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Review Article

Formulation, Optimization and Dissolution of Piper Nigrum Nanosuspension

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ABSTRACT

The aim of present study was to enhance the dissolution rate of Piper nigrum, plant extract by formulating its nanosuspension. Piperine which is the main bioactive constituent of Piper nigrum is responsible for its bioactivities. Nanoprecipitation approach was used for the formulation of nanosuspension. The present research was divided into different phases. The first phase of this study was consisting of formulation and optimization of formulative parameters for the preparation of a stable nanosuspension of Piper nigrum. Nanosuspension formulation was prepared by using sodium lauryl sulphate, hydroxy propyl methyl cellulose, poly vinyl alcohol and polysorbate 80. The formulated nanosuspension was further accessed to identify particle size, Polydispersity index, and zeta potential value. Characterization and stability studies of optimized Nanosuspension were the next phase. The formulated nanosuspension was characterized by using scanning electron microscope. Storage stability of formulated nanosuspension was determined after three months of storage at room temperature and in the refrigerated condition. In vitro dissolution studies were performed for coarse plant extract and nanosuspension at different pH values (6.8, 7.0, 7.2). Antioxidant, antimicrobial and antifungal activities of optimized nanosuspension were also determined with respect to coarse plant extract. Results of formulated nanosuspension showed that HPMC is the suitable stabilizer for the Piper nigrum nanosuspension. The mean particle size of Piper nigrum nanosuspension is 341.0nm with polydispersity index of 0.190. Piper nigrum showed improved results of dissolution rate for nanosuspension as compared to its coarse suspension at pH 7.0. Results of all bioactivities illustrated better potential of nanosuspension as compared to coarse plant extract. In conclusion, our study showed that the nanotechnology can be used as an effective approach to enhance the dissolution rate of Piper nigrum nanosuspension.

Keywords: Piper nigrum-Piperine; Nanosuspension; Optimization; Dissolution

INTRODUCTION

Medicinal plants are an essential component of research developments in the pharmaceutical industry. Such research emphases on the isolation and direct use of active medicinal constituents, or on the development of semi-synthetic drugs, or still again on the active screening of natural products to yield synthetic pharmacologically-active compounds. According to WHO (World Health Organization) about 80% of world's population depends upon herbal medication for primary health care.

P. nigrum has been used for therapeutic purposes in many parts of the world since ancient times. Medicinal uses of P. nigrum include antibacterial, antifungal, antiapoptotic, antidepressant, antidiarrheal, anti-inflammatory, antimutagenic, antioxidative, antipyretic, antispasmodic, antitumor, to recover appetite and digestive power, anti-cold, anti-cough, dyspnea, for curing from throat diseases, anti-intermittent fever, anticolic, anti-dysentery, get rid of worms and piles. The methanolic extract of Piper longum displays a significant protection against Adriamycin induced cardiotoxicity by virtue of its antioxidant and free radical scavenging capacity [1].

Piperine is a code bioactive compound of Piper nigrum and Piper

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longum. It has been shown to have antioxidants properties and antihypertensive and hepatic protective effects. In recent years, piperine has thrilling increasing interest among researchers owing to its excellent bio-enhance effect and low toxicities. In several studies, piperine has to be effective bioavailability enhancer of several drugs and other pharmacological active substances such as curcumin, resveratrol, theophylline, in animals and in human volunteers. For example, it was reported that co-administration of piperine and curcumin to human and rats could increase the bioavailability of curcumin by 2000% and 154% respectively.

Piperine, is the main pungent alkaloid present in the fruits of black pepper *(Piper nigrum)* and long pepper; *Piper longum*. Piperine has been reported to have variety of pharmacological properties such as antipyretic, analgesic and anti-inflammatory, cytoprotective, antioxidant and anticonvulsant effects. Piperine also has been reported to exhibit an antidepressant and memory enhancing effects in animal models. Numerous reports have shown that piperine inhibits the intestinal efflux transporter P-glycoprotein (P-gp) which plays an important role in drug absorption and disposition and the major drug metabolizing enzyme CYP3A4.

Nanotechnology has determined drug delivery approaches to improve the existing issues of conventional drug delivery systems. Nanotechnology mainly refers to the study of materials and structures at the nanosized level. Nanocarriers including natural and synthetic polymeric nanoparticles, metal nanoparticles, liposomes, transferosomes, ethosomes, niosomes, virosomes, cochleate, cubosomes, solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC), lyotropic liquid crystalline nanoparticles, microemulsions, nano emulsions, and quantum dots have been investigated for numerous disease conditions with numerous drug candidates.

Nanosuspensions can be defined as colloidal dispersions of nano-sized drug particles that are produced by an appropriate method and stabilized by a suitable stabilizer. Nanosuspensions have exposed their potential to tackle the problems related with the delivery of poorly water-soluble and poorly water- and lipid-soluble drugs, and are unique since their simplicity and the advantages they confer over other approaches. This review focuses on the various aspects of nanosuspensions and their potential as a promising strategy in drug delivery.

The aim of this research was to formulate, optimize and characterize the nano suspension of *Piper nigrum* nano suspension with enhanced dissolution rate. The stability studies of nanosuspension were also done during this research. One of the objectives of this research was to evaluate antimicrobial and antioxidants activities of Piper nigrum nanosuspension and coarse plant extract.

