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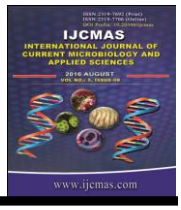

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Comparative Study of Antioxidant Potential in Hairy Roots and Field grown Roots of *Solanum nigrum* L.

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A B S T R A C T

Field grown roots (FGR) and hairy roots (HR) were compared for total phenolics, flavonoids and antioxidant capacity using three different solvents. The aqueous and ethanolic extract showed high phenolic (9.036±0.613) and flavonoids (25.392±0.687) content in HR compared to FGR respectively. High alkaloid content was observed in ethanolic extract of HR (23.628±0.907). The aqueous extract showed maximum DPPH, ABTS and SO radical scavenging antioxidant activity in HR compared to FGR. The highest Frap value (1.88) was observed for aqueous extract in HR. The ethanol and water was found to be best for the extraction of flavonoids and antioxidant compounds respectively. HR showed high content of biochemical than the FGR.

Introduction

Solanum nigrum (black nightshade) is a medicinal plant member of the Solanaceae family. This family comprises many genera, well known for their therapeutic properties. It occurs on a wide range of soils but prefers soil rich in nitrogen. *Solanum nigrum* Linn. is commonly used in the traditional medicine as a remedy for treating various ailment such a pain, inflammation and fever (Zakaria *et al.,* 2006.). *Solanum nigrum* is also used as antitumorigenic, antioxidant (Lee *et al*., 2003), anti-inflammatory, diuretic, antipyretic agent (Zakaria *et al.,* 2006), hepatoprotective (Raju *et al.,* 2003), anticancer, immunomodulatory (Jian *et al.,* 2009), larvicidal (Ahmed *et al.,* 2002), hepatoprotective (Raju *et al.,* 2003) and antibacterial (Rani *et al.,* 2004). It has been used against sexually transmitted diseases as well (Atanu *et al.,* 2011). The species exhibits a high level of morphological plasticity and several subspecies have been identified. The plant and berries can form a sticky mass during harvesting operations.

The leaves are used to heal open wounds and is known to possess hypotensive effect. The toxicity of black nightshade due to the alkaloid Solanine has caused varying degrees of poisoning in humans, cattle, pigs and goats (Cooper and Johnson, 1984). Although it is considered a rich source of one of the most popular plant poisons, it has also proven to be a reservoir of phytochemicals with phamacological prospects (Lee and Lim, 2006). Solasodine has been reported as a valuable steroidal precursor for the supplementary source of the commercial synthesis of several steroidal drugs (Rodriguez *et al*., 1979; Sree *et al*., 1982). It could be primarily obtained from various plants of genus Solanum (Crabbe and Fryer, 1982). Subroto *et al.,* (1994) reported that the solasodine content was increased by 5 times in hairy root compared to normal plant. Due to the importance and presence of solasodine in hairy roots, few reports are available on hairy root induction in solanum species (Khatodia and Biswas, 2014; Pawar and Maheshwari, 2004; Ooi, 2012). The active components are polyphenolics, polysaccharides such as gallic acid, cathechin, caffic acid, rutin (Chauhan *et al.,* 2012).

In present study, we have intended to evaluate and compare the antioxidant potential between HR and FGR with the help of total phenolics, flavonoids and alkaloids and have also performed the free radical scavenging activity by DPPH, ABTS, FRAP and SO assays. There is no report available on comparative study in hairy root and field grown roots for antioxidant activity.

Materials and methods

Reagent and standards

Ascorbic acid, ferric chloride, aluminium trichloride, sodium bicarbonate, 1,10phenanthroline, folin-ciocalteu reagent, rutin, colchicines, DPPH, TPTZ and ABTS were purchased from Sigma chemicals USA and NADH, NBT and PMS from Hi-Media.

Sample Preparation

Extraction of samples was performed in a similar way as described by Chaturvedi *et al*., 2011. The dry FGR and HR of *Solanum nigrum* (Fig. 1) was crushed using liquid nitrogen. Fine root powder was suspended in 100 ml of three different solvents (Water, Methanol and Ethanol) and kept on shaker for overnight. The extracts were filtered through the muslin cloth and centrifuged at 10,000 rpm for 10 minutes. The supernatant was collected and condensed to 10ml on rotary evaporator and stored at $4^{O}C$. Further extracts were tested for Total Phenolics, flavonoids and antioxidant activity.

