ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: https://ijrps.com

Comparative study on the free radical scavenging potency of *Vigna radiate* sprouts and its zinc oxide nanoparticle

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Article History:

Received on: 06.01.2018
Revised on: 22.05.2018
Accepted on: 27.05.2018

Keywords:

UV,
FTIR,
SEM,
ABTS,
Reducing potential

ABSTRACT

Seeds and sprouts of *Vigna radiata* (VR), commonly known as Mung bean, is a nutritionally potent common food. Though the seeds and sprouts of VR are not traditionally used as folklore medicine, various pharmacological activities are evaluated scientifically. The present study aims to Synthesize and characterize Zinc oxide nanoparticles from sprouts of VR and compare its free radical scavenging potency against an ethanolic extract of *Vigna radiata* (VR) and its sprouts (VRS). Zinc oxide nanoparticle from VRS is synthesized and characterized by UV, FTIR spectroscopy and SEM. The free radical scavenging potency of the synthesized nanoparticle, VR, VRS is evaluated by ABTS radical scavenging assay and reducing potential. UV, FTIR and SEM image of synthesized Zinc oxide nanoparticle reflects the morphology of nanoparticle. In *In-vitro* free radical scavenging activity, ZnO Nanoparticle does not exhibits its reducing potential activity. ABTS radical scavenging activity of VRS is significantly better than that of VR and ZnO Nanoparticle ($p<0.01$). In conclusion, ethanolic extract of *Vigna radiata* sprouts is a potent free radical scavenger than that of ethanolic extract of *Vigna radiata* and Zinc oxide nanoparticle synthesized from *Vigna radiata* sprouts.

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ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v9i3.1598

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INTRODUCTION

Nanotechnology, an advanced Engineering field, exhibits its development starts with food, clothes, extends their applications up to agriculture, medicine etc., (Gurjas *et al.*, 2012). Nanomedicine, an important application of nanotechnology, comprises of nanomaterials and biological devices. Many devices such as a biosensor, nanoelectronic instruments, pacemakers, monitoring apparatus and advanced ECG machines, all these terrific machines are the invention of nanomedicine. Nanomaterials are more useful for *In vivo* and *In vitro* biomedical research and applications. The most advanced form of nanomedicine uses the nanorobots and nano instruments as surgeons. These kinds of machines might repair damaged cells, or get into the cells and replace or assist damaged intracellular structures at individual stage (Wagner, 2006) nanomedicine spreads its applications in the development of numerous devices like chemotherapy, biochips, insulin pumps, needleless injectors and drug injecting systems. Interaction of nanomaterials with biology leads to the development of drug delivery vehicles.

Green synthesis of nanoparticles refers to the concept of synthesizing nanoparticles from metal salts by using the reducing property of biologically active compounds like secondary metabolites of plants. Biologically active reducing compounds are used to produce metal nanoparticle with effective therapeutic potential (Meiwan *et al.*, 2011). Gold, silver, Zinc oxide, the copper nanoparticle can be synthesized by using herbal extract. Plant extracts are the best reducing agents due to the active compound like polyphenol, which has reduced potential due to the –OH group.

Owing to very high surface area-to-volume ratio, metal nanoparticles possess effectiveness to carry drugs to specific target organs. Small sized hollow sphere metallic nanoparticles can carry drugs to the target region and mediate their effects. 40 nm sized nanoparticle is commonly used for drug delivery (Iram 2014*).*

Zinc oxide (ZnO) nanoparticle or nanopowders exhibit antibacterial, anti-corrosive, antifungal and UV filtering properties. One of the most important features of ZnO nanomaterials is low toxicity and biodegradability (Kyung *et al*., 2018). Majority of studies indicate that ZnO nanoparticles do not penetrate the skin and cause recognizable illness (Nohynek *et al*., 2008). Zn^{2+}plays an important role in various metabolic reactions. It is slowly dissolved in both acidic and alkaline media thereby exhibits its stable activity in normal and tumour cells. Thus, ZnO nanomaterials are more important in biomedical applications (Liu *et al*., 2006).

