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Appraisal of Bioenhancers in Improving Oral Bioavailability: Applications to Herbal Medicinal Products

Francis A. Oladimeji[1], Adebanjo J. Adegbola[2] and Cyprian O. Onyeji[2*]

[1]Department of Pharmaceutics, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria.
[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

The oral route of administration is associated with some challenges such as poor aqueous solubility and low intestinal permeability of many active pharmaceutical ingredients resulting in decreased drug absorption and subsequent poor bioavailability. In recent years, research on bioenhancers has started receiving increased attention and this approach can complement the traditional methods of solubility enhancement. Bioenhancers boost the bioavailability of drugs when co-administered at low doses with the drug. A large variety of compounds have been clearly demonstrated to possess significant bioenhancing activity with different mechanisms of action. Some compounds considered as bioenhancers isolated from plants include piperine, gallic acid, niaziridine, sinomenine, genistein, and lysergol. There are several reports of herbal medicinal products (HMPs) that require large doses to be therapeutically effective and some studies have identified that the active compounds in such products have poor bioavailability. Thus, there is every need to optimise dosage formulations of HMPs. The application of the concept of bio-enhancement and knowledge of their mechanism of action can lead to the selective use of suitable bioenhancers

**Corresponding author: E-mail: conyeji@oauife.edu.ng, cyponyeji@yahoo.com;*

resulting in reduction in therapeutic dose of the HMP, thus reducing the possibilities of toxicity. This review was aimed at updating knowledge on different natural bioenhancers and also provides an overview of currently marketed HMPs containing bioenhancers. The article highlights different strategies that can be employed to improve the bioavailability of HMPs. The bioactive constituents of the HMP were identified and facts on their physicochemical properties, metabolic fate, as well as factors that impact on their gastrointestinal absorption, were examined with a view to providing information to serve as a guide in developing suitable bioavailability enhancement strategies for other HMPs.

Keywords: Bioavailability improvement; bioenhancers; herbal medicinal products.

1. INTRODUCTION

1.1 Bioavailability and Biopharmaceutic Classification Systems (BCS)

For a drug to undergo absorption, it must be available as an aqueous solution at the site of absorption. This implies that aqueous solubility and dissolution rate are very important properties of any drug and impact on its oral bioavailability [1]. A major challenge in the development of formulations of poorly water-soluble drugs is how to increase their bioavailability and it is reported that about 70% of all new chemical entities (NCE) have poor water solubility and fail to have commercial viability due to their limited bioavailability [2]. The drug molecules have to traverse biological membrane at the absorption site to enter the systemic circulation. Hence, the gastrointestinal permeability of the drug is also a fundamental factor that contributes to determining the rate and extent of drug absorption and its bioavailability [3]. The importance of solubility and permeability in controlling bioavailability has been demonstrated in their being used to construct the Biopharmaceutic Classification Systems (BCS). BCS is a scientific framework for classifying a drug substance based on its solubility and intestinal permeability and they have been found useful in combination with dissolution rate data to predict absorption profile of drug products [4-6]. It is important to consider the BCS class of a drug substance to be able to make an informed judgment on the best approach to follow when improvement of bioavailability of a drug is desired. According to United States Food and Drug Administration (USFDA) BCS guidance, a drug substance is considered highly soluble when the highest dose strength is soluble in 250 mL or less of aqueous media over the pH range of 1- 6.8 at 37 ± 1°C [7]. The drug permeability may be determined directly from the extent of intestinal absorption of a drug substance in humans. It can also be estimated indirectly from the measurements of the rate of the mass transfer across the intestinal membranes in vitro or by using in vivo models. A drug substance is considered to be highly permeable when the systemic bioavailability or the extent of absorption in humans is determined to be 85 percent or more of an administered dose based on a mass balance determination (along with evidence showing stability of the drug in the GI tract) or in comparison to an intravenous reference dose [7].

The BCS class 1 (high solubility/high permeability) usually do not have bioavailability problems since their absorption is neither dissolution nor permeability rate-limited. However, Active Pharmaceutical Ingredients (APIs) in this BCS class can only enjoy bioavailability waiver if they satisfy all the requirements of high solubility, high permeability and rapid dissolution [7]. Examples of drug substances in this class include diltiazem, metoprolol, paracetamol, propranolol etc [8]. The BCS class II drugs have low aqueous solubility and high permeability, hence, dissolution process is the rate-limiting step in the drug absorption process. Bioavailability of this class of drugs can be enhanced by using techniques that increase the solubility and dissolution rates of the compound. Studies from our laboratories and by several other investigators indicate that different methods are used for the improvement of the solubility of poorly water-soluble drugs and these include micronisation, crystal form modification, salt formation, use of surfactant, complexation with cyclodextrin and other complexing agents, solid dispersion, and others [3,9-12]. With the advancement in technology, novel techniques such as cryogenic technology, nano-suspension, and supercritical processing have also been developed for solubility enhancement [1,13-16]. It is noteworthy that several researches on solubility enhancement have been focused on the BCS class II drugs [17,18]. For the BCS class III drugs (High solubility/low permeability), there

can be bioavailability waivers for the APIs that have high solubility and rapid dissolution rate. In addition, the test product formulation should be qualitatively the same and quantitatively very similar [7]. However, since the absorption of some APIs in this class can be limited by its rate of intestinal permeability, the use of absorption enhancers or bioenhancers which can improve their permeability has the potential of enhancing bioavailability of the drug. In the case of BCS class IV compounds (low solubility/low permeability), the drug absorption is both dissolution and intestinal permeability rate-limited. The bioavailability of this class of drug substances may be achieved through enhancement of solubility and dissolution rate in addition to the use of absorption enhancers. Examples of drugs in this class include ritonavir, saquinavir, taxol, cyclosporine A and several others [8].

1.2 Bioavailability with Bioenhancers

It is evident from the BCS that the classes III and IV drugs have low intestinal permeability, thus, approaches that can increase the permeability of these compounds are likely to enhance their bioavailability. The use of bioenhancers can be regarded as an innovative concept. Bioenhancers are agents or substances that boost the bioavailability of drugs when co-administered at low doses with the drug. An ideal bioenhancer should be safe, effective, readily available and economical. In addition, it should not produce its own pharmacological effects at the dose used, should be rapid-acting with predictable and reproducible activity and should be compatible with other APIs [19,20]. When used in a judicious manner bioenhancers can lead to reductions in drug dosages due to enhanced bioavailability, with resultant decreases in drug treatment cost, toxicity and adverse effect profiles [21]. From historical viewpoint, bio-enhancement originated from Ayurvedic medicine as reported by Bose in 1929 who first described the use of a condiment, long pepper, to improve the antiasthmatic effect of the leave of *Adhatodavasica* species [22].

1.3 Bioavailability and Herbal Medicinal Products

From time immemorial, phytomedicine has been playing an important role in pharmacotherapy and presently, about 50 percent of useful drugs are obtained from natural sources [23]. Several reports indicate that the use of Herbal Medicinal Products (HMPs) as part of Complementary and Alternative Medicines is very widespread in large sections of the population in developing countries [24,25]. Also, as complementary and alternative medicines are now becoming popular in developed countries [26,27], reports show that over the past two decades, there has been an increased prevalence in use of HMPs in these countries [28,29]. The revival of interest in HMPs has been ascribed to a number of factors such as the perception that herbal products are safer because they are natural in origin. Other reasons include the cost-effectiveness and the belief that herbal medicines might be effective in the treatment of certain diseases where conventional therapies have proven to be ineffective [27,30, 31]. In spite of their significant therapeutic effects and less adverse effects profiles, there are numerous cases of herbal products with remarkable in vitro activities but with disappointingly poor in vivo efficacy. These are revealed through animal and clinical trial studies conducted on HMPs indicating that the herbal drugs have poor bioavailability [32,33]. Thus, several plant extracts and phytoconstituents demonstrate reduced in vivo actions due to the inappropriate molecular size, poor aqueous solubility and/or poor intestinal permeability of the bioactive constituents resulting in decreased absorption and consequently poor bioavailability. For example, extract of the plant *Azadirachta indica*, which is widely used in indigenous medicine for the treatment of malaria exhibits high in vitro activity against various strains of *Plasmodium falciparum* but required very high doses (e.g. 800 mg/kg body weight) to exhibit significant in vivo activity [34,35]. Similarly, *Silybum marianum* commonly known as milk thistle has Silymarin as the major bioactive compound isolated from its seed which has numerous applications including use for the oral therapy of chronic liver disorder. But, its efficacy is limited by poor aqueous solubility, resulting in poor bioavailability [36]. In addition to other techniques used for improving solubility and bioavailability of APIs, it has been demonstrated that the efficacy of HMPs with poor in vivo activity can be improved by formulating the product with a herbal bioenhancer [37]. The need for bioenhancers is especially applicable when the bioactive constituents have limited intestinal permeability. In essence, a better understanding of the physicochemical characteristics of the bioactive constituents of HMPs will assist in designing a formulation with optimal bioavailability.

The objective of this review was to update knowledge on different natural agents being used as bioenhancers and to discuss their possible mechanisms of action with the overall aim of providing an insight into how these pharmacological advances can be applied in optimization of bioavailability of formulations of HMPs. Also, the article highlights different other strategies that can be employed to improve the bioavailability of HMPs. Since herbal medicine is increasingly playing a significant role in the healthcare industry, it is appropriate that the products also receive due attention to the orthodox medicines.

2. ROLE OF BIOENHANCERS

2.1 Classification of Bioenhancers

A large variety of both natural and synthetic compounds have been clearly demonstrated to possess significant bioenhancing activity and they have different mechanisms of action [38-40]. Bioenhancers are categorized either based on their origins (natural-from plants and animals; and synthetic), chemical classes or mechanism of actions. Some compounds considered as bioenhancers isolated from plants include piperine, gallic acid, niaziridine, sinomenine, genistein, and lysergol. Apart from the isolated molecules, whole plant or parts of the plant are also used as bioenhancers. Examples of parts of plants used as bioenhancers include the rhizomes of *Zingiber officinale* and the seeds of *Carum carvi* [16]. Piperine is the world's first bioavailability enhancer first reported in 1979 and there are currently several reports on its bioavailability enhancement of various drugs and nutraceuticals [39,40]. Since bioenhancers have different mechanisms of bioavailability improvement, it is pertinent to clarify that the term 'absorption enhancers' is used for a class of bioenhancers that promote bioavailability by specifically enhancing the intestinal permeability of the drug [41]. Based on their mechanisms of action, there are three major classes of bioenhancers [38,42] and these include:

(a) Regulators of GIT functions: *Aloe vera*, Niaziridin, *Zingiber officinale* (Ginger), Glycyrrhizin (from Liquorice).
(b) Inhibitors of cytochrome P450: Piperine, Naringin, Gallic acid and its esters.
(c) Inhibitors of P-glycoprotein (P-gp) and other efflux pumps: Piperine, *Carum carvi*, Genistein, Sinomenine, Naringin.

2.1.1 Inhibitors of cytochrome P450 and P-gp

Piperine (1-piperoyl piperidine) is an alkaloidal component of Black pepper (*Piper nigrum*) and long pepper (*Piper longum*). The fruits of these two herbs generally contain volatile oil (1 – 2.5%) and total alkaloids (5 – 9%) with the major alkaloids consisting of piperine, piperidine, and piperetine. It has been documented that most of the pharmacological properties of the fruits are credited to the piperine [43]. Ethnopharmacological survey indicates that piperine/pepper is commonly used as antirheumatic, analgesic, diuretic, antispasmodic and antiseptic [44] or a preservative and a perfume in some climes. However, when used as a bioenhancer, these pharmacological effects are not relevant.

Studies have established piperine to be useful as a bioenhancer at a low dose of 10-15 mg [45]. This property was first utilized to enhance the bioavailability of rifampicin by more than 50% in an antitubercular regimen [46]. Thus, studies have shown that piperine increases the bioavailability and efficacy of numerous drugs including phenytoin, sulfadiazine, propranolol, carbamazepine, fexofenadine, ampicillin Trihydrate, nevirapine, metronidazole, omeprazole and many other drugs. It was concluded that piperine significantly enhanced the oral bioavailability of these drugs, possibly by decreasing the elimination and/or by increasing its absorption through the inhibition of P-glycoprotein (P-gp) at the gut and kidneys, in addition to inhibition of CYP3A4 and other drug-metabolizing enzymes especially UDP-glucuronosyl transferase (UGT) in the gut [47-53]. Piperine also mediates bioavailability enhancement by enhancing the blood supply in the enteric vessel due to its local vasodilatory effect [54], improvement of lipid solubility through increase in the secretion of bile acid and modification of intestinal epithelial cells [45].