MATERIALS AND METHODS

Medicinal plant collection & extract preparation

In this study, the medicinal plant *Piper nigrum* was selected for the formulation, optimization and for enhancing dissolution rate. The medicinal plant, *Piper Nigrum* was collected from local market of District Okara, and washed with distilled water to remove dirt and dried under shade. Dried material was ground into fine powder and sieved to remove larger particles. Excessive fat/oil contents of plants were removed by defating them with n-Hexane by using Soxhlet extractor. Ethanolic extract of plants was prepared by using Soxhlet apparatus. Plant material, Piper nigrum (20g) was added in thimble of Soxhlet extractor and 200ml of ethanol was taken in round bottom flask. Plant material was allowed to extract for about 6-8 hours until complete extraction. The resultant ethanolic extract was filtered and concentrated by using hot plate. Concentrated ethanolic extract obtained was stored in falcon tubes for further use.

Preparation of Nanosuspension

Nano precipitation method was used for the preparation of nanosuspensions by adopting the method of Zafar with some modifications. Briefly, plant extract (0.25g) was completely dissolved in n-hexane (10ml) as an aqueous phase and filtered. For the preparation of organic phase, the plant extract (20mg) was dissolved in Ethanol (10ml) as an organic phase. The resulting organic phase was slowly injected (1ml/min) with the help of syringe into aqueous phase containing stabilizer with continuous stirring at 1000 rpm for 6 hours at room temperature.

The same procedure was applied to form different nanosuspension by using different surfactant with same concentration of surfactant 250mg and plant extract 250mg._ This process has been repeated three times with different stabilizers. All the prepared nanosuspensions were stored at room temperature.

Optimization of Formulative Parameters

In the present study various formulative parameters such as stabilizer, conc. of stabilizer and amount of plant extract were optimized for the formation of stable nanosuspensions with minimum particle size [2]. Initially, screening of stabilizers takes place to determine the best possible stabilizer for each plant extract keeping all other parameters constant. After finalizing the stabilizer, remaining parameters (conc. of stabilizer) were optimized.

Selection of Stabilizer

For the formulation of stable nanosuspensions four different stabilizers (PVA, SLS, HPMC and Tween 80) were used. All the four stabilizers (Poly vinyl alcohol, hydroxypropyl methyl cellulose, Tween 80 and sodium lauryl sulphate) were screened by using 20mg plant extract, 20mg stabilizer and solvent to antisolvent ratio was fixed to 1:1

Amount of the plant extract

Amount of the plant extract was kept constant i.e., 0.25g. Experimental conditions used for the preparation of *Piper nigrum* nanosuspensions of are given in (Table 1).

Characterization of Nanosuspension

Nanosuspensions were characterized by employing following techniques:

Physical Appearance

The nanosuspensions were physically evaluated by checking their

Table 1: Optimization conditions for the preparation of stable nano suspension of Piper Nigrum.

Sr. No	Amount of plant extract (mg)	Amount of stabilizer (mg)	Plant to stabilizer ratio
1	250mg	250mg	1:1
2	250mg	500mg	1:2
3	250mg	125mg	1:0.5

stability and clarity**.** The nanosuspension that was physically more stable after preparation was considered as optimized nanosuspension and characterized by different methods.

Particle size, Polydispersity Index and Zeta Potential

Mean particle size (Z-average-nm) and Polydispersity Index (PDI) of the prepared nanosuspensions were measured by Dynamic Light Scattering (DLS) technique employing Malvern Zetasizer (Nano ZS). For measuring particle size and PDI, selected nanosuspension was added to the glass cuvette and placed in sample holder unit and measurement was carried out by using software. Zeta potential was also measured similarly by using quartz cuvette.

Scanning Electron Microscopy (SEM)

Surface morphology of optimized Nanosuspension was evaluated by using SEM. The solid nanosuspension (obtained after evaporating excess solvent using hot plate) was used for this purpose [3]. A scanning electron microscope (JEOL, JSM-6400, Japan) equipped with secondary electron detector was employed to get digital images at an accelerating voltage of 15kv.

Stability Studies

Physical stability of optimized Nanosuspension was evaluated at two different temperatures, at 4°C (in refrigerator) and at 25-30°C (at room temperature) for a period of three months. Freshly formulated Nanosuspensions were used as control.

In-vitro Dissolution Studies of Optimized Nanosuspension

In-vitro dissolution behaviors of optimized nanosuspension as compared to coarse plant extract was determined by adopting the method of Gera et al, 2009 with some modifications. A semipermeable membrane was used for in vitro dissolution testing of coarse herbal extract and nanosuspensions. For dissolution testing nanosuspension was added to a semipermeable (egg) membrane and this membrane was placed in 900 ml of 0.1 M phosphate buffer (pH=7.2) as dissolution media in a beaker placed at magnetic stirrer. Temperature of the dissolution medium was maintained at 37±0.5 °C and stirring rate was set at 50rpm during the whole experiment.

