



Synthesis and Characterization of Herbal Nano-suspensions and Evaluation of their *In-vivo* Antihypertensive Potential with Especial Focus on Piperine

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AM and ZZ select the contents and design the article authors SN and TR removed plagiarism authors MI and SH helped out in formatting and setting according to requirements authors IK and MHAT made table and figures. All authors read and approved the final manuscript.

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ABSTRACT

Poor aqueous solubility is the principle obstacle in accomplishing suitable oral bioavailability for huge proportion of drug composites in drug development today's. Nano-suspension is an emerging field of research in the scientific community to provide a new solution for poorly water-soluble active constituents. The objective of the nano-suspension is to reduce the drug particle size (100-200 nm) range, which enhance solubility and bioavailability of biopharmaceutical active compounds. Crude extract of *Piper nigrum* shows various biological activities but poor aqueous solubility so, there is a need to isolate the piperine from *Piper nigrum* and prepare its nano-suspension to reduce particle size and enhance its bioavailability. This review defines the principles behind nanosizing, the

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synthesis and characterization of piperine nano-suspension as well as the recent practice with application of such formulations *in-vivo* can be used as a better alternative to treat cardiovascular diseases with improved therapeutic efficacy compared to extract.

Keywords: Nano-sizing; bioavailability; formulation; characterization; nano-suspension; *in-vivo* ACE inhibition; piperine.

1. INTRODUCTION

Nanotechnologies are used to overcome conventional methods via different parameters like solubility, saturation stability at room temperature, compatibility with solvent, excipient, and photo stability. These parameters play an acute role in the formulation of drugs. Poor water solubility is a major problem for pharmaceutical scientists because more than 40% new chemical things are poor water soluble. Poor solubility of a drugs can be enhanced by formulating emulsion, solid dispersion, complex formation with cyclodextrin, nano-emulsion [1], micronization, surfactant dispersion, solid dispersion [2], and other conventional techniques can enhance solubility but it is less effective for poor water soluble drugs like liposomes and complexation with cyclodextrin techniques [2]. All above described conventional methods possess certain drawbacks such as less enhancement in solubility and rapid loss of physical and chemical properties of a drug when dissolve in a suitable solvent [3].

Nano-suspensions is favored in order to enhance bioavailability of active entities like Naproxen and Bupravaquone [2] by using precipitation technique (solvent-anti-solvent method) frequently needs bulky amount of co-solvents which led to toxic effect and increases the drug bioavailability [4]. Nano-suspension is a biphasic system which contains drug particles which are suspended less than 1 μ m in size [2] in the presence of surfactant or polymer [5]. Saturation solubility and solution velocity increase bioavailability and solubility of poorly soluble drugs by decreasing particle size to nanometer (1-1000 nm range) [6].

Many plants extracts, flavonoid's, alkaloids, and chemical compounds has been used by numerous researchers to cure different disease like *Querciten* nano-suspension used for anti-inflammation [7], *Andrographis Paniculata* nano-suspensions used for long acting intramuscular administration [8], *Teniposide* for breast cancer therapy [9], *Hypericum perforatum* for anti-hepatitis B virus (HBV) activity and Hepato-

protective [10], *Andrographis* for liver diseases [11], *Rabdosia rubescens* for tumor [12], Amphotericin B for *Balamuthia mandrillaris* [13], estrogen receptor- β 005 (ER- β 005) for analgesic efficacy, *Teniposide* and *Camptotheca Acuminate* nano-suspension for antitumor [5].

Medicinal plants contains numerous secondary metabolite's such as alkaloids, flavonoids, and phenolic compounds have been used to inhibit ACE activity and lower the hypertension [14]. [15] used flavonoid-rich Apple peel extract to study its ACE inhibitory property. Ginger rhizomes [4], Legumes [16], Bell peppers [17] and Kiwi fruit [18] demonstrate good ACE inhibitory potential. [19] reported the ACE inhibitory activity of Black pepper fruit. Cardiovascular diseases caused by over production of angiotensin converting enzyme (ACE), metabolic syndrome, hypertension and obesity are major risks of cardiovascular disease [20].