Determination of total phenolic content (TPC)

The total phenolics content from all the extracts was determined using Singleton and Rossi (1965) method. The reaction mixture was prepared by adding 0.125 ml of 1% plant extract and 1.8 ml of Folin-Ciocalteu reagent. The assay mixture was incubated for 5 min at room temperature. 1.2 ml of aqueous sodium carbonate was added to the mixture and was kept in dark for 90 min at room temperature. Phenolic content of these samples was determined spectrophotometrically using a UV visible spectrophotometer at 760 nm. The total phenolics content was quantified with standard curve of gallic acid and total content was expressed as mg of gallic acid equivalents per g of roots.

Determination of total flavonoids content (TFC)

The total flavonoids content from all the extracts was determined using Luximan-

Ramma *et al.,* (2002) method. The reaction mixture contains 1.5 ml of 1% extract and 1.5 ml of 2% methanolic aluminum chloride. The reaction mixture was incubated for 10 min at room temperature. The absorbance was measured at 368nm in UV-visible spectrophotometer. The total flavonoids content was quantified with standard curve of rutin and total content was expressed as mg of rutin equivalents per g of roots.

Determination of total alkaloids content (TAC)

The total alkaloid content from all the extracts was determined using the method of Singh *et al*., (2004). The reaction mixture contains 1% extract, 1 ml of 0.05M, 1-10 phenanthroline reagent and 1 ml of 0.025 M ferric chloride. After addition of all reagent mixture was incubated at 70^{o}C in water bath for 30 min. The absorbance was recorded at 510 nm. The total alkaloids content was quantified comparing with standard curve of colchicine and total content was expressed as mg of colchicine equivalents per gm of root.

Determination of DPPH radical scavenging activity

The free radical scavenging activity was estimated using DPPH as described by Ghatak *et al.,* 2014. The assay mixture contains 0.5mL of 0.3mM DPPH solution and 100µL respective extracts. The mixture was kept in dark at 37˚C for 30 min. The absorbance was measured at 517nm by spectrophotometer. The ability to scavenge DPPH radical was calculated using following formula:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of the control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Ferric reducing antioxidant power (FRAP) assay

FRAP assay was carried out according to the method of Jain *et al*., 2014. FRAP reagent was prepared using sodium acetate buffer (1.6g sodium acetate and 8 ml acetic acid make up to 100mL) (pH 3.6), 10 mM 2,4,6-Tripyridyl-s-Triazine (TPTZ) solution in 40 mM HCl and 20 mM ferric chloride solution in proportion of 10:1:1 (v/v) respectively. The FRAP reagent was prepared fresh. 100µL extracted samples were added to 3 mL of the FRAP reagent and mixed well. The absorbance was measured at 593 nm at 0 min and after 4 min using FRAP reagent as blank. Standard curve of ascorbic acid was prepared. FRAP value of sample was calculated.

ABTS radical scavenging assay

ABTS assay was carried out using the method described by Pai *et al*., 2015. Stock solutions of 7 mM ABTS and 2.4 mM potassium persulfate/ ammonium persulfate were prepared. The working stock was prepared by mixing both solutions in equal quantities and incubated in dark for 12h-16h at 30ºC. The incubated solution was diluted by methanol to obtain 0.706 OD at 734 nm. Each plant extract were mixed with diluted ABTS solution in equal quantity and the OD was taken at 734 nm after 7 min. by spectrophotometer. The ABTS scavenging capacity of the extract was calculated as:

$$\text{ABTS radical scavenging activity (\%)} = \frac{\text{Abs. of control} - \text{Abs. of sample}}{\text{Abs. of Control}} \times 100$$