Aliquot (5 ml) was withdrawn from dissolution media at predetermined time intervals (0, 15, 30, 45, 60, 75, 90, 120min) and same volume of the pre warmed (37°C) dissolution medium (0.1 M phosphate buffer, pH 7.4) was added to the dissolution vessel immediately in order to maintain the sink conditions. Concentration of dissolved drugs was determined spectrophotometrically [4]. Piperine was the standard compound for *P. nigrum* coarse plant extract and its nanosuspension. Samples analyzed with respect to piperine were evaluated at 342nm wavelength (λ_{max} of piperine). Percentage release of coarse plant extracts and optimized nanosuspension were compared. Concentration of active constituents was evaluated from the regression equation generated from the suitably constructed calibration curve of quercetin and piperine. Experiments were conducted in triplicate and results were presented as percentage of drug dissolved for coarse plant extract and nanosuspension.

In-vitro bioactivity studies

Among in-vitro activities antioxidant, anti-bacterial and antifungal activities were determined. Detailed methodology of these is given as follows:

Determination of Antioxidant Activity

Antioxidant activity of all the coarse plant extracts and their respective nanosuspensions were assessed by using DPPH assay.

Preparation of DPPH Solution

DPPH solution (0.1mM) was prepared by dissolving 0.00395g of DPPH (1-1- diphenyl 2-picryl hydrazine) in a small amount of methanol and made up the volume up to 100ml with distilled water.

DPPH Radical Scavenging Activity

The DPPH free radical scavenging potential was evaluated by following the method of Zafar *et al.* (2015). Five different concentrations of coarse plant suspensions and respective nanosuspensions in the range of 0.02- 0.1 mg/ml were prepared. Aliquot (3ml) of these concentrations was taken and freshly prepared DPPH solution (0.1mM, 1.0 mL) was added to it. These solutions were allowed to stand at room temperature for 30 minutes. The absorbance of the resulting solutions was taken at 517nm by using UV-Vis spectrophotometer (Shimadzu, Japan). Decrease in absorbance with increase in concentrations showed high free radical scavenging activity. Ascorbic acid was used as standard compounds to compare results. A blank solution was also run-in similar way. All the experiments were repeated thrice and averaged results were used. Following formula was applied to calculate %age inhibition of DPPH radical.

%age inhibition of DPPH = $[1-A_1/A_0] \times 100$

Where

A_1 = Absorbance of samples

A_0 = Absorbance of control

Determination of Antimicrobial Activity

Antimicrobial potential of plant extracts and their respective nanosuspensions were determined by disc diffusion method by following the method of Zia-ud

-Den and shahid, (2017) using two bacterial strains (*Escherichia coli, and Bacillus subtilis*) and one fungal strain (*Aspergillus Niger*).

Antibacterial Activity (Assay Protocol)

Nutrient agar (28.08 g/L) medium was added in petri plates and inoculated with the bacterial cultures. Very small filter paper discs were impregnated with 30 µL samples (20mg/ml) of the plant suspension and nanosuspension. Methanol and rifampicin were employed as negative and positive control respectively. Discs were placed flatly on the growth media and petri dishes were incubated at 37°C for 24 hours. The herbal extracts possessing antibacterial potential inhibited the growth of bacteria and results in the form of clear zones. The zones of inhibition were measured in millimeters using zone reader.

Antifungal Activity (Assay Protocol)

Potato Dextrose Agar (PDA) (39.06 gm/L) was added in petri dishes and inoculated with the fungal species. Appropriately cut discs of filter paper were impregnated with 30µL samples (20mg/mL) of plant extracts and Nanosuspensions [5]. Fluconazole (5µL, 15mg/250µL) was used as a positive control. The plates were

incubated at 2°C for 48 hours and the antifungal activity was assessed by measuring the zones of inhibition employing zone reader.

RESULTS AND DISCUSSION

Preparation and Optimization Studies

In the present study, nanoprecipitation method was used for the preparation of nanosuspensions because of its simplicity, reproducibility, rapidity and cost effectiveness. For the preparation of stable nanosuspension, important process parameters like stabilizer and amount of stabilizer were optimized.

Selection of Stabilizer

The objective of screening of stabilizers was to select appropriate stabilizer for the formulation of stable nanosuspension. The stabilizers used in the preparation of nanosuspensions should adsorb onto the surfaces of drug nanosuspensions and provide steric or electrostatic stabilization effect, powered by the force of hydrophobic moieties in the stabilizers. Another important function of the stabilizer is to provide a substantial mechanical and thermodynamic barrier at the interface that slow down the coalescence of formulated nanoparticles and hence prevents Ostwald ripening. Furthermore, the type of stabilizer may have remarkable influence on the size of nanoparticles.

In order to select a suitable stabilizer different stabilizers Polyvinyl Alcohol (PVA), Sodium Lauryl Sulphate (SLS), Polysorbate 80 (P-80), and Hydroxypropyl Methyl Cellulose (HPMC) were employed for the formulation of nanosuspensions. During stabilizer selection fixed concentration of each stabilizer (1%) was used with selected amount of plant extract (0.25g) and antisolvent to solvent ratio (1:10). The stabilizer which provided physically stable nanosuspension was selected for further studies.

The nanosuspension of *Piper nigrum* extract prepared by using different stabilizer showed variable responses such as Physical appearance, Particle size, zeta potential and PDI. Only one of the above stabilizers showed better stability as compared to other stabilizer used.