ACE is associated with kinin nitric oxide and renin-angiotensin system. In RASS, angiotensin converting enzyme (ACE) converts angiotensin-1 into effective vasoconstrictor angiotensin-2. In kinin nitric oxide system bradykinin and hypotensive peptide inactivates by angiotensin converting enzyme [21]. When angiotensin-1 change into angiotensin-2 cause's hypertension thus, inhibition of ACE benefits to control high blood pressure. Therefore, inhibition of angiotensin converting enzyme (IACE) is a useful healing method in the management of hypertension. Therefore, in field research ACE inhibition has significant role in progress of herbal medicinal drugs to control hypertension. Numerous positive challenges used for the manufacture of Captopril as ACE inhibitors, amlodipine [20], lisinopril and enalapril [22]. Recently, natural sources are of researchers interest to overcome the side effects of synthetic ACE-1 [23].

Moreover, construction of synthetic ACE inhibitors have some certain side effects [21]. Consequently, there is a need to the synthesis of natural herbal plant medicines instead of synthetic medicines. Natural plant properties may

have good hypotensive potential and have rare or no side-effects which provide advanced, safer and cost-effective ACE inhibitors for the anticipation and medication of high blood pressure [24].

Furthermore, it is investigated that all herbal medicinal extracts do not exhibit all biological significant effect. Because some medicinal plants (crude extracts) exhibit good *in-vitro* angiotensin converting enzyme inhibition activity but have less *in-vivo* ACE inhibition in animal trials. In order to overcome this problem, most active ingredients (alkaloids and flavonoids) extracted from different native plants to enhance their *in-vitro* as well as *in-vivo* ACE inhibition.

Keeping in view the above facts, current research was focused on the isolation of piperine from Black pepper and prepare its nano-suspension to enhance dissolution rate and improved its bioavailability. *In-vitro* angiotensin converting enzyme (ACE) inhibitory potential, drug entrapment efficiency, *in-vitro* drug release and *In-vivo* antihypertensive activities of the developed nano-suspension was determined and subsequent objectives have been deliberate for this study.

2. REVIEW OF LITERATURE

2.1 Nano-suspension Approach

Nano-suspension is a biphasic or colloidal dispersion of drug delivery system containing drug particles which are less than 1µm in size or 1-1000 nm range and small amount of surfactant or polymer to stabilize the drug [5]. Nano-suspension has low processing cost, high drug loading and diminutive side effect by excipients [25]. To improve surface to volume ratio of nano-suspension, drug particles have greater saturation solubility, simple formulating composition, easier solid dosage form, management path and quicker dissolution rate that enhanced the bioavailability [5].

2.2 Need for Nano-suspension

Drug formulations having poor water solubility always persisted a stimulating task for pharmacists. Drugs that are produced in drug discovery programs have almost of 40% poor water solubility [26]. Drugs having low permeability, little stability and poor solubility in GI tract, cause poor oral bioavailability [2]. Along with these synthetic drugs, there are several natural substances including plant ingredients

(alkaloids, flavonoids, tannins, steroids, essential oils and flavor's) are pharmaceutically important but suffering from poor aqueous solubility [27]. Therefore, to increase water solubility of nutraceuticals in drug formulations and functional foods, there is a positive requirement to develop advanced formulation technologies [28,29].

Drugs having poor solubility can be formulated effectively by using several techniques for example dissolution in surfactant or polymer solution, pH adjustment, emulsions, solid dispersion, liposomes and using cosolvents [26,30,31,32]. However, given techniques are not much effective due to their less stability and dense particle size [33], unsatisfactory drug loading [34] and greater treatment cost [26,7]. Moreover, these formulation techniques use extracts in huge amount [35] and are not beneficial for drugs which have poor solubility both in aqueous and organic solvents. Nano-suspension can be used to untangle the difficulty related with these conventional techniques and can be used in superior way to enhance the solubility and bioavailability of drugs due to their and greater benefits as compared to other techniques [2].