Other herbal bioenhancers that have similar bioenhancing mechanisms of action like piperine include:

(a) Naringin: Naringin is a CYP3A4 inhibitor [55]. It is the major flavonoid glycoside found in grapefruit, apples and onions. Due to its ability to inhibit intestinal CYP3A4 and P-gp, it has been found to enhance the bioavailability of a number of CYP3A4 and P-gp substrates such as paclitaxel, diltiazem, verapamil, saquinavir and cyclosporine A [56,57].

(b) Gallic acid: Gallic acid is a CYP3A4 inhibitor [58] and its esters like propyl gallate, octyl gallate and lauryl gallate have been demonstrated to increase the bioavailability of orally administered pharmaceutical compounds which are substrates of CYP3A4 [57,58].

(c) Sinomenine: Sinomenine, a monoterpene glucose alkaloid extracted from *Sinomenium acutum* is known to be a P-gp inhibitor [59]. Co-administration of sinomenine markedly increased the oral absorption of paeoniflorin through a decrease in the efflux transport of the drug by P-gp in the small intestine [59]. Paeoniflorin, used in the treatment of inflammation and arthritic conditions, has a very low oral bioavailability which is in the range of 3 – 4% [60].

(d) Genistein: Genistein, an isoflavone found in a number of dietary plants like soyabean (*Glycine max*) and kudzu (*Pueraria lobata*), is known to be a P-gp inhibitor [40,61]. The intestinal absorption of paclitaxel, a substrate of P-glycoprotein, was significantly augmented when co-administered with genistein [61].

(e) *Carum carvi:* *Carum carvi* (family – Apiaceae) also called Caraway/cumin has carvone as its major constituent which is obtained from the dried and crushed seeds. At a dose in the range of 5 – 100 mg/kg body weight, this extract significantly enhanced bioavailability of a wide range of drugs of various therapeutic classes including antimicrobial, antileprosy, anti-inflammatory, anti-arthritic, cardiovascular, antihistaminics, CNS drugs, antiulcers, nutraceuticals and herbal formulations. Its mode of action is by inhibition of P-gp efflux pump [38,40,62].

2.1.2 Regulators of GIT functions

Ginger, the rhizome of *Zingiber officinale*, has a long history of medicinal use for conditions such as diarrhoea, nausea, and stomach ache, in addition to a range of other pharmacological activities [63,64]. Ginger contains numerous constituents but the major is gingerols which can be converted to shogaols, zingerone, and paradol [65]. These compounds along with the volatile oil constituents have strong effects on GIT mucous membrane resulting in regulating the intestinal function to facilitate absorption [42]. Ginger is used in the range of 10-30 mg/kg body weight as bioenhancer [66].

The extracts of ginger have been found to be selective in their abilities to enhance bioavailability of a wide range of drugs of different therapeutic classes and the enhancement can vary from 20% to 200% [67, 68]. These drugs include azithromycin, ketoconazole, erythromycin, cefadroxil, amoxycillin, cloxacillin, rifampicin, ethionamide, zidovudine and fluorouracil [47]. These drugs are not necessarily substrates of particular drug metabolising enzymes or drug transporter systems. Therefore, unlike piperine and other bioenhancers which are inhibitors of CYP3A4 and P-gp, and whose bioavailability enhancement are manifested on drugs that are substrates of these systems, the bioenhancing effect of ginger extract is not predictable.

Other herbal Bioenhancers with action on GIT membrane similar to the effect of ginger include:

(a) Aloe Vera Extract: The gel and whole leaf extracts of *Aloe vera* have been demonstrated to enhance the oral absorption of vitamin C and vitamin E [69]. The bioenhancing mechanism of action of these products is thought to be attributed to their polysaccharides constituents. Polysaccharides of natural origin such as chitosan act as penetration enhancers through a process of a transient opening of the tight junctions between adjacent epithelial cells. Thus, they are capable of enhancing the intestinal absorption of concurrently administered drugs. It has been shown that the *Aloe vera* gel and whole leaf extract significantly increased the transport of the macromolecular peptide drug, insulin, across the Caco-2 cell monolayers [70]. Limited information is currently available on the drug absorption enhancement activities of *A. vera* extracts.

(b) Liquorice: Liquorice is the name given to the root of the plant *Glycyrrhiza glabra*. The extract of this plant contains a phytoconstituent, Glycyrrhizin, that has been established to have bioenhancing activity. The compound is reported to cause a 2 to 6 fold enhancement in GIT absorption of various drugs such as rifampicin, tetracycline, nalidixic acid, ampicillin and vitamins B1 and B12 [71]. In a study to elucidate the molecular mechanism of bioenhancing action of glycyrrhizin, the influence of the compound on the functional properties of biomembranes was investigated using a

model system of human erythrocytes. Glycyrrhizin produced about 60% increase in the permeability and decreased elasticity modulus of cell membranes even in micromolar concentrations [72]. Structurally, glycyrrizin is a saponin and saponins have surfactant properties that can act on the paracellular route [73]. The absorption enhancing effect of glycyrrhizin is further enhanced in the presence of other enhancers [74].

(c) Nitrile glycosides: Niaziridin and niazirin are nitrile glycosides isolated from the pods of *Moringa oleifera* (Family - Moringaceae). They have been found to increase the bioavailability of commonly used antibiotics (eg rifampicin, tetracycline and ampicillin), vitamins, and nutrients by facilitating their absorption through the gastrointestinal membrane [47,75]. For example, in a study on the influence of active fraction isolated from pods of *Moringa oleifera* on pharmacokinetic disposition of rifampicin, the results revealed that co-administration of the extract resulted in significantly increased rifampicin plasma concentrations [76].

3. HERBAL MEDICINAL PRODUCTS WITH BIOENHANCERS

Applications of novel techniques for improvement of bioavailability of drugs have been extended to HMPs and these include herbal liposomal formulations, microspheres, nanoparticles, transferosomes, lipid-based herbal formulations, and other herbal vesicular formulations [77]. In addition, there are studies reporting clear evidence of improvement of oral absorption and bioavailability of HMPs by concomitant use of bioenhancers. The literature is replete with studies indicating bioavailability enhancement of orthodox medicines by bioenhancers [16] but an extensive review of the literature revealed comparatively limited data on the deliberate application of this technique in HMPs with poor bioavailability.

The emphasis of this review is to focus more on the enhancement of bioavailability of HMPs. The bioactive phytoconstituents of the HMP are identified and information on their physicochemical properties, metabolic fate as well as factors that impact on their gastrointestinal absorption are examined. These can serve as guide for the application of the bioavailability enhancement technique to other HMPs with intrinsic poor bioavailability of its active constituents. There are numerous reports of decreased absorption and subsequent poor bioavailability resulting in reduced in vivo activities of several medicinal plant extracts and phytoconstituents. The factors contributing to decreased gastric absorption and subsequent poor bioavailability include the nature of physicochemical properties of the active constituents such as poor aqueous solubility, large molecular weight, excessively high aqueous solubility, instability in the GIT fluid as well as metabolism at the gut epithelial cells and activities of drug transporters [34-36].

3.1 HMPs with Piperine (and Other Bioavailability Improvement Approaches)

3.1.1 Curcumin

Curcumin, commonly called diferuloyl methane, has been identified as the active principle derived from the rhizome (called turmeric) of the herb *Curcuma longa*. It is a hydrophobic compound that has been used traditionally in the management of a variety of ailments due to its wide spectrum of pharmacological activities [78]. Curcumin has structurally related compounds which are all curcuminoids but are often referred to simply as curcumin and their chemical structures are presented in Fig. 1. Commercially available curcumin generally contains three major curcuminoids: curcumin (77%), demethoxycurcumin (7%) and bisdemethoxy-curcumin (3%) [78].

Early studies of the pharmacokinetics of curcumin in animal models have shown that it is poorly absorbed from intestine after oral administration of different doses of the compound [79]. The hydrophobicity of the compound results in its poor aqueous solubility and this class of compounds in the BCS is associated with reduced bioavailability. In a study to determine whether curcumin is a substrate of P-gp and CYP3A isozymes, the compound was co-administered with drugs (celiprolol and midazolam) that are known substrates of these systems in a rat model. Results indicated that the plasma blood levels of celiprolol (a P-gp substrate) and midazolam (substrate of CYP3A) were significantly increased by curcumin, confirming that curcumin is a substrate for both P-gp and CYP3A4 [80]. Since the mechanisms by which piperine acts as a bioenhancer is by reducing drug metabolism in the gut through

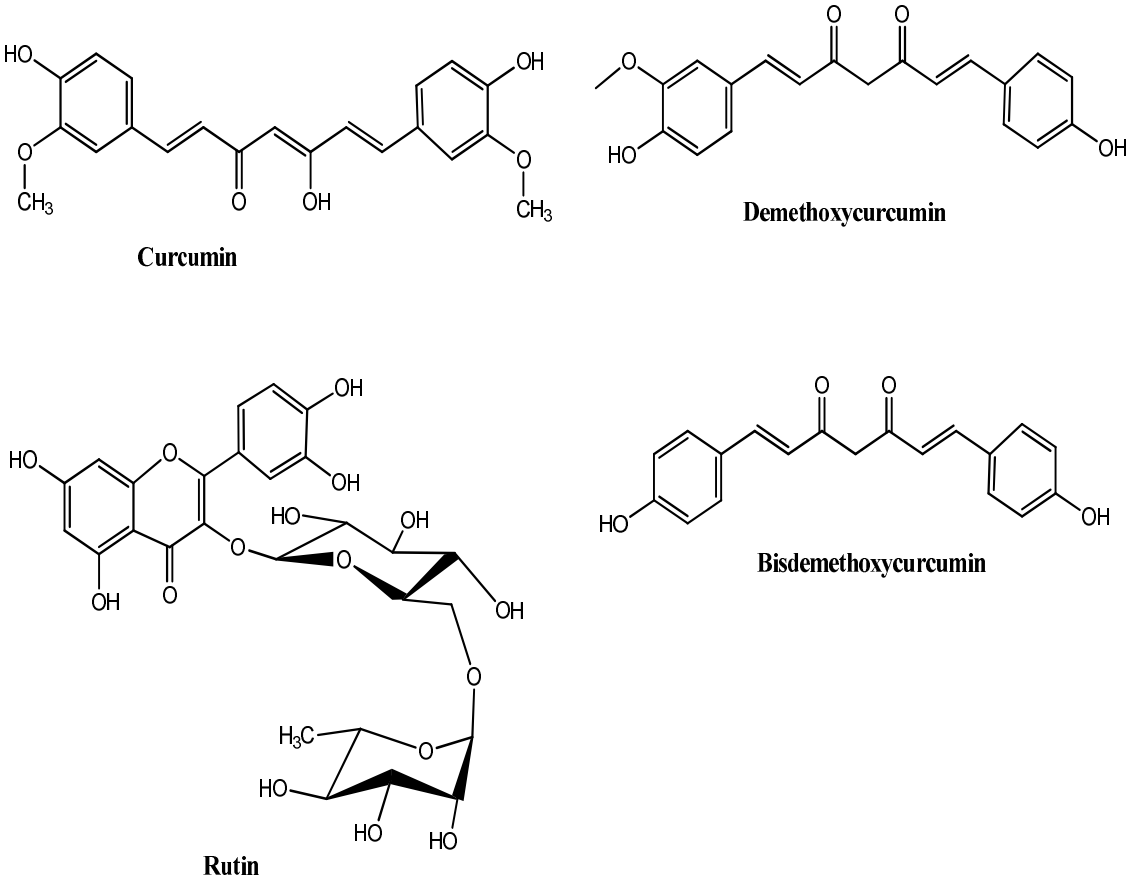


Fig. 1. Chemical structures of Curcumin and its derivatives, and Rutin

inhibition of CYP3A activity in gut epithelial cells and also by inhibiting P-gp transport system [52], inhibition of these two systems by piperine in gut epithelial cells is expected to lead to increasing in curcumin absorption. This hypothesis was validated in a study on the effect of piperine on the bioavailability of curcumin in which it was demonstrated that concomitant administration of piperine significantly increased the extent of absorption and bioavailability of curcumin in both rats and humans with no adverse effects. In humans, the increase in bioavailability was as high as 2000% [81]. Also, there was a potentiation of curcumin antidepressant activity following concomitant administration of this HMP with piperine. This approach has been found useful in the management of depression [82,83]. It is reasonable to project that a poorly absorbed HMP with constituents having similar physicochemical characteristics as curcumin and are also substrates of P-gp and CYP3A4 can be made more bioavailable by concurrent use with piperine.