Only nanosuspension prepared by Hydroxypropyl Methyl Cellulose (HPMC) was stable and clear when it was prepared freshly. It remained stable after three months of its preparation [6]. On the other side the other nanosuspension were physically stable at the time of preparation but became unstable after storage in three months.

From above results in (Table 2) it is shown that HPMC was choose as a stabilizer for formulation and further optimization of *Piper nigrum* nanosuspension. HPMC is large and nonionic stabilizers mainly stabilize the drug particles in nanosuspensions by steric hindrance and ensure an appropriate viscosity for nanosuspension. It is a polymeric molecule containing many methoxy and hydroxypropyl groups. The stability of HPMC stabilized nanosuspension may be ascribed to its hydrophobic groups, which have good affinity for drug particles. In the present situation, these hydrophobic groups may adsorb on the surface of drug (*P. nigrum*) and provided effective steric barrier against particle growth.

HPMC was found best stabilizer for the preparation of *Piper nigrum* nanosuspension as it provided most stable nanosuspension when freshly prepared and even remain stable after three months of its preparation.

Table 2: Screening of stabilizers for the formulation of Piper nigrum Nanosuspensions.

Nanosuspension code	Stabilizer used	Physical stability	Stability after 3 months (room temp)
P.N 1	HPMC	Stable	Stable
P.N 2	PVA	Stable	Unstable
P.N 3	SLS	Stable	Unstable
P.N 4	P.80	Stable	Unstable

OPTIMIZATION OF STABILIZER AMOUNT

HPMC for Black Pepper

HPMC was found best stabilizer for the preparation of Piper nigrum Nanosuspension as it provided most stable Nanosuspension when freshly prepared and even remains stable after three months of its preparation (Table 3).

CHARACTERIZATION OF NANOSUSPENSIONS

Particle size and Polydispersity Index

In order to increase the bioavailability of *Piper nigrum*, a piperine was successfully formulated by a simple nanoprecipitation method. The particle size of freshly prepared nanosuspension of *Piper nigrum* are 341.0 mm with a PDI value of 0.190. They appeared physically as clear and stable suspension [7].

A minute difference in particle size (373.9nm) and a little bit large variation in PDI value (0.240) were observed nanosuspension of *Piper nigrum* at room temperature (25-30°C).

Zeta Size and Potential Analysis

From following the (Figure 1), In order to increase the bioavailability of Piper nigrum, a piperine was successfully formulated by a simple nanoprecipitation method. The particle size of freshly prepared nanosuspension of *Piper nigrum* are 341.0 mm with a PDI value of 0.190.

They appeared physically as clear and stable suspension. With such particle size and their PDI value, the prepared nanosuspension of *Piper nigrum* showed a zeta potential of -28.9 ±11.1mv.

The particle size of prepared nanosuspension at room temperature of (25-30°C) was 373.9 with a PDI value of 0.240. The physical appearance of these nanosuspension was clear and stable.is the particle size of prepared nanosuspension at refrigerated conditions of (2-8°C) was 367.3 with a PDI value of 0.213. The physical appearance of these Nanosuspensions is stable (Figure 2).

SEM analysis of Piper nigrum Nanosuspension

Scanning electron microscope photographs of *Piper nigrum* at one resolution are presented in (Figure 3). From the figure, it was shown that the average particle size was less than 1µm which revealed that after lyophilization no aggregation of particle was present and the stabilizer HPMC that is used for the formulation of nanosuspension

Table 3: Optimization of plant extract and amount of stabilizer.

Sr. No	Amount of plant extract (g)	Amount of stabilizer (g)	Plant to stabilizer ratio
1	0.25	0.50	1:2

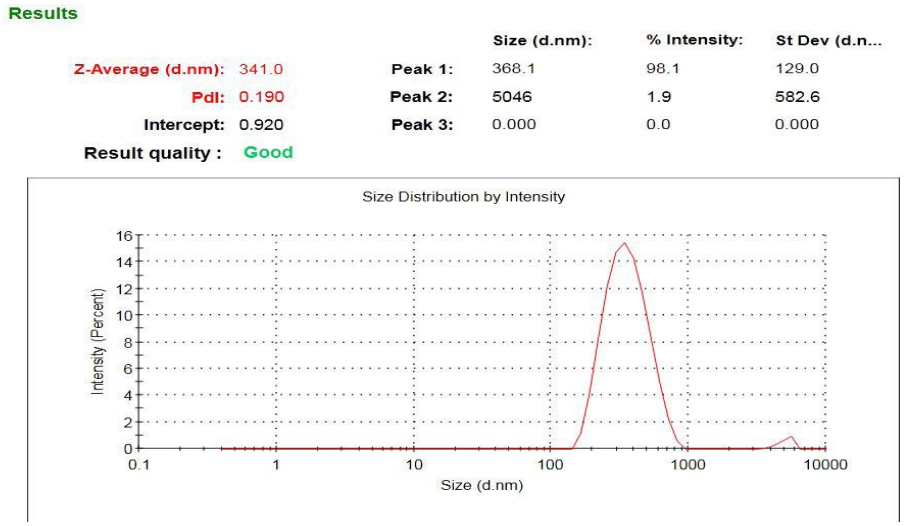

Figure 1: Particle size and PDI value.

