ | =/= |
| Sterol | +++ | ++ | ++ | +++ |
| Proteins | +++ | =/= | =/= | =/= |
| Carbohydrates | +++ | =/= | =/= | =/= |

+Presence of phytochemicals; ++Definite presence; +++Definite heavy presence; ++++Definite heaviest presence; =/=Not done

**Table 2: Level of different antioxidants in aqueous, ethanol and chloroform extracts of *Solanum nigrum* per 100 g**

| Antioxidant | Ethanol leaf | Aqueous leaf | Chloroform leaf |
|---|---|---|---|
| Vitamin A (mg) | 20.16±0.1673 | 25.28±0.2683 | 65.24±1.135 |
| Vitamin C (mg) | 140.6±1.342 | 180.6±1.342 | 103±6.708 |
| Vitamin E (mg) | 20.8±0.1225 | 23.784±0.1307 | 26.88±0.08367 |
| Polyphenol (mg) | 600.6±2.608 | 776.6±2.608 | 100.6±2.608 |
| Superoxide radical scavenging% | 97.32±0.5762 | 97.24±0.6066 | 96±0.7071 |
| Reducing Power OD at 700 nm | 1.04±0.00707 | 0.8±0.00707 | 0.33±0.00707 |
| Protein(g) | 4.02±0.1095 | 0.62±0.1095 | 3.02±0.1095 |
| Sterol(mg) | 100.6±1.342 | 100.6±1.342 | – |
| Carbohytrate (g) | 2.092±0.0444 | 2.004±0.3967 | 1.132±0.0989 |

Values are mean±SD of 10 replications

### Protein Profile

Table 2 shows the excellent protein content in ethanolic extract of *S. nigrum*. It may have more hydrophobic amino acids and lesser hydrophilic amino acids containing proteins. So they are extracted more in ethanol than in aqueous extract amino acids, peptides, such as carnosine and anserine, and proteins are common food components. Amino acids were found to be efficient antioxidants in model experiments. Their application is advantageous in mixtures with other inhibitors as they often act as synergists of phenolic antioxidants and as chelating agents. Amino acids convert hydroperoxides into imines, and sulphur containing amino acids reduce hydroperoxides into the respective inactive hydroxylic derivatives. Methionine and selenomethionine were found to be more active than α -tocopherol in olive oil.[43]

### Carbohydrate Profile

Table 2 shows that *S. nigrum* has high carbohydrates in aqueous extract indicating that carbohydrates in *S. nigrum* were soluble in water. These are likely to contribute to the radical scavenging activity and reducing power of the extract. Simple carbohydrates have antioxidant properties.

Hydroxyl radicals generated by a fenton reaction induce damage on simple carbohydrates with a consequent free radical scavenging activity. Carbohydrate activities were measured by different methods as spin-trapping of hydroxyl radical and electron paramagnetic resonance detection and 1, 1-diphenyl-2-picrylhydrazyl quenching. Carbohydrate damage was evaluated in a fenton system by measuring the reactive substances to thiobarbituric acid, by their decreased detection with an HPLC test, and by a gas chromatographic determination of formic acid from sugar oxidation. Different intensities of damage and scavenging were found according to molecular structure, and some hyphotheses on the carbohydrate action against free radicals were attempted. The assayed disaccharides were shown to be more active toward and less damaged by hydroxyl radical than monosaccharides.[44]