3.1.2 Rutin

Rutin also known as quercetin-3-rutinosoid or sophori is a flavonol glycoside comprising quercetin (a flavanol) and a disaccharide called rutinose (α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranose). The chemical structure is presented in Fig. 1. It is found in a wide variety of plants including many citrus fruits and is often sold as an herbal supplement. Quercetin, the active moiety of rutin has been reported to exert numerous pharmacological activities including antidiabetic, anti-inflammatory, antioxidant, cardiovascular, and it has been used therapeutically to decrease capillary fragility and for management of many chronic diseases [84-86].

Considering the size and polarity of flavonoids, some medicinal chemistry rules would predict difficulty in their ability to cross bio-membranes. Thus, generally, flavonoids are poorly absorbed from the small intestine. The high molecular

weight of rutin (664.58) also limits the intestinal permeability of the compound. Studies have shown that trans-epithelial transport of rutin is poor and mainly by passive diffusion [87,88]. For this class of compounds, techniques that enhance solubility and intestinal permeability are promising approaches to bioavailability improvement. The oral bioavailability of rutin in human studies showed that the compound is poorly orally absorbed with only 20 – 30% of the dose absorbed [89]. There are numerous reports on studies aimed at improving the bioavailability by applying various excipients and techniques including use of cyclodextrin complexation, surfactants, nano-phytosomes, chitosan, and nanoemulsion [20,90-92].

A contribution to the poor oral absorption of rutin is the metabolism of the compound in the gut. The microflora in lower gut hydrolyzes rutin to quercetin and isoquercetin [93]. But, the enzyme Uridine Diphosphate-Glucoronosyl-Transferase (UGT) conjugates the part of the free quercetin with glucuronide producing a bulky and inert adduct which is un-absorbable, hence, reducing the extent of absorption of quercetin [94]. Since piperine has been shown to be an inhibitor of UGT [51], this bioenhancer was demonstrated to improve the neurocognitive efficacy of quercetin in mice [95] and this is an indication of enhanced bioavailability of quercetin by piperine. Studies have also shown that piperine can enhance the antioxidant and anti-inflammatory properties of quercetin, and quercetin exhibited stronger neuroprotective effects when given in combination with piperine [96]. An in vitro study demonstrated that piperine enhanced antioxidant effect of rutin and the authors suggested that the findings provide more insight into the mechanism of the hepato-protective action of rutin [97]. It is not likely that piperine enhanced bioavailability and hence the efficacy of rutin or quercetin through modulating P-gp activity because rutin and related compounds have been shown not to be substrates for efflux transporters [89]. From the foregoing, the bioavailability of HMPs containing phytoconstituents with similar physicochemical properties and metabolic fate as rutin might be expected to be enhanced when co-administered with piperine.

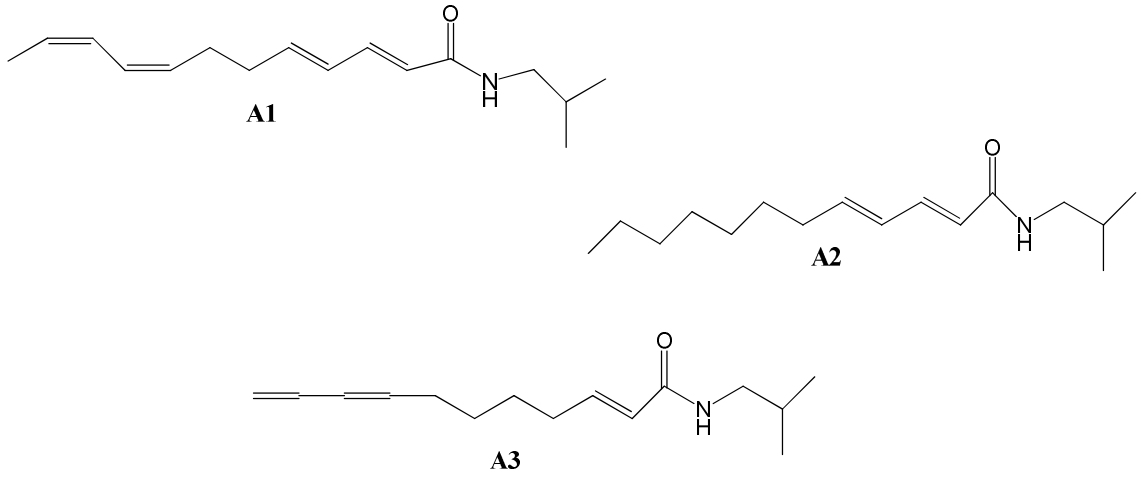
3.1.3 Echinacea

Preparations from the genus Echinacea which are from Echinacea species (*Echinacea angustifolia, Echinacea pallida* and *Echinacea purpurea*) are among the most widely used herbal medicines and have a long history of medicinal use for a variety of conditions especially in the prevention and treatment of upper respiratory tract infections attributed to the immunostimulatory and anti-inflammatory properties of the HMP. The fresh or dried underground parts (roots, rhizomes) of these species are used and the marketed echinacea products may contain one or more of these three species [98-100]. The major constituents that are responsible for the bioactivities of this HMP are hydrophilic components which include caffeic acid derivatives (cichoric acid, echinacoside, and chlorogenic acid) and lipophilic components which are alklyamides [98]. Their chemical structures are presented in Fig. 2.

Studies to enhance the bioavailability of echinacea constituents have been reported. Due to the hydrophilicity of caffeic acid derivatives, their intestinal permeability has been shown to be poor while the lipophilic alklyamides diffuse rapidly [101]. Echinacoside, representing caffeic acid derivatives is a substrate of P-gp and inhibition of this transporter by verapamil resulted in the oral bioavailability enhancement of the compound [102]. Also, the essential oils in clove oil acting as absorption enhancers augmented the intestinal absorption of echinacoside [102]. Since these caffeic acid derivatives are substrates of P-gp and CYP3A4 [102,103], it is expected that absorption of these compounds may be enhanced by piperine which inhibits the activities of this transporter and isozyme at the gut epithelial. This assumption was validated by studies indicating augmentation of bioavailability of echinacea extract by concurrent administration of piperine [42].

3.1.4 Boswellia

Boswellia serrata (Family: Burseraceae) is a deciduous middle sized tree mostly concentrated in parts of Asia and Africa. The dried exudate from the bark of this tree is an oleo-gum-resin which has a wide range of pharmacological activities and is reported to be useful in the treatment of bronchitis, asthma, cough, inflammatory conditions and various intestinal problems [104-106]. The major fraction of the gum-resin consists of terpenoids of which the biologically active phytoconstituent is boswellic acid which is a pentacyclic triterpene acid [107]. α- and β-Boswellic acids were the first to be isolated followed by several other of its derivatives such as 3-acetyl-α-boswellic acid, 3-acetyl-β-boswellic acid, 11-keto- β-boswellic acid and 3-acetyl-11-keto- β-boswellic acid [106]. Some of these structures are presented in Fig. 3.



Dodeca-2E,4E,8Z,10Z-tetraenoic acid isobutylamide (A1);
Dodeca-2E,4E-dienoic acid isobutylamide (A2);
Undeca-2E-en-8,10-diynoic acid isobutylamide (A3)

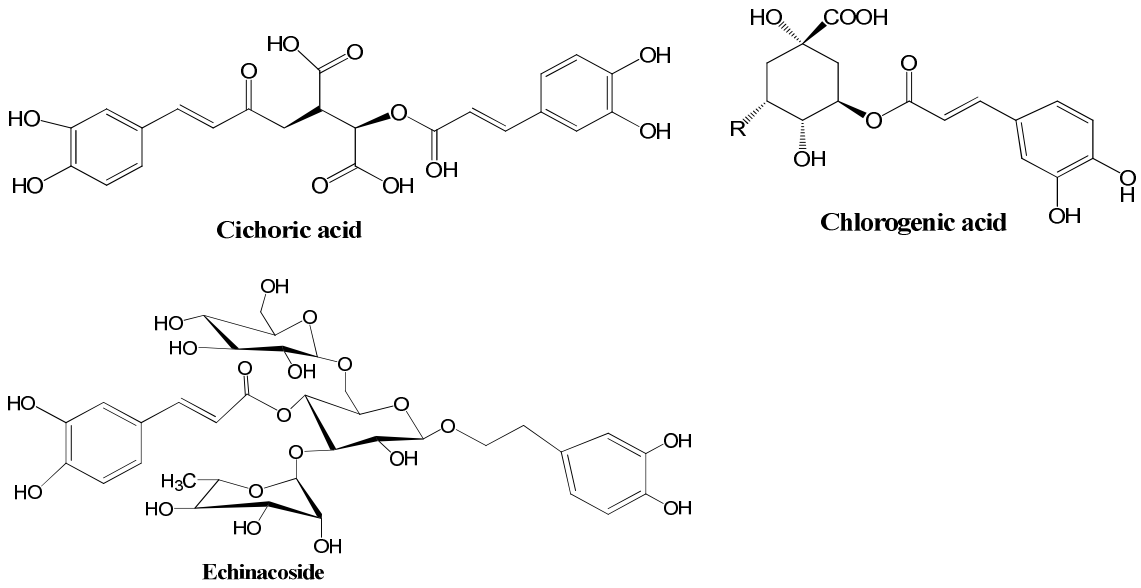


Fig. 2. Chemical structures of some of the bioactive constituents of Echinacea

Being pentacyclic triperpenes, boswellic acid and its derivatives are lipophilic and the poor aqueous solubility results in low oral bioavailability of these compounds. In addition, the keto derivatives of boswellic acid undergo extensive first pass metabolism and this contributes to the low oral bioavailability [108, 109]. Many attempts have been made to produce drug delivery systems with improved absorption such as loading boswellic acids into liposomes, solid lipid nanoparticles as well as delivery as niosomes, phytosomes and nano-micelles [110].

Studies indicate that CYP3A4 plays a predominant role in the hydroxylation reaction of Keto-boswellic acid in the GIT of humans [111] and this contributes to a reduction in drug absorption. Coadministration with piperine has the potential to improve the bioavailability of keto-boswellic acid as it is an inhibitor of CYP3A4. It has been demonstrated that piperine significantly enhanced the bioavailability of boswellia extracts when concurrently administered [112]. This observation can also be applied to other HMPs containing phytoconstituents with similar metabolic fate.

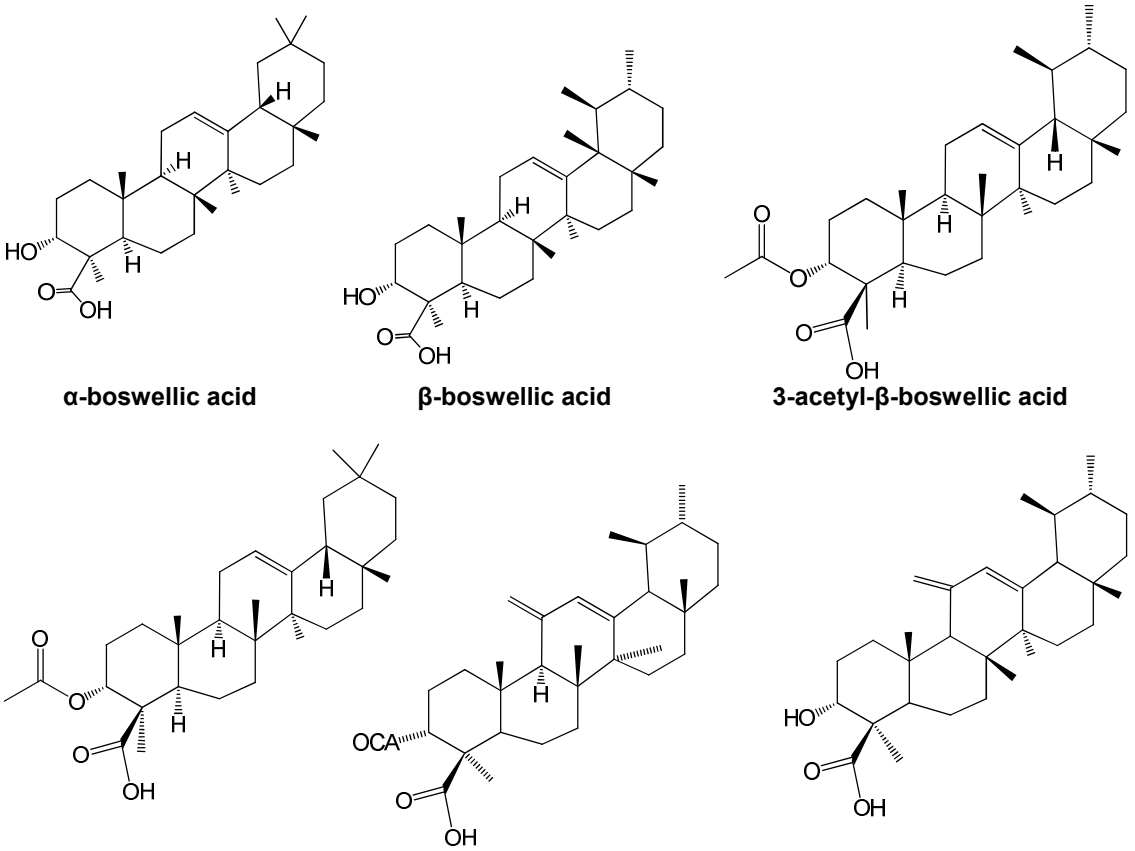
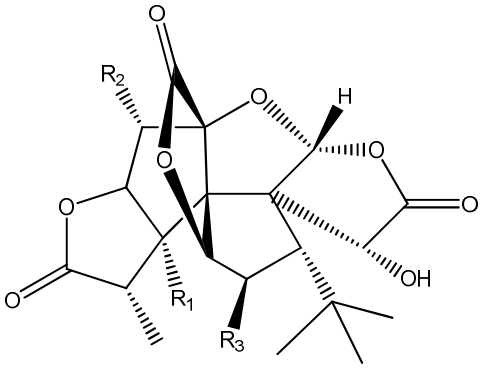