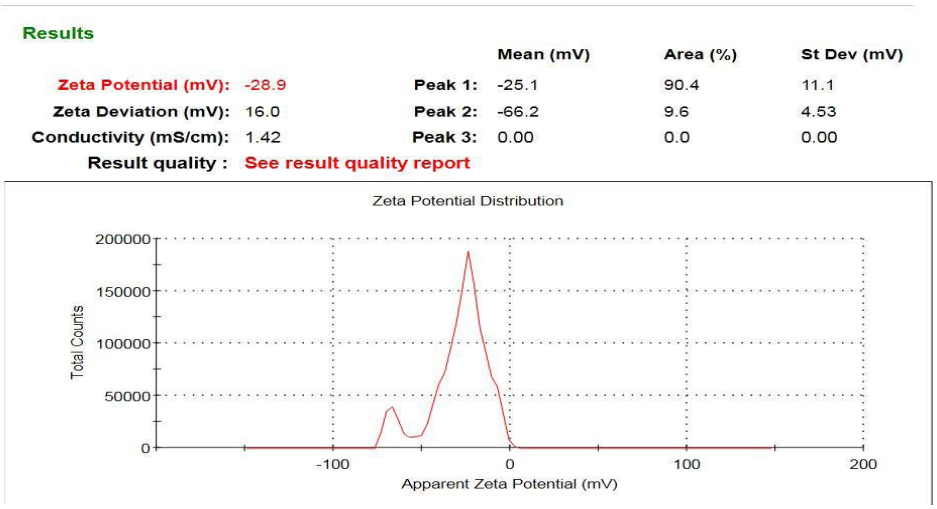

Figure 2: Zeta potential trend for Piper nigrum nano suspension.

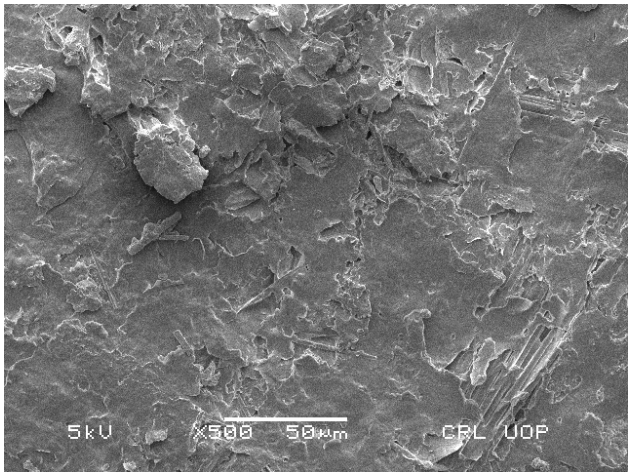

Figure 3: SEM image of Piper nigrum nano suspension at one resolution.

provide better stability of nanosuspension after lyophilization. The particles shown in the figure was uniform and somewhat good characteristics of surface. HPMC which is a polymeric stabilizer controlled the particle size of *Piper nigrum* nanosuspension and also increased the stability. Present outcomes are in good conformity with many previous findings in which HPMC provide better and uniform characteristics to lyophilized Nanosuspensions.

SEM images showed irregular shaped particles with non-uniform particle size. The precipitated drug from ethanol water system shows as needle like crystals. The lyophilized formulation has the flaky appearance.

Physical Stability Studies of Nanosuspensions

The particle size of prepared nanosuspension at room temperature of (25-30°C) was 373.9 with a PDI value of 0.240. The physical appearance of these nanosuspension was clear and stable. The particle size of prepared nanosuspension at refrigerated conditions of (2-8°C) was 367.3 with a PDI value of 0.213. The physical appearance of these nano suspensions is stable.

Results of stability testing of optimized *Piper nigrum* nanosuspension revealed in (Table 4) that nanosuspension remain stable at refrigerated condition [8]. On the other hand, the nanosuspension present in room temperature shows clear and stable physically. The insignificant increase in the particle size (367.3nm with PDI value of 0.213) of nanosuspension was seen at refrigerated condition (2-8°C).

In-vitro Dissolution Studies

The most important feature of nanoparticles is the increase in the dissolution velocity, not only because of increase in surface area but also because of increase in saturation solubility. Keeping in view the importance of dissolution studies, prepared nanosuspension

Table 4: Physical stability studies of Black pepper nano suspensions.

Stability	Parameters	Results
Freshly prepared nano suspension	Size (z-average nm)	341.0
	PDI	0.190
	Physical appearance	Clear and stable
Room Temperature (25-30°C)	Size (z-average nm)	373.9
	PDI	0.240
	Physical appearance	Clear and stable
Refrigerated condition (2-8°C)	Size (z-average nm)	367.3
	PDI	0.213
	Physical appearance	Stable

Table 5: Dissolution profile of P.N nano suspension and plant extract at pH 6.8.

Time	P. Ext	Nano
0	0	0
15	9.28	10.69815385
30	13.74	19.46738462
45	20.69	28.852
60	26.87	33.62123077
75	34.22	39.92892308
90	35.84	48.39046154
105	36.58	51.15969231
120	37.73	51.32892308

was subjected to dissolution testing to evaluate their in-vitro release behaviors. For determining the dissolution rate Phosphate buffer of pH 6.8, 7.0 and 7.2 were employed as dissolution medium and the concentration of released drug was determined spectrophotometric ally.