ZnO nanomaterials have a very large surface area which provides the flexible platform for drug delivery. They are highly toxic towards bacterial and cancer cells. McCormick. (2001) Has reported that ZnO nanoparticle exhibits a promising role in gene therapy and gene delivery. Green synthesis of ZnO nanoparticles from various herbs are reported earlier (Parthasarathy *et al*., 2011). The antioxidant and anticancer activity of ZnO nanoparticle synthesized has been reported earlier by Jeba *et al*., (2017).

Seeds of *Vigna radiata* belongs to the family *Fabaceae*, commonly known as mung bean, is a rich source of nutrients. Its sprouts are used by dieticians as a salad for breakfast to accomplish the balanced diet. Mung beans are well known for their detoxification activities. Mung bean protein, tannin, and other polyphenols are thought to combine with organophosphorus pesticides, mercury, arsenic, and other heavy metals, promoting the excretion of sediments from the body (Zhang, 1995).

Medicinal plants are the principal source of pharmaceutical agents (Mohanasundaram *et al*., 2011) and contain flavonoids, phenolic acids, organic acids and lipids. Twelve phenolic acids have been identified from mung bean seeds and sprouts (Dongyan *et al*., 2014). Flavone, isoflavone, flavonoids, and isoflavonoids are the important metabolites found in the mung bean (Wang *et al*., 2008) Twenty-one organic acids, including phosphoric and citric acid, and 16 lipids, including γ- tocopherol, were reported to be the major components of mung beans by gas chromatography/mass spectrometry (GC/MS) (Bowles, 1990). Various pharmacological activities like antioxidant, antimicrobial, anti-ulcer, anti-diabetic, anti-hypertensive, anti-tumour effect of mung bean and its sports are reported earlier (Ali *et al*., 2014)

The present study aims to synthesize and characterize ZnO nanoparticle from sprouts of *Vignaradiata* and to evaluate and compare the free radical scavenging potency of Vigna radiate sprout extract tagged ZnO nanoparticle against Vitamin C.

MATERIALS AND METHODS

Preparation of sprout from *Vigna radiata seeds*

Vigna radiata seeds were purchased from the grocery, Chennai. 5.0 gm of washed seeds were soaked in 100.0 ml of water for 4 h. The water was removed and soaked seeds were allowed to stand at room temperature for 8 h at the night time. The sprouts developed were used for the preparation of Zinc oxide nanoparticle.

Preparation of ethanolic extract from seeds (VR) and sprouts (VRS) of *Vigna radiata*

100.0 g of sprouts and seeds were individually crushed into coarse powder and soaked in 1000.0 ml of ethanol. The mixture was allowed to stand at room temperature for 72 h. The extract was filtrated and concentrated under reduced pressure using a rotary vacuum evaporator. The trace of the final solvent was removed by drying at hot air oven. The concentrated extract was stored at refrigerator until used.

Synthesis of Zinc oxide nanoparticle from *Vigna radiata seeds* (ZnONP)

10.0 g of sprouts were grinding with distilled water and the volume was made to 50.0 ml with distilled water. The mixture was kept in a water bath and the temperature was maintained at 60-70^{0}C. The extract was filtered using Whatman No.1 filter paper. 10.0 ml of filtrate was added to 300.0 ml of 4.0 mM of Zinc sulphate and stirred well at room temperature for 10 min. 1.0 M NaOH was added to the mixture in drops and pH was adjusted to 7.0. The content was then incubated at room temperature overnight. The content was filtered using Whatman No.1filter paper. The residue obtained was kept in the oven until it was completely dried. The crystals obtained were stored at room temperature for future use.

The bioreduction of ZnO nanoparticles using VRS aqueous extracts was monitored by measuring the UV-Visible spectroscopy. The UV-Visible absorption spectra of the reaction media were recorded at room temperature in a quartz cuvette (1 cm path length) and at the wavelength ranging from 200 to 700 nm using a Systronics Double Beam Spectrophotometer (Model 2202, Systronics Ltd.). Fourier transform infrared spectroscopic measurements were done using Shimadzu, IR-Prestige-21 spectrophotometer. Scanning electron microscopy (SEM) analysis of synthesized zinc oxide nanoparticles was done using a Quanta FEI 450 SEM machine.

