Life Science Informatics Publications

Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences

Journal Home page http://www.rjlbpcs.com/

RJLBPCS

ISSN 2454-6348

Original Research Article **DOI:10.26479/2019.0501.51**

PHYTOCHEMICAL ANALYSIS AND FREE RADICAL SCAVENGING ACTIVITY OF GREEN-SYNTHESIZEDLIV-PRO-08 FORMULATION

Anandhi Eswaran, Suriyavathana Muthukrishnan*, Kavitha Rani Mari, Punithavathi Manogaran

Department of Biochemistry, Periyar University, Salem, India.

ABSTRACT: Nowadays nanotechnology research is emerging as cutting edge technology interdisciplinary with physics, chemistry, biology, material science and medicine. Plant-mediated synthesis of nanoparticles has earned wide interest owing to its inherent features such as rapidity, simplicity, eco-friendliness and cheaper costs. Synthesis of NPs through biological route is preferred due to its environment-friendly and economic aspects. The present study demonstrates an eco-friendly and low-cost method of biosynthesis of NPs using the dried seeds of *Nigella sativa*, *Entada pursaetha* and dried fruit of *Ficus glomerata* (Liv- Pro-08) confirmed by the color transformation and Ultraviolet-visible(UV-visible) spectroscopy. These biologically synthesized NPs were tested for which were analyzed for different characteristics such as phytochemical and free radical scavenging activity to understand its potential benefits. The antioxidant potential of the green synthesized Liv-Pro-08 was analyzed by DPPH, Nitric oxide radical scavenging activity, Hydrogen peroxide scavenging activity. Phytochemical analysis reveals that the seeds are a rich source of flavonoids, phenolics, saponins, alkaloids and other secondary metabolites. The present study explored that the Liv-Pro-08 which are efficient producers of NPs, and could act as safe and cost-effective with potential antioxidant activities. These findings encourage studying suggest that synthesized AgNPs can be developed into a promising drug candidate for biomedical applications.

KEYWORDS: Liv-pro-08,green synthesis, radical scavenging.

Corresponding Author: Dr. Suriyavathana Muthukrishnan* M. Phil., Ph.D.,

Department of Biochemistry, Periyar University, Salem, India.

Email Address: suriyabio14@gmail.com, suriyaveda@yahoo.co.in

1. INTRODUCTION

Medicinal plants are the local heritage with global importance world endowed with a rich wealth

© 2019 Life Science Informatics Publication All rights reserved

of medicinal plants. These plants have made a best contribution to the development of ancient material medical [1]. It has been shown that *in vitro* screening methods could furnish the needed preliminary observations necessary to select crude plant extracts with potentially useful properties for further chemical and pharmacological exploration [2]. Plants such as vegetables, fruit, spices, medicinal herbs, etc., have been used to cure many diseases since ancient time. Today in this modernistic world, even though synthetic drugs are readily available and highly effective in curing various diseases, there are people who still prefer using traditional folk medicines because of their less harmful effects. Natural remedies from medicinal herbs are found to be safe and effective. Many plants species have been used in folkloric medicine to treat various illness. Even today compounds from plants continue to play a major role in primary health care as therapeutic remedies in many developing countries [3]**.** Standardization of plant materials is the need of the day. Several pharmacopoeias containing monographs of the plant materials narrate only the physicochemical parameters. Henceforth modern methods describing the identification and quantification of active constituents in the plant material may be useful for proper standardization of herbals and its formulations. Oxidative stress has been identified as the main cause of the development and progression of several diseases. Intake of exogenous antioxidants or boosting endogenous antioxidant defenses of the body is a promising way of combating the undesirable effects of reactive oxygen species (ROS) induced oxidative damage. Plants have an inborn ability to biosynthesize a wide range of non-enzymatic antioxidants capable of attenuating ROS- induced oxidative damage. Most of phenolic compounds from most of the tested herbs were primarily identified and analyzed and mainly included phenolic acids, flavonoids, tannins, saponins, alkaloids, glycosides. These medicinal herbs exhibited far efficient antioxidant activity and contained significantly higher levels of phenolics than common vegetables and fruits. Plant mediated food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids and phytoestrogens have been recognized as having the potential to reduce disease threat. *Nigella sativa,* one of species of the Ranunculaceae family, is a seed that is frequently added to bread and pickles as a flavoring agent. The seeds have been used as a natural remedy for many ailments over many centuries [4&5]. *E pursaetha* belongs to Mimosaecea family. The *Entada* species have two amorphous saponins. The seeds have various medicinal uses, e.g., as a topical application in an ointment for the treatment of jaundice. *Ficus glomerata* (family: Moraceae) is more commonly known as an atthi in Tamil and a cluster fig in English. The fruits are effective against leprosy, diseases of the blood, fatigue, bleeding nose, cough, diabetes, and leucoderma . Both the root and the bark are credited with hypoglycemic activity [6]. *N sativa, E. pursaetha,* and the dry fruit *F. glomerata* have been part of a high nutritional diet in many parts of the world, especially *N sativa* [7] and *F. glomerata*, which are used as spices in cooking and as a carminative and diuretic by Asians all over the world. The plant

