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# Evaluation of Pharmacokinetic Profile of Cirpusins®, Extract of *Cyperus rotundus* in Presence of Bioavailability Enhancer, BioPerine®

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**Abstract**

Obesity, a condition accompanying abnormal or excessive accumulation of fat in the body has become a major public health concern globally. Various herbs hailed from traditional systems of medicine like Ayurveda have gained importance in recent years to combat obesity. One such herb *Cyperus rotundus* (nutgrass), is known for its beneficial effects in obesity, in Ayurveda. Cirpusins® a patented extract from the dried rhizomes of *Cyperus rotundus* (standardized for minimum of 6% total identified stilbenes - Piceatannol, Scirpusin A & Scirpusin B) and BioPerine®, a bioavailability enhancer, obtained from the dried fruits of *Piper nigrum* (standardized to minimum 95% piperine). In this study, we examined the pharmacokinetic profile and bioavailability of Cirpusins® in Sprague-Dawley rats at two different doses (45 and 90 mg/kg) in the presence and absence of BioPerine® respectively. The results signifies that addition of BioPerine® (0.45 mg/kg) with Cirpusins® (45 mg/kg) enhances the bioavailability by reducing the effective dose of Cirpusins® (90 mg/kg) to half.

**Keywords:** Obesity, Bioavailability, Pharmacokinetics, Cirpusins®, BioPerine®, Scirpusin A, Scirpusin B

## 1. Introduction

Obesity, a multifactorial disorder has become a major public health concern that significantly contributes to the global burden of chronic diseases (Mokdad et al., 2003). Obesity is caused by imbalance in energy consumption and expenditure which is a challenging condition of excess body fat (Engin, 2017). The incidence of obesity has nearly tripled worldwide since 1975 with nearly 40% of the adult population being overweight, according to World Health Organization (WHO,2021). Obesity is associated with comorbidities like diabetes, hypertension, hyperlipidaemia, cancer, and sleep apnoea, and is considered as an independent risk factor for cardiovascular diseases (Poirier et al., 2006). The primary options available to control obesity are diet control, exercise, and lifestyle management. Natural products help regulate various mechanisms in obesity, such as appetite suppression, modulating adipogenesis, and regulating lipid hydrolyzing enzymes such as pancreatic lipase and lipoprotein lipase (Gooda Sahib et al., 2012).

One such perennial herb native to India is *Cyperus rotundus* (nutgrass) belonging to the Cyperaceae family, which has been used traditionally over many centuries for various ailments. *Cyperus rotundus* has been proven for various pharmacological activities such as analgesic, antimalarial, anti-inflammatory, antidiarrheal, antidiabetic, wound healing, neuroprotective and antioxidant activities (Kamala et al., 2018 & Al-Snafi, 2016). It is known as 'Musta' in Ayurvedic system of India, and proclaimed by Ayurvedic Pharmacopoeia of India for its beneficial effects in obesity. The major organic chemicals isolated from various parts of the *Cyperus* species include quinonoid pigments, sesquiterpenoids, flavonoids and stilbene derivatives. Several monoterpenoids, amino acids and fatty acids are also reported. Stilbenoids are phenolic compounds that are reported to have several health benefits. Scirpusins are dimerized stilbenes; Scirpusin A is a dimerized resveratrol isolated for the first time from the rhizome of *Scirpus fluviatilis* in 1978 along with resveratrol and 3, 3', 4, 5'-tetrahydroxystilbene (Nakajima et al., 1978). Piceatannol (trans-3',4',3,5-tetrahydroxystilbene), commonly found in berries, grapes, rhubarb, and white tea, is very

similar in structure to resveratrol, except for an additional hydroxyl group. Scirpusin B is one of the many possible dimeric structures of Piceatannol (Chou et al., 2018).

Cirpusins® is a patented extract, prepared from the dried rhizomes of *Cyperus rotundus*, standardized to contain a minimum of 6% total identified stilbenes (Piceatannol, Scirpusin A, and Scirpusin B). Cirpusins® has been scientifically evaluated for its anti-obesity potentials *in vitro* and *in vivo*. Clinical findings showed that Cirpusins® are a safe and effective supplement to manage body weight in obese individuals. The lipid profile and the biochemical parameters were normalized with the supplementation of Cirpusins®. In a preclinical study on high-fat diet induced obesity, Cirpusins® showed reduction in body weight, and visceral fat weight along with normalized lipid levels, corticosteroids and circulating leptins. Cell based studies confirmed that Cirpusins® can significantly inhibit adipogenesis *in vitro* (Majeed et al., 2022).

Bioavailability refers to 'the ability of a drug or other substance to be absorbed and used by the body'. To increase this absorbing ability of an ingested substance, 'bioenhancers' play an important role which can enhance bioavailability and bioefficacy of a particular drug with which it is combined (Kesarwani et al., 2013).