ABTS radical scavenging activity

Re et al. (1999) determined the ABTS radical scavenging activity of VR, VRS and ZnONp. A stock solution of ABTS radical cation was prepared by dissolving ABTS (7 mM, 25 ml in deionized water) with potassium persulfate ($K_2S_2O_8$) (140 mM, 440 µl). The mixture was left to stand in the dark at room temperature for 15-16 h (the time required for formation of the radical) before use. For the evaluation of ABTS radical scavenging activity, the working solution was prepared by the previous solution and diluting it in ethanol to obtain the absorbance of 0.700 ± 0.02 at 734 nm. Different concentrations of the VR, VRS, ZnONP were mixed with the ABTS working solution (1.9 ml) and the reaction mixture was allowed to stand at room temperature for 20 min, then the absorbance was measured by using a UV-visible spectrophotometer at 734 nm.:

ABTS radical scavenging effect (%) = [(Control O.D. –Test O.D). /Control O.D] × 100

Total reduction capacity (Sherikar and Mahanthesh, 2015)

Different concentrations of VR, VRS ZnONP, was mixed with phosphate buffer (2.5 ml, 0.2 mol/L, pH 6.5) and potassium ferricyanide (2.5 ml, 1%). Then, the mixture was incubated at 50°C for 20 min. At the end of the incubation, TCA (2.5 ml, 10%) was added to the mixture, which was centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was collected and mixed with 2.5 ml of Millipore water and ferric chloride (0.5 ml, 0.1%). The absorbance was measured at 700 nm against a blank. Increased absorbance of the reaction mixture indicates increased reducing power. The test was performed in triplicate and the results were averaged.

Statistical Analysis: Values are Mean ±SD of triplicate. The significant difference is observed using one way ANOVA and significant difference is observed at $p<0.01$.

RESULTS AND DISCUSSION

Reactive oxygen species (ROS) are formed from oxygen. It plays an important role in signal transduction and maintaining homeostasis. An excess amount of ROS may damage the cell structure. Ionising radiation can cause the development of free radicals like ROS, RNS which may cause various disorders like cancer, atherosclerosis, Diabetes mellitus etc., (Taibur *et al*., 2012). Any drug, which acts as an antioxidant, can protect the cell from the development of numerous diseases.