Fig. 3. Chemical structures of the six major bioactive constituents of *Boswellia serrata* extracts

3.1.5 Ginkgo Biloba

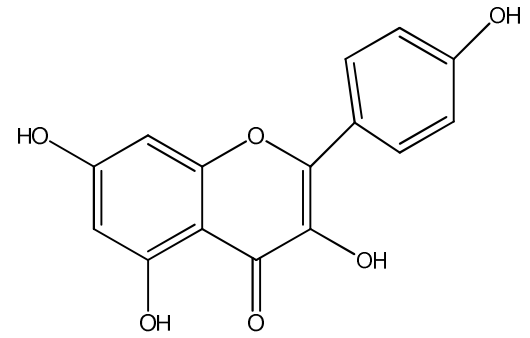
Ginkgo biloba is a large tree originally native to China but it is now cultivated worldwide. The leave extract has been used in traditional medicine for centuries to treat circulatory disorders, asthma, tinnitus, vertigo, and cognitive problems, and it is one of the most commonly taken phytomedicines globally [113,114]. The pharmacological properties of this HMP are attributed to its constituents of terpenoids, flavonoids and proanthocyanidins [115]. These active components have been identified as bilobalide, ginkgolides A, B, C, quercetin, kaempferol, rutin hydrate, and isorhamnetin [116]. Their chemical structures are depicted in Fig. 4.

As earlier highlighted with quercetin, flavanoids have poor oral absorption. Thus, different approaches have been investigated for improvement of the bioavailability of Ginkgo extract and these included the use of phospholipid complexes and solid dispersions [117]. The major pathway of metabolism of flavonoids in humans is hydrolysis in the intestinal microflora followed by phase II conjugation reaction where UGTs are the major contributors [118]. Based on the knowledge of mechanisms of bioenhancing activity of piperine, being an inhibitor of UGT, a concurrent administration with piperine is expected to enhance the oral absorption of the Ginkgo flavanols. In addition, studies have shown that Ginkgo flavonols quercetin, kaempferol and isorhamnetin are substrates of P-gp. This efflux pump contributes to limiting the bioavailability of the flavanols [119]. Since piperine also acts by inhibiting activity of P-gp, this is an additional pathway through which it can enhance bioavailability of Ginkgo flavanols. These expectations have been validated in studies which demonstrated that piperine significantly enhanced the bioavailability of Ginkgo extracts when concurrently administered [112].

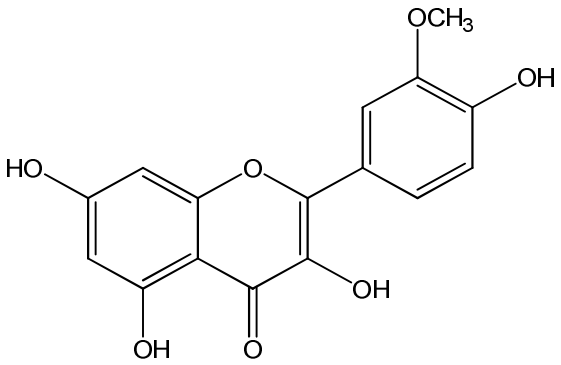


Ginkgolides A, B and C

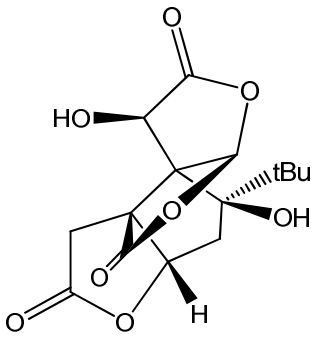
	R^1	R^2	R^3
A	OH	H	H
B	OH	OH	H
C	OH	OH	OH



Kaempferol



Isorhamnetin



Bilobalide

Fig. 4. Chemical structures of the major bioactive constituents of *Gingo biloba* extracts

The physicochemical properties and metabolic fate of the Ginkgo terpene lactones (bilobalide and ginkgolides) are different from those of the flavanols. The intestinal absorption of the terpene lactones is dictated by their intermediate membrane permeability and they are not substrates of CYP3A4, UGT and P-gp at the gut [120-121]. Thus, augmentation of the oral absorption of these terpene lactones by piperine could plausibly be through the non-specific mechanism of enhancing the blood supply in the enteric vessel due to the local vasodilatory effect of piperine [54].

3.1.6 Other Bioenhancers with action as Piperine

Other herbal bioenhancers that have similar bioenhancing mechanisms of action like piperine (Genistein, Naringin, Sinomenine, *Carum carvi*) might be expected to also augment oral absorption of phytoconstituents that are substrates of CYP3A4, UGT and P-gp. It will be worthwhile to subject this proposition to further research.

3.2 HMPs with Ginger (and Other Bioavailability Improvement Approaches)

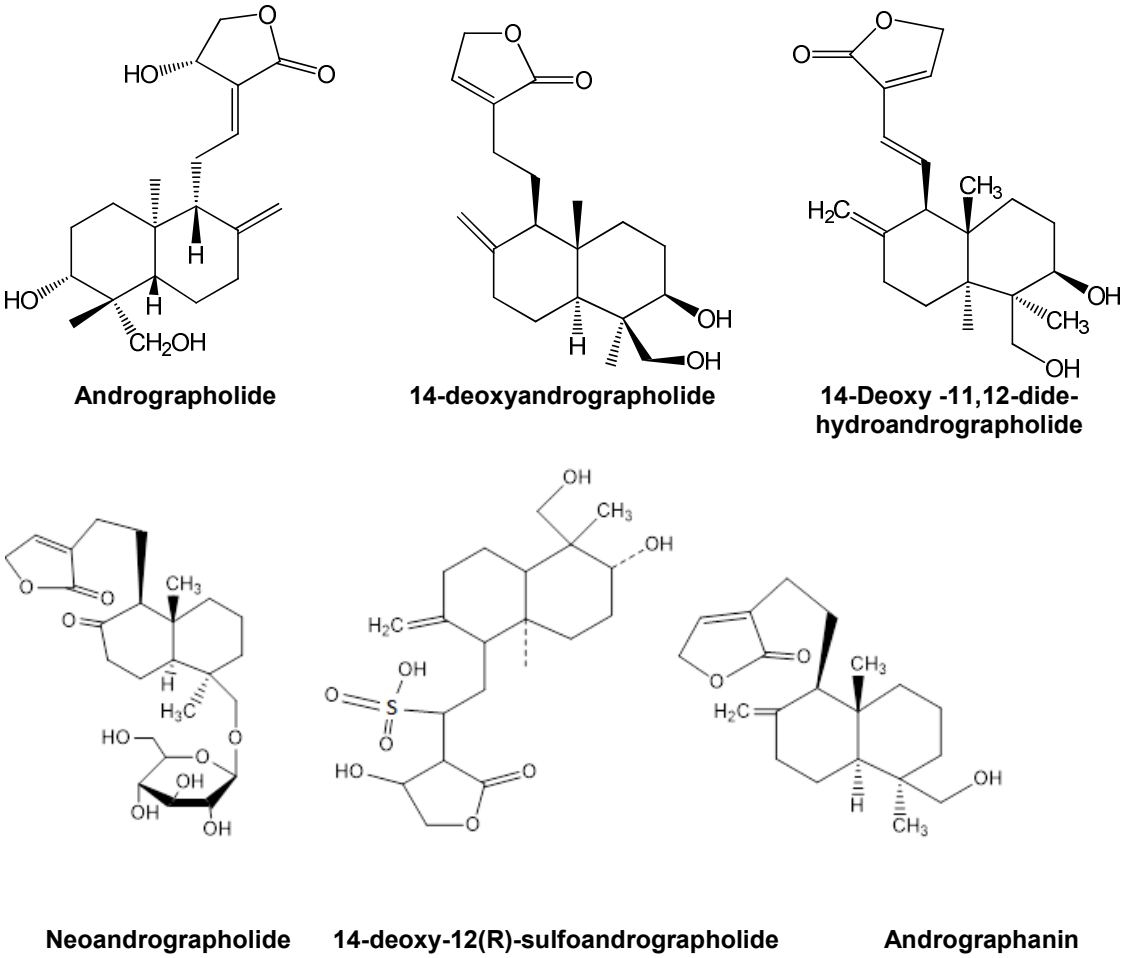
3.2.1 *Andrographis paniculata*

Andrographis paniculata belongs to the family Acanthaceae and is an important medicinal plant widely used around the world [122]. The aerial part of this herb is most commonly used and has been reported to have a broad range of pharmacological effects including anticancer,

antihepatitis, antihyperglycemic, immunomodulatory, antibacterial, anti-inflammatory, antioxidant and antimalarial [123-125]. The phytochemicals extracted from this herb consist of diterpenoids, flavonoids, quinicacids, xanthones, andandrographoloid with its derivatives [126, 127]. The major bioactive compounds are the andrographoloids which are diterpene lactones [125]. The chemical structures of some bioactive phytoconstituents isolated from *Andrographis paniculata* are presented in Fig. 5.

Andrographis paniculata extracts are standardized using andrographolide contents. Andrographolide has poor aqueous solubility and its oral bioavailability at therapeutic doses is very low [127-129]. The poor oral absorption of the product at the clinically useful dose necessitated reformulation of the extract to enhance its bioavailability. Several strategies have been used to improve the bioavailability of andrographolide from the herbal extract [130] and these include cyclodextrin complexation [131], preparation as herbosome [132,133], niosome formulation [134], nanoemulsion formulation [135], liquid and solid self-microemulsifying drug delivery systems [136], pH-sensitive nanoparticle delivery system [129], and solid lipid nanoparticle formulation [137].

In addition to these approaches, the incorporation of ginger, acting as a bioenhancer, significantly enhanced the bioavailability of *Andrographis paniculata* extract by up to 55%, through regulation of intestinal function to facilitate absorption [42,67,68]. Addition of piperine further increased the bioavailability to 70%, which suggests that the oral absorption



Andrographolide **14-deoxyandrographolide** **14-Deoxy -11,12-dide-hydroandrographolide**

Neoandrographolide **14-deoxy-12(R)-sulfoandrographolide** **Andrographanin**

Fig. 5. Chemical structures of some bioactive phytoconstituents of *Andrographis paniculata*

can be bioenhanced through other mechanisms. Reports indicate that andrographolide is a substrate of P-gp [138], hence, inhibition of intestinal P-gp by piperine has the potential of potentiating the bioavailability of the compound [39]. Thus, the poor oral bioavailability of andrographolide is attributed to not only its poor aqueous solubility but also to its rapid gut biotransformation and efflux by P-gp [139].