One such 'bioenhancer' is *Piper nigrum extract* (BioPerine®), obtained from the dried fruits of *Piper nigrum* (black pepper), standardized to minimum 95% piperine. Several studies have confirmed that BioPerine® significantly improves the bioavailability when co-administered with various compounds such as curcumin, resveratrol, ginseng, β-carotene, coenzyme Q10, minerals (elemental iron, selenium), vitamins (vitamin B6, vitamin C) through their increased absorption (Majeed et al., 2017). Overall, piperine is well known for enhancing the efficacy of pharmacologically active ingredients. Backed by several clinical trials, BioPerine® is a very well recognized natural bioenhancer in the market, which can be used in conjunction with several natural extracts, drugs, vitamins, minerals, and antioxidants (Majeed et al., 2017).

The objective of the present study was to determine the pharmacokinetic profiling of Cirpusins® at two doses (45 mg/kg and 90 mg/kg) and see whether addition of BioPerine® (0.45 mg/kg) improves the bioavailability at a lower dose making it equipotent at half a dose of currently being used dose of 90mg/kg. Thus, reduced concentration of the dose will reduce the risk of physiological side-effects and will also have an impact on reduction of pricing in therapies that benefits more people due to its low cost.

## 2. Materials and Methods

### *2.1 Plant Extracts, Chemicals, and Reagents*

The test sample Cirpusins® is prepared from the dried rhizomes, standardized to contain a minimum of 6% total identified stilbenes - Scirpusin A, Scirpusin B and Piceatannol (Figure 1) by HPLC. Piceatannol ($C_{14}H_{12}O_4$, 244.24 $gmol^{-1}$) is a stilbenol that is trans-stilbene in which one of the phenyl groups is substituted by hydroxy groups at positions 3 and 4, while the other phenyl group is substituted by hydroxy groups at positions 3 and 5. It is a stilbenol, a member of resorcinols, a member of catechols and a polyphenol. It derives from a hydride of a trans-stilbene. Scirpusin A ($C_{28}H_{22}O_7$, 470.50 $gmol^{-1}$) is a mixed dimer of resveratrol and

Piceatannol. Chemically it is (E)-4-(3-(3,5-dihydroxyphenyl)-6-hydroxy-4-(4-hydroxystyryl)-2,3-dihydrobenzofuran-2-yl) benzene-1,2 diol. Scirpusin B ($C_{28}H_{22}O_8$, 470.50 $gmol^{-1}$) is a dimer of Piceatannol and chemically it is 5-[(2S,3S)-2-(3,4-dihydroxyphenyl)-4-[(1E)-2-(3,4-dihydroxyphenyl)ethenyl]-6-hydroxy-2,3 -dihydro-1-benzofuran-3-yl]benzene-1,3-diol, a natural molecule found in *Cyperus rotundus*, *Senna garrettiana* and *Passiflora edulis*. Besides these some other stilbenols like Cyperus phenols (Cyperus phenol A, B, C and D) and resveratrol have also been identified, but these were not quantified in our product. Many secondary metabolites such as quinones, flavonoids, saponins, alkaloids, phenolic acids (salicylic acid, protocatechuic acid, caffeic acid and p coumaric acid) also may be present in the product as they reported in the plant *Cyperus rotundus.*

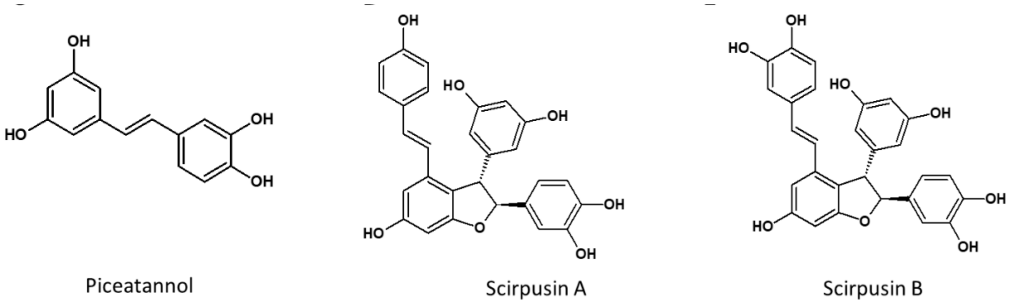



Figure 1. Chemical structures of Piceatannol, Scirpusin A, and Scirpusin B.

Also, BioPerine® prepared from the dried fruits, was standardized to contain a minimum of 95% piperine by HPLC. Both the test samples have been obtained through solvent extraction process. The HPLC chromatogram of the compound, Cirpusins® at 320 nm is shown in Figure 2. All other chemicals/reagents and solvents used throughout the experiment were of analytical grade.