© 2019 Life Science Informatics Publication All rights reserved

metabolites induce the synthesis of metallic nanoparticles in ecofriendly manner. The plant mediated nanoparticles have the potential to be used in various fields such as pharmaceuticals, therapeutics, sustainable and renewable energy and other commercial products. The plant derived metallic nanoparticles have impact on diagnosis and treatment of various diseases with controlled side effects. In future, the plants have broad perspective for the synthesis of metallic nanoparticles in healthcare and commercial products. Even though, there are two reasons to be explored in the biological production of nanoparticles, they are, to identify the active metabolite which is involved partially/fully in the reduction reaction and laboratory scale production of metallic nanoparticles to the extent of large scale production, and need to elucidate their functional mechanism against the pathogenic diseases.In this study, We selected the formulation namely Liv-Pro-08 constituting *Nigella sativa* and *Entada pursaetha* seeds and *Ficus glomerata* fruits in specific proportion and the green synthesis of Silver, Copper, Magnesium& Zinc nanoparticles done in order to evaluate the phytochemical and free radical scavenging ability of those synthesized nanoparticles compared with that of crude extract.

2. MATERIALS AND METHODS

2.1 Collection of samples

Nigella sativa and *Entada pursaetha* seeds and *Ficus glomerata* fruits were collected from Kolli hills, Namakkal district, Tamil Nadu, India.

2.2Processing of plant sample

The fruits and seeds of *Nigella sativa* and *Entada pursaetha* seeds and *Ficus glomerata* collected were dried under shade and then ground into fine powder form (80 mesh sieve size) by electrical grinder. Powdered sample stored in clean paper bags for further use.

2.3 Preparation of aqueous extract from Liv-Pro-08 polyherbal formulation

The aqueous extract of Liv-Pro-08 polyherbal formulation prepared by taking respective ratio of 10 g of powdered samples in 100 ml of distilled water for 30 minutes. The mixture was kept in an incubator shaker at 40 °C with 140 rpm for 48 hours which then filtered through a whatman filter paper 1 and the filtrate obtained was evaporated, concentrated at room temperature and stored at 4 °C. The extract acquired after double extraction with solvent was dark yellowish brown in color.

2.4 Synthesis of silver nanoparticles by Liv-Pro-08 extract

Silver nanoparticles are synthesized by incubating 88 mL of 1 mM $AgNO_3$ resolution with 12 mL of aqueous extract of Liv-Pro-08was accessorial and the therefore resulting solution became brown in colour. Then extract filtered through nylon mesh (spectrum), followed by Millipore hydrophilic filter (0.22 μm) and used for further experiments [8]. An impression setup was additionally maintained while not Liv-Pro-08extract and colour intensity of the extracts was measured at 420 nm.

© 2019 Life Science Informatics Publication All rights reserved

2.5 Synthesis of Mg nanoparticles by Liv-Pro-08 Extract

0.2 M Aqueous solution of magnesium oxide (MgO) was prepared and used for the synthesis of magnesium nanoparticles. 10 mL of Liv-Pro-08 extract was added into 90 mL of aqueous solution of 0.2 M magnesium oxide for reduction into Mn^{+} ions and kept at room temperature for 30 mins.

2.6 Synthesis of Zn nanoparticles by Liv-Pro-08 Extract

For the synthesis of Zn nanoparticles 50 mL of Liv-Pro-08 extract was taken and boiled to 60-80 degree Celsius using a stirrer heater. 5g of Zinc oxide was added to the solution as the temperatures reached 60 degree Celsius. The mixture is then boiled until it reduced to a deep yellow colored paste was then collected in a ceramic crucible and heated in an air heated furnace at 400 degree Celsius for 2 hours. A light yellow colored powder was obtained was carefully collected and stored for characterization purposes. The material was mashed in a mortar pestle so as to get a finer nature for nature for characterization.