3.2.2 *Withania somnifera*

Withania somnifera (family Solanaceae) also known as ashwagandha or Indian ginseng has been an important herb in the ancient system of Indian traditional medicine. The main parts of the plant used therapeutically are the roots. Results from animal studies and clinical trials support the use of this herb for treatment of anxiety, cognitive and neurological disorders, inflammation, hyperlipidemia and Parkinson's disease [140]. It has several other pharmacological activities including immunomodulatory, aphrodisiac and sedative properties [141]. The roots extracts of *Withania somnifera* consist mainly of steroidal lactones known as withanolides, which the medicinal properties of the herbal product are attributed to [142,143]. The major withanolides are withanolide A and D, and Withaferin A with their derivatives. Their chemical structures are presented in Fig. 6. Other biologically active chemical constituents are alkaloids of which withanine is the main constituent with other minor alkaloids such as somniferine, cuscohygrine, isopelletierine, anaferine andanahydrine [144].

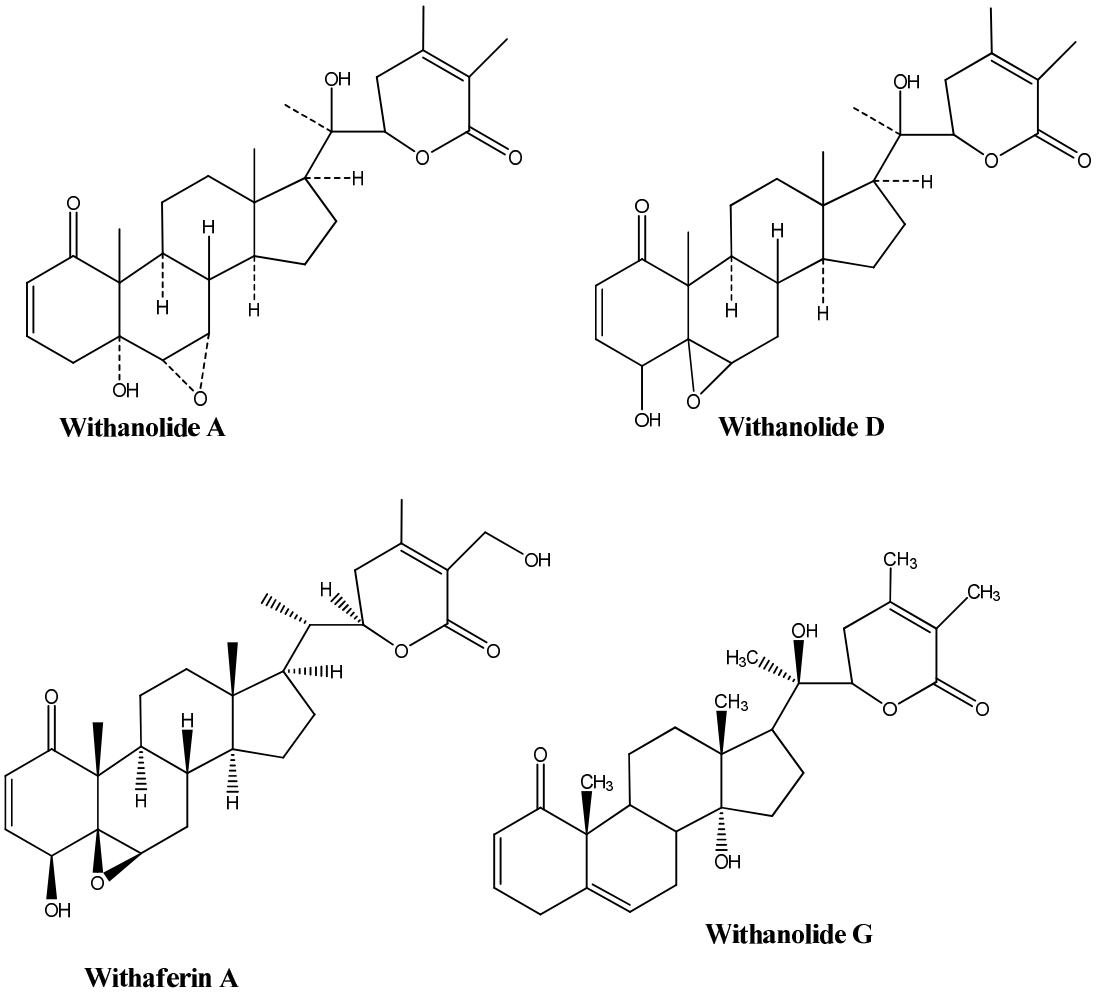


Fig. 6. Chemical Structures of some bioactive phytoconstituents of *Withania somnifera*

Steroidal lactones including withanolides generally have poor aqueous solubility and hence have bioavailability problems. Several approaches have been investigated aimed at enhancing the solubility and bioavailability, and thus increase the pharmacological activity of this HMP. These include formulation of polymeric nanoparticles [145], phytosomes [146,147], and preparation of enteric coated dosage form of the herbal extract which protects the composition from hydrolysis in the acidic medium of the stomach, thus enhancing the absorption [148].

Incorporation of ginger into *Withania somnifera* extract increases its absorption by up to 64% [42]. This action is mediated via the established activity of ginger as a potent bioavailability enhancer which operates by regulation of intestinal function resulting in increased GIT drug absorption [42,67,68].

3.2.3 *Picrorhiza kurroa*

Picrorhiza kurroa (family scrophulariaceae), also commonly known as Kutki (Nepali language), is an important alpine herb that grows in limited regions and altitude and found especially in the Himalaya region. The roots and rhizomes of this plant are used in traditional medicine for treatment disorders of liver and upper respiratory tract, dyspepsia, diarrhea, and dysentery, among other ailments [149]. The HMP has also been shown to possess other pharmacological activities including antioxidant and anti-inflammatory [150,151]. The major bioactive constituents which its therapeutic effects are attributed to are irioid glycosides called picroside I and picroside II [152]. The chemical structures are presented in Fig. 7.

Like most irioid glycosides, the picrosides have poor intestinal permeability attributable to their high hydrophilic nature. Also, the glycosides undergo hydrolysis in the GIT and the released aglycones have poor bioavailabilty due to their limited aqueous solubility [153]. As a result of the poor bioavailability, this herbal product is recommended to be administered in higher quantities resulting in increased treatment cost [154]. Review of the literature shows that limited strategies have been developed to improve bioavailability of these biomolecules and these include a nano-encapsulation formulation of the plant extract [155]. Another study is an invention which reports on formulation of phytosomes of picroside II with phospholipid which significantly improves the bioavailability of picroside II [156].

Ginger extract was found to significantly increase the bioavailability of *Picrorhiza kurroa* product by as much as 56 % and this was increased further to 87% when piperine was added to the formulation [67,68]. Both are bioenhancers acting through different mechanisms [42].

3.2.4 *Tinospora cordifolia*

Tinospora cordifolia (family Menispermaceae) grows throughout tropical Asia and its extracts have a broad range of pharmacological activities notable of which are anti-diabetic, anti-spasmodic, anti-inflammatory, anti-oxidant, anti-malarial, hepatoprotective, immunomodulatory and anti-neoplastic activities [157]. A variety of phytoconstituents isolated from the root, stem and whole plant have been identified to belong to different chemical classes, and the major group of compounds considered as the bioactive constituents are protoberberine alkaloids (egberberine, tembetarine, isocolumbin); diterpenoid lactones (eg furanolactone, tinosporon, tinosporide); steroids (eg β – sitosterol, δ-sitosterol,ecdysterone, giloinsterol) and glycosides (egcordifolioside, cordioside, tinocordiside, syringin) [157,158]. The chemical structures of some of these bioactive constituents are shown in Fig. 7.

It is apparent from the chemical structures of the various bioactive constituents of *T. cordifolia* that they have different degrees of hydrophilicity or lipophilicity. For example, steroids are lipophilic and generally have poor aqueous solubility while glycosides are hydrophilic and are associated with poor intestinal permeability. This entails that formulation modalities which can improve aqueous solubility and/or GIT permeability have the potential of enhancing the bioavailability of some constituents of this HMP. Few studies are reported on the bioavailability improvement of *T. cordifolia* extract. Using an everted gut model, when compared to control, about a 4-fold enhancement in permeability coefficient of *T. cordifolia* extract was obtained with nanoemulsion formulation of this HMP [159]. The authors concluded that the nanoemulsion formulation will result in enhancement of therapeutic efficacy through improved permeation of the active constituents across the GIT [159]. In another study, a significant enhancement of bioavailability of *T. cordifolia* product was observed when *Carum carvi* extracts was incorporated into the product [62]. *Carum carvi* extract has been reported to

enhance the bioavailability of several synthetic and other herbal drugs and this bioavailability enhancing activity was found to be consistent from 5 to100 mg/kg body weight irrespective of the amount of the drug(s) present in the formulation [62]. The mode of bioavailability enhancement action is thought to be by inhibition of P-gp mediated efflux of the absorbed compounds back into the GIT [38,40] since some constituents of *T. cordifolia* extract are substrates of P-gp [159].

Ginger extract was found to significantly increase the bioavailability of *T. cordifolia* product by up to 67% and this was increased further to 112% when piperine was added to the formulation [67, 68]. As earlier stated, ginger has a strong effect on GIT mucous membrane leading to regulation of intestinal function to facilitate absorption [42]. Piperine, being a P-gp inhibitor just like *Carum carvi* extract [38,39] could have potentiated the bioavailability by inhibition of P-gp efflux of *T. cordifolia* constituents.

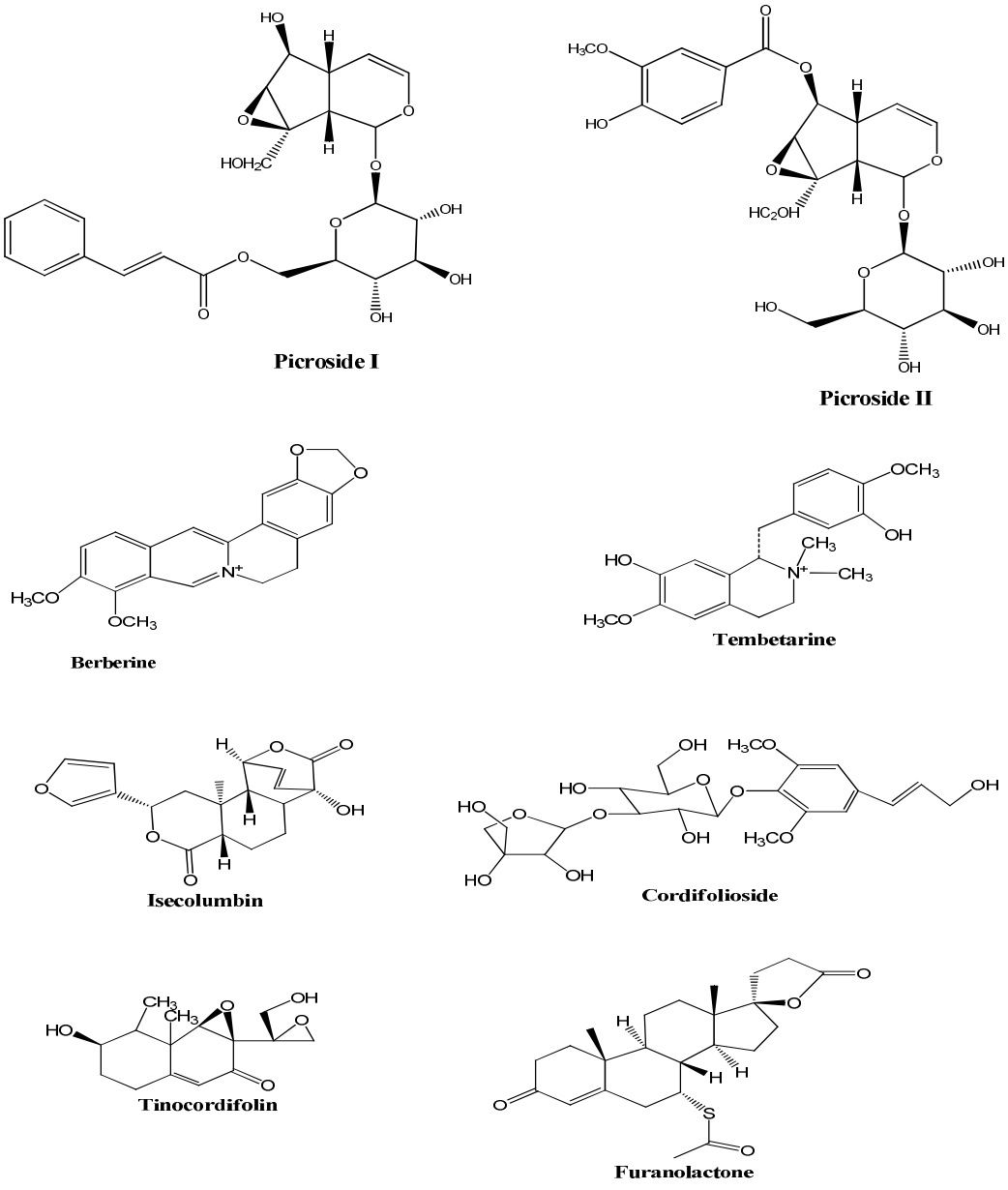


Fig. 7. Chemical Structures of bioactive phytoconstituents of *Picrorhiza kurroa* and *Tinospora cordifolia*

4. CONCLUSION

Phytomedicine has been playing an important role in pharmacotherapy. However, there are numerous reports of poor bioavailability resulting in reduced in vivo activities of several medicinal plant extracts and phytoconstituents [34-36]. Improving the bioavailability of these HMP will go a long way in the optimisation of efficacies of the products. Extensive research work has been focused on the enhancement of oral bioavailability of the poorly absorbed orthodox medicines but this review has shown that HMPs are receiving relatively poor attention in this regard. The present review highlighted different strategies that can be employed to improve the bioavailability of HMPs with emphasis on the utility of bioenhancers derived from herbs.