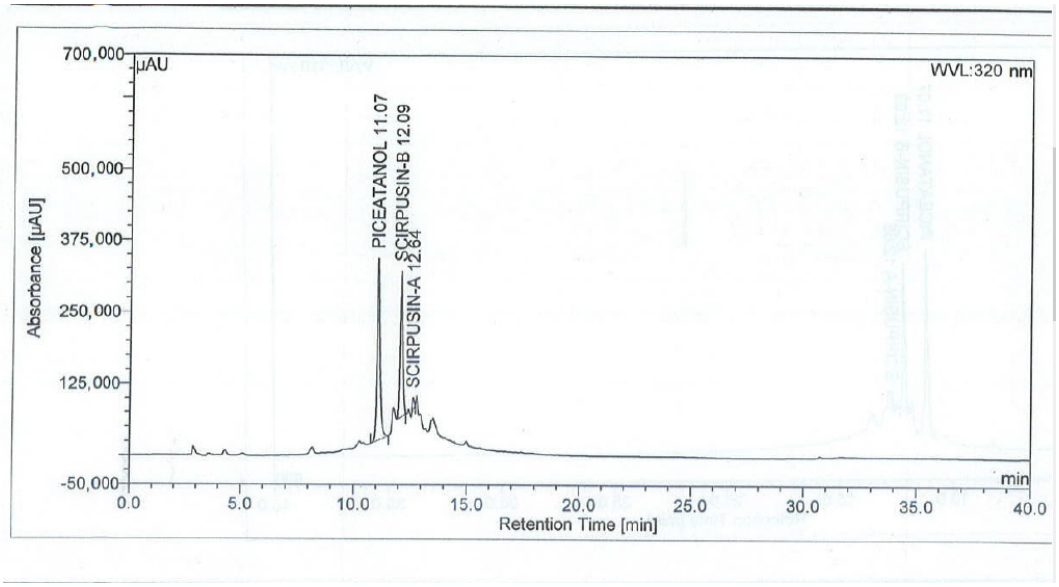



Figure 2. HPLC chromatogram of Cirpusins®.

*2.2 Animals*

Experimental healthy young adult female Sprague-Dawley rats weighing 180-200 g, aged between 8 to 10 weeks were used in this study. The animals were housed in a polypropylene cage, kept in clean and sieved paddy husk. The temperature was maintained at 22±3°C with a relative humidity of 30-70%, air-conditioned with adequate fresh air supply (12-16 air changes per hour). All animals were acclimatized for a period of five days before the experiment under 12 h light and 12 h dark cycle. They were fed with pelleted feed *ad libitum* and deep bore-well water passed through reverse osmosis and exposed to UV rays in Aquaguard water filter. The animals were fasted overnight with free access to water during the experiment and were divided into two groups. The groups were further subdivided into 3 subgroups of 3 animals each.

*2.3 Justification of Dose*

The grouping and respective doses are explained in Table 1. The justification of the dose is based on the effective dose in the clinical study conducted in obese individuals at 525 mg twice a day to manage body weight (Majeed et al., 2022). The 525 mg twice a day human dose comes to approximately 90 mg/kg p.o. bw in rats.

Table 1. Grouping and Dosage used in the study

| **Test item + Dose (Human Equivalent Dose)** | **Test item + Dose (Rat Equivalent Dose)** | **Sub-groups** | **No. of animals** | **Blood sampling Time points (8)** | | |
|---|---|---|---|---|---|---|
| Cirpusins®+ BioPerine® (500 mg + 5 mg) | Cirpusins®+BioPerine® (45 mg /kg + 0.45 mg/kg bw) | Group 1 | 3 | 0 min | 1 h | 12 h |
| | | Group 2 | 3 | 15 min | 2 h | 24 h |
| | | Group 3 | 3 | 30 min | 4 h | - |
| Cirpusins® (1000 mg) | Cirpusins® (90 mg/kg bw) | Group 1 | 3 | 0 min | 1 h | 12 h |
| | | Group 2 | 3 | 15 min | 2 h | 24 h |
| | | Group 3 | 3 | 30 min | 4 h | - |

The test items were administered as a single dose through oral route by gavage to each animal, using gavaging needle fitted onto a disposable syringe of appropriate size. Prior to dosing and during the study period, the animals were observed for clinical signs and pre-terminal deaths periodically at 15$^{th}$ min, 30$^{th}$ min, 1$^{st}$ h, 2$^{nd}$ h, 4$^{th}$ h, 12$^{th}$ h and 24$^{th}$ h. The cage-side observations like changes in the skin and fur, eyes, mucous membranes, also respiratory, circulatory, and autonomic and central nervous systems, motor activity and behavior pattern of animals were observed and recorded. Observations were also directed at observation of tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma. The animals were subjected to detailed veterinary examination prior to test item administration. Changes in skin and fur, eyes, mucous membrane, occurrence of secretions and excretions, autonomic

activities, response to handling, changes in gait, posture, clonic or tonic movements, stereotypic and bizarre behaviors of animals were recorded. At the end of the experimental period all the animals were sacrificed by carbon dioxide asphyxiation method using chamber and subjected to necropsy. The external and internal gross pathological observations were recorded.