In-vitro Dissolution Profile of Piper Nigrum Nanosuspension and Coarse Extract

As plants contain a large number of bioactive phytoconstituents and it is immensely difficult to evaluate the concentration of all these constituents, so in the ongoing research only one key bioactive component was used as standard compound to compare results. For *P. nigrum* nanosuspension, pipeline, which is the major bioactive constituent of *P. nigrum* fruit, was used as a standard compound and results were expressed as "pipeline equivalent". However, to evaluate the role of size reduction on dissolution profile of nanosuspensions, coarse extract of respective plants was used as reference.

In-vitro dissolution profile of Piper nigrum Nanosuspension and Coarse Extract At pH 6.8

The results shown in (Table 5 and Figure 4) that in vitro dissolution profile of *Piper nigrum* nanosuspension and coarse plant at 60 min is 33.62 of *Piper nigrum* nanosuspension at 6.8pH. with the passage of time at 120min is 51.32 for *Piper nigrum* nanosuspension while of coarse plant is 37.73.

As compared to other pH that is under study, at pH 6.8 it shows lowest dissolution profile for *Piper nigrum* nanosuspension.

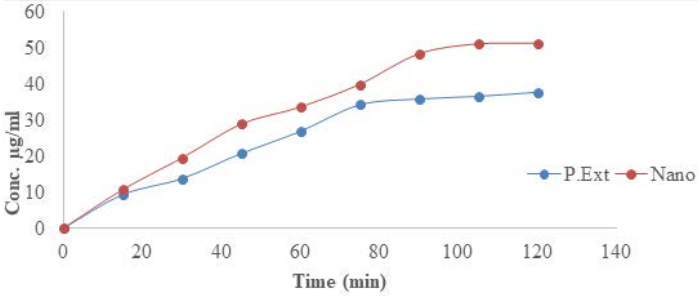

Figure 4: In vitro dissolution graph of Piper nigrum nano suspension and coarse plant at 6.8 PH.

Table 6: Dissolution profile of P.N nanosuspension and plant extract at pH 7.0.

Time	P. Ext	Nano
0	0	0
15	9.73	11.69815385
30	14.3	19.46738462
45	17.79	28.852
60	25.67	33.62123077
75	33.72	45.92892308
90	37.54	55.39046154
105	39.58	59.15969231
120	39.73	60.32892308

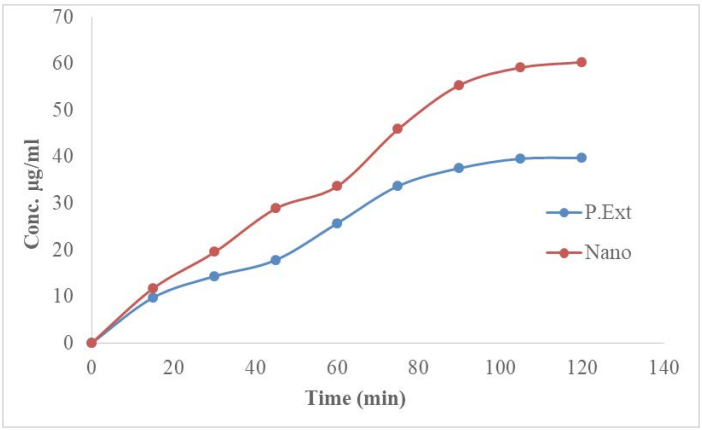

Figure 5: In vitro dissolution graph of Piper nigrum nanosuspension and coarse plant at 7.0 PH.

In-vitro dissolution profile of Piper nigrum Nanosuspension and Coarse Extract At pH 7

Table 6 represents the tabular form of dissolution rate of nanosuspension and coarse plant extract at pH 7.0. The concentration of coarse plant extract and nanosuspension is 65% (39.73µg/ml) and about 95% (60.32µg/ml) at 120 mins respectively. The given graph of (Figure 5) describes that maximum dissolution rate at about 95% in nanosuspension at 120mins. It also describes that this concentration in nanosuspension is less than the the percentage of graph at 7.2 pH.

In-vitro Dissolution Profile of Piper nigrum Nanosuspension and Coarse Extract At pH 7.2

From (Table 7 and Figure 6), the greater concentration of piperine in dissolution medium of was observer for pH 7.2. Concentration of piperine after 15 mins was 13.8 for nanosuspension and 10.3 for coarse plant. The concentration of piperine in the dissolution

Table 7: Dissolution profile of P.N nano suspension and plant extract at pH 7.2.