2.3 Advantages of Nano-suspension

Advantages of nano-suspension are recognized to their decrease particle size, increase dissolution rate, increase solubility, greater adhesiveness to cells and more habitation time in the GI tract [36]. Nano-suspension technology is mostly suitable for hydrophobic drugs and enhance the bioavailability [25], solubility and chemical and physical strength of drugs. Drug dose saving is also possible due to greater bioavailability. Moreover, this technology can be suitably used for high scale manufacturing due to accomplishing passive drug targeting and more drug loading [37,38,25].

2.4 Formulation Techniques and Characterization of Nano-suspensions

Two approaches are used to prepare nano-suspension of desired material [39]. Wet Ball-milling, media-milling, high pressure-homogenization are examples of top-down route and solvent-anti solvent precipitation and ultrasonication techniques are examples of bottom-up technique [40]. In this technology powdered micronized drug used as a starting material and suspended in dispersion phase having stabilizer to stabilize the suspended drug presence of

organic solvents and thermodynamically instability of particles restricts this approach [19].

Precipitation method is known as via Humid a Paratum" (v. h. p.) is the example of bottom up technique. At first, a poor water soluble pharmaceutical active API macromolecules or drug dissolved in a suitable organic solvents ethanol, chloroform and acetone which is known as solvent system which is usually water miscible [41]. Secondly, make water-stabilizer solution which is known as anti-solvent system. Stabilizers are selected on the bases of highly Hydrophilic-Lipophilic Balance (HLB) like SLS, Pluronic's, Tween-80 and Spans has high HBL [42]. Then gently this solution is injected drop wise into an anti-solvent solution containing stabilizer at 1000 rpm on magnetic stirrer. This method is simple and less costly [43].

Hydroxycamptothecin (HCPT) nano-suspensions were developed by using ultra-sonication method using probe sonicator (20 cycles/3 sec). dynamic light scattering (DLS), (SEM), and (XRD) were characterized by ultra-sonication method. For HCPT nano-suspension sonicator were used for 1hr under space at room temperature [32]. It was found that HCPT nano-suspensions exhibited significance increase HCPT concentrations in the body and enhanced antitumor activity [5].

Media-milling is a top-down approach. It contained microcer media-mill chamber lined with zirconia a shaft [44] used to prepare paliperidone palmitate (PP) nano-suspensions Particle size of paliperidone palmitate (PP) was measured as 1061 ± 6 nm (A) and 605 ± 9 nm (B), respectively. Prepared nano-suspensions was characterized and suitable tested particle size of nano-suspensions can be proposed for the intramuscular administration in order to yield larger beneficial effect [45]. It has advantages due to less batch to batch variations and simple highly scale up method but it has disadvantages, the impurity of final product due to the corrossions of treasures [46].

Amphotericin B as nano-suspensions were developed by using high pressure homogenization method to improve a nano-particulate brain delivery system. Formulated nano-suspension characterized by laser diffraction and photon correlation spectroscopy for nebulization and zeta potential characterization [44]. Tested nano-suspensions were confirmed for antifungal and amoebicidal activity. This technique were less cost effective

and reduce energy densities [47]. The results revealed that nano-suspensions formulated by homogenizer covered with polysorbate-80 and sodium chelate dramatically enhanced drug brain delivery. It is a time consuming technique [46].

2.5 Characterization of Nano-Suspensions

Nano-suspensions characterization has a vital role in understanding the *in-vivo* and *in-vitro* concert of organized constructions. Primarily biological concert of nano-formulations depends upon particle charge, particle magnitude, particle dispersal, particle morphology and crystalline state. These parameters also liable for stability, safety, proficiency and act of nano-suspension [48,49]. Nano-suspension can be characterized by following technologies: zeta size, zeta potential saturation solubility, size distribution, morphology and crystalline state.