### *2.4 Pharmacokinetic Study of Cirpusins® in Female Rats*

The blood samples were collected from individual animals by orbital venous plexus in Eppendorf tube containing dipotassium ethylenediaminetetraacetic acid (K2-EDTA) as anticoagulant, at time intervals (0 min, 15 min, 30 min, 1, 2, 4, 12 and 24 hours) after administration of test item. Blood samples were centrifuged (3000 rpm for 10 minutes) to obtain the plasma within one hour after the collection.

### *2.5 Instrumentation and LC-MS /MS Method*

The LC-MS/MS analysis was performed using the LC-MS/MS instrument, Water ZEVO TQS analyst 4.1 version. The LC-MS/MS system consisted of an HPLC system and a tandem mass spectrometer with (+) ESI. The analytical column Acquity UPLC BEH (C18, 2.1 x 100) was used for chromatographic separation. The mobile phase was a mixture of 5 mM Ammonium formate: Acetonitrile (Gradient). The injection volume was 10 µL. MRM transition for Scirpusin A: 470.98>56.84 (q1 to q2); Scirpusin B 487.16>101.83 (q1 to q2) and Piceatannol: 260.64>182.72 (q1 to q2).

### *2.6 Preparation of Plasma Samples*

The plasma samples for LC-MS/MS analysis were prepared by the protein precipitation method. At the time of analysis, plasma samples were removed from the deep freezer, were thawed at room temperature. The samples were prepared by adding plasma (50 µL) and acetonitrile (150 µL) as precipitating agent. The samples were vortexed for 2 min and centrifuged at 4000 rpm for 7 min. After centrifugation, the supernatant layer was separated and was injected to the LC-MS/MS system for analysis.

### *2.7 Preparation of Calibration Curve*

A calibration curve is the relationship between instrument response and known concentrations of the analyte. A calibration curve should be generated for each analyte in the sample. A sufficient number of standards should be used to adequately define the relationship between concentration and response. A calibration curve should be prepared in the same biological matrix as the samples in the intended study by spiking the matrix with known concentrations of the analyte. The number of standards used in constructing a calibration curve will be a function of the anticipated range of analytical values and the nature of the analyte/response relationship. Concentrations of standards should be chosen based on the concentration range expected in a particular study. A calibration curve should consist of a blank sample (matrix sample processed without internal standard/molecule of interest for analysis), and six to eight non-zero samples covering the expected range, including lowest limit of quantification (LLOQ).

*2.8 Ethics*

The animal experiment was in accordance with the guidelines of committee for the purpose of control and supervision of experiment on animals (CPCSEA Registration Number-377/PO/ReBi/S/01/CPCSEA). All procedures involving animals were conducted humanely and were performed by or under the direction of trained or experienced personnel. The Institutional Animal Ethic Committee (IAEC), Karnataka, India reviewed and approved the study protocol.

## 3. Statistical Analysis

Pharmacokinetic parameters such as peak plasma concentration ($C_{max}$), time to peak concentration ($T_{max}$), and area under the plasma concentration-time curve ($AUC_{0-t}$), were calculated based on concentration over time by using pk1 and pk2 software.

## 4. Results

*4.1 Clinical Observations*

During the study period, no treatment related changes in body weight gain, clinical signs of toxicity, pre-terminal mortality and abnormal behaviors were observed in any of the groups. All animals were found to be normal in appearance and healthy, upon detailed clinical veterinary examination (Table 2). None of the animals revealed any gross pathological changes both externally and internally on the organs.

Table 2. Findings of clinical veterinary examination of individual female animals of both the groups

| **Test group** | **Cirpusins® + BioPerine® (45 mg/kg+ 0.45 mg/kg)** | **Cirpusins® (90 mg/kg)** |
|---|---|---|
| **Skin and Fur** (0,1,2,3) | 0 | 0 |
| **Eyes** (0,1,2,3,4) | 0 | 0 |
| **Mucous membrane** (0, 1,2,3) | 0 | 0 |
| **Occurrence of secretions and excretions** | | |
| - Salivation (0,1,2) | 0 | 0 |
| - Urine staining (0,1) | 0 | 0 |
| - Fecal staining or diarrhea (0,1, D) | 0 | 0 |
| - Nasal discharge (0,1) | 0 | 0 |
| **Autonomic activity** | | |
| - Lacrimation (0,1,2) | 0 | 0 |
| - Piloerection (0,1) | 0 | 0 |
| - Pupil size or Pupillary response (0,1) | 0 | 0 |
| - Unusual respiratory pattern (0,1, S) | 0 | 0 |
| **Response to handling** (0,1) | 0 | 0 |