Time	P. Ext	Nano
0	0	0
15	10.3	13.815385
30	14.58	21.38462
45	19.28	29.852
60	24.79	35.62123077
75	28.27507692	44.92892308
90	28.42123077	57.39046154
105	29.69815385	61.15969231
120	29.8	62.32892308

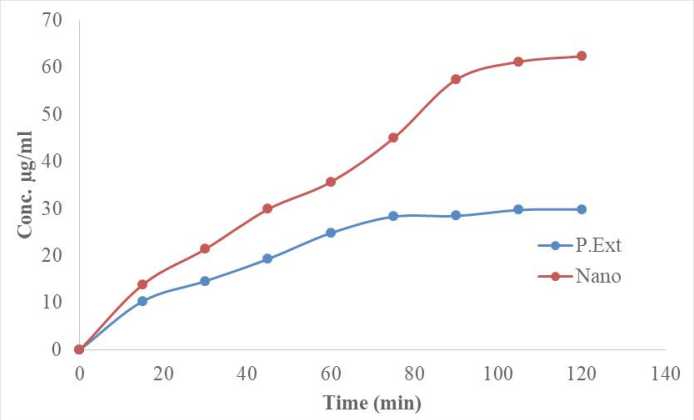

Figure 6: In vitro dissolution graph of Piper nigrum nano suspension and coarse plant at 7.2 pH.

medium with the passage of time was increase and after 60 mins increased dissolution was seen for nanosuspension (35.6) as compared to plant extract (24.7). The same trend was seen after 120 mins, where maximum amount of piperine was seen (62.3) in the nanosuspension. This is remarkable a greater concentration of nanosuspension as compared to plant extract after 120 mins**.**

By all these results at we can see that on different pH, different dissolution rates can be observed, which vary due to pH caused by anionic cationic interaction of component particle with medium or due to some Vander wall interaction. In conclusion we examine that in all three pH of 6.8, 7.2 and 7.0. At pH 7.2 coarse plant extract and nanosuspension shows highest dissolution rate which determines that it's most appropriate medium for its absorption in any solvent and medium. Results of Dissolution profile of *Piper nigrum* nanosuspension and coarse plant extract is demonstrated in table

Antimicrobial Activity of Coarse Plant Suspensions and Nanosuspensions

Both these antibacterial and antifungal activities were examined by *Escherichia coli* and *Aspergillus Niger* respectively [9]. Various strains were used for evaluating antimicrobial activity which includes *Piper nigrum* coarse suspension and nanosuspension, fluconazole, Rifampicin and methanol.

Results of antimicrobial activities of *Piper nigrum* coarse suspension and nanosuspension are given in (Table 8). Comparative study of coarse suspension and nanosuspension revealed significantly higher antifungal activity ($p<0.05$) for nanosuspension. However, significantly ($p<0.05$) greater antifungal activity was observed for

Table 8: Antimicrobial activities of *Piper nigrum*.

Plant /Standard	Treatment	Antifungal activity (mm)	Antibacterial activity (mm)
Strain used		**Aspergillus Niger**	**Escherichia coli**
P. nigrum	**C. Sus**	**3.7**± 0.03	**2.5 ± 0.04**
	Nano	**5.9**± 0. 19	**3.8**± 0.05
Fluconazole		**43.5 ± 0.23**	
Rifampiciln		-	**37.5 ± 0.13**
Methanol		-	**0**

Table 9: Antioxidant potential of Piper nigrum coarse plant suspension and nanosuspension.

	IC_{50} values (µg/mL)
Ascorbic acid	189.06
Nano suspension	127.8
Coarse suspension	211.2

Fluconazole as compared to both treatments. The antibacterial activity, greater ($p<0.05$) inhibition zones was observed for nanosuspension as compared to coarse suspension against both bacterial stains (*E. coli, A Niger*). However, Rifampiciln illustrated significantly ($p<0.05$) enhanced antibacterial activity as compared to both suspensions. The study concluded that nano formulation increased the antimicrobial activities many times greater than normal coarse extract of *Piper nigrum*. Its due to enhancing bioavailability of particles by forming their nano formulation as it increases activity ratio as compare to coarse extracts.

Values are expressed as mean ±SD (n=3) indicating the diameter of zone of inhibition in mm

Nano=nanosuspension, **C. Sus=**coarse suspension

In-vitro Antioxidant Potential of Coarse Plant Suspensions and Respective Nano Suspensions by DPPH Assay

Antioxidant potential of coarse plant suspensions and their respective nanosuspensions was evaluated by employing DPPH radical scavenging assay. Other methods can also be used for the evaluation of antioxidant potential, but DPPH is most widely used mainly because it is unaffected by side reactions like chelation of metal ions and enzyme inhibition reactions. DPPH radical, possessing deep violet color, shows absorption maxima at 515–528 nm and when it accepts proton from any hydrogen donor species such as phenolics, it loses its chromophore nature and changes into yellow color. This conversion is directly related to the number of phenolic compounds or degree of hydroxylation of the phenolic compounds. Half maximal Inhibitory Concentration (IC50) value is the concentration of the sample that can scavenge 50% of DPPH free radical in DPPH free radical scavenging method. The value of IC50 is inversely proportion to the free radical scavenging activity/ antioxidant property of the sample.

These medicinal plant species have significantly high phenolic content and a large number of flavonoids, flavonoids as well as antioxidant activity as compared to synthetic antioxidants that have side effect and have been reported to be carcinogenic [10].