2.6 Zeta Size Analysis

Particle size distribution are elementary physiochemical characterizing constraint which influence the biopharmaceutical properties of nano-formulations [25]. Zeta-sizer is used to measure particle size. He-Ne used as a light beam to determine the particle size at wavelength 632.8 nm. For perfect considerations all samples were diluted with deionized water before measurement. Tiny sized particles due to large superficial area rise the disbanding of drug. photon correlation spectroscopy, dynamic light scattering, laser diffraction or countercurrent multisizer, electron microscopy and field flow fraction methods is used to quantify particle size and distribution of particles [46]. Allowing to ostwald ripening process small size particle distribution is important to maintain physical stability of prepared nano-suspensions and elude particle development. Greater particle size distribution revealed higher PDI value. Normally 0.1 to 0.25 PDI values require a contracted particle distribution, where more than 0.5 PDI value show wide distribution [37].

2.7 Saturation Solubility

Nano-formulations by falling particle size improve the action of drug and saturation solubility properly. Due to variation in surface tension which subsequently decrease the particle size and increased the saturation solubility. Saturation solubility and estimation of dissolution rate supports to govern *in-vitro* nano-suspensions action [48].

Table 1. Summary of previous reported prepared nano-suspensions along with formulated techniques and characterization

Nano suspension	Technique	Polymer/surfactant	Zeta potential (mv)	Particle size (nm)	Benefits	References
Medoximil	Combination of ball milling and probe sonication	Poloxer 188	-8.3	79	Anti-hypertensive	9
		Poloxer 407	-13.11	274		
		Poloxer 407	-19.13	469.5		
Meloxicam	Media milling	HPMC	-33.10	221		72
Naproxen	Precipitation ultrasonication	HPMC	-33.07	530.05	Anti-inflammatory	51
Quercetin	Evaporative Precipitation into aqueous solution	Pluronic F68 and lecithin	-23.13	251.5	Anti-inflammation, anti-oxidation, antitumor activities Heart, liver and renal diseases	25
			-22.37	192.45		
Furosemide	HPH	Pluronic F68	-30.36	237.79	Anti-hyperglycemic	51

2.8 Crystalline State

X-ray-diffraction and differential scanning calorimeter techniques used to assess' crystalline state of drug nanoparticles. Information of change in melting temperature, thermodynamic and enthalpy change of drug nano-suspensions given by DSC [18].

2.9 Surface Morphology

A brief idea during nano-suspension preparation a drug that have some morphological fluctuations characterized by surface morphology. electron microscopy, atomic force microscopy, transmission electron microscopy, scanning and light microscopy can be used to define the nano-suspensions morphology [50]. Light microscope is used to see minute particles that cannot be differentiate with naked eye. In case of TEM, samples should be sectors very high pitched. A very fine beam of electrons is used to scan samples and Sample morphological features were determined in case of SEM. Two dimensional image of particles provides by SEM. contrasting SEM, atomic force microscopy gives 3D picture of nanoparticles exterior. Moreover, this technique can be done underneath

traditional procedures rather there is no specific handling given for samples [26].

2.10 Biological Activities of Medicinal Plants

Due to their dramatic therapeutic and healing effects medicinal plants have been used since ancient times. Secondary metabolites of medicinal plants like phytochemicals or phenolics particularly phenolic acids, alkaloids, flavonoids, steroids, tannin, reserpine, quercetin, piperine and ajmalicine have excellent curative properties. These bioactive secondary metabolites play an important role for the inhibition of diseases cardiovascular [20], brain stroke, oxidative stress [51], anticancer, neuroprotective, antimicrobial, cerebral-protective microbes and hypertension [52].

The hypertension is a chronic disease. Due to their chronic effects hypertension especially in human beings is one of the main issue in the worldwide. There are many risks of hypertension but angiotensin converting enzyme (ACE) is one of the major risk. Angiotensin Converting Enzyme (ACE) is also called glycosylated zinc dipeptidyl-carboxypeptidase. The main function

of angiotensin converting enzyme is to maintain borderline of arterial blood pressure and electrolyte balance through RAAS. Renin-Angiotensin-Aldosterone (RAAS) converts decapeptide inactive angiotensin-1 into the active octapeptide vasoconstrictor angiotensin-2 as well as into an important vasodilator bradykinin [17].