2.7 Synthesis of Cu nanoparticles by Liv-Pro-08Extract

0.249g of copper sulphate salt in 1000 mL of distilled water to get 1.0 mM solution of copper sulphate and stored. 50 mL of extract and 50 mL of CuSO4 solution was taken in two separate beakers and heated at 60 ˚C for 30 min in water bath. On keeping the solution overnight, the copper nanoparticles formed were separated out from the solution by centrifugation (6000 rpm, 10 min) followed by redispersion of pellet in deionized water.

2.8 Phytochemical analysis

Plants are found to be the sources of many chemical compounds, most of which accounts for their various uses by man. The medicinal values of the plants rely in the presence of certain chemical substances that produce a definite physiological effect on the human body. Phytochemical screening was done according to the published standard method [9]with slight modifications.

2.9 Quantitative Determination of Phytochemical Constituents

2.9.1 Determination of Total Phenol Content(TPC)

By using the tannic acid as a standard phenolic compound, the total phenolic content of the extract of a selected samples was characterized by standard method with minor modifications [10]. The extract diluted with distilled water to a known concentration in order to get the readings within the standard curve range of 0.0 to 600µg of tannic acid/ml. 250 µl of diluted extract which is also called as tannic acid solution combined with 1 ml of distilled water in a test tube followed by the addition of 250µl of Folin-Ciocalteu reagent. The samples were mixed well and then allowed to for 5 min at room temperature in order to allow complete the reaction with the Folin-Ciocalteu reagent. Then, 2.5 ml of 7 % sodium carbonate solution was added and the final volume was made up to 6 ml with distilled water. The absorbance of the blue color solution was measured at 760 nm using spectrophotometer after incubating the samples for 90 min.

© 2019 Life Science Informatics Publication All rights reserved

2.9.2 Determination of Alkaloids

To the 5 g of the dried powder of each sample weighed into a 250 ml beaker add 200 ml of 10 % acetic acid in ethanol. The mixture is covered was allowed to stand for 4 hours. Then purified and the extract was observed on a water bath until it goes to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the complete precipitation. The total solution grant to determine and the precipitated was accumulated was washed with a dilute ammonium hydroxide and later filtered. The residue was the alkaloid, which was dried, weighed and percentage was calculated [9].

2.9.3 Determination of Saponins

20 g of each sample was taken into a conical flask and added 100 ml of 20 % aqueous ethanol. The samples were heated in water bath for 4h with continuous stirring at 55 °C. The mixture filtered and the sediment re-extracted with another 200 ml 20 % ethanol. The extracts were reduced to 40 ml over water bath at 90°C. The concentrate transferred into a 250 ml separating funnel added with 20 ml of diethyl ether and shaken vigorously. Then the ether layer was throwing away the aqueous layer was recovered. The purification process was done repeatedly after that 60 ml of n-butanol was added. Then add the combined n-butanol extracts were washed two times with 10 ml of 5% aqueous sodium chloride. The left over solution was heated in a water bath. It gives the evaporation and the samples were kept dried in the oven to a constant weight and saponin content was measured as percentage [11]

2.9.4 Determination of Flavonoids

10 g of plant sample extracted frequently with 100ml of 80 % aqueous methanol at room temperature. The complete solution was filtered through Whatman filter paper No 41. The filtrate was allowed to evaporated into dryness over a water bath and weighed to a constant weight [12].

2.10 Determination of the antioxidant property of Liv-Pro-08 NPs and aqueous extract of Liv-Pro-08-*In vitro* study

The NPs and crude extract of *Liv-Pro-08* were subjected to various free radical scavenging assays: 1,1-diphenyl-2-picrylhydrazyl [DPPH], Hydrogen Peroxide scavenging activity, Nitric oxide radical scavenging activity as mentioned in figure (1 - 3)

2.10.1 DPPH radical quenching activity

Various concentrations of extract of the sample (4.0 mL) were mixed with 1.0 mL methanolic solution containing DPPH radicals, resulting in the final concentration of DPPH being 0.2 mM.[13] The mixture was shaken vigorously and left to stand for 30 minutes, and the absorbance was measured at 517 nm. Ascorbic acid was used as a control. The percentage of DPPH decolorization of the extract sample was calculated according to the equation:

DPPH scavenging effect (%) = (1 – As /Ac) × 100

© 2019 Life Science Informatics Publication All rights reserved

IC_{50} value (mg extract/mL) was the inhibitory concentration at which DPPH radicals were scavenged by 50%. Ascorbic acid was used for comparison.

