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GREEN SYNTHESIS OF ZnO NANOPARTICLES FROM *FOENICULUM VULGARE*, ITS CHARACTERIZATION AND BIOLOGICAL POTENTIAL AGAINST BACTERIA

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ABSTRACT

The present research deals with synthesis, characterization, and biological potential of *Foeniculum vulgare* mediated zinc oxide nanoparticles (ZnO NPs). Extract of *Foeniculum vulgare* (a medicinal plant) was used to synthesize nanoparticles by reaction of extract with Zinc acetate (2M) solution. Synthesized ZnO Nps were characterized by various advance techniques such as FTIR, SEM, EXD, SEM and XRD. Debye–Scherrer equation ($D = k\lambda / \beta \theta \cos$) was used to calculate mean diameter of synthesized Nps utilizing XRD technique. Formation of ZnO Nps was confirmed by SEM analysis of resultant white colored stable Nps of reaction. The size of synthesized Nps was determined by SE technique which was found to be 22.94 nm. Phytochemical analysis reveals high content of secondary metabolites in NPs as compare to crude extract. The plant was observed with high content of flavonoids followed by phenolic compounds and then alcoholic compound. Order of different phytochemical was found to be flavonoids > Phenolic compound > alcoholic compounds. Plant's crude extract and plant mediated ZnO Nps were analyzed for biological potential with refence of antibacterial and antioxidant analysis. DPPH and TRP test were performed for antioxidant potential which showed positive response while antibacterial potential was evaluated by well diffusion method. Significant antibacterial potential was observed against *Escherichia coli* and *Staphylococcus aureus* bacterial strain. However, plant mediated ZnO NPs were not observed with potential to kill population of *Listeria monocytogenes* bacterial specie.

Key words: Medicinal Plants, *Foeniculum vulgare,* ZnO Nps, antibacterial activity

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INTRODUCTION

Nanotechnology is gaining attraction all over the scientific world. It stands among rapidly growing and dynamic field of research based on Nano-scale materials (Wilczewska *et al.*, 2012). The building blocks of nanotechnology are nanoparticles (NPs) which may be synthesized chemically, physically, and biologically (Iravani *et al.,* 2014). Biological methods are known as green methods because such synthesis is eco-friendly (Bello *et al.,* 2017), non-toxic and less harmful as compare to chemical and physical methods. Biological method may include synthesis by microbes or by mean of plant. Preparation of Nps from plant material is gaining attraction due to use of less chemical throughout entire procedure because natural products present in plants have capability to act as reducing and capping agent (Valli *et al.,* 2012) during synthetic procedure. Merging of nanotechnology and medicinal potential of plant is bringing revolution in pharmaceutical industry. Another attracting cause towards nanotechnology is enormous and effective use of nanoparticles for various purposes. Applications of NPs depend on size and structure of nanoparticles (Kelly *et al.,* 2003).

Foeniculum vulgare is a medicinal plant, commonly known as fennel. The plant belongs to family Apiaceae which is also called Umbelliferae (Mahfouz *et al.,* 2007). The plant is found to be cultivated in every country of world (Muckensturm *et al*.,1997). Many researchers have reported microbial potential in plant to cure infection caused by variety of microbes such as fungus, bacteria and protozoans (Dua *et al*., 2013; Kaur *et al*., 2009: Manonmani *et al*., 2011: Orhan *et al*., 2012; Morales *et al*., 2012). Many scientists reported various biological potentials of plant such as anti-oxidant (Choi *et al*., 2004: Ruberto *et al*., 2000), anti-tumor (Pradhan *et al*., 2008), anti-cancer (Zaahkouk *et al*., 2015), anti-fungal (Miraj *et al*., 2016-Mimica-Dukić *et al*., 2003), anti-inflammatory (Yang *et al*., 2014), and anti-bacterial (Kaur *et al*., 2009). Isolated essential oil from *F. valgare* plant is reported to possess hepato-protective potential (Ozbek *et al*., 2003). Koppula *et al*., (2013) has documented nerve relaxing power of fennel by reducing stress. They also reported memory enhancing potential (Koppula *et al*., 2013). The plant is found to be nutritive beneficial due to presence of essential fatty acid (Carvalho *et al*., 2010) and other natural products. Flavonoids and phenolic compounds have also been

isolated from extract of *F. vulgare* (Kunzemann *et al*., 1977).