### Sterol Profile

Table 2 indicates that *S. nigrum* leaf has excellent sterol content both in organic and aqueous extracts. This may be due the presence of sterol which is extractable with organic and aqueous solvents. *S. nigrum* was found to have steroidal saponins and steroidal alkaloid which contributes total sterol content. *S. nigrum* has β-sitosterol- an important group of antioxidant. Plant sterols inhibit the intestinal absorption of cholesterol. Functional food ingredient derived from phytosterols has been clinically proven to have significantly lower Low Density Lipoprotein (LDL) or "bad" cholesterol when consumed in different foods, and hence they have a hypocholesterolemic action. They also inhibit endogenous synthesis of cholesterol by inhibiting and repressing the rate limiting enzyme hydroxy methyl glutaryl CoA (HMG-coA) reductase in cholesterol synthesis. Steroidal glycosides in plant foods have estrogenic antiestrogenic actions and are known as phytestrogen. These have antibacterial and antifungal actions and also produce typical estrogen responses with a biological activity 1/500 to1/1000 of estradial. Studies proved that phytestrogens lower the incidence of osteoporosis, breast and uterine cancer.[45]

### Phytochemicals Profile

Saponins are natural surfactants or detergents. It can be used as foaming agents for beverages. Saponins have astypic activity which can be utilized in dissolving thrombus. Recent studies have suggested that the low serum cholesterol levels of Masai tribes in East Africa who consume a diet high in animal products, cholesterol and saturated fat with saponins rich-herbs. Saponins act by binding with bile acids and cholesterol. So it cleans or purges fatty compounds from the body, lowering the blood cholesterol level. Digitalis is a saponin used as heart tonic to strengthen contractions of the heart muscle. Saponins are active antifungal agents.[46]

The alkaloid present in *S. nigrum* is an Indole alkaloid. The aromatic nitrogen heterocyclic is a potent cancer fighter- anticancer agent, blocking carcinogenic substances before they reach their cellular targets and eliminating DNA damage in cell nuclei. It may also turn out to be an important chemical tool in fighting breast cancer because it inhibits estrogen induced growth of cancer cells and converts the more dangerous forms of estrogen to safer forms.[47]

Isoflavones have hormone like role and act as phytestrogens. They benefit humans in three ways as cancer enzyme inhibitor, as antioxidants and as immune system enhancers or stimulants. Phytestrogens compete with estrogen for binding to estrogen receptors. Their use could have beneficial effects on preventing osteoporosis and sex hormone-mediated malignancy such as breast and prostate cancer. Clinical trials identified the potential efficacy of isoflavones in the prevention of coronary heart disease, osteoporosis, breast and prostate cancer.[48,49]

### FRSA Profile

Table 2 shows that excellent superoxide radical scavenging ability found in almost all extracts of *S. nigrum*. Super oxide anions are one of the potent reactive oxygen species (ROS) which are produced from molecular oxygen due to oxidative enzymes[50] of body as well as via non enzymatic reaction such as autoxidation by catecholamine.[51] In the present study superoxide radical reduces NBT to a blue coloured formazan that is measured at 560 nm.[52] Table 2 shows the excellent reducing power of *S. nigrum*. In the reducing power ($Fe^{3+}$ to $Fe^{2+}$ transformation ability) assay, the presence of antioxidants in the extracts would result in the reduction of $Fe^{3+}$ to $Fe^{2+}$ by donating an electron. Amount of $Fe^{2+}$ complex can be then monitored by measuring the formation of Perl's Prussian blue at 700 nm.[53] Antioxidant activity has been reported to be concomitant with development of reducing power. This shows that extracts might contain reductones, like ascorbic acid, reducing sugar, thiol group containing protein which could react with free radicals to stabilize and terminate radical chain reaction.

## CONCLUSION

These findings suggest that *Solanum nigrum leaves* exhibit elevated free radical scavenging activities. It also chelates iron and has enhanced reducing power. These *in vitro* assays indicate that this plant extract is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses, cancer, ageing, ischemic heart disease in dissolving thrombus, microbial infections and hormone replacement therapy (HRT) justifying their use in traditional medicine The plant extracts have assumed an increased importance in medicine and in health care industry. Therefore, further investigations need

to be carried out to isolate and identify the antioxidant compounds present in the plant extract. Furthermore, the *in vivo* antioxidant activity of this extract needs to be assessed prior to clinical use.

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