2.10.2 Nitric oxide radical scavenging activity

Nitric oxide radical generated from sodium nitroprussidewas measured [14]. Briefly, the reaction mixture (5.0 mL) containing sodium nitroprusside (5 mM) in phosphate-buffered saline (pH 7.3), with or without the plant extract at different concentrations, was incubated at

25C for 3 hours. The nitric oxide radical thus generated interacted with oxygen to produce the nitrite ion, which was assayed at 30 minute intervals by mixing 1.0 mL incubation mixture with an equal amount of Griess reagent. The absorbance of the chromophore (purple azo dye) formed during the diazotization of nitrite ions with sulfanilamide and subsequent coupling with naphthyl ethylene diamine dihydrochloride was measured at 546 nm.

2.10.3 Hydrogen peroxide radical scavenging assay

The ability of the extract to scavenge hydrogen peroxide was determined in accordance with modified method given by [15]. A hydrogen peroxide solution (2mmol/l) was prepared in phosphate buffer (pH 7.4). Extracts of (1–10 μg/ml) were added to hydrogen peroxide solution (0.6 ml). Absorbance of hydrogen peroxide at 230 nm was determined after 10min against a blank solution containing phosphate buffer without hydrogen peroxide and compared with ascorbic acid as reference compound.

H_2O_2 scavenging effect (%) = (1 – As/Ac) ×100

Where, Abs (control): Absorbance of the control and Abs (test):absorbance of the extracts/standard.

2.11 Statistical analysis

The experiments were carried out in triplicate and the results are given as mean ± standard deviation (SD) n=3. A sample analysis test was performed for the comparison between the means of samples and standard.

3. RESULTS AND DISCUSSION

3.1 Qualitative analysis of phytochemicals in green synthesized Liv-Pro-08 compared to aqueous extract

The results of qualitative analysis on phytochemical constituents of extract of green synthesized Liv-Pro-08 compared to aqueous extract investigated were summarized and shown in Table 1demonstrated the presence of various phytochemicals in various forms of extracts of aqueous and green synthesized NPs.

© 2019 Life Science Informatics Publication All rights reserved

Table 1: Qualitative analysis of phytochemicals in green synthesized Liv-Pro-08 compared to aqueous extract

Phytochemicals	Aqueous Extract Liv-Pro-08	AgNP Liv-Pro-08	CuNP Liv-Pro-08	ZnNP Liv-Pro-08	MgNP Liv-Pro-08
Flavonoids	+	+	+	+	+
Phenols	+	+	+	+	+
Saponions	+	+	+	+	+
Tannins	_	+	+	-	-
Cardiac glycosides	+	+	-	+	+
Terpenoids	+	-	-	+	+
Protein	+	+	_	+	+
Carbohydrate	+	+	+	+	+
Alkaloids	-	+	+	+	+

+ indicates presence & - indicates absence

3.2 Quantitative Estimation of Phytochemical of green synthesized Liv-Pro-08

The results of quantitative analysis on four major groups of phytochemical constituents of extract green synthesized Liv-Pro-08 compared to crude extractanalyzed were depicted in Table 2that reveals the quantitative assessment of secondary metabolites (flavonoids, alkaloids, saponins & TPC). The total phenolic content value of extract of crude and green synthesized Liv-Pro-08measured by Folin'sciocalteau reagent in terms of tannic acid equivalent is 13.16±0.31 mg/mL. The results strongly suggest that the phenolics are important component of this plant and some of the pharmacological effect could be attributed to the presence of this invaluable component. It is believed that phenolic compounds contribute to quality and nutritional value in terms of modifying color, taste, aroma, and flavor and also in providing health beneficial effects.

Table 2: Quantitative Estimation of Phytochemical of green synthesized Liv-Pro-08

Phytochemicals	Crude Extract Liv-Pro-08	AgNP Liv-Pro-08	CuNP Liv-Pro-08	ZnNP Liv-Pro-08	MgNP Liv-Pro-08
Flavonoids	17.25±0.47	19.71±0.91	13.31±0.64	11.71±0.21	12.71±0.27
Total Phenol Content	10.36±0.24	13.16±0.31	12.60±0.26	16.16±0.11	12.19±0.13
Saponins	11.17±0.32	15.11±0.27	12.21±0.19	14.11±0.22	10.1±0.17
Alkaloids	13.25±0.36	16.47±0.32	14.27±0.26	11.58±0.27	12.24±0.43

Values are expressed as mean± SD of three replicates

© 2019 Life Science Informatics Publication All rights reserved

3.3 Determination of the antioxidant property of green synthesized Liv-Pro-08 – *In vitro* study

The green synthesized NPs and aqueous extract of the Liv-Pro-08 was subjected to various experimental procedures Free radical scavenging assays:(1,1-diphenyl-2- picrylhydrazyl [DPPH], Nitric oxide radical scavenging activity, hydrogen peroxide radical scavenging activity.