Superoxide radical scavenging assay

The superoxide radical scavenging assay was performed as per Mandal *et al.*, 2009. The assay mixture contains 1 mL of root extract, 0.5mL (0.3mM) Nitro blue tetrazolium (NBT), 0.5ml Nicotinamide adenine dinucleotide (NaDH) (0.936mM), TrisHCl Buffer (16mM) (pH-8.0) and Phenazinemethosulphate (PMS) (0.12 mM). The reaction mixture was kept for incubation at 25ºC for 5 min. After incubation absorbance was measured by spectrophotometer at 560nm. The super-oxide scavenging activity was calculated as:

Superoxide radical scavenging activity (%) =

$$\frac{\text{Abs. of control} - \text{Abs. of sample}}{\text{Abs. of Control}} \times 100$$

Statistical analysis

All the observations were taken in triplicate and the data was presented as ± standard deviation (SD). Analysis of Variance (one way ANOVA) was further performed using SPSS software.

Result and Discussion

Total phenolic content

FGR (6.950±0.436 mg GAE g $^{-1}$) and HR (9.036±0.613 mg GAE g $^{-1}$) showed high phenolic content in aqueous extract where as lowest phenolics was observed in ethanolic extracts (0.786±0.022 and 2.892±0.613mg GAE g $^{-1}$) respectively (Table no. 1). Water found to be best solvent compared to other for the extraction of phenolic compounds. We also observed that HR show high phenolic content as compared to the FGR. Similar results also reported by Jayachitra A and Kritiga N (2012), in which aqueous extract of *Solanum nigrum* showed 14 mg of phenolics. In another study *Solanum melongena* also showed similar result in which they found 0.611 mg of phenolic content (Somavati *et al.*, 2014). Ghosal M and Mandal P (2012) also reported the same result, in which two plants, *Solanum anguivi and Solanum incanum* showed 1.606 and 2.306 mg of phenolic content respectively.

Total flavonoids content

FGR (18.747±0.954 mg RE g $^{-1}$) and HR (25.392±0.687 mg RE g $^{-1}$) showed high flavonoids content in ethanolic extract where as lowest flavonoids was observed in methanolic extracts (7.675±0.376 and 9.640±0.277 mg RE g $^{-1}$) respectively (Table no. 2). Ethanol was found to be best solvent compared to other for the extraction of flavonoids compounds. The HR showed high flavonoids content than the FGR. Ghosal M and Mandal P (2012) reported the same in which two plants *Solanum anguivi and Solanum incanum* showed 0.101 and 0.207 mg of flavonoid content respectively. In another study of *Solanum muricatum* the flavonoids content was found to be 53.60 mg (Sudha *et al.*, 2011).

Total alkaloid content

The highest alkaloids content was observed in HR. The ethanolic extract showed high alkaloid content in HR (23.628±0.907 mg CE g $^{-1}$) as well as in FGR (12.036±1.134 mg CE g $^{-1}$) where as lowest alkaloids was observed in methanolic extract (5.621±1.026 and 4.02±0.641 mg CE g $^{-1}$) respectively (Table no. 3). For the extraction of alkaloids the ethanol solvent was found best compared to other solvent. HR showed high alkaloid content than the FGR. Sundari *et al.*, (2013) reported the same kind of result in three different species of *Solanum, S. trilobactum, S. torvum* and *S. xanthocarpum* showed (6.12, 5.30 and 4.70 mg/g) of alkaloids content respectively.

Estimation of DPPH radical scavenging activity

The DPPH method has been widely used to evaluate the free radical scavenging ability of antioxidants. Plants are the potential source of natural antioxidants and produce antioxidative compounds in order to survive and counteract with reactive oxygen species (Huda *et al.,* 2009). The method is based on scavenging of DPPH radicals by antioxidant compounds from plant that decolourizes the DPPH solution. The colour change can be proportional to the potency and concentration of antioxidants (Saeed *et al.,* 2012). The present study shows that DPPH radical scavenging activity was highest in aqueous extracts of FGR (51.339±1.107%) and HR (86.377±1.310%) (Fig.2). Methanolic and ethanolic extracts showed less antioxidant activity. The HR showed high DPPH radical scavenging activity than the FGR. The aqueous extracts were found best in DPPH radical scavenging activity in both FGR and HR. Similar study was performed on *Solanum nigrum* plant by Sharma *et al.,* (2014) in which they showed that chloroform extract have highest (68.74±0.37%) radical scavenging capacity. In comparison to *Saraka asoka* in which Methanolic exract showed higest (94.4±1.2%) DPPH activity (Ghatak *et al.,* 2015) where as 88% DPPH activity was observed in Citrullus colocynthis (Kumar *et al.,* 2008). In another study six plants species of *solanum* were campaired for DPPH activity in which ethyl acetate extract of *Solanum anguivi* showed maximum activity (Gandhhipan and Rengasamy, 2012).