Among metallic nanoparticles, ZnO Nps are found to be less toxic (Xia *et al*., 2008, Rasmussen *et al*., 2010). These NPs have been reported with anti-bacterial activities (Premanathan *et al*., 2011). ZnO NPs are reported to possess potential to fight against serious uncontrolled disease such as cancer (Hanley *et al*., 2008; Akhtar *et al*., 2012). The plant is observed to reduce concentration of free radicals, thus, preventing various diseases resulting from oxidative reactions (Das *et al*., 2013). The reported therapeutic potentials of ZnO NPs prove antibacterial, antioxidant and anticancer potential of Zinc-oxide nanoparticles. So, these NPs can be millstone for discovery of effective and advance drugs for number of serious diseases.

In current research, we investigated synthesis of ZnO NPs by eco-friendly procedure involving *in-situ* reduction of Zn by *F. vulgare* extract. We also reported biological potential of green synthesized Zinc-oxide nanoparticles. (ZnO-NPs) in term of antibacterial and antioxidant activity.

MATERIAL AND METHOD

Field area: *F. vulgare* (selected medicinal plant) was collected in August 2016 from Hazarganji, the region of Balochistan. The plant *F. vulgare* is identified with flora of Pakistan (Nasir, 1972).

Extract preparation and Nps synthesis: Plant samples were dried under shade for 5 days. Fully dried plant material was subjected for grinding to get fine powdered sample. 20 g of powdered material was dissolved in 200 ml distilled water with ratio 10:100. The mixture obtained was placed in oven for heating about 2-3 minutes. The extract was weighed and treated with 0.02M $C_4H_6O_4Zn.2H_2O$ (Zinc Acetate Dihydrate) solution. The step was followed by heating and constant stirring on hot plate for two hours. During synthesis, pH of mixture was maintained till 12 with help of NaOH (2M solution). Appearance of white colored precipitate indicates the synthesis of ZnO NPs which were later washed with methanol solution of commercial grade AA.

Bacterial Culture preparation: Selected bacterial species for current research were i.e., *Listeria monocytogenes*, *Escherichia coli, Klebsiella pneumonia* and *Bacillus subtilis.* All these species were provided by Microbiology department of Quaid e Azam University, Islamabad-Pakistan. All bacterial strains were maintained in nutrition agar broth. All species were subculture regularly in agar broth and stored at 4 ^{0}C.

Characterization: Structural features of *F. vulgare* mediated nanoparticles were characterized by FTIR, EDX, XRD and SEM. All analysis were made in triplicates. Data was analyzed through origin software.

Shimadzu-Model, XRD 6000, X-ray diffractometer was used to record XRD pattern on scanning mode to check out phase formation and to evaluate purity of green synthesized ZnO Nps. For this analysis, powder sample was used which was placed in XRD instrument operating at 40 kV with 30 mA current.

Size of green synthesized NPs was calculated by Debye–Scherrer equation $D = k\lambda / \beta \theta \cos$ where λ is x-ray wavelength which was 1.5418 A^0.

Reaction mixture for green synthesis of Nps was placed in centrifuge at speed 45 rmp for 40 mins for separation of solid NPs from mother liquor followed by filtration. Obtained Nps were dissolved in solvent and small amount of this solution was utilized for SEM and EDX analysis. Sample was analyzed in SEM using HITACH, Model S-3400 N instrument. ZnO Nps sample was placed in test tube and coated with sputter coater to prevent charging while solid dried sample of NPs was used for FTIR analysis using Shimadzu FTIR 8400s.