3.3.1 DPPH radical scavenging activities of Liv-Pro-08 NPs in comparison with ascorbic acid and crude extract

The scavenging capacity of DPPH free radical is widely used to analyze the antioxidant potential of naturally derived foods and plants [16]. The DPPH radical scavenging potential of the different extracts is represented in Table 3 indicate all the extracts showed an inhibitory potential against DPPH free radical. The inhibitory percentages vary from 21.07±1.68 for the aqueous extract from the Liv-Pro-08 to 83.70 ± 4.42 for the vitamin C. The AgNPs from the Liv-Pro-08 has the highest and significant inhibitory potential among the extracts samples tested at the different concentrations compared to the other extracts of CuNPs, MgNPs and ZnNPs indicating DPPH Values were increased in dose dependent manner[17]. Plant acts as electron donors because of their content in phenolic compounds[18] may justify the DPPH radical scavenging power noted in the extracts tested which corroborates previous study which explained that DPPH scavenging properties of plant extracts increase with the concentration of extracts [19,20&21] The 2,2-diphenyl-1-picrylhydrazyl (DPPH) activity results showed the effective free radical % scavenging potential of AgNPs and crude extract as 35.44± 3.71 to 74.10±2.14 respectively (Figure.1) indicate enhanced scavenging activity with the increase in concentration similar to chitosan and torolex coated gold nanoparticles [22] silver nanoparticles, selenium, platinum [23]have been reported.

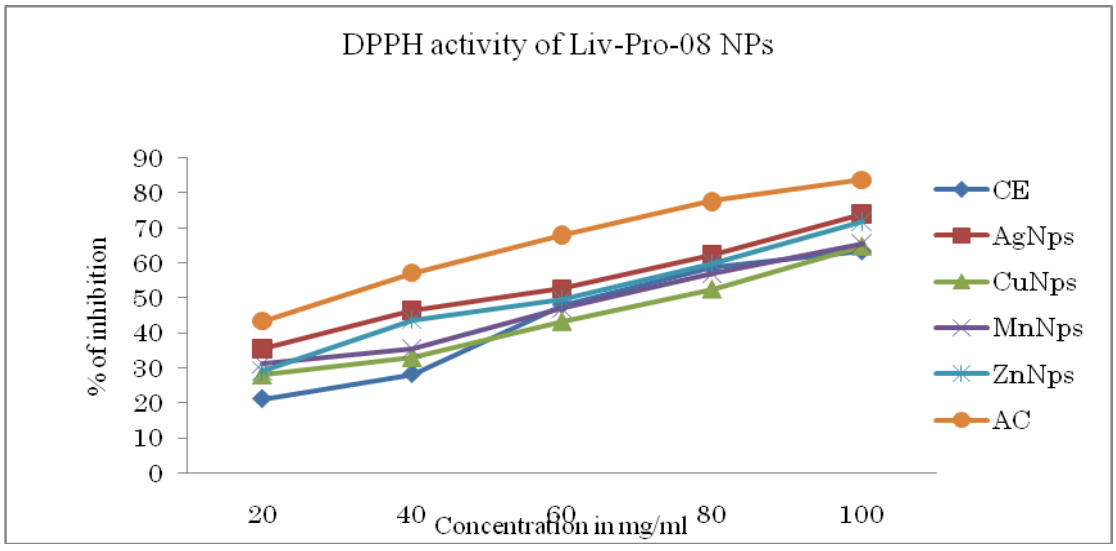
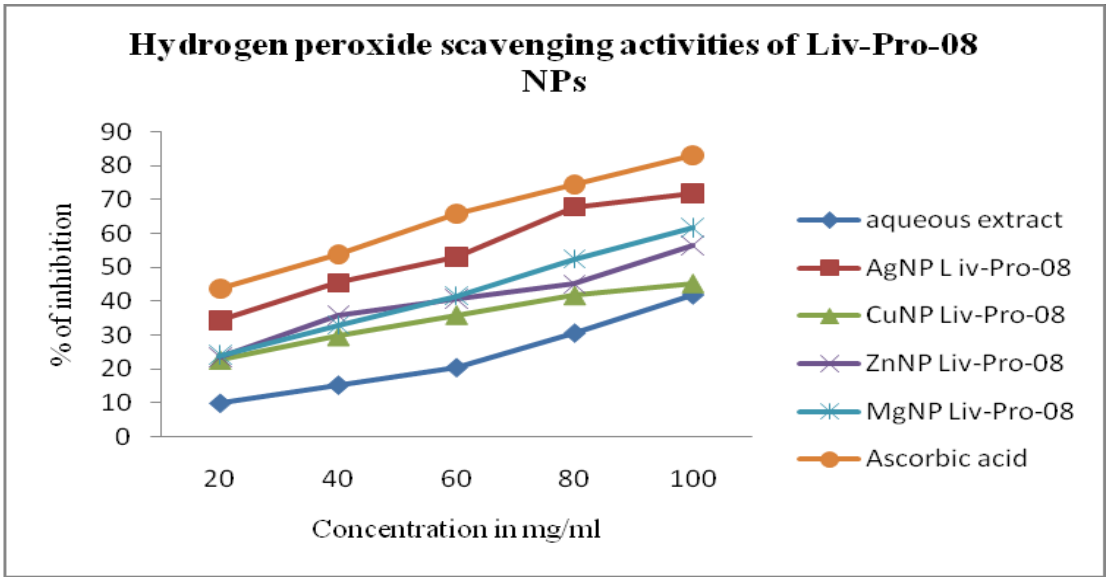