It was found expedient to identify the bioactive phytoconstituents of the HMPs such that information on their physicochemical properties, metabolic fate and factors that impact on their gastrointestinal absorption were examined to serve as guide for application in the bioavailability enhancement of other HMPs with poor bioavailability of their active constituents. It is essential to understand the reason behind the poor bioavailability of bioactive moieties before selecting an appropriate technique or bioenhancer. The benefits of incorporation of suitable bioenhancers to drug products with poor bioavailability cannot be over-emphasized since this is associated with a reduction of dosage, drug toxicity and cost of the whole treatment.

Thus, there is a need for more research endeavours on a combination of HMPs with suitable bioenhancers since this has the potential of not only enhancing efficacy but also reducing drug treatment cost.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kumar S, Dilbaghi N, Rani R, Bhanjana G, Umar A. Novel approaches for enhancement of drug bioavailability. Rev Adv Sci Eng. 2013;2:1–22.
2. Kawabata Y, Wada K, Nakatani M, Yamada S, Onoue S. Formulation design for poorly water-soluble drugs based on biopharmaceutics classification system: Basic approaches and practical applications. Int J Pharm. 2011;420:1- 10.
3. Khadka P, Ro J, Kim H, Kim I, Kim JT, Kim H, et al. Pharmaceutical particle technologies: An approach to improve drug solubility, dissolution and Bioavailability. Asian J Pharm Sci. 2014;9:304–16.
4. Roy K, Mao HQ, Huang SK, Leong KW. Oral gene delivery with chitosan-DNA nanoparticles generate immunologic protection in a marine model of peanut allergy. Nature Med. 1999;5:387-91.
5. Yu LX, Amidon GL, Polli JE, Zhao H, Mehta MU, Conner DP, et al. Biopharmaceutics classification system: the scientific basis for biowaiver extensions. Pharm. Res. 2002;19:921-5.
6. Wagh MP, Patel JS. Biopharmaceutical classification system: Scientific basis for biowaiver extensions. Int J Pharm Pharm Sci. 2010;2:12-19.
7. U.S. Department of Health and Human Services, Center for Drug Evaluation and Research (CDER), USFDA. Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System Guidance for Industry; 2017.
8. Chavda HV, Patel CN, Anand IS. Biopharmaceutics Classification System. Sys Rev Pharm. 2010;1(1):62–9.
9. Beg S, Swain S, Rizwan M, Irfanuddin M, Malini DS. Bioavailability enhancement strategies: basics, formulation approaches and regulatory considerations. Curr Drug Deliv. 2011;8(6):691-702.
10. Hetal T, Bindesh P, Sneha T.A review on techniques for oral bioavailability enhancement of drugs. Int J Pharm Sci Rev Res. 2010;4(3):203–23.
11. Onyeji CO, Omoruyi SA, Oladimeji FA. Dissolution properties and characterization of halofantrine –2-hydroxypropyl- β –cyclodextrin binary systems. Die Pharmazie. 2007;62:858–63.

12. Onyeji CO, Oladimeji FA, Omoruyi SI, Soyinka JO. Physicochemical Characterization and Dissolution properties of the binary systems of Pyrimethamine and 2-hydroxypropyl-beta-cyclodextrin. Afr J. Biotech. 2009;8(8):1651–9.
13. Gunasekaran T, Haile T, Nigusse T, Dhanaraju DM. Nanotechnology: an effective tool for enhancing bioavailability and bioactivity of phytomedicine. Asian Pac J Trop Biomed. 2014;4(Suppl 1):S1-S7.
14. Breedveld P, Beijnen JH, Schellens JH. Use of P-glycoprotein and BCRP inhibitors to improve oral bioavailability and CNS penetration of anticancer drugs. Trends Pharmacol Sci. 2006;27:17-24.
15. Kang MJ, Cho JY, Shim BH, Kim DK, Lee J. Bioavailability enhancing activities of natural compounds from medicinal plants. J Med Plants Res. 2009;3:1204-11.
16. Randhawa GK, Kullar JS, Rajkumar. Bioenhancers from mother nature and their applicability in modern medicine. Int J Appl Basic Med Res. 2011;1(1):5-10. DOI: 10.4103/2229-516X.81972
17. Kumar S. Drug carrier systems for solubility enhancement of BCS class II drugs: A critical review. Crit Rev Therap Drug Carrier Syst. 2013;30:217-56.
18. Kumar S, Dilbaghi N, Rani R, Bhanjana G, Umar A. Novel approaches for enhancement of drug bioavailability. Rev Adv Sci Eng. 2013;2:1–22.
19. Raut SV, Nemade LS, Desai MT, Bonde SD, Dongare SU. Chemical penetration enhancers: for transdermal drug delivery systems. Int J Pharm Rev Res. 2014;4(1): 33-40.
20. Patil AG, Jobanputra AH. Rutin-Chitosan Nanoparticles: Fabrication, Characterization and Application in Dental Disorders. Polymer-Plastics Tech Eng. 2015;54(2): 202–8.
21. Navin A, Bedi KL. Bioenhancers: Revolutionary concept to market. J Ayurveda Integr Med. 2010;1(2):96-9.
22. Gangwar AK, Ghosh AK. Medicinal uses and pharmacological activity of Adhatoda vasica. Int J Herb Med. 2014;2(1): 88-91.
23. Kingston DGI. Modern natural products drug discovery and its relevance to biodiversity conservation. J Nat Prod. 2011;74:496-511.
24. Tilburt JC, Kaptchuk TJ. Herbal medicine research and global health: An ethical analysis Bulletin of the World Health Organization. 2008;86:594-9.
25. Kasilo O, Trapside J-M, Mwikisa C, Lusamba-Dikassa P. On behalf of the WHO Regional Office for Africa, Brazzaville. An Overview of the Traditional Medicine Situation in the African Region; 2010. Available:http://ahm.afro.who.int/issue13/pdf/AHM%2013%20Special%20Issue%20Pages%207to15.pdf
26. Anquez-Traxler C. The legal and regulatory framework of herbal medicinal products in the European Union: A focus on the traditional herbal medicines category. Drug Inf. J. 2011;45:15–23.
27. Ekor M. The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol. 2014;4(177):1-9.
28. Hunt KJ, Coelho HF, Wider B, Perry R, Hung SK, Terry R, et al. Complementary and alternative medicine use in England: results from a national survey. Int J Clin Pract. 2010;64(11):1496-502.
29. Nordeng H, Bayne K, Havnen GC, Paulsen BS. Use of herbal drugs during pregnancy among 600 Norwegian women in relation to concurrent use of conventional drugs and pregnancy outcome. Complement. Therap Clin Pract. 2011;17(3):147-51.
30. Bandaranayake WM. Quality control, screening, toxicity, and regulation of herbal drugs. In: Modern Phytomedicine. Turning Medicinal Plants into Drugs; Ahmad I, Aqil F, Owais M (eds.), (Weinheim: Wiley-VCH GmbH & Co. KGaA). 2006;25-57. Available:http://dx.doi.org/10.1002/9783527609987.ch2
31. Lynch N, Berry D. Differences in perceived risks and benefits of herbal, over-the-counter conventional, and prescribed conventional, medicines, and the implications of this for the safe and effective use of herbal products. Complement Therap Med. 2007;15(2):84-91.
32. Anand P, Kunnumakkara AB, Newman RA, Aggarwal BB. Bioavailability of Curcumin: Problems and promises. Mol Pharm. 2007;4(6):807-18.
33. Azmi AS, Ahmad A, Banerjee S, Rangnekar VM, Mohammad RM, Sarkar FH. Chemoprevention of pancreatic cancer: Characterization of Par-4 and its modulation by 3, 3'diindolylmethane (DIM). Pharm Res. 2008;25:2117-24.

34. Adebayo JO, Krettli AU. Potential antimalarials from Nigerian plants: A review. J Ethnopharmacol. 2011;133:289–302.
35. Isah AB, Ibrahim YK, Iwalewa EO. Evaluation of the antimalarial properties and standardization of tablets of *Azadirachta indica* (Meliaceae) in mice. Phytother Res. 2003;17:807–810.
36. Yang KY, Du Hyeong Hwang AMY, Kim DW, Shin YJ, Bae O-N, Kim YI, et al. Silymarin-loaded solid nanoparticles provide excellent hepatic protection: Physicochemical characterization and in vivo evaluation. Int J Nanomed. 2013;8: 3333-43.
37. Kesarwani K, Gupta K. Bioavailability enhancers of herbal origin: An overview. Asian Pac J Trop Biomed. 2013;3(4):253-66.
38. Muttepawar SS, Jadhav SS, Kankudate AD, Sanghai SD, Usturge DR, Chavare SS. A review on bioavailability enhancers of herbal Origin. World J Pharmacy and Pharm Sci. 2014;3(3):667-677.
39. Jain G, Patil UK. Strategies for enhancement of bioavailability of medicinal agents with natural products. Int J Pharm Sci Res. 2015;6(12):5315-24.
40. Javed S, Ahsan W, Kohli K. The concept of bioenhancers in bioavailability enhancement of drugs – a patent review. J Scientific Lett. 2016;1(3)Suppl.:143-65.
41. Shaikh MS, Derle ND, Bhamber R. Permeability enhancement techniques for poorly permeable drugs: A review. J Appl Pharm Sci. 2012;2((6):34-9.
42. Dudhatra GB, Mody SK, Awale MM, Patel HB, Modi CM, Kumar A, et al. A comprehensive review on pharmacotherapeutics of herbal bioenhancers. The Scientific World J; 2012. Article ID 637953, 33 pages. DOI: 10.1100/2012/637953
43. Evans WC. Trease and Evans' Pharmacognosy, 14th edn. W. B. Saunders, London. 1997;363–64.
44. Gorgani L, Mohammadi M, Najafpour GD, Nikzad M. Piperine—The bioactive compound of black pepper: From isolation to medicinal formulations. Comprehensive Rev Food Sci food Safety. 2017;16. Available:https://doi:10.1111/1541-4337.12246
45. Mhaske DB, Sreedharan S, Mahadik KR. Role of piperine as an effective bioenhancer in drug absorption. Pharm Anal Acta. 2018;9:592. DOI: 10.4172/2153-2435.10000592
46. Atal N, Bedi KL. Bioenhancers: Revolutionary concept to market. J Ayurvedic Integ Med. 2010;1(2):96-9.
47. Drabu S, Khatri S, Babu S, Lohani P. Review Article Use of Herbal Bioenhancers to Increase The Bioavailability of Drugs. Res J Pharm Biol Chem Sci. 2011;2(4): 107–12.
48. Pattanaik S, Hota D, Prabhakar S, Kharbanda P, Pandhi P. Pharmacokinetic interaction of single dose of piperine with steady-state carbamazepine in epilepsy patients. Phytother Res 2009;23(9):1281-6. Available:http://dx.doi.org/10.1002/ptr.2676
49. Jin MJ, Han HK. Effect of piperine, a major component of black pepper, on the intestinal absorption of fexofenadine and its implication on food-drug interaction. J Food Sci. 2010;75(3):H93-96. Available:http://dx.doi.org/10.1111/j.1750-3841.2010.01542.x
50. Kasibhatta R, Naidu MU. Influence of piperine on the pharmacokinetics of nevirapine under fasting conditions: A randomised, crossover, placebo-controlled study. Drugs Res Dev. 2007;8(6):383-391. Available:http://dx.doi.org/10.2165/00126839-200708060-00006
51. Srinivasan K. Black pepper and its pungent principle-piperine: A review of diverse physiological effects. Crit Rev Food Sci Nutr. 2007;47(8):735-48.
52. Wadhwa S, Singhal S, Swati Rawat S. Bioavailability Enhancement by Piperine: A Review. Asian J Biomed Pharm Sci. 2014; 4(36):1-8.
53. Boddupalli MB, Anisetti NR, Ramani R, Malothu N. Enhanced pharmacokinetics of omeprazole when formulated as gastro retentive microspheres along with piperine. Asian Pac J Trop Dis. 2014;4(Suppl 1): S129-33.
54. Bhardwaj RK, Glaeser H, Becquemont L, Klotz U, Gupta SK, Fromm MF. Piperine, a major constituent of black pepper, inhibits human P-glycoprotein and CYP3A4. J Pharmacol Exp Ther. 2002;302:645-50.
55. Gescher AJ and Steward WP: Curcumin: The story so far. Eur J Cancer. 2005; 41(13):1955–68.
56. Lim SC, Choi JS. Effects of naringin on the pharmacokinetics of intravenous paclitaxel