Antioxidant activity: Antioxidant activity was evaluated by Saeed *et al* 2012 with slight modification. For this assay, different concentration of methanolic crude extract and NPs were mixed with DPPH solution followed by spectroscopic analysis. Different concentration 0.02, 0.04, 0.06, 0.08, and 0.1mg/ml of crude extract and NPs were prepared through dilution using distilled water as solvent. In 1.5 ml of each concentration, 1ml of DPPH solution (0.1mM) was added. The mixture was placed in dark for 30 min at 37 ^{0}C. Each sample was analyzed at 517 nm by UV. Vis's spectrophotometer. Solution of ascorbic acid of same concentration ranging from (0.02-0.1 mg/ml) was use as control for anti-oxidant assay. The percentage of inhibition or scavenging of free radicals was determined by the following formula:

Percentage of radical capturing activity = (Abs(control)–Abs(sample))/Abs(control)× 100 %

Antibacterial activity: Antibacterial potential was evaluated against four human pathogenic bacteria names *Escherichia coli* and *Staphylococcus aureus*, *Listeria monocytogenes* and *Salmonella typhi* by standard well diffusion method. For this assay, measured quantity of 10 mg/ml of ZnO NPs was dissolved in DMSO solution. Bacterial colony was made to grow for overnight in nutrient broth having nutrient agar. For anti-bacterial potential of synthesized Nps, 10µg of ZnO Nps was dissolve in radical releasing solution which was DMSO which also act as negative control. For analysis Zinc Acetate Dihydrate solution of 0.02 M concentration was taken as positive control. Triplicated observation was made for each revealing antioxidant potential and results were expressed in term of Zone of Inhibition (ZOI).

RESULT AND DISCUSSION

Uses of medicine to cure diseases with no fear of harmful effect raise the need to identify the bioactive potential components of medicinally specified plants. Such bioactive components are called natural products and can prove milestones for pharmaceutical companies. A natural product of medicinal plants act as capping and reducing agent while synthesizing Nps and eliminate use of many chemical and proves to be safer with therapeutic application.

The detailed study on biosynthesis of zinc oxide nanoparticles by plant extract of *F. vulgare* was carried out and documented in this work. The research involves reduction of aqueous zinc ions into zinc oxide nanoparticles when added to medicinal plant extract of *F. vulgare.* Colour change from yellow to white. This change in colour indicated the formation of ZnO NPs.

Perkin–Elmer FTIR spectrophotometer was utilized for FTIR analysis to confirm the surface groups of the nanoparticles qualitatively. Mean diameter of plant mediated ZnO Nps was determined by XRD considering the line width of the plane and refraction peak using Debye–Scherrer equation while topology and size of green synthesized particles were determined by SEM analysis. This observation showed synthesis of high density poly-dispersed ZnO NPs of flower shape. ZnO NPs were observed to have 22.94 nm sizes. The presence of metal in NPs was analyzed by EDX technique.

FTIR analysis: Fig 1 contains FTIR spectra of ZnO Nps synthesized by *F. vulgare* plant extract. A reduced hydroxyl peak (transmittance peak) at 3550 cm $^{-1}$ was observed which indicates the synthesis of ZnO Nps and corresponds to –OH stretching band vibration. Peak for C—H bond appeared at 2550 cm $^{-1}$. Another peak was observed at 1500 cm $^{-1}$ which reflects stretching vibration of C—C bond. Spectra also confirmed the presence of carbonyl functional (—C=0) group by peak at 1010 cm $^{-1}$.

XRD analysis: XRD spectra (fig 2) showed distinct diffraction peaks at 31.75, 34.54, 36.21, 47.67, 56.66, 62.76 and 68.16 corresponding to (100), (002), (101), (102), (110), (103), (200), (112) and (201). These observations indicated hexagonal wurtzite structure for synthesized ZnO NPs. The XRD data *Foeniculum vulgare* mediated ZnO NPs was matched with the database of Joint Committee on Powder Diffraction Standards (JCPDS) file No. 36-1451. The average size of the ZnO Nps formed in the bio reduction method was determined to be 22.94 nm by using $D = k\lambda / \beta \theta \cos$ equation.