Therefore, inhibition of ACE is important because hypertension (high blood pressure) is the main issue in worldwide. ACE inhibition is used to address curing of hypertension. Many plant extracts like: Apple peel (flavonoids rich), Ginger rhizomes [53], Legumes [16], Kiwi fruit [18] and Black pepper fruit [54] used to study ACE inhibitory property [15]. The results revealed that secondary metabolites except (one genistein and two quercetin) all apple flavonoids showed good ACE inhibitory activity ($p < 0.05$).

2.11 In-vivo Antihypertensive Effect

In-vivo analysis refers to experimentation using an entire, living organism as opposed to a partial or dead organism. Clinical trials and animal studies are two dissimilar methods of *In-vivo* research. *In-vivo* testing is often employed over *In-vitro* because it is suitable for detecting the overall effects on a living subject experimentation. Experimental models of human diseases were used to study pathophysiological parameters elaborate blood pressure and estimate antihypertensive agents. Nowadays, in most laboratories different class of rats are carried out as a trial models which are available for therapeutic studies of hypertension. Animal models used for assessment of hypertension comprises of dietary hypertension, endocrine hypertension, renovascular hypertension, neurogenic hypertension, mid genetic hypertension and psychogenic hypertension [55,11].

Renovascular hypertension is a commonly used model of hypertension. In this model, rennin angiotensin system plays a vital role [56]. Experimentally, renal hypertension is produced by renal artery constriction, which activates peripheral RAAS and sympathetic nervous system. The activation of sympathetic nervous system elevates the renin secretion from kidney and increase the conversion of angiotensinogen to angiotensin-I is further converted into angiotensin-II by angiotensin converting enzyme (ACE). Hence, through ACE angiotensin-1 is further transformed into angiotensin-2. Angiotensin-2 is an effective vasoconstrictor

resulting increased in blood volume and hypertension due to release of aldosterone that leading to salt and water preservation [57,58].

Goldblatt method is most effective method for presenting renovascular hypertension. Renovascular increase in blood pressure process was described by Goldblatt et al, in 1934. According to this method, a partial narrowing of renal arteries in dogs and rats cause hypertension [55,11]. This type of hypertension has also been induced in monkeys, rabbits and rats. In rabbits and rats, to contract the renal arteries, a U-shaped silver ribbon pin is used. Retroperitoneal approach can be used in dogs, the process used in dogs is carried out by an oblique lateral abdominal cut impartial beneath and equivalent to the costal margin [55,56].

Ethanollic extract of *Cydonia oblonga* Mill leaves lower blood pressure in rats significantly in dose dependent manner. Hypertension cause through two kidney and 2K I C one clip Goldblatt model. Ethyl acetate extract of *Acorus calamus* rhizomes significantly reduce systolic and diastolic blood pressure in (EAC) administrated rats compared to 2K IC rats [37]. Phenolic compound, curcumin extracted from rhizomes of *Curcuma longa* contains antioxidant, antimicrobial, cardio-protective and anti-inflammatory belongings. The antihypertensive effect of curcumin was examined through (2 KI C) 2 kidney- 1 clip induced hypertension in male Sprague-Dawley rat's model, [37]. Recently, my study of interest is specific alkaloids (Piperine) extraction and their therapeutic effect as antihypertensive potential.

2.12 Alkaloids

In present plants, secondary metabolites belong to maximum amount of alkaloid. They confined oxidation state of nitrogen negative and make them water soluble and has salt formation capability. By preventing ion channel alkaloids distort the bio-membranes role. Alkaloids stop translation-process in different organisms and used as antibiotics. Alkaloids have the aptitude to prevent the vital process at cellular and tissue level in animals [59]. They accomplished the upsetting of intestinal process by hindering hydrolytic enzymes actions. Alkaloids weaken the appropriate functioning of liver and kidney reproductive system [60,61]. While many alkaloids are toxic, they have biological and physiological

Table 2. Summary of plant taxonomic and individual species associated with *in-vitro* ACE inhibitory activity, including medium of extraction and specific activity of extractable solids revealed by [14]