Estimation of FRAP radical scavenging activity

FRAP assay measures the amount of antioxidants based on its ability to reduce Fe3+ to Fe2. In FGR the aqueous extract showed highest (1.076±0.135) frap value where as lowest (0.280±0.036) frap value was observed for ethanolic extract (Fig. 2). In case of HR the highest frap value was observed for aqueous extract (1.884±0.097) and lowest for Methanolic extract (0.536±0.043) (Fig. 3). Similar kind of result was observed in *Saraca asoca*, high frap value (2.83±0.8) was observed for ethanolic extract (Ghatak *et al*., 2014). In comparison of both roots the HR shows high frap value than the FGR. From the above result the aqueous solvent was found to be best for FRAP antioxidant activity. Similar result were reported in *Helicterus isora*, in which they found aqueous solvent is best for FRAP antioxidant activity (Jain *et al.,* 2014). In another study the ethanolic extract of *Saraca indica* showed highest frap value (Gayatri *et al.,* 2013).

Estimation of ABTS radical scavenging activity

The ABTS radical cation is generated by the oxidation of ABTS with potassium persulfate, its reduction in the presence of hydrogen-donating antioxidants is measured spectrophotometrically at 734 nm (Saeed *et al.,* 2012). The aqueous extract of FGR showed highest (69.61±0.739 %) ABTS scavenging activity followed by Methanolic and ethanolic extract (Fig. 4). In HR aqueous extract showed highest (92.751±1.452 %) ABTS radical scavenging activity (Fig. 3). In both FGR and HR the highest and lowest antioxidant activity was observed in aqueous and ethanolic extract respectively. Comparatively HR showed high antioxidant activity than the FGR. These result were similar to the study performed by Somawati *et al*., (2014), they showed that aqueous extract of *solanum melongena* shows 40.45% activity. In other study the Methanolic extract of *Solanum*

pseudocapsicum also showed 49.66% antioxidant activity (Badami *et al.,* 2005). Sudha *et al.,* (2011) showed that ethyl acetate extract of *Solanum muricatum* have the 98% antioxidant activity. In another study the ethanolic and aqueous extract of *Solanum nigrum* showed 94.06 and 85.62 % ABTS radical scavenging antioxidant activity respectively (Gbadamosi and Afolayan, 2016).

Estimation of Superoxide radical scavenging activity

In the PMS-NADH-NBT system, superoxide anion was derived from dissolved oxygen by PMS-NADH coupling reaction and reduces NBT. The decrease of absorbance at 560 nm with antioxidants indicates the consumption of superoxide anion in the reaction mixture (Muruhan *et al.,* 2013). In the present study the aqueous extract of HR and FGR (62.68±2.078 and 52.31±1.801%) showed maximum superoxide radical scavenging activity respectively (Fig. 5). The ethanolic and methanolic extract showed lowest SO activity respectively. The HR were found best for Superoxide radical scavenging activity in comparison with FGR. The aqueous solvent was observed best for extraction of antioxidant compounds. The same study was performed by Gandhiappan *et al.,* (2012), in which they found 9.427% activity for *S. anguivi*. In another study the same result was observed for *S. surattense*, they observed 65% SO scavenging activity (Muruhan *et al.,* 2013). Backialakshmi *et al.,* (2015) also reported that ethanolic extract of *S nigrum* shows 50% Superoxide radical scavenging activity at 5.9 µg/ml.