Figure 1: DPPH scavenging activity of Liv-Pro-08 NPs in comparison with ascorbic acid and aqueous extract

© 2019 Life Science Informatics Publication All rights reserved



Values are expressed as mean± SD of three replicates

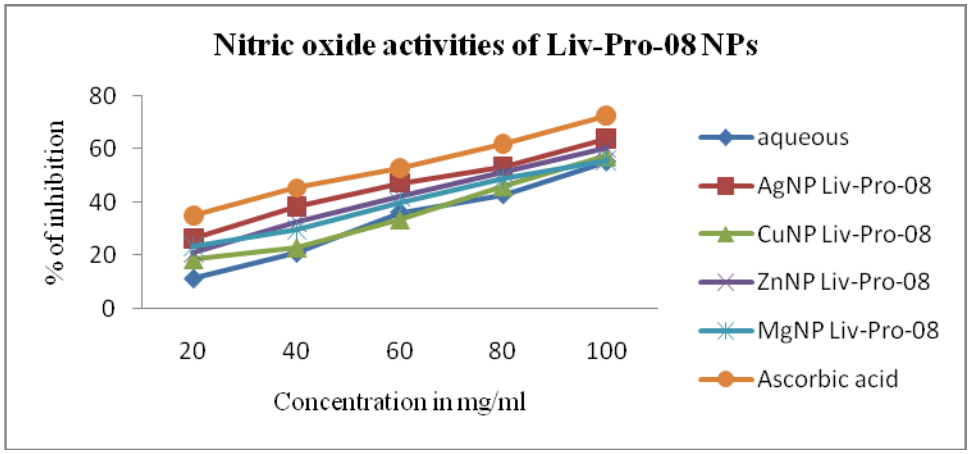
Figure 2: The Hydrogen peroxide scavenging activity of Liv-Pro-08 NPs in comparison with ascorbic acid and aqueous extract

3.3.2 The Hydrogen peroxide scavenging activity of Liv-Pro-08 NPs in comparison with ascorbic acid and aqueous extract

H_2O_2 is measured as one of major inducers of cellular aging and could attack numerous cellular energy-producing systems [24].Hydrogen peroxide (H_2O_2) is a byproduct of respiration made in all living cells which was harmful and must be removed as soon as it is produced in the cell. The production of even low levels of H_2O_2 in biological systems may be important. Cells produce enzyme catalase to remove hydrogen peroxide. Hydrogen peroxide scavenging activity depends upon the phenolic content present in the extract, which can donate electrons to H_2O_2 and thereby neutralizing it in to water. Activity of Hydrogen peroxide scavenging in greensynthesized nanoparticles and crude extract is shown in figure.2 shows activity in dose dependent manner. The obtained results are compared with standard ascorbic acid. The Hydrogen peroxide radical induces severe damage to adjacent biomolecules such as lipids, proteins and DNA[25]. Geetha RV *et al* reported that ethanolic extract of the *Aesculushippocastanum* was capable of scavenging H_2O_2 in a concentration dependent manner. At a concentration of 100µg,the test extract showed 71.69±2.84 scavengingactivity. Thus, the present study demonstrated thesignificant antioxidant activity of the extracttested [26]. However, AgNPs exhibited higher H_2O_2scavenging activity than CuNPs,ZnNPs & MgNP. suggest that AgNPs was an excellent platform to scavenge the ROS and there is an immense potential for the biologically synthesized AgNPs as a natural basis of antioxidants [12 & 27].