SEM: etermination of morphology of plant synthesized NPs was achieved by SEM analysis. For this analysis, dried sample of reaction mixture was used. A representative SEM micrograph of synthesized ZnO NPs is showed in fig 3. SEM analysis reveals spherical structures with a size of 22.94 nm.

EXD analysis: EXD analysis was carried out to determine presence of metal in sample of green synthesized NPs. Fig 4 (A and B) shows EXD spectra for *Foeniculum vulgare* mediated NPs. These spectra contain a peak at 1.23 keV, 8.88 keV and 9.57 keV which are absorption peak for Zn element. Another optical

absorption peak is also visible which is at 0.66 keV, this one is due to optical absorption of O element.

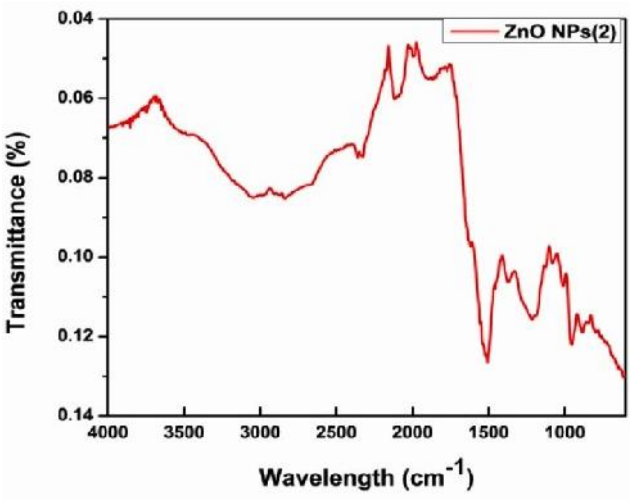

Figure 1. FTIR spectra for Foeniculum vulgare

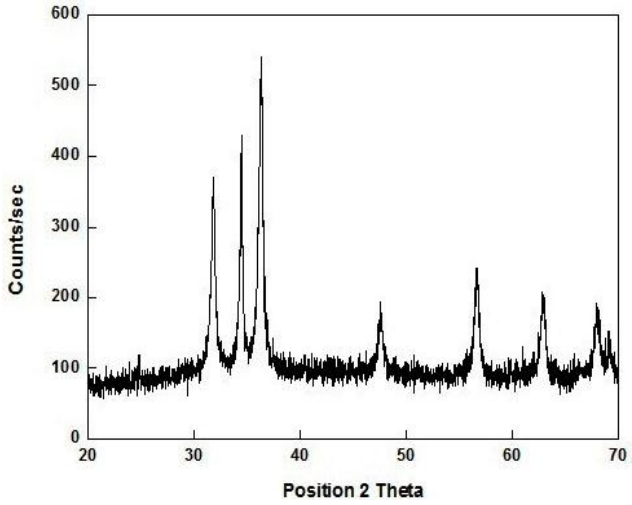

Figure 2. Foeniculum vulgare Mediated ZnO Nps.