Table.1 Total phenolics content

Solvent/plant material	Water	Ethanol	Methanol
Field grown root	6.950±0.436	0.786±0.022	1.071±0.085
Hairy root	9.036±0.613**	2.892±0.613**	4.229±0.085**

Values represent mean ± standard deviation (SD), **Indicates significance at p<0.001

Table.2 Total flavonoid content

Solvent/plant material	Water	Ethanol	Methanol
Field grown root	11.301±0.259	18.747±0.954	7.675±0.376
Hairy root	15.910±1.939**	25.392±0.687**	9.640±0.277**

Values represent mean ± standard deviation (SD), **Indicates significance at p<0.001

Table.3 Total alkaloid content

Solvent/plant material	Water	Ethanol	Methanol
Field grown root	4.647±0.319	12.036±1.134	4.02±0.641
Hairy root	9.250±0.812**	23.628±0.907**	5.621±1.026

Values represent mean ± standard deviation (SD), **Indicates significance at p<0.001

Fig.1 a. *Solanum nigrum* L., b. Entire *Solanum nigrum* plant with roots, c. Hairy roots, d. PCR confirmation of hairy roots.

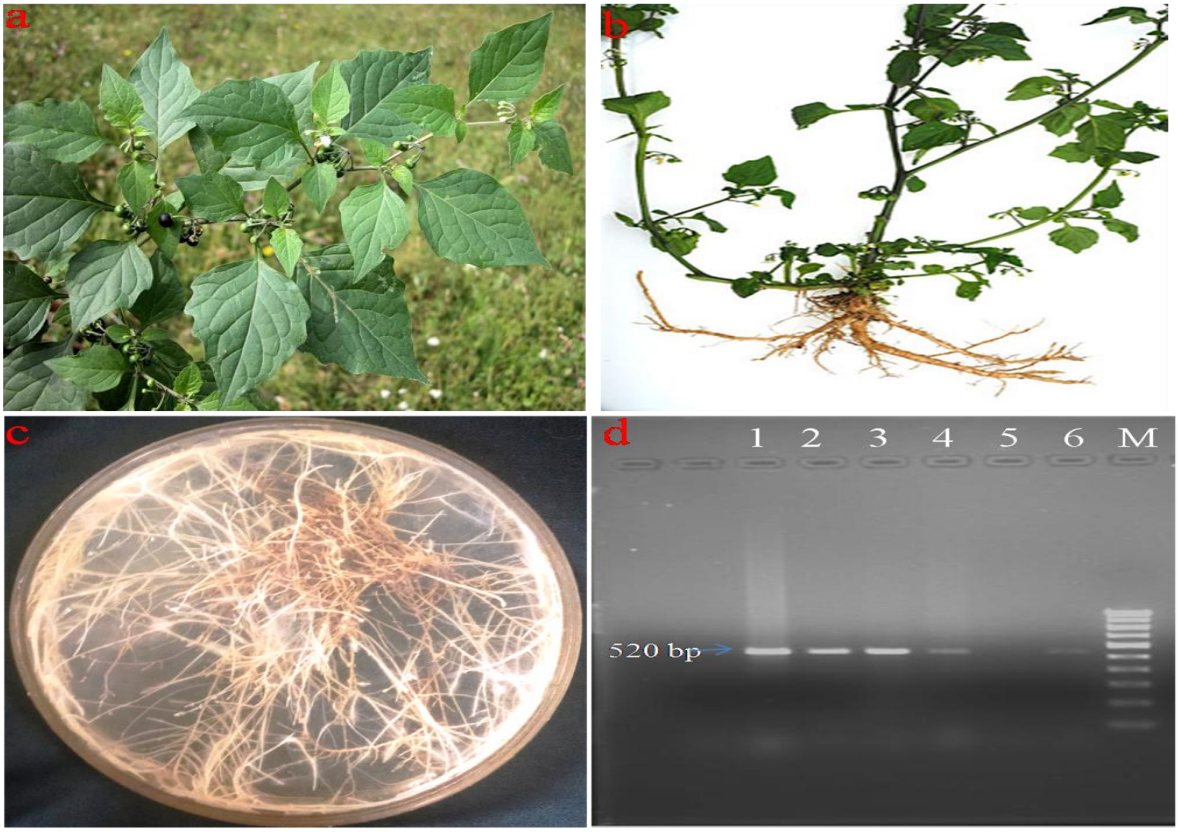
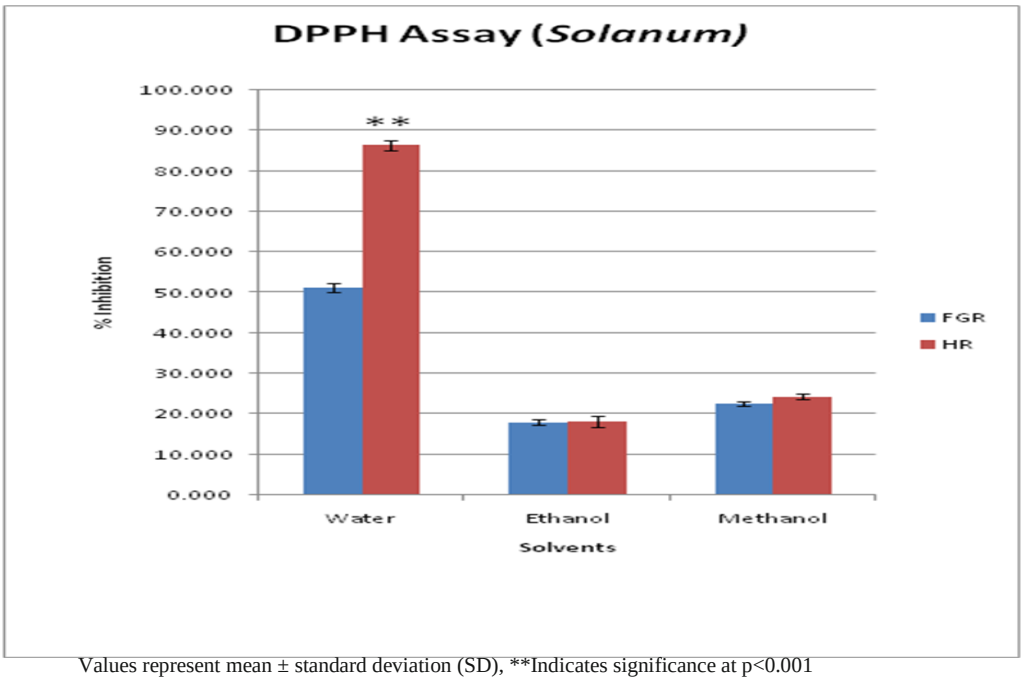
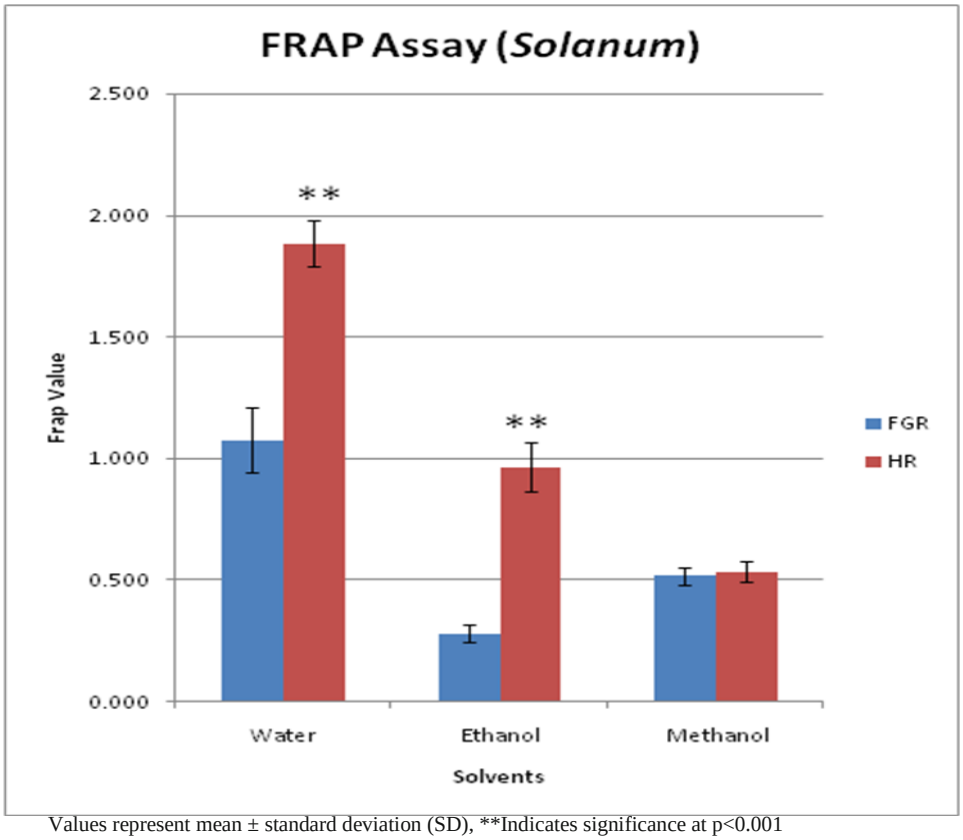