in rats.Biopharm Drug Dispos. 2006;27: 443-7.
57. Tatiraju DV, Bagade VB, Karambelkar PJ, Jadhav VM, Kadam V. Natural bioenhancers: An overview. J Pharmacog Phytochem. 2013;2(3):55-60.
58. Wacher VJ, Benet ZL. Use of gallic acid esters to increase bioavailability of orally administered pharmaceutical compounds. U.S. Patent US 6180666 B1; 2001.
59. Chan K, Liu ZQ, Jiang ZH, Zhou H, Wong YF, XU HX, et al. The effects of sinomenine on intestinal absorption of paeoniflorin by the everted rat gut sac model. J Ethnopharmacol. 2006;103(3): 425-32
60. Takeda S, Isono T, Wakui Y, Matsuzaki Y, Sasaki H, Amagaya S, et al. Absorption and excretion of paeoniflorin in rats. J Pharm Pharmacol. 1995;47:1036–40.
61. Li X, Choi JS. Effect of genistein on the pharmacokinetics of paclitaxel administered orally or intravenously in rats. Int J Pharm. 2007;337:188-93.
62. Qazi G, Bedi K, Johri R, Tikoo M, Tikoo A, Sharma S, et al. Assignee: Council of Scientific and Industrial Research. Bioavailability enhancing activity of Carumcarvi extracts and fractions thereof. US20070020347A1; 2007.
63. Yamahara J, Huang Q. Gastrointestinal motility enhancing effect of ginger and its active constituents. Med Hypotheses. 1990;39:342.
64. Rahmani AH, Al shabrmi FM, Aly SM. Active ingredients of ginger as potential candidates in the prevention and treatment of diseases via modulation of biological activities. Int J Physiol Pathophysiol Pharmacol. 2014;6(2):125-36.
65. Jolad SD, Lantz RC, Solyom AM, Chen GJ, Bates RB, Timmermann BN. Fresh organically grown ginger (*Zingiber officinale*): composition and effects on LPS-induced PGE2 production. Phytochem. 2004;65(13):1937–54.
66. Qazi GN, Tikoo L, Gupta AK, Ganju K, Gupta DK, Jaggi BS, et al. Bioavailability enhancing activity of *Zingiber officinale* and its extracts/ fractions thereof. European patent EP 1465646; 2002.
67. Qazi GN, Bedi KL, Johri RK. Bioavailability enhancing activity of Zingiberofficinale and its extracts/ fractions thereof. United States Patent Number, US2003/0170326A1; 2003.
68. Qazi GN, Tikoo L, Gupta AK, Ganju K, Gupta DK, Jaggi BS, et al. Bioavailability enhancing activity of Zingiber officinale Linn and its extracts/fractions thereof. World Intellectual Property Organization, International Publication Number, WO03049753A1; 2003.
69. Vinson JA, Al Kharrat H, Andreoli L, Effect of *Aloe vera* preparations on the human bioavailability of vitamins C and E. Phytomed. 2005;12(10):760–5.
70. Sharma K, Mittal A, Chauhan N. *Aloe vera* as penetration enhancer. Int J Drug Dev Res. 2015;7(1):31–43.
71. Khanuja SPS, Kumar S, Arya JS, Shasany AK, Singh M, Awasthi S, et al. Composition comprising pharmaceutical/nutraceutical agent and a bio-enhancer obtained from *Glycyrrhiza glabra*. United States Patent, Number 6979471; 2002.
72. Selyutina OY, Polyakov NE, Korneev DV, Zaitsev BN. Influence of glycyrrhizin on permeability and elasticity of cellmembrane: Perspectives for drugs delivery. Drug Deliv. 2014;1–8. DOI: 10.3109/10717544.2014.919544
73. Imai T, Sakai M, Ohtake H, Azuma H, Otagiri M, In vitro and in vivo evaluation of the enhancing activity of glycyrrhizin on the intestinal absorption of drugs. Pharm Res. 1999;16:80-6.
74. Imai T, Sakai M, Ohtake H, Azuma H, Otagiri M. Absorption enhancing effect of glycyrrhizin induced in the presence of capric acid. Int J Pharm. 2005;27:11–21.
75. Khanuja SPS, Arya JS, Ranganathan T, Kumar S, Saikia D, Kaur H, et al. Nitrile glycoside useful as a bioenhancer of drugs and nutrients, process of its isolation from moringa oleifera. United States Patent, Number 6858588; 2003.
76. Pal A, Bawankule DU, Darokar MP, Gupta SC, Khanuja SPS. Influence of Moringa oleifera on pharmacokinetic disposition of rifampicin using HPLC-PDA method: A pre-clinical study. Biomed Chromatogr. 2010;25(4):641-5.
77. Ambwani S, Tandon R, Ambwani TK, Malik YS. Current knowledge on nanodelivery systems and their beneficial applications in enhancing the efficacy of herbal drugs. J Exp Biol Agric Sci. 2018;6(1):87–107.
78. Pankti SP, Roy SP, Patel N, Kashmira J, Gohil KJ. Curcumin as an antidepressant: A review. Research and Reviews: J Pharmacog Phytochem. 2014;2(2):29–33.

79. Ravindranath V, Chandrasekhara N. Absorption and tissue distribution of curcumin in rats. Toxicol. 1980;16:259–265.
80. Zhang W, Tan TM, Lim LY. Impact of curcumin-induced changes in P-glycoprotein and CYP3A expression on the pharmacokinetics of peroralceliprolol and midazolam in rats. Drug Metab Dispos. 2007;35(1):110-15.
81. Shoba G, Joy D, Joseph T, Majeed M, Rajendran R, Srinivas PSR. Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers. Planta Medica. 1998;64(4): 353–56.
82. Bhutani MK, Bishnoi M, Kulkarni SK. Studies on antidepressant effect of curcumin with piperine. Pharmacol Biochem Behavior. 2009;92:39-43.
83. Kulkarni SK, Bhutani MK, Bishnoi M. Anti-depressant like effect of curcumin and its combination with piperine inunpredictable chronic stress-induced behavioral, biochemical and neurochemical changes. Pharmacol Biochem Behav. 2009;92:39-43.
84. Pu F, Mishima K, Irie K, Motohashi K, Tanaka Y, Orito K, et al. Neuroprotective effects of quercetin and rutin on spatial memory impairment in an 8-arm radial maze task and neuronal death induced by repeated cerebral ischemia in rats. J Pharmacol Sci. 2007;104:329–34.
85. Kim HP, Son KH, Chang HW, Kang SS. Anti-inflammatory plant flavonoids and cellular action mechanisms. J Pharmacol Sci. 2004;96:229–45.
86. Al-Dhabi NA, Arasu MV, Park CH, Park SU. An up-to-date review of rutin and its biological and pharmacological activities. EXCLI Journal. 2015;14:59-63.
87. Scalbert A, Williamson G. Dietary intake and bioavailability of polyphenols. J Nutr. 2000;130:2073S–85S.
88. Rastogi H, Jana S. Evaluation of physicochemical properties and intestinal permeability of six dietary polyphenols in human intestinal colon adenocarcinoma Caco-2 cells. Eur J Drug Metab Pharmacokinet. 2014;1–11.
DOI: 10.1007/s13318-014-0234-5
89. Hollman PC1, van Trijp JM, Buysman MN, van der Gaag MS, Mengelers MJ, de Vries JH, et al. Relative bioavailability of the antioxidant flavonoid quercetin from various foods in man. FEBS Lett. 1997; 418(1-2):152-6
90. Hooresfand Z, Ghanbarzadeh S, Hamishehkar H. Preparation and characterization of rutin-loaded nanophytosomes. Pharm Sci. 2015; 21(Suppl 1):145-51.
DOI: 10.15171/PS.2015.29
91. Miyake K, Arima H, Hirayama F, Yamamoto M, Horikawa T, Sumiyoshi H, et al. Improvement of solubility and oral bioavailability of rutin by complexation with 2-Hydroxypropyl-β-cyclodextrin. Pharm Dev Tech. 2000;5(3):399–407.
92. Macedo AS, Quelhas S, Silva AM, Souto EB. Nanoemulsions for delivery of flavonoids: Formulation and in vitro release of rutin as model drug. Pharm Dev Tech. 2014;19(6):677–80.
93. Scholz S, Williamson G. Interactions affecting the bioavailability of dietary polyphenols in vivo, Int J Vitam Nutr Res. 2007;77:224–35.
DOI: 10.1024/0300-9831.77.3.224
94. Heim K, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. J Nutr Biochem. 2002; 13(10):572-84.
95. Rinwa P, Kumar A. Quercetin along with piperine prevents cognitive dysfunction, oxidative stress and neuro-inflammation associated with mouse model of chronic unpredictable stress. Arch Pharm Res; Jul 16, 2013. Advance Online Publication.
DOI: 10.1007/s12272-013-0205-4
96. Singh S, Jamwal S, Kumar P. Neuroprotective potential of Quercetin in combination with piperine against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurotoxicity. Neural Regeneration Res. 2017;12(7): 1137–44.
97. Yadala P, Viswanathswamy AHM. In vitro antioxidant and cytotoxic activity of rutin and piperine and their synergistic effect. Int J Pharm Pharm Sci. 2016;8(5):78-82.
98. Barnes J, Anderson LA, Gibbons S, Phillipson DJ. Echinacea species (*Echinacea angustifolia* (DC.) Hell., *Echinacea pallida* (Nutt.) Nutt., *Echinacea purpurea* (L.) Moench): A review of their chemistry, pharmacology and clinical properties. J Pharm Pharmacol. 2005;57: 929–54.
99. Wichtl M, (ed.). Herbal drugs and phytopharmaceuticals. A handbook for

practice on a scientific basis, 3rd Edn. Medpharm Scientific Publishers, Stuttgart; 2004.