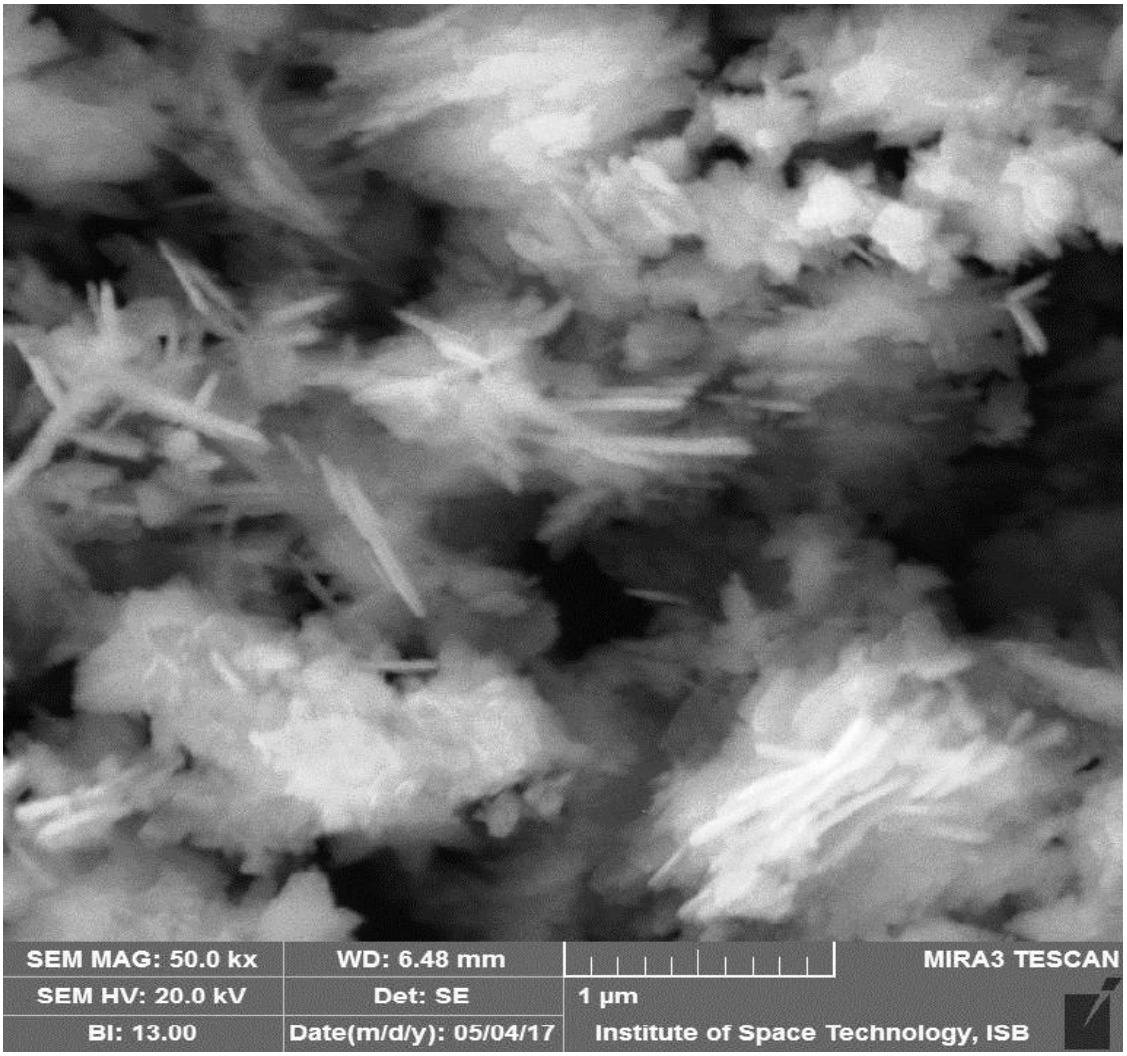

Figure 3. SEM result for Foeniculum vulgare -Mediated ZnO Nps

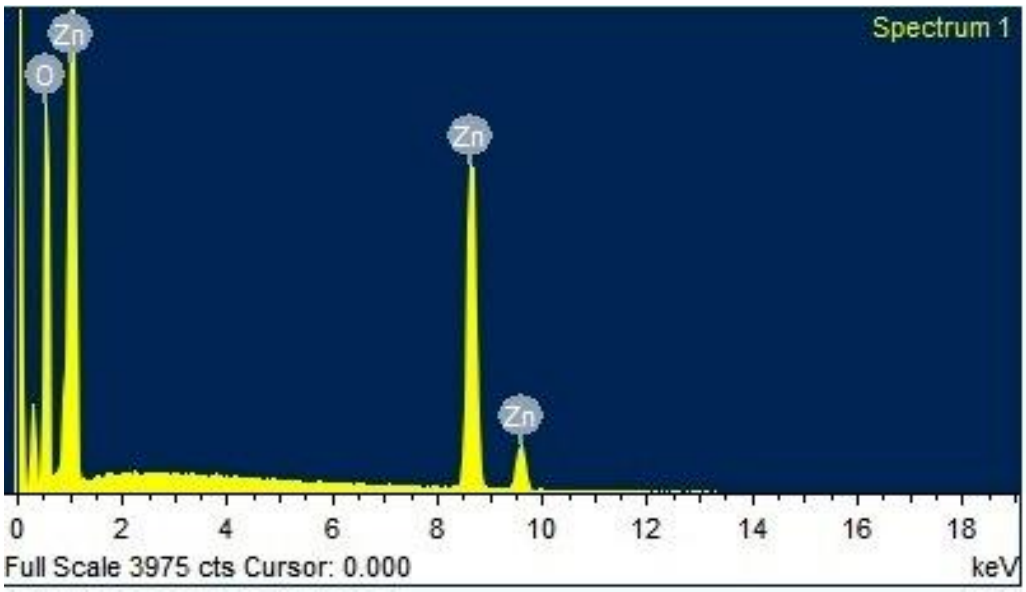

Fig 4 A. EXD spectrum showing presence of Zn and O element

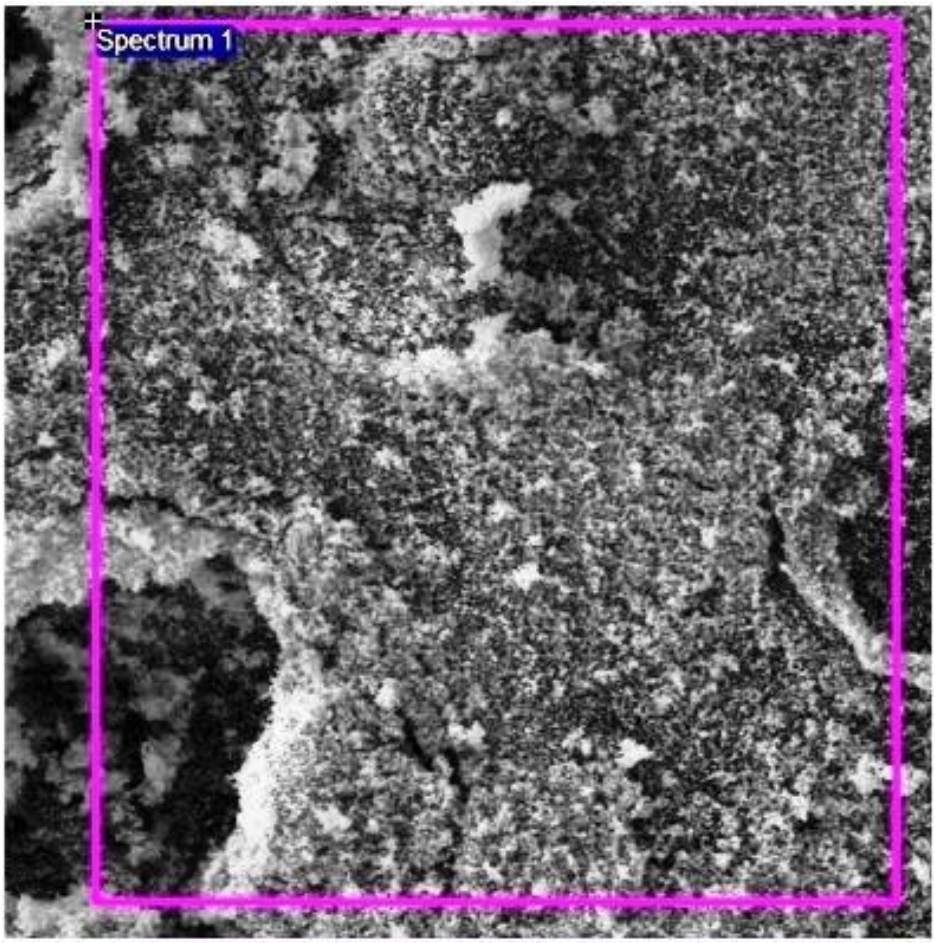

Fig 5 A. EXD spectrum of plant mediated ZnO NPs

The data obtained from EXD of ZnO Nps synthesized by plant extract of *F. vulgare* is shown in table 1 which shows presences of 24.31 % oxygen element and 75.69 % Zinc element.