Results of antioxidant potential of *Piper nigrum* coarse plant suspension and nanosuspension were expressed in terms of amount required for 50% inhibition of DPPH radical (IC50). Results are given in (Table 9). Ascorbic acid that is a natural antioxidant was

used as standard compound to compare the results. Ascorbic acid possessed IC50 value of 189.06 µg/mL. As the IC50 value is inversely proportional to the amount of nanosuspension, therefore lower the Value of IC50 value of nanosuspension, the higher will be antioxidant potential of nanosuspension. Therefore, in the IC50 value of nanosuspension is lower so the radical scavenging potential will be for nanosuspension. Present results clearly demonstrated the enhanced DPPH radical scavenging potential of nanosuspensions as compared to respective coarse plant suspensions. However, no prominent difference was observed between the IC50 values of nanosuspensions and standard ascorbic acid.

The difference in scavenging potential of various plant extracts can be attributed to the presence of varying amount of bioactive phytochemicals like phenolic and flavonoid contents.

The enhanced antioxidant activity of nanosuspensions was probably due to greater solubility of nanosuspensions as compared to coarse water extract. Furthermore, this enhanced solubility of nanosuspensions can be used to reduce the dose of these extracts and ultimately to improve the bioavailability. By considering IC50, we can conclude that antioxidant activity power of *Piper nigrum* nanosuspension is excellent and this activity is responsible to protect from the side effects induced by free radicals. This study demonstrated that *Piper nigrum* has powerful and excellent antioxidant activity as compared with ascorbic acid.

DISCUSSION

During this work, the whole process is done in four different steps. The formulation of nanosuspension, selection of suitable stabilizer, optimization of formulative parameter for a stable nanosuspension, these are carried out in the first step of research work. Selection of a suitable stabilizer shows that Hydroxypropyl Methyl cellulose (HPMC) was the best stabilizer for Piper nigrum. Optimization of nanosuspension showed the mean particle size of 341.0 nm with PDI value of 0.190.

The results showed that large amount of stabilizer completely cover the particle surface and prevents their aggregation during nano formulation. A gradual increase in particle size is observed by increasing the amount of stabilizer i.e., HPMC. An effective amount of polymer increases particle size by increasing the size of polymer and prevents diffusion between solvent and antisolvent during nano precipitation.

The second step was that the optimized nano suspension was characterized by SEM. The images and results taken by SEM showed good surface characteristics of Piper nigrum nano suspension; however, larger and non-uniform particles are also present at certain places. This may be due to aggregation between nanoparticles. HPMC seems to be a perfect stabilizer for Piper nigrum because the stability of nanosuspension was better with it, even after lyophilization. The particles are uniform and have a spherical shape and having smooth surface due to the presence of HPMC.

After this, the stability studies of nanosuspension were carried out at different Temperature. The *Piper nigrum* nanosuspension was carried out with room temperature, high temperature and at refrigerated condition. The better stability was seen at Room temperature (25°C), where average zeta potential was 373.9nm with a high PDI value of 0.240.

In vitro dissolution studies of optimized nanosuspension with comparison of coarse plant suspension and nanosuspension are the third step of the research. A greater concentration of Piperine was observed in the graph for nanosuspension after 120 mins. This increasing dissolution rate of nanosuspension maybe from the increasing surface area and decreasing particle size according to Noyes Whitney's equation. Present study showed the better performance of nanosuspension as compared to coarse plant suspension. Drug nanoparticles or drug nanosuspensions consist of drug particles between 200 and 500 nm stabilized with/without additives. The higher dissolution rate can be seen at the pH of 7.0. The pH actually tells about the basicity, alkalinity or neutral state of sample.

In vitro bioactivities testing of optimized nanosuspension of *Piper nigrum* were performed with the comparison of coarse plant suspension. This is the fourth step of this research work. The results of in vitro bioactivities such as Antimicrobial, Antioxidant showed effectiveness of nanosuspension as compared to coarse plant suspension. In biological activities, the *Piper nigrum* nanosuspension showed increased antifungal activities than its coarse suspension against fungal strains *A. Niger and E. coli*. The nanosuspension of *Piper nigrum* can prove to be a step to treat the fungal diseases due to its enhanced antifungal potential.

Results of antioxidant activity demonstrated the enhanced DPPH radical scavenging potential of nanosuspension as compared to native plant suspension. The enhanced radical scavenging potential of *Piper nigrum* nanosuspension can be a way to improve its medical benefits.

CONCLUSION

The formulation of *Piper nigrum* nanosuspension was prepared and study with the use of nanoprecipitation method. The particle size and other parameters were highly dependent on the whole process and formulation parameters. The optimized nano formulation showed a mean particle size of 360.7nm with polydispersity index. HPMC was found a best stabilizer for the preparation of nanosuspension of *Piper nigrum* because it is suitable for a stable nanosuspension of Piper nigrum. The irregular cubic shape of nanoparticles of *Piper nigrum* changed into some smooth, regular and spherical nanoparticles. The formulated nanosuspension of Piper nigrum looks stable physically.

The prepared nanosuspension showed enhancement in dissolution rate as compared to its coarse plant, which provide the stability and efficacy of nanosuspension as compared to coarse plant. The nanosuspension can be used as oral, pulmonary, parental, and ocular ways, since it is a simple technique, but it increased the dissolution rate and stability of medicinal plants. In vitro dissolution test ensures the product quality and performance and also assist in the regulatory process of Nanotechnology. This study revealed that the nanosuspension of Piper nigrum can be easy, rapid and better approach to increase the dissolution rate.

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DECLARATION OF INTEREST

The authors declare no conflicts of interest.

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