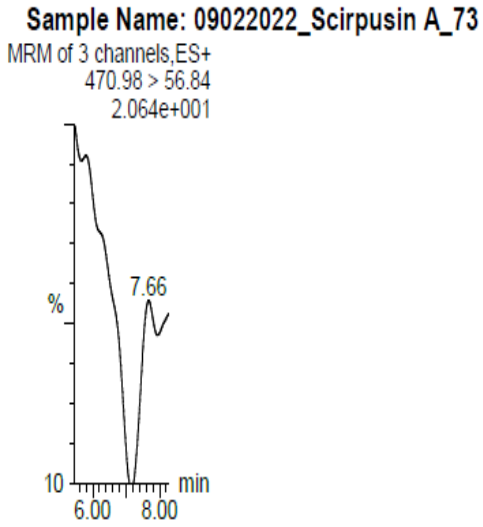
| | | |
|---|---|---|
| **Changes in gait** (0,1,2) | 0 | 0 |
| **Posture** (0,1,2) | 0 | 0 |
| **Clonic or Tonic movements** (0,1,2) | 0 | 0 |
| **Stereotypic behavior** | | |
| - Repetitive circling (0,1) | 0 | 0 |
| - Excessive grooming (0,1) | 0 | 0 |
| **Bizarre behavior** | | |
| -Self-mutilation (0,1) | 0 | 0 |
| -Walking backwards (0,1) | 0 | 0 |

n=3; "0" - Absence of clinical sign

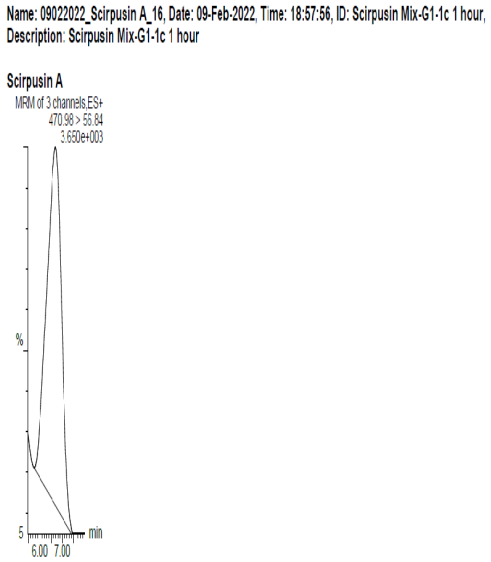
*4.2 In vivo Pharmacokinetic Study*

The LC-MS/MS method was applied to estimate the three chemical constituents after administration of Cirpusins® in female Sprague-Dawley rats. LC-MS/MS chromatogram of three chemical constituents (Scirpusin A, Scirpusin B and Piceatannol) were shown in Figure. 3 (A to E).