100. Manayi A, Vazirian M, Soodabeh SS. Echinacea purpurea: Pharmacology, phytochemistry and analysis methods. Pharmacog Rev. 2015;9(17):63–72. DOI: 10.4103/0973-7847.156353
101. Matthias A, Penman KP, Matovic NJ, Bone KM, De Voss JJ, Lehmann RP. Bioavailability of Echinacea Constituents: Caco-2 Monolayers and Pharmacokinetics of the Alkylamides and Caffeic Acid Conjugates. Molecules 2005;10(10):1242-51. DOI: 10.3390/10101242
102. Shen JY, Yang XL, Yang ZL, Kou JP, Li F. Enhancement of absorption and bioavailability of echinacoside by verapamil or clove oil. Drug Design Dev Therapy 2015;9:4685–93.
103. Gorski JC, Huang SM, Pinto A, Hamman MA, Hilligoss JK, Zaheer NA, et al. The Effect of Echinacea (*Echinacea purpurea* Root) on Cytochrome P450 Activity *in vivo*. Clin Pharmacol Therap. 2004;75(1):89-10. Available:https://doi.org/10.1016/j.clpt.2003.09.013
104. Upaganlawar A, Ghule B. Pharmacological Activities of *Boswellia serrata* Roxb. - Mini Review. Ethnobot Leaflets. 2009;13:766-74.
105. Alam M, Khan H, Samiullah L, Siddique KM. A review on Phytochemical and Pharmacological studies of Kundur (*Boswellia serrata* Roxb ex Colebr.) -A Unani drug. J Appl Pharm Sci. 2012;2(3): 148-56.
106. Hussain H, Al-Harrasi A, Csuk R, Shamraiz U, Green IR, Ahmed I. Therapeutic potential of boswellic acids: A patent review (1990-2015). Expert Opinion on Therapeutic Patents. 2017;27(1):81-90.
107. Al-Harrasi A, Ali L, Rehman NU, Hussain H, Hussain J, Al-Rawahi A, et al. Nine triterpenes from Boswellia sacra Flückiger and their chemotaxonomic importance. Biochem Syst Ecol. 2013;51:113-16.
108. Barthe L, Woodley J, Houin G. Gastrointestinal absorption of drugs: Methods and studies. Fundam Clin Pharmacol. 1999;13:154–68.
109. Reising K, Meins J, Bastian B, Eckert G, Mueller WE, Schubert-Zsilavecs M, et al. Determination of boswellic acids in brain and plasma by high-performance liquid chromatography / tandem mass spectrometry. Anal Chem. 2005;77:6640-5.
110. Mehta M, Satija S, Nanda A, Garg M. Nanotechnologies for Boswellic acids. Am J Drug Discovery. 2014;4(1):1–11.
111. Cui Y, Tian X, Ning J, Wang C, Yu Z, Wang Y, et al. Metabolic profile of 3-acetyl-11-keto-β-boswellic acid and 11-keto-boswellic acid in human preparations, in vitro species differences, and bioactivity variation. AAPS J. 2016;18(5):1273-88.
112. Majeed M, BadmaevV, Rajendran R. Use of piperine as a bioavailability enhancer - US Patent 5,744,161(to Sabinsa Corp, New Jersey); 1998.
113. Ernst E. The risk-benefit profile of commonly used herbal therapies: Ginkgo, St. John's wort, ginseng, echinacea, saw palmetto, and kava. Annals of Internal Med. 2002;36(1):42–53.
114. Kennedy DO, Wightman EL. Herbal extracts and phytochemicals: Plant secondary metabolites and the enhancement of human brain function. Adv Nutr. 2011;2:32–50.
115. Brondino N, De Silvestri A, Re S, Lanati N, Thiemann P, Anna Verna A, et al. A Systematic Review and Meta-Analysis of Ginkgo biloba in Neuropsychiatric Disorders: From Ancient Tradition to Modern-Day Medicine. Evidence-Based Complem Altern Med; 2013. Article ID 915691, 11 pages. Available:http://dx.doi.org/10.1155/2013/915691
116. Ding S, Dudley E, Plummer E, Tang J, Newton RP, Brenton AG. Quantitative determination of major active components in Ginkgo biloba dietary supplements by liquid chromatography/mass spectrometry. Rapid Communications in Mass Spect. 2006;20(18):2753-60. Available:https://doi.org/10.1002/rcm.2646
117. Chen Z, Suna J, Chen H, Xiao Y, Liu D, Chen J, et al. Comparative pharmacokinetics and bioavailability studies of quercetin, kaempferol and isorhamnetin after oral administration of Ginkgo biloba extracts, Ginkgo biloba extract phospholipid complexes and *Ginkgo biloba* extract solid dispersions in rats. Fitoterapia. 2010;81(2):1045-52.
118. Chen Z, Zheng S, Li L, Jiang H. Metabolism of Flavonoids in Human: A Comprehensive Review. Curr Drug Metabol. 2014;15(1):48-61.

119. Wang Y, Cao J, Zeng S. Involvement of P-glycoprotein in regulating cellular levels of Ginkgo flavonols: Quercetin, kaempferol, and isorhamnetin. J Pharm Pharmacol. 2005;57(6):751-8.
120. Gaudineau C, Beckerman R, Welbourn S, Auclair K. Inhibition of human P450 enzymes by multiple constituents of the Ginkgo biloba extract. Biochem Biophys Res Commun. 2004;318(4): 1072-8.
Available:https://doi.org/10.1016/j.bbrc.2004.04.139
121. Li L, Zhao Y, Du F, Yang Y, Xu F, Niu W, et al. Intestinal absorption and presystemic elimination of various chemical constituents present in GBE50 extract, a standardized extract of *Ginkgo biloba* leaves. Curr Drug Metabol. 2012;13(5): 494-509.
122. Akbar S. *Andrographis paniculata*: A review of pharmacological activities and clinical effects. Altern Med Rev. 2011; 16(1):66–77.
123. Hossain MS, Urbi Z, Sule A, Rahman KMH. *Andrographis paniculata* (Burm. f.) Wall. ex Nees: A review of ethnobotany, phytochemistry, and pharmacology. Scientific World Journal; 2014. Article ID 274905, 28 pages.
Available:http://dx.doi.org/10.1155/2014/274905.
124. Joselin J, Jeeva S. *Andrographis paniculata*: A review of its traditional uses, phytochemistry and pharmacology. Med Aromat Plants. 2014;3(4):1-15.
DOI: 10.4172/2167-0412.1000169
125. Nyeem MAB, Mannan MA, Nuruzzaman M, Kamrujjaman KM, Das SK. Indigenous king of bitter (*Andrographis paniculata*): A review. J Med Plants Studies. 2017;5(2): 318-24.
126. Xu C, Chou GX, Wang ZT. A new diterpene from the leaves of *Andrographis paniculata* Nees. Fitoterapia. 2010;81(6): 610–3.
127. Xu C, Chou GX, Wang CH, Wang ZT. Rare noriridoids from the roots of *Andrographis paniculata*. Phytochem. 2012;77:275–9.
128. Ou WP, Ye SM, Mi HQ, Hong X. Comparison of bioavailability between chuanxinlian capsule and chuanxinlian Pill. Chin J Integr Med. 2000;1:8–10.
129. Chellampillai B, Pawar AP. Improved bioavailability of orally administered andrographolide from pH-sensitive nanoparticles. Eur J Drug Metabol Pharmacokinetics. 2011;35(3-4):123–29.
130. Pawar A, Rajalakshmi S, Mehta P, Shaikh K, Bothiraja C. Strategies for formulation development of andrographolide. RSC Advances. 2016; 73:1–57.
DOI: 10.1039/C6RA12161F
131. Ren K, Zhang Z, Li Y, Liu J, Zhao D, Zhao Y, et al. Physicochemical characteristics and oral bioavailability of andrographolide complexed with hydroxypropyl-b-cyclodextrin. Pharmazie. 2009;64:515–20.
132. Maiti K, Mukherjee K, Murugan V, Saha BP, Mukherjee PK. Enhancing bioavailability and hepatoprotective activity of andrographolide from Andrographis paniculata, a well-known medicinal food, through its herbosome. J Sci food and Agric. 2010;90(1):43-51.
133. Jain PK, Khurana N, Pounikar Y, Gajbhiye A, Kharya MD. Enhancement of absorption and hepatoprotective potential through soya-phosphatidylcholine-andrographolide vesicular system. J Liposome Res. 2013; 23(2):110-8.
134. Tu YS, Sun DM, Zhang JJ, Jiang ZQ, Chen YX, Zeng XH, et al. Preparation and characterisation of andrographolide niosomes and its anti-hepatocellular carcinoma activity. J microencap. 2014; 13(4):307–16.
135. Yen CC, Chen YC, Wu MT, Wang CC, Wu YT. Nanoemulsion as a strategy for improving the oral bioavailability and anti-inflammatory activity of andrographolide. Int J Nanomed. 2018;2018(13):669-80.
136. Sermkaew N, Ketjinda W, Boonme P, Phadoongsombut N, Wiwattanapatapee R. Liquid and solid self-microemulsifying drug delivery systems for improving the oral bioavailability of andrographolide from a crude extract of *Andrographis paniculata*. Eur J Pharm Sci. 2013;50(3-4):459-66.
137. Parveen R, Ahmad FJ, Iqbal Z, Samim M, Ahmad S. Solid lipid nanoparticles of anticancer drug andrographolide: Formulation, *in vitro* and *in vivo* studies. J Drug Dev Ind Pharm. 2014;40(9):1206-12.
138. Daodee S, Wangboonskul J, Jarukamjorn K. Membrane transport of andrographolide in artificial membrane and rat small intestine. Pakistan J Biol Sci. 2007;10(12): 2078–85.
139. Ye L, Wang T, Tang L, Liu W, Yang Z, Zhou J, et al. Poor oral bioavailability of a promising anticancer

agent andrographolide is due to extensive metabolism and efflux by P-glycoprotein. J Pharm Sci. 2011;100(11):5007-17.
140. Gupta GL, ARana AC. Withania somnifera (Ashwagandha): A Review. Pharmacog Reviews. 2007;1(1):129–36.
141. Uddin Q, Samiulla L, Singh VK, Jamil SS. Phytochemical and Pharmacological Profile of *Withania somnifera* Dunal: A Review. J Appl Pharm Sci. 2012;02(01): 170-5.
142. Ali M, Shuaib M, Ansari SH. Withanolides from the stem bark of *Withania somnifera*. Phytochem. 1997;44:1163–8.
143. Mishra LC, Singh BB, Dagenais S. Scientific basis for the therapeutic use of *Withania somnifera* (ashwagandha): A review. Altern Med Rev. 2000;5:334–46.
144. Narinderpal K, Junaid N, Raman BA. Review on Pharmacological Profile of *Withania somnifera* (Ashwagandha). Research and Reviews: J Botanical Sci. 2013;2(4):1–9.
145. Haripriya S, Vadivel E, Ilamurugu K, Venkatachalam R. Preparation of Withaferin-A loaded PLGA nanoparticles by modified emulsion diffusion evaporation technique. Int J Nanoparticles. 2010;4(2): 163–8.
146. Keerthi B, Pingali PS, Srinivas P. Formulation and Evaluation of Capsules of Ashwagandha Phytosomes. Int J Pharm Sci Rev Res. 2014;29(2):138-42.
147. Ahmad H, Arya A, Agrawal S, Samuel SS, Singh SK, Valicherla GR, et al. Phospholipid complexation of NMITLI118RT+: Wayto a prudent therapeutic approach for beneficial outcomes in ischemic stroke in rats. Drug Deliv. 2016;23(9):3606–18.
148. Benny Anthony. A process to enhance the bioactivity of ashwagandha extracts. US patent No. WO2017068600A; 2017.
149. Bhattacharjee S, Bhattacharya S, Jana S, Baghel DS. A review on medicinally important species of picrorhiza. Int J Pharm Res and Bioscience. 2013;2(4):1-16.
150. Chander R, Kapoor NK, Dhawan BN. Picroliv, picroside-I and kutkoside from *Picrorhiza kurrooa* are scavengers of superoxide anions. Biochem Pharmacol. 1992;14:180–183.
DOI: 10.1016/0006-2952 (92)90054-M.
151. Singh GB, Bani S, Singh S, Kaul A, Khajuria A, Sharma ML, et al. Anti-inflammatory activity of the iridoids kutkin, picroside-I and kutkoside from *Picrorhiza kurroa*. Phyther Res. 1993;7:402–7.
152. Tiwari SS, Pandey MM, Rawat AKS. TLC densitometric quantification of Picroside (Picroside-I and Picroside-II) in *Picrorhiza kurroa* and its substitute *Picrorhiza scrophularii* flora and their antioxidant studies. Biomed Chromatography. 2012; 26(1):61–8.
153. Park EJ, Lee HS, Oh SR, Lee HK, Lee, HS. Pharmacokinetics of verproside after intravenous and oral administration in rats. Arch Pharm Res. 2009;32(4):559–64.
154. Hussain A, Shadma W, Maksood A, Ansari SH. Protective effects of Picrorhiza kurroa on cyclophosphamide-Induced immune-suppression in mice. Pharmacog Res. 2013;5(1):30–5.
155. Jia D, Barwal I, Thakur S, Yadav SC. Methodology to nanoencapsulate hepatoprotective components from *Picrorhiza kurroa* as food supplement. Food Bioscience. 2015;9(1):28-33.
156. Shuxiang FYZ. Assignees: Beijing Xingyi Pharmaceutical Co., Ltd. Picroside II phospholipid composition and preparation method. US Patent - CN101890034A; 2010.
157. Joshi V, Inamdar S, Acharya A, Rajesh Kowti R. Screening, Fabrication and Evaluation of Pharmaceutical Dosage Forms using P-Glycoprotein Modulators. Int J Pharm Sci Nanotech. 2017;10(2): 3688–98.
158. Uppuluri S, Ali SL, Nirmala T, Babu SDS. A review on *Tinospora cordifolia*. International Curr Pharmaceutic Rev Res. 2013;4(2):61-68.
159. Kumar A, Jaiswal M. Enhanced intestinal permeability of *Tinospora cordifolia* extract through nanoemulsion formulation: *In-vitro* and *ex-vivo* studies. J. Nanopharm Drug Del. 2014;2:1–10.

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