Table 1. Data of composition for *Foeniculum vulgare* mediated ZnO NPs.

Element	Weight%	Atomic%
O K	24.31	56.75
Zn K	75.69	43.25
Totals	100.00	

Phytochemical analysis: Phytochemical screening was completed with plant's crude extract as well as for plant mediated metal bases ZnO NPs. Total phenolic content (TPC), total flavonoids content (TFC) and total alcoholic content (TAC) were determined. Results are expressed in table 2.

Table 2. Phytochemical analysis of crude extract and Nps.

Phytochemical	*F. Vulgare* mediated ZnO Nps	*F. Vulgare* crude extract
TPC	**10.47**	**8.20**
TFC	**10.94**	**9.82**
TAC	**10.86**	**9.79**

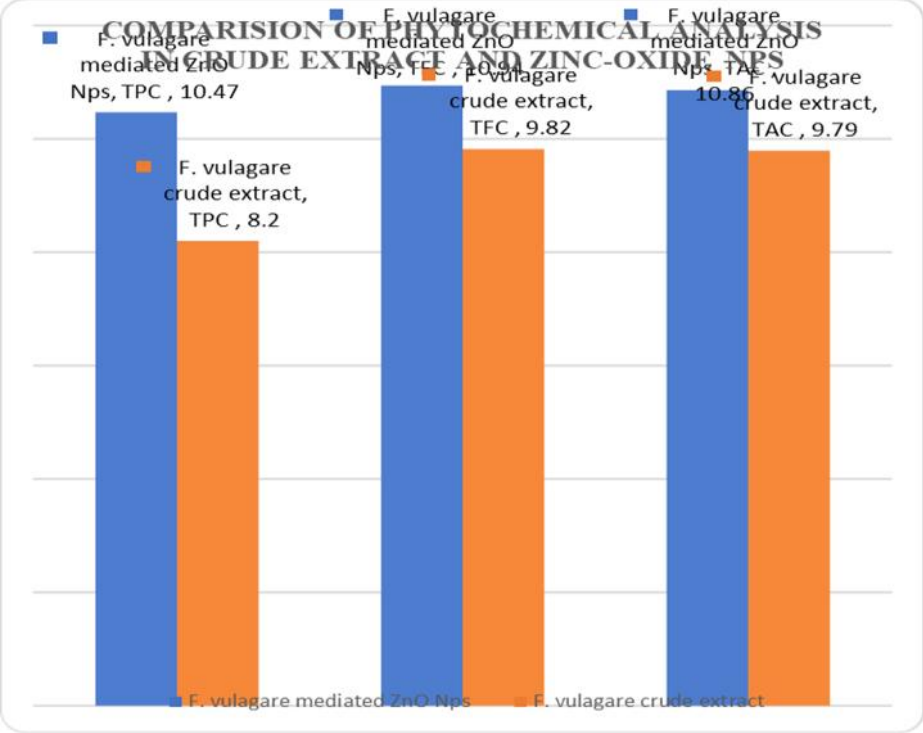

Figure 4. Graphical representation of phytochemical analysis

Fig 6 contains graphical comparison of TPC, TFC and TAC. Observation leads to conclusion that phytochemical content is higher in ZnO Nps as compare to crude extract, more phytochemical concentration reveals higher content of flavonoids as compare to phenolic and alcoholic compounds. Order of phytochemicals according to concentration was found to be as: Flavonoids> Alkaloids > Phenolic compounds.