**A: Blank Plasma Solution**




**B: Sample Solution of Scirpusin A**



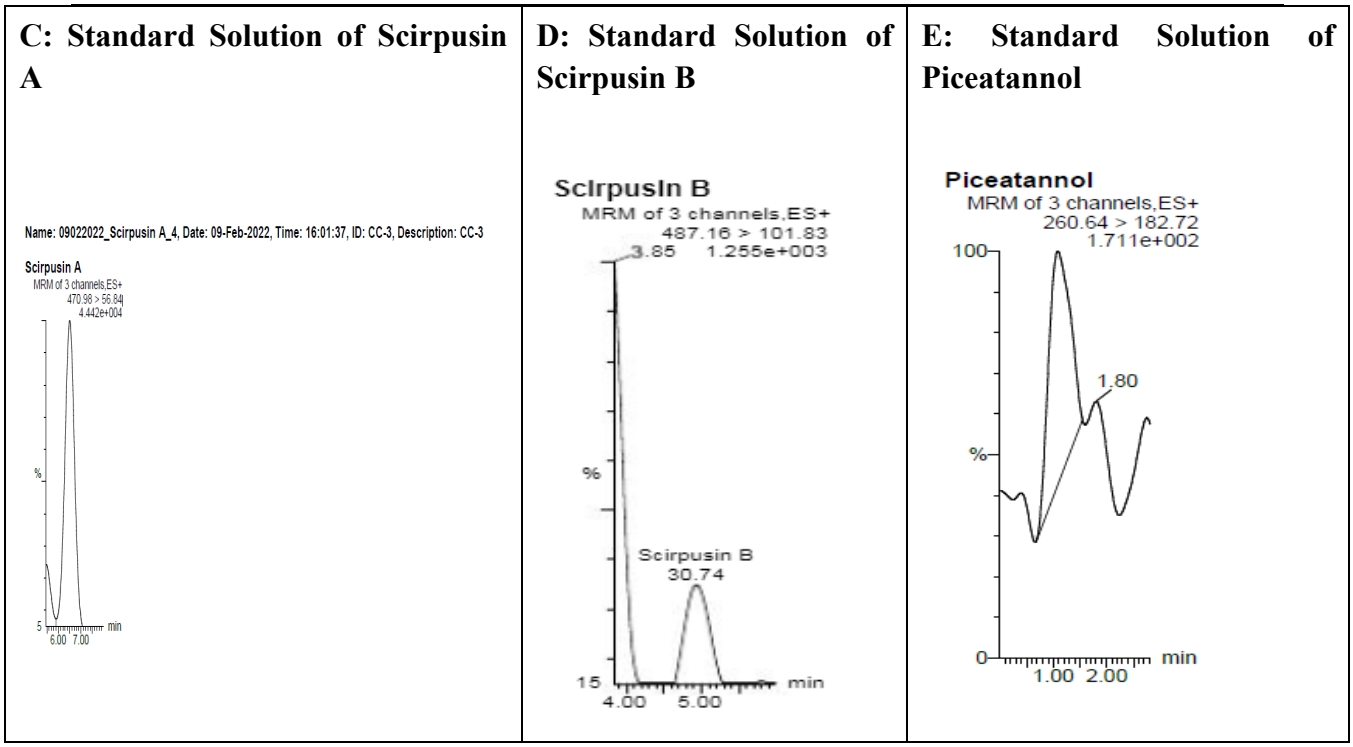
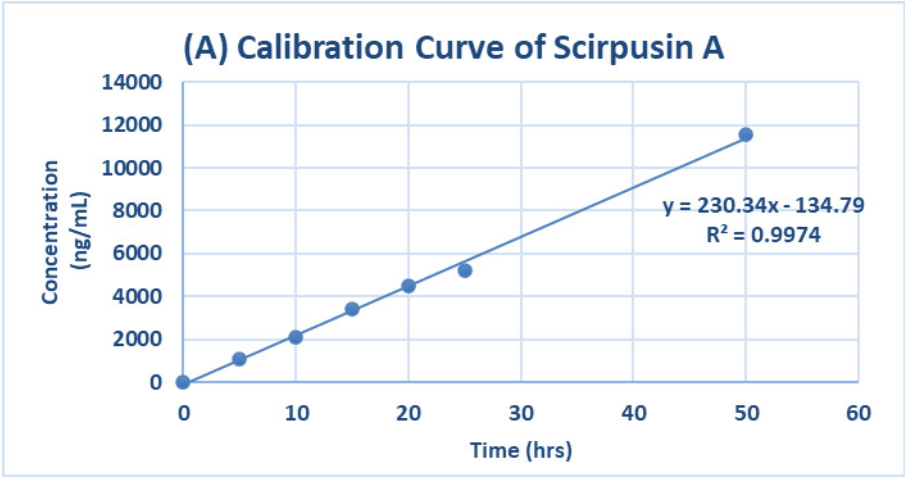
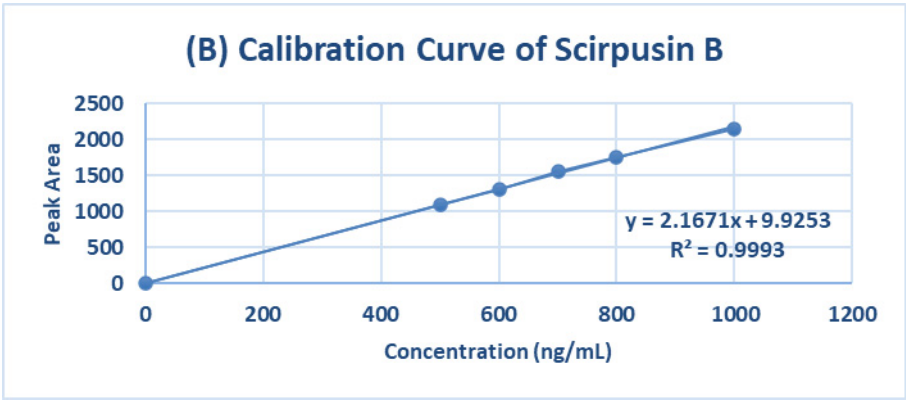



Figure 3. LC-MS / MS chromatogram of Scirpusin A, Scirpusin B and Piceatannol

The concentration range for the calibration curve of

a) Scirpusin A was 5 to 50 ng/mL with $r^2$=0.997; LLOQ = 5 ng/mL b), Scirpusin B was 500 to 1000 ng/mL with $r^2$=0.999; LLOQ = 500 ng/mL, c) Piceatannol was 1 to 25 mcg/mL with $r^2$ =0.994; LLOQ = 1.0 mcg/mL (Figure 4).