Family	Species	Common name	Plant parts	Medium of extraction	IC ₅₀ or %ACE inhibition
Acanthaceae	Andrographis echiodides	False water willow	Aerial	Acetone	55.51% at 00.42
	Asystasia gangetica	Chinese violet	Leaf	Methanol	51.31% at 11
	Ustica flava	Worm-bar	Leaf	Water	54%
Agavaceae	Agave Americana	Century plant	Leaf	Ethanol	82.61% at 0.52
Apocynaceae	Ceropegia rupicola	Bukira	Aerial	Methanol	0.1100
Araliaceae	Panax ginseng	Asian ginseng	Root	Water	5.41
Berberidaceae	Epimedium brevicornum	Epimedium	Leaf	Methanol	84.12% or 0.82
Bignoniaceae	Mansoa hirsta	Not defined	Leaf	Dichloromethane, methanol	53% or 0.431
Casuarinaceae	Casuarina equisetifolia	She-oak	Fruit	Water	94.014% or 0.533
Combretaceae	Terminalia chebula	Black myrobalan	Fruit	Acetic acid	68.050% or 0.833
Diptero-carpaceae	Shorea rubasta	Sal tree	Stem	Water	0.294
Ericaceae	Vaccinium myrtillus	Bilberry	Leaf	Water	0.0037
Fabaceae	Glycine max	Soy bean	Seed	Water	95.23%
Gentianaceae	Exacum offline	Persian violet	Aerial	Methanol	0.402
Lamiaceae	Melissa officinalis	Lemon balm	Leaf	Water	81.96%
	Rabdosia coetsa	Mint type	Whole plant	Cold ether	71.35%
Moraceae	Musanga ceropioides	Umbrella tree	Leaf	Methanol	100%
Paeoniaceae	Paeonia suffruticosa	Tree peony	Root	Methanol	61.31%
Rutaceae	Citrus limon	Lemon	Leaf	Water	71%
Simaroubaceae	Ailanthus excels	Tree of heaven	Leaf	Methanol	54.011%

Table 3. Exported and Imported medicinal plants and their used parts presented by [62]

Exporting of herbals		Importing of herbals	
Scientific Name	Parts Used	Scientific Name	Parts used
<i>Glycyrrhiza glabra</i>	Root	<i>Juniperus communis</i>	Fruit
<i>Withania somnifera</i>	Vegetable rennet	<i>Myrica nagi</i>	Bark
<i>Piper nigrum</i>	Fruit	<i>Hyllanthus amarus</i>	Fruit
<i>Myrica nagi</i>	Leaf	<i>Strycnos nux-vomica</i>	Bark and seed
<i>Piper longum</i>	Fruit	<i>Aloe-Vera</i>	Dried leaf
<i>Rubia cordifolia</i>	Madder root	<i>Ricinus communis</i>	Seed
<i>Curcuma amada</i>	Rhizome	<i>Cinnamomum iners</i>	Bark and leaf
<i>Curcuma longa</i>	Rhizome	<i>Curcuma aromatica</i>	Rhizome
<i>Curcuma aromatica</i>	Turmeric	<i>Garcinia indica</i>	Fruit
<i>Cassia lanceolata</i>	Leaves	<i>Gloriosa superba</i>	Seed

effects that make them appreciated medicines against various diseases, including cancer, diabetes, cardiac dysfunction and malaria. alkaloids are used in pain relief and local anesthesia [59].

2.13 Extraction and Characterization of Alkaloids from Plants

Researchers from whole world have tried to find advanced and developed techniques for the extraction and the approximation of alkaloids due to more attention in these appreciated class of secondary metabolites [63].

Alkaloids have varied chemical structure. Pure alkaloids are frequently soluble in organic solvents. However, salts of alkaloids are water soluble [64]. After extraction, alkaloids remain in salt form and transformed into bases. Plants contain alkaloids in the form of organic acid or salts. A quinolone alkaloid extracted from *Curcuma longa* root tuber. The structures of quinoline alkaloids are identified by spectral evidences. Two alkaloids (8-Hydroxy- 7-((4-nitrophenyl) (phenyl amino) methyl) quinoline-3-yl) propan-2-one and 8-Hydroxy- 7-((4-nitrophenyl) (phenyl amino) methyl) quinoline-3-yl) were isolated from *Nyctanthes arbor-tristis* leaves [65,66].