Fig.2 Percent DPPH radical scavenging activity of *Solanum nigrum*.



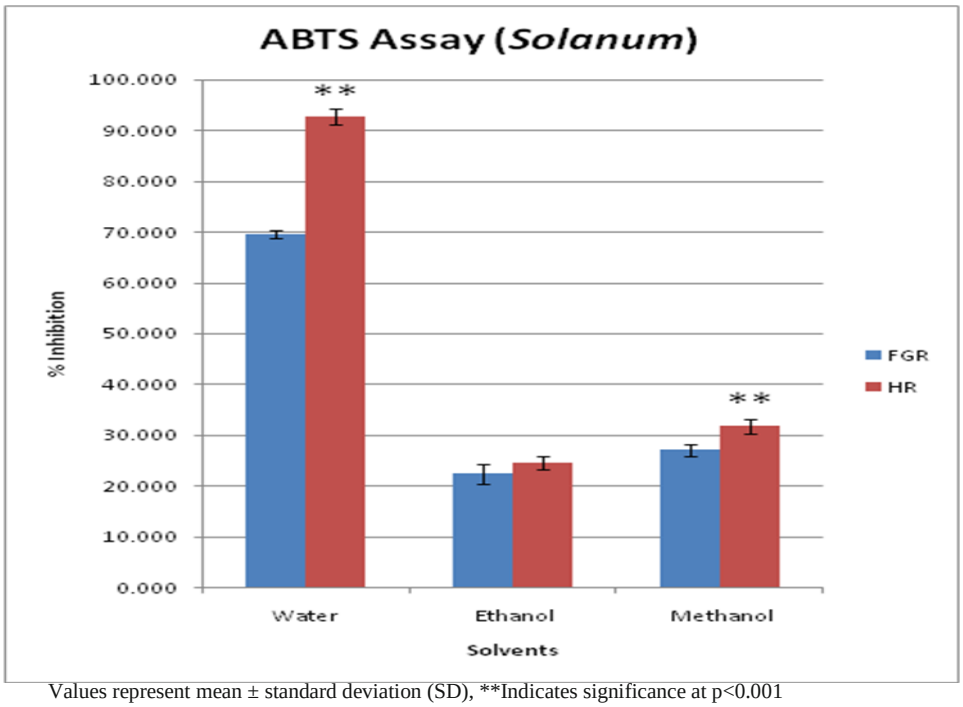
Values represent mean ± standard deviation (SD), **Indicates significance at p<0.001

Fig.3 FRAP radical scavenging activity of *Solanum nigrum*.