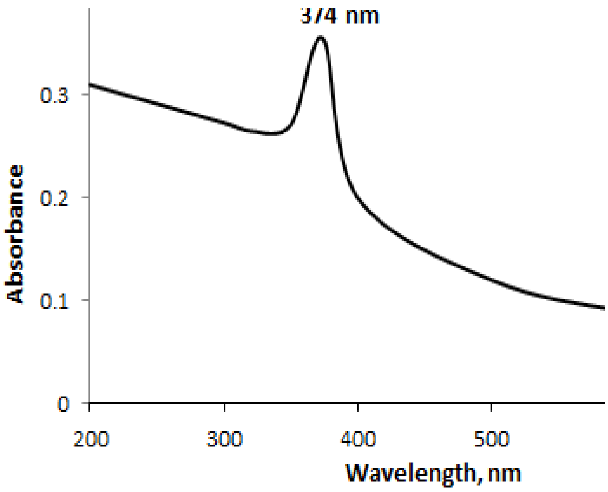

Figure 1: UV Spectrum of ZnONP from *Vigna radiata* sprout

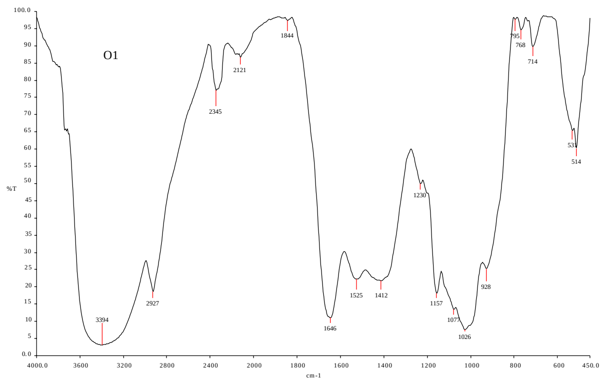

Figure 2: FTIR Spectra of ZnONP from *Vigna radiata* sprout

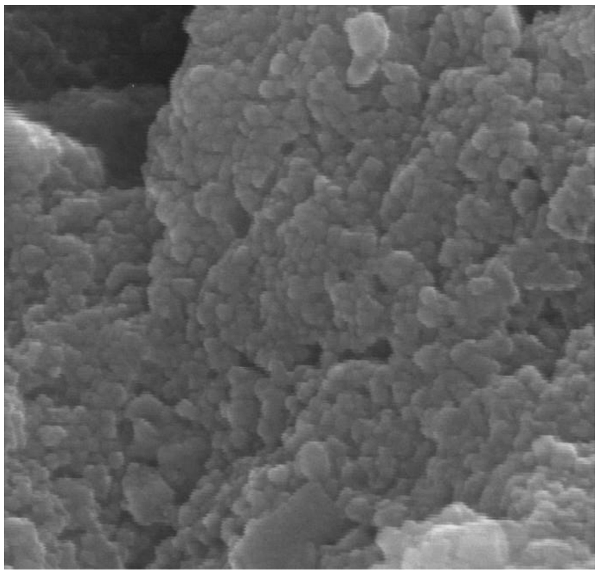

Figure 3: SEM Image of ZnONP from *Vigna radiata* sprout

In ancient days, Indian Pharmacopoeia is fully dominated with herbal medicine. Nowadays, many commercially available synthetic drugs are originated from herbal medicine. Nowadays, research on pharmacology associated with pharmacognosy

Table 1: ABTS radical scavenging ability of an ethanolic extract of *Vigna radiata* seeds (VR), sprouts (VRS), and ZnONP from *Vigna radiata* sprout

Conc. (µg/ml)	%Inhibition		
	ZnONP	VR	VRS
5	14.6 ± 0.2	14.7 ± 0.3	19.2 ± 0.4
10	15.9 ± 0.4	21.4 ± 0.2	23.5 ± 0.3
20	17.6 ± 0.6	23.8 ± 0.3	31.3 ± 0.31
50	18.7 ± 0.8	25.0 ± 0.3	40.0 ± 0.4
100	19.1 ± 0.3	27.1 ± 0.2	53.5 ± 0.3
200	21.6 ± 0.5	53.3 ± 0.3	80.8 ± 0.3
400	30.8 ± 0.5	77.2 ± 0.2	99.0 ± 0.1
800	47.0 ± 0.3	94.5 ± 0.2	99.4 ± 0.0
1600	70.8 ± 0.3	99.3 ± 0.04	99.793±0.071
IC_{50}	850.8 ± 12.3 [a]	187.62 ± 9.8 [b]	93.4 ± 5.6 [c]

Note: Values are Mean ± SD of triplicate. The significant difference is observed using One way ANOVA. [a, b, c] Significant difference is observed at the level of p<0.01

Table 2: Reducing potential of ethanolic extract of *Vigna radiate* seeds (VR), sprouts (VRS), and zinc oxide nanoparticle encapsulated with *Vigna radiata* sprout (ZnONP).

Conc. (µg/ml)	Optical Density		
	ZnONP	VR	VRS
20	No change in optical density	0.1267±0.015	0.16±0.01
40		0.136±0.015	0.18±0.02
60		0.15±0.01	0.203±0.015
80		0.156±0.0057	0.223±0.015
100		0.18±0.01	0.24±0.01
200		0.2±0.01	0.486v0.015
500		0.223±0.015	0.53±0.01 *

Note: Values are Mean ± SD of triplicate. The significant difference is calculated by Student's 't' test between the optical density of seed and sprout extract at 500 µg. * - Values are differed significantly at p<0.01.

and nanotechnology is leading the research track. Research evaluating the antioxidant activity of herbal extracts and nanoparticles isa usual and necessary research work. Antioxidant activity of various herbs has been reported earlier (Khaled, 2014). Likewise, the antioxidant activity of ZnO nanoparticles synthesized from various herbs like *Ruta graveolens* has also been reported (Lingaraju *et al*., 2016).