A new alkaloid persicaside was isolated from methanolic seeds extract of *Prunus persica*. It was recrystallized and purified by using chromatographic techniques. Structure of persicaside resolved from NMR and MS spectra's (Qumar, 2016). Piperine (major alkaloid) was extracted from ethanolic extract of *Piper nigrum* fruit through reflux method [67].

2.14 Alkaloids as ACE Inhibitors

Alkaloids are the least studied class of secondary metabolites for angiotensin converting enzyme inhibition potential. Different extracts of *Fritillaria ussuriensis* were assessed for angiotensin converting enzyme inhibition (ACEI) efficiency. These extracts were purified by different chromatographic techniques to isolate pectisidine, verticine and verticinone alkaloids [66]. Separated alkaloids effectively inhibited ACE activity in a dose dependent manner. [68] and [69] also suggested that the ACE inhibition efficiency of some Indonesian medicinal plants power due to the presence of alkaloids and terpenoids.

2.15 *Piper nigrum* (Black Pepper)

Piper nigrum as a drug was first defined by Hippocrates rather than a spice. *Piper nigrum* was commonly known as Black pepper. It belongs to family Piperaceae and kingdom Plantae occurs in approximately all habitable limestone soil, rainfall regions and generally used as a household. A major constituent of black pepper is an alkaloid piperine. It is present in highly humid areas [70]. This plant is one of the major plants to treatment of different diseases antihypertensive, hepatoprotective, antiasthmatic, hypolipidemic and has a maximum therapeutic efficiency in biological system [71]. Skin disorder, anti-inflammatory, anti-cancer, antihypertensive and nervous system benefits in stroke patients [72]. Based on the reported multiple usage of black pepper, it can also be used to inhibit the Angiotensin Converting Enzyme (ACE). Through [Hippuryl-Histidyl-Leucine (Bz-Gly-His-Leu) substrate ACE activity is determined [53].



Fig. 1. Structure of *P. nigrum* fruit powder and extracted crystals of piperine

2.16 Piperine (Alkaloid of *Piper nigrum*)

Piper nigrum fruit consists of a greater number of alkaloids, flavonoids and consists of many other constituents. *Piper nigrum* contains many alkaloids. But it contains most plentiful an active alkaloid piperine used as spices [70]. Piperidine is the IUPAC name of piperine with chemical formula $C_{17}H_{19}NO_3$. It contains melting point of 128 to 130°C and isolated in yellow crystalline form. It is a very weak base and decomposes into an alkali piperidine and piperic acid. It has three isopiperine *cis-trans* isomer, chavicine *cis-cis*-isomer and *trans-cis* isomer isochavicine isomers. Chavicine isomer is used as taste in peppers. Piperine and its isomers formation shown in Fig. 2.

It is investigated that, piperine is a major component of *Piper nigrum* and used as a spice in peppers. The occurrence of chavicine and isopiperine has not been established in pepper extracts but isochavicine is used as photolytic revolution of piperine. Chavicine with piperine used as a spice in peppers (*Piper nigrum* and *Piper longum*)

and is a combination of many minor alkaloids [73].

2.17 Therapeutic Effect of Piperine as Bioenhancer

Major alkaloid *P. nigrum* is piperine, which act as bioavailability accompaniment of many drug and other ingredients that are of medical significance. By reducing ROS and free radicals piperine works as defensive agent inhibit lipid peroxidation against oxidative damage. Piperine give protection against LDL oxidation because it acts as hydroxyl radical scavenger and used as antioxidant beneficially. Piperine has capability to lessen the level of free fatty acids, triglycerides, cholesterol and phospholipids due their hypolipidemic effect [74]. Except antioxidant and antibacterial affect piperine also possesses anti-inflammatory action by inhibiting the production of point inflammatory intermediates [75]. Ethanolic extract of piperine shows significant protection against cardio-toxicity and hypertension. Hypertension prompted by adriamycin because of its antioxidative and free radical scavenging nature [74].