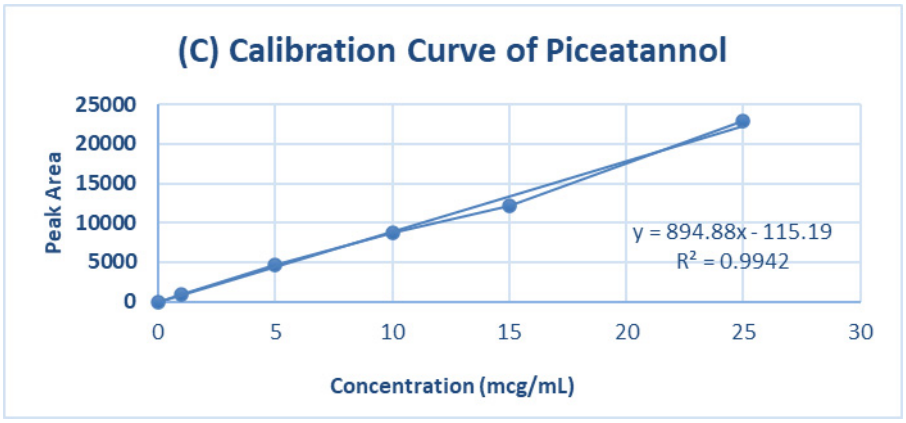




Figure 4. Calibration curve of (A) Scirpusin A, (B) Scirpusin B, and (C) Piceatannol in rat plasma

The concentrations of Scirpusin B and Piceatannol were detected as below the lowest limit of quantification (BLOQ). Hence, pharmacokinetic parameters of Scirpusin B and Piceatannol were not determined. The mean plasma concentration *vs*. time curves of Scirpusin A in Cirpusins® (90 mg/kg) and of Cirpusins® (45 mg/kg) with BioPerine® (0.45 mg/kg) are shown in Figure 5. The pharmacokinetic parameters of Cirpusins® (90 mg/kg) and Cirpusins® (45 mg/kg) in combination with BioPerine® are shown in Table 3.

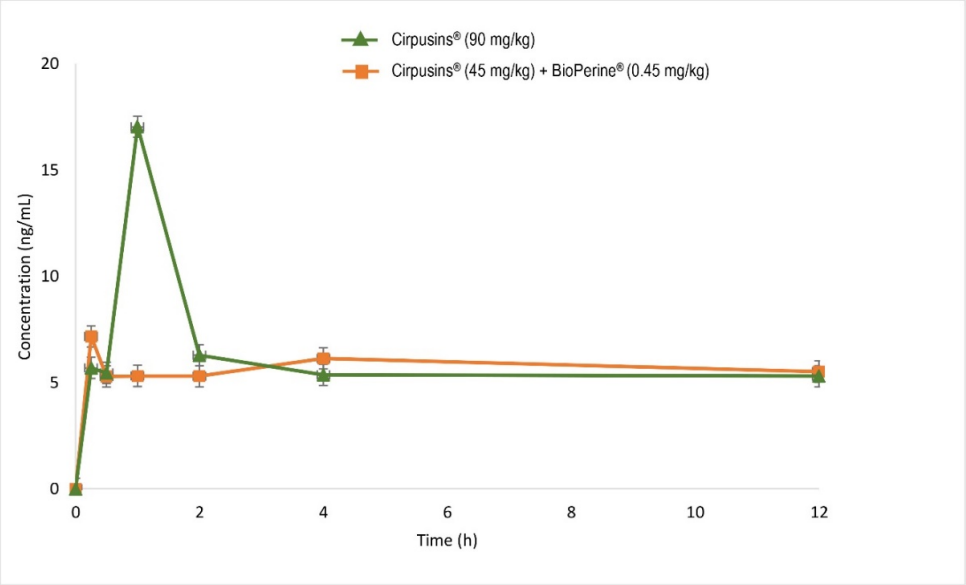



Figure 5. Mean plasma concentration *vs.* Time profile (0-24 h) of Scirpusin A in Cirpusins® (45 mg/kg)+ BioPerine® (0.45 mg/kg) and Cirpusins® (90 mg/kg)

Table 3. Pharmacokinetic parameters of Scirpusin A in Cirpusins®+ BioPerine® and Cirpusins®

| **Pharmacokinetic Parameters** | **Cirpusins®+BioPerine® (45 mg/kg+ 0.45 mg/kg)** | **Cirpusins® (90 mg/kg)** | **Bioavailability of Scirpusin A in Cirpusins® + BioPerine® (45 mg/kg + 0.45 mg/kg) compared to Cirpusins® (90 mg/kg)** |
|---|---|---|---|
| **$C_{max\ (ng/mL)}$** | **7.1676** | **17.0316** | **96.30 %** |
| **$T_{max\ (h)}$** | **0.25** | **1** | |
| **$K_{el\ (h)}$** | **0.0133** | **0.0650** | |
| **$t_{1/2\ (h)}$** | **52.0565** | **10.6621** | |
| **$AUC_{0\text{-}24\ (ng.h/mL)}$** | **101.5342** | **105.4335** | |
| **$AUC_{0\text{-}\infty\ (ng.h/mL)}$** | **515.7682** | **186.8997** | |

$C_{max}$: Maximum observed concentration; $T_{max}$: Maximum observed time; $K_{el}$: Elimination rate constant; $t_{1/2}$: Half-life; AUC: Area under the curve; $AUC_{0\text{-}24}$ = Area under the curve 0-24 h; $AUC_{0\text{-}\infty}$: Area under the curve zero to infinity.