Antioxidant analysis: Anti-oxidant potential was evaluated in term of total radical scavenging potential (TRP) and DPPH assay. Plant extract and plant mediated nanoparticles both were used for analysis and observed significant response in both cases. TRP analysis was almost have similar result but DPPH test showed significant response in crude extract as compare to Nps.

Table 3. Anti-Oxidant assay of crude extract and ZnO Nps.

Plant Name	TRP	DPPH
***F. vulgare* mediated ZnO NPs**	**17.53**	**32.27**
***F. vulgare* crude extract**	**17.42**	**85.45**

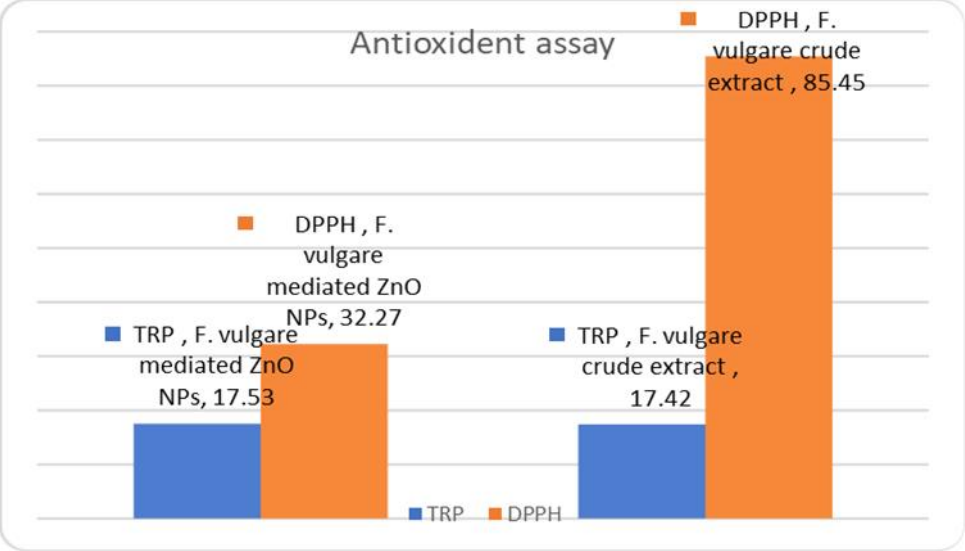

Figure 5. Graphical representation of TRP and DPPH assay.

Antibacterial analysis: Antibacterial assay was conducted for four bacterial strain *S. aureus, E. coli, L. monocytogenes* and *S. typhi* expressed in table 4. Plant mediated ZnO Nps were effective against *S. aureus* and *E. coli*. Although crude extract was able to kill more population of *S. aureus* as compare to plant mediated Nps. Observation showed no effect of NPs on *L. monocytogenes* specie.

Table 4. Antibacterial assay of *green synthesized* ZnO Nps and its crude extract.

Bacteria	Zinc oxide Nps	Plants Extracts
Staphylococuss aureus	13mm	16mm
Listeria monocytogenes	None	14mm
Escherichia coli	13mm	12mm
Salmonila	10mm	11mm

Conclusion: Spherical ZnO Nps of 22.94 nm were synthesized from methanolic extract of *F. vulgare,* a medicinally significant plant, using zinc acetate and sodium hydroxide chemicals. Basic composition of ZnO Nps was 24.31 % with regard of oxygen element (O) and 75.69 % with regard of Zinc (Zn). Comparative phytochemical study of plant mediated ZnO Nps and plant's crude extract showed higher content of TFC as compare to TAC and TPC. Bio-potential was also observed in green synthesized Nps. Zincoxide nanoparticles showed significant potential as anti-oxidant agent. These particles showed positive response against *S. aureus* and *E. coli.* Nps were observed with no potential to kill *L. monocytogenes* while crude extract of plant was effective to eradicate all bacterial strain significantly.

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