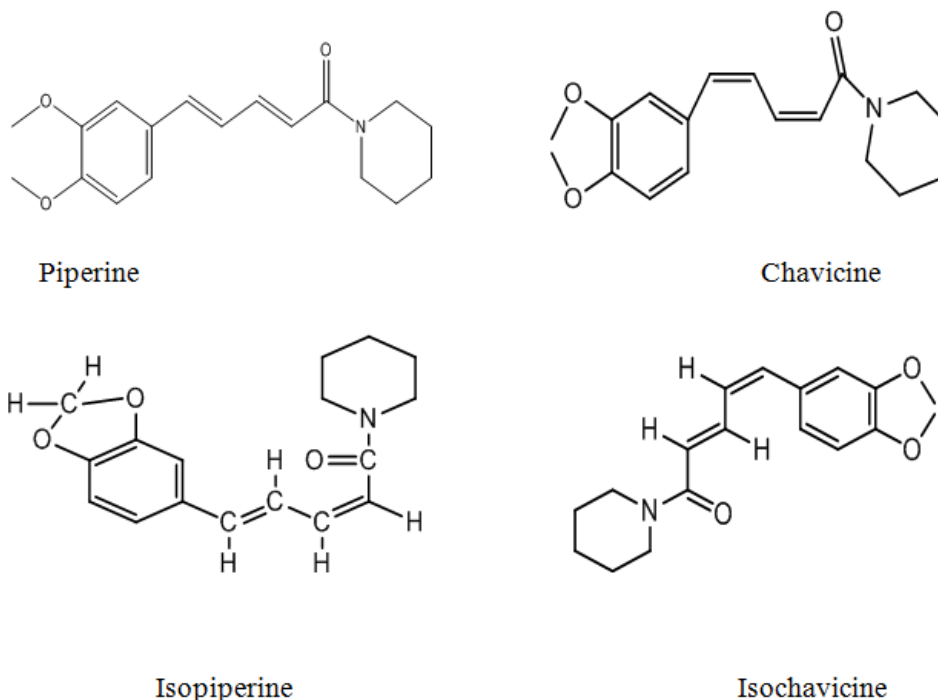


Fig. 2. Piperine and its isomers

Table 4. Piperine and its various physiochemical and biological aspects revealed by [76]

Drug	Class	Reference
Ampicillin	β -lactam antibiotic	[77]
Norfloxacin	Antibacterial agent	[77]
Nevirapine	NNRTI	[78]
Metronidazole	Nitro imidazole antibiotic	[55]
Diclofenac sodium	NSAID's	[79]
Pentazocine	Opioid analgesic	[79]
Carbamazepine	Anticonvulsant	[80]

Table 5. Bioenhancer effect of piperine with some medicines depicted by [76]

Drug	Class	Reference
Rifampicin	Bactericidal antibiotic	[81]
Tetracyclines	Broad- spectrum antibiotic	[76]
Sulfadiazine	Sulphonamide	[76]
Oxytetracyclines	Antibiotic	[76]

3. CONCLUSION

Poor aqueous solubility is the principle obstacle for scientists working on oral drug delivery formulation of drug composites and lead to employment of innovative formulation technologies. While nano-sized particles have been employed in pharmaceutical industry for numerous periods. Various reports in the review have revealed advantages of nano-formulation as compared to other technologies (such as liquid filled capsules) in improving *in-vivo* oral bioavailability over micronized API. The objective of the nano-suspension is to reduce the drug particle size into nanometer range, which enhance solubility, dissolution rate and bioavailability of biopharmaceutical active compounds. Piperine nano-suspension effectively inhibited ACE activity in a dose dependent manner due to reduced particle size, which enhance the bioavailability and API dissolution rate of active constituents. Due to nano-sizing piperine nano-suspension can be used as a better alternative to treat cardiovascular diseases with improved therapeutic efficacy as compared to extract.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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