Compared statistically Cirpusins®+ BioPerine® *vs* Cirpusins® (p values $C_{max}$ 0.489; $AUC_{0\text{-}24}$ 0.732; $AUC_{0\text{-}\infty}$ 0.835).

Results from the study elucidated that the group treated with Cirpusins® (90 mg/kg) showed the values of $C_{max}$, $T_{max}$, $AUC_{0\text{-}24h}$ and $t_{1/2}$ were 17.0316 ng/mL, 1 h, 105.4335 ng.h/mL and 10 h respectively whereas the group treated with Cirpusins® (45 mg/kg) along with the addition of BioPerine® (0.45 mg/kg) the $C_{max}$, $T_{max}$, $AUC_{0\text{-}24h}$ and $t_{1/2}$ were 7.1676 ng/mL,

0.25 h, 101.5342 ng.h/mL and 52 h respectively as described in Table 3. The data demonstrated that $AUC_{0\text{-}24h}$ with Cirpusins® (45 mg/kg) along with the addition of BioPerine® was almost similar to Cirpusins® (90 mg/kg) without BioPerine®. Thus, the bioavailability of Scirpusin A in Cirpusins® (45 mg/kg) combination with BioPerine® was 96% with respect to Cirpusins® (90 mg/kg). Overall, the data demonstrated that addition of BioPerine® (0.45 mg/kg) has enhanced the bioavailability of Cirpusins® (45 mg/kg) equivalent to that of Cirpusins® (90 mg /kg).

## 5. Discussion

Natural products have been a significant manifestation of the human evolution, for both as medicine as well as for health and wellness. Bioenhancers play an important role to significantly enhance the bioavailability of supplemented actives through increased absorption. Black pepper is one such spice, which has been an essential part of human diets and commerce. BioPerine® is a natural bioavailability enhancer, standardized for minimum 95% piperine. The synergistic interaction with a range of nutrients, minerals, vitamins, and other modern therapeutic agents is a renowned and well-established property of piperine. Piperine has been attributed to add nutritive value to a variety of phytonutrients and other drugs by enhancing their bioavailability and thermogenesis. The mechanism by which piperine enhances the drug bioavailability is explained through modulation of P-glycoprotein-mediated drug/nutrient efflux, modulation of metabolic enzymes involved in biotransformation of drugs/nutrients, enhanced intestinal absorption, and thermogenesis (Majeed et al., 2017). In this study, we evaluated the pharmacokinetic profile of Cirpusins® and whether addition of BioPerine® improves the bioavailability at a lower dose making it equipotent as the double dose.

Cirpusins® involves the benefits of the three pharmacologically active molecules (Piceatannol, Scirpusin A, and Scirpusin B) and is scientifically evaluated in obese individuals as an effective supplement at 525 mg twice a day to manage body weight (Majeed et al., 2022). Based on the effective dose in the anti-obesity study (Majeed et al., 2022), this experiment was conducted to compare, Cirpusins® (45 mg/kg) with BioPerine® (0.45 mg/kg) will be as effective as Cirpusins® (90 mg/kg) in improving the bioavailability.

The animals were grouped into two doses: Cirpusins® (90 mg/kg) and Cirpusins® (45 mg/kg) with BioPerine® (0.45 mg/kg). Oral administration in Sprague-Dawley rats resulted in no significant change in body weight. All animals were found to be normal and healthy with no abnormal behaviours or clinical signs or pre-terminal mortality. None of the animals showed any gross pathological changes both externally and internally, thus signifies that the test item did not have any adverse effects on the animals. To determine the pharmacokinetic parameters, blood samples were collected from the orbital venous plexus of animals, after administration of test item at different time intervals.

The plasma concentration of Scirpusin B and Piceatannol were detected as BLOQ and pharmacokinetic parameters of these constituents were not determined. Thus, the pharmacokinetic parameters of Scirpusin A alone was determined. It was found that $AUC_{0\text{-}24h}$ in Cirpusins® (45 mg/kg) with BioPerine® (0.45 mg/kg) was equivalent to Cirpusins® (90 mg/kg) without BioPerine®. The bioavailability of Cirpusins® (45 mg/kg) with BioPerine® was 96% compared to Cirpusins® (90 mg/kg), emphasizing that the Scirpusin A in Cirpusins®

with BioPerine® was equivalent to double the dose without BioPerine®. Hence, addition of BioPerine® enhanced the bioavailability of Scirpusin A with respect to administration of Cirpusins®. This study paves way for future clinical studies on Cirpusins® in combination with BioPerine® to evaluate its enhanced pharmacological efficacy, thereby minimizing the dosage of the active with maximum benefits.

## 6. Conclusion

The current *in vivo* and pharmacokinetic study has shown that addition of BioPerine® enhances the bioavailability and bioefficacy of Cirpusins® by reducing the effective dose of Cirpusins® from 90 mg/kg bw to half, i.e. the dose of 45 mg/kg bw.

## Conflict of interest

The authors have no conflict of interest to disclose.

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