© 2019 Life Science Informatics Publication All rights reserved

3.3.3 The Nitric oxide activities of Liv-Pro-08 NPs in comparison with ascorbic acid and aqueous extract



Values are expressed as mean± SD of three replicates.

Figure 3: The Nitric oxide activities of Liv-Pro-08 NPs in comparison with ascorbic acid and aqueous extract

Nitric oxide (NO), being a potent pleiotropic mediator inphysiological system and a diffusible free radical in thepathological conditions, reacts with superoxide anion and form apotentially cytotoxic molecule, the 'peroxynitrite (ONOO-)'. Itsprotonated form, peroxynitrous acid (ONOOH), is a very strong oxidant [28]. The main way of damage is the nitration or hydroxylation of aromatic compounds, particularly tyrosine. Underphysiological conditions, peroxynitrite also forms an adduct withcarbon dioxide dissolved in body fluid and responsible for oxidative damage of proteins [29] in living systems.Aqueouswas found to be lessscavenging on nitric oxide (55.20±4.00), when compared to dose independent ability of AgNPs(64.12±2.82) and to ascorbic acid, the reference compound. Studies were made on total reduction ability of Fe3+ to Fe2+ transformation in the presence of both extracts and found increasing in showing reduction ability in a dose dependent manner, with increasing concentrations. Since the reducing capacity of a compound serve as a significant indicator of its potential antioxidant activity, the reducing ability of phyto extracts are measured in this study. The antioxidant activity has been reported to be concomitant with development of reducing power [30]. A similar results were found with silver nanoparticles synthesized by using plant leaves *Excoecari aagallocha* [31].

4. CONCLUSION

In this study, antioxidant activity of the aqueous and green synthesized NPs of Liv- Pro-08 has been investigated using three different assays. The phytochemical potential of green synthesized Liv-Pro-08 nanoparticles exemplified with margin enhanced invitro antioxidant scavenging activity and exhibited wide range of secondary compounds indulged in herbal formulation Liv-Pro-08.

© 2019 Life Science Informatics Publication All rights reserved

ACKNOWLEDGEMENT

We are grateful to thank Periyar University, Salem for granting University Research Fellowship to carry out this part of work.

CONFLICT OF INTEREST

We do not have any conflict of interest for the published data.

REFERENCES

1. Suriyavathana. M. Studies on phytochemical analysis and antioxidant activity of selected medicinal plants from kolli hills. J. Pharm Res. 2010; 3(2):260-262
2. Mathekaga. AD and Meyer JJM. Antibacterial activity of South African *Helichrysum* species. South Afr J Bot. 1998;64:293-5.
3. Bobbarala V, Bramhachari PV, Ravichand J, Reddy YHK, Kotresha D, Chaitanya KV. Evaluation of hydroxyl radical scavenging activity and HPTLC fingerprint profiling of *Aeglemarmelois (L.)* Correa extracts. J Pharm Res. 2011; 4(1): 252-255.
4. Choudhury PA, Kumar A, Reddy AVR, Garg AN. Thermal neutron activation analysis of essential and trace elements and organic constituents in Trikatu: an Ayurvedic formulation. J RadioanalNucl Chem. 2007; 274:411-419.
5. Randhawa MA, Al-Ghamdi MS. A review of the pharmacotherapeutic effects of Nigella sativa. Pakistan J Med Res. 2002;41:77-83.
6. Rao BR, Murugresan T, Sinha S, Saha BP, Pal M, Mandal SC. Glucose lowering efficacy of Ficus racemosa bark extract in normal and alloxan diabetic rats. Phytother Res. 2002;16: 590-592.
7. Hedrick UP. Sturtevant's edible plants of the world. New York: Dover Publications;1972.
8. Parashar UK, Saxena PS and Srivastava A. Bioinspired synthesis of silver nanoparticles. Digest Journal of Nanomaterials and Biostructures. 2009; 4(1):159 –166.
9. Harborne JB. Phytochemical Methods. Chapman and Hall Ltd., London, 1973,49-188.
10. Makkar H, Bluemmel M, Borowy N, Becker K. Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. J Sci Food Agric. 1993; 61:161-165.
11. Obdoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the crude extracts of some Homostatic plants in Edo and Delta States of Nigeria. Glob J Pure Appl Sci. 2001; 8b:203-208.
12. Arumugam P, Ramamurthy P, Santhiya ST, Ramesh A. Antioxidant activity measured in different solvent fractions obtained from *Menthaspicata*Linn: An analysis by ABTS. + decolorization assay. Asia Pac J ClinNutr. 2006; 15(1):119- 124.
13. Singh RP, Chidambara Murthy KN, Jayaprakash GK. Studies on antioxidant activity of pomegranate peel and seed extracts using in vitro models. J Agric Food Chem.2002;