In the present study, ZnO nanoparticles have been synthesized from VRS and characterized by UV spectra, FT-IR spectra and SEM. UV spectrum of Zinc oxide nanoparticle shows the maximum absorbance at 372 nm (Fig 1). Zinc oxide nanoparticle synthesized from *Oleaeuropea* shows maximum absorbance at 374 nm (Khoushika and Brinda, 2017).

FTIR spectra (Table 2) of Zinc oxide nanoparticle exhibits wave number at various cm^{-1} as follows: 3394, 2927, 2345, 2121, 1844, 1646, 1525, 1412, 1230, 1157, 1077, 1026, 928, 795, 768, 714, 531, 514 cm^{-1} (Fig 2). In FTIR, wave number 3394 cm^{-1}shows O-H, 2927 cm^{-1}shows C-H, 2121 cm^{-1} and 928 cm^{-1}shows C-C, 1230 cm^{-1}, 1157 cm^{-1}, 1077 cm^{-1}, 1026 cm^{-1}shows C-O, 1646 cm^{-1}shows C=O, 795 cm^{-1}shows N-O, 768 cm^{-1},714 cm^{-1} for N-H, 531 cm^{-1},514 cm^{-1} shows S-S, 2324 cm^{-1}shows P-H. The synthesized Zinc oxide nanoparticle consists of the above mentioned functional groups.

FTIR spectra of ZnO nanoparticle synthesized from *Ixora Coccinea* has been reported earlier (Snehal *et al*., 2016). Kumari *et al*., (2017) have reported the FT-IR spectra of silver nanoparticle synthesized from legume of Vigna radiate seeds

SEM image of Zinc oxide nanoparticle synthesized from VRS extract is furnished in Fig 3. A large number of nanosheets of ZnO nanoparticles are overlapped together and form a flower-like structure with the size of 500 nm. The dense morphology observed in Fig 3 might be due to overlapping of thin sheets of ZnO where the individual sheets appear to have a lateral dimension of less than one micrometre.

SEM image of ZnO nanoparticle synthesized from *Passiflora caerulea* fresh leaves has been reported earlier by (Santhosh Kumar *et al*., (2017). Kumari et al. (2017) have reported SEM image of silver nanoparticle synthesized from VR.

Research evaluating the antioxidant activity of herbal extracts and nanoparticles is usual, most urgent and important. ABTS radical scavenging is a more sensitive and specific method to predict the free radical scavenging effect of any test compound (Ilhami *et al*., 2010). ABTS radical scavenging activity of various herbs is reported earlier (Ece *et al*., 2013). ABTS radical scavenging potency of VR and VBS has been reported earlier by Zhaohui *et al*., (2016)

In the present study, the VRS inhibits 50 % of ABTS radical generation at the concentration of 93.46 µg (Table 1), which is significantly lower than other samples ($p<0.01$) include VR and ZnONP This might be due to the presence of high phenolic content of ethanolic extract of VRS rather than VR extract (Table 1). Less ABTS radical scavenging activity of ZnONP might be due to the utilization of VRS have been used as a reducing agent for the formation of nanoparticles and they might not be available to scavenge ABTS like free radical.

Free radicals are scavenged by means of reduction. Compounds with high reducing ability can exhibit potent free radical scavenging activity. In the present study, reducing the potential ability of VRS was found to be significantly higher than that of ethanolic extract of VR at 500 µg/ml ($p<0.01$, Table 2). ZnONP does not exhibit any potential reducing ability. In the present study, a positive correlation has been observed between the ABTS radical scavenging activity of ZnONP and reducing potential.

CONCLUSION

In conclusion, ethanolic extract of *Vigna radiata* sprouts are more potent antioxidant than that of aqueous extract of ethanolic extract of *Vigna radiate* sprouts and ZnONP synthesized from Vigna *radiata* sprouts.

ACKNOWLEDGEMENT

Authors are grateful to Dr. Ishari Ganesh, Chairman, Vels University, his able administrative colleagues for allowing us to complete this work. The authors would like to thank the Faculty of the Department of Biochemistry, Vels University, for their valuable suggestions and assistance to complete this work successfully

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