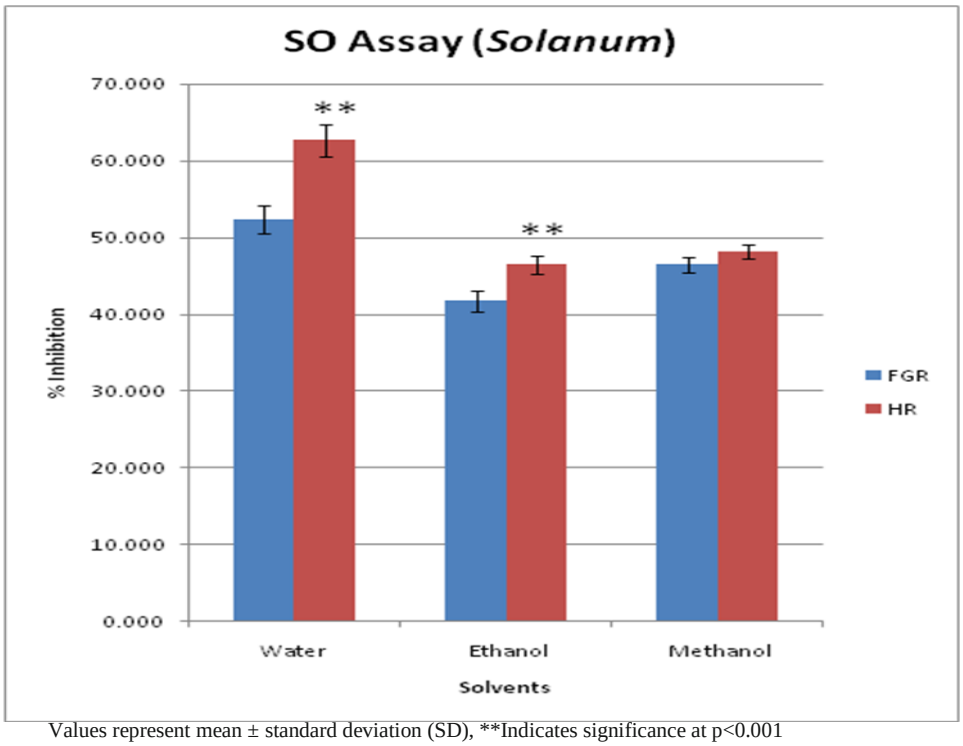
Values represent mean ± standard deviation (SD), **Indicates significance at p<0.001

Fig.4 Percent ABTS radical scavenging activity of *Solanum nigrum*.



Values represent mean ± standard deviation (SD), **Indicates significance at p<0.001

Fig.5 SO radical scavenging activity of *Solanum nigrum*.



Values represent mean ± standard deviation (SD), **Indicates significance at p<0.001

Hairy Root Culture technique was developed as the new source for large scale secondary metabolite production (Flores *et al.,* 1987; Rhodes *et al.,* 1987) and production of phytochemicals (Shanks and Morgan, 1999). Hairy roots (HRs) are characterized by rapid growth and extensive branching in growth regulator free medium. In general, they exhibit genetic stability and, in certain cases, they have the capability of synthesizing secondary metabolites normally present in roots and organs of the species of origin. For this reason, hairy roots have been induced in several medicinal and aromatic plants and cultured for the production of secondary compounds (Bonhomme *et al.,* 2000; Murthy *et al.,* 2008; Khatodia *et al.,* 2014). The secondary metabolites play an important role in antioxidant capacity of any plant and it has been proved by present study. In the present study the HR showed high antioxidant activity than the FGR which indicates the high content of secondary metabolite present in hairy root. Also it the first report of comparative study for antioxidant activity in HR and FGR. Many reports are available on presence of Solasodine an precursor of commercially important steroid (Subroto *et al* 1994; Jacob *et al* 2004; Loc *et al*.,), in solanum species so the present report will help for the production of root biomass and secondary metabolite (solasodine) on large scale.

In conclusion, in the present study FGR and HR is compared for antioxidant activity. We observed significant increase in accumulation of different important Phytochemicals like phenolics and flavonoids in hairy roots. We also observed a significant increase in antioxidant radical scavenging activity of the HR in comparison of FGR. HR may be considered as good source of natural antioxidant as compared to FGR. Further investigation also lead us to

conclude that Water is the best solvent system for the extraction of antioxidant compounds compared to other solvent system. It has been proved that HR accumulate higher amount of metabolites than the FGR. The result from this investigation indicates that HR of *S. nigrum* exhibits excellent scavenging activity against free radicals such as DPPH, FRAP, ABTS and Superoxide anion. The present findings of the study suggested that HR of *Solanum nigrum* could be a potential source of natural antioxidant that could replace the use of toxic synthetic antioxidants.

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