© 2019 Life Science Informatics Publication All rights reserved

50:86-89

14. Garret DC. The quantitative analysis of drugs, champman and Hall, Japan.1964; 3:456-458..
15. Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis 1989; 10: 1003-1008.
16. Alam N, Hossain M, Khali M, Moniruzzaman M, Sulaiman S, Gan S. High catechin concentrations detected in *Withaniasomnifera* (ashwagandha) by high performance liquid chromatography analysis. BMC Complementary and Alternative Medicine. 2011;11:65.
17. Mohamed S. Abdel-Aziz, Mohamed S. Shaheen , Aziza A. El-Nekeetys, Mosaad A. Abdel-Wahhab,Antioxidant and antibacterial activity of silver nanoparticles biosynthesized using *Chenopodium murale* leaf extract, J of Saudi Chemical Society. 2014; 18, 356–363
18. Duthie S, Dobson V. Dietary flavonoids protect human colonocyte DNA from oxidative attack in vitro. Eur J Nutr. 1999;38:28–34.
19. Gul M, Ahmad F, Kondapi A, Qureshi I, Ghazi I. Antioxidant and antiproliferative activities of *Abrusprecatorius* leaf extracts - an in vitro study. BMC Complement Alternat Med. 2013; 13(53).
20. Kumar S, Kumar R, Dwivedi A, Pandey AK: In Vitro Antioxidant, Antibacterial, and Cytotoxic Activity and In Vivo Effect of *Syngonium podophyllum and Eichhornia crassipes* Leaf Extracts on Isoniazid Induced Oxidative Stress and Hepatic Markers. BioMed Research International. 2014.
21. Hilmi Y, Abushama M, Abdalgadir H, Khalid A, Khalid H. A study of antioxidant activity, enzymatic inhibition and in vitro toxicity of selected traditional sudanese plants with anti-diabetic potential. BMC Complement Alternat Med 2014; 14(149).
22. Z. Nie, K.J. Liu, C.-J. Zhong, L.-F. Wang, Y. Yang, Q. Tian, Y. Liu. Enhanced radical scavenging activity by antioxidant-functionalized gold nanoparticles: a novel inspiration for development of new artificial antioxidants. Free Radic. Biol.Med.2007; 43(9):1243-1254.
23. J.P. Saikia, S. Paul, B.K. Konwar, S.K. Samdarshi. Nickel oxide nanoparticles: a novel antioxidant. J. Colloid. Surf. B. Biointerfaces.2010; 78 (1): 146-148.
24. J. Liu , L. Jia , J. Kan , C.H. Jin . In vitro and in vivo antioxidant activity of ethanolic extract of white button mushroom (*Agaricus bisporus*) Food Chem. Toxicol. 2013; 51:310 316.
25. Sunita Patil, P Rajiv, Rajeshwari Sivaraj. An Investigation Of Antioxidant And Cytotoxic Properties Of Green Synthesized Silver Nanoparticles, Indo Ame J.Pharm Sci, 2015; 2 (10): 1453-1459.
26. R. V. Geetha, Anitha Roy, Sitalakshmi. In Vitro Antioxidant and free Radical Scavenging activity of the Ethanolic extract of *Aesculus hippocastanum*,Int. J. Drug Dev. & Res. 2013; 5(3): 403-407.

© 2019 Life Science Informatics Publication All rights reserved

27. Saumya S M & Mahaboob Basha P. In Vitro evaluation of free radical scavenging activities of *Panax ginseng* and *Lagerstroemia speciosa*: A comparative analysis. Int. J. Pharm and Pharma Sci. 2011; 3(1): 165-169.
28. Malinski T. Nitric oxide and nitroxidative stress in Alzheimer's disease. J. Alzheimers Dis 007; 11: 207-218.
29. Szabó C, Ischiropoulos H, Radi R. Peroxynitrite: Biochemistry, pathophysiology and developm ent of therapeutics. Drug discovery. 2007; 6: 662.
30. Yang JH, Lin HC, Mau JL. Antioxidant properties of several commercial mushrooms. Food Chem 2002; 77: 229-235.
31. Arun S, Saraswathi U, Singaravelu. Investigation on the free radical scavenging activity of biogenic silver nanoparticle. Int J Pharm Bio Sci, 2015; 6(2):439 -445.

© 2019 Life Science Informatics Publication All rights reserved