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Assessment of bioavailability of Guduchi (*Tinospora cordifolia*) Kashaya in healthy volunteers

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Abstract
Although Ayurvedic medicines are widely used for the prevention, diagnosis, treatment, and management of disease, it has been facing constant challenges like quality control, Standardization, and pharmacokinetic profile of biomarkers in the formulation. Pharmacokinetics which deals with the absorption, distribution, metabolism and excretion of the biomarkers or the new drug entity is one of the regulatory requirements for an investigation new drug approval. Hence bioactive guided pharmacokinetic approach method is needed for Ayurvedic system of medicine to determine the pharmacokinetic of relevant markers in the formulation having number of markers. With this background, the present study has been taken with the objective to explore the importance of pharmacokinetics of the drug and evaluate the bioavailability of Guduchi Kashaya in blood plasma using LCMS MS analysis [1].

Keywords: Guduchi, *Tinospora cordifolia*, bioavailability, LCMS analysis

Introduction
Ayurveda is the most ancient system of traditional medicine of the world which is being practiced in India. Although Ayurvedic medicines are widely used for the prevention, diagnosis, treatment and management of disease, it has been facing constant challenges like quality control, standardization and pharmacokinetic profile of biomarkers in the formulation [2, 3]. Pharmacokinetics which deals with the absorption, distribution, metabolism and excretion of the biomarkers or the new drug entity is one of the regulatory requirements for an investigation of new drug approval. Plants have been the earliest source of medicine from time immemorial. A drug has a pivotal role in instituting the therapy to any patient. Hence it has been considered next to physician in the quadruples of treatment by Acharya Charaka [4]. Guduchi also known as *Tinospora cordifolia* used for its medicinal value since Vedic periods and having claimed as a highly potential drug in Ayurveda, for preservation of health, prevention of disease and in curative measures is attributed with a wide range of pharmacological activities and is commonly used non-controversial drug [5]. It is a traditional plant used in Ayurvedic treatments and known best for its uses in Fever, bleeding disorders, rejuvenation etc. [6] Currently, plant is of great interest to researchers across the globe because of its reported medicinal properties like immunomodulatory, hepatoprotective, anti-diabetic, anti-spasmodic, anti-inflammatory, anti-arthritic, antioxidant activities [7]. The plant mainly contains alkaloids, glycosides, steroids, mixture of fatty acids and polysaccharides.
Guduchi in its various dosage forms like Guduchi Satva, Swarasa, Kashaya, Vati, Arishta and in compound formulations are being used in the treatment. As we all know any drug taken through oral route has a delayed effect when compared to drug administered through different routes like sublingual administration of Swarna Garbha Pottali or any rasaushadhis. But the fate of the kashta aushadhi-plant origin is not known, and the dose is fixed accordingly as said by acharyas, but the complete pharmacokinetics and mechanism of action of Guduchi is yet unexplored. Since little is known about the fate of Guduchi Kashaya in human participants the bioavailability studies for Guduchi Kashaya through oral route was taken up. When Kashaya was subjected to HPLC, the study revealed 20β-hydroxy ecdysone compound, and this was further taken for the study to trace in the blood sample.

Materials and Methods [8, 9, 10]
Collection of Drug
The stem of the plant *Tinospora cordifolia* Miers-Guduchi were collected from Botanical Garden of JSS AMC, Mysore. The sample compared with specimen voucher preserved in the museum was authenticated by Dept of PG studies Dravyaguna, JSSAMC, Mysore.

Collection of blood samples
For collection of blood samples, disposable syringes with needles (Dispovan, 2 cc), medicated cotton, disinfectant 6 ethanol and small band-aids used. For storage of blood prior to analysis, heparinised vacutainers of specification BD vacutainer sodium heparin (NH) 75 USP units blood collection tubes used. Pipettes are used for transfer of blood from vacutainers and plasma is separated and stored in -20 °C.

Preparation of the trial drug
The raw drug was Cleaned thoroughly and cut into pieces, shade dried and course powdered.

Preparation of Kashaya
Guduchi Kashaya was prepared as per general Kashaya Paaka Vidhi of Sharangadhara Samhita [1].
One part of coarse powder was boiled with 8 parts of clean portable water over mandagni and reduced to one fourth. Kashaya was filtered using 4 folded clean and dry cora cloth.

Selection of volunteers
Volunteers' height, weight, previous health history, biochemical parameters were all checked and those who had normal reports were selected for the study.

Administration of Kashaya to volunteers [11, 12]
Six healthy volunteers were selected with equal distribution of gender and age, 3 male and 3 female volunteers of age between 25-45, residents of Mysore. The present study was proposed using blood level data upon single dosing. The participants were asked to report JSS Ayurveda Hospital at 7.30 am, the individuals were kept fasting for twelve hours prior to the drug administration and two hours afterwards. One blood sample was collected by aseptic technique from cubital vein from each participant by disposable syringe and labelled as Zero-Hour blood sample. A standard bland diet upma for breakfast and rice, dal for lunch was provided to all the volunteers and intake of milk and beverages was restricted.
Later Subjects were given 100ml freshly prepared Guduchi Kashaya, subjects were at rest during the sampling and were continuously monitored for BP, pulse and frequency of micturition throughout during the study. After 2 hours of administration the blood sample was again collected, and breakfast was given. Then at 4th hour & 6th hour blood was collected and Lunch was given. And 12th hour last blood sample was collected. Along with the study subjects blank blood sample was collected from the individual who has not taken Kashaya. The collected blood samples were stored and separated plasma from them time to time, separated blood plasma was taken to Himalaya drug company and stored at -20 °C. This stored plasma was later subjected to LCMS analytical study for tracing the metabolite which was found in the Kashaya.

Observations [13, 14, 15]
The present study carried out under 4 headings:
1. Pharmacognostic study.
2. Physico-chemical analysis.
3. Pharmacokinetic study: Preliminary Phyto-chemical study.
4. Analytical study-LCMS.

Pharmacognostic study
Macroscopic features:

1. Guduchi Kashaya

Table 1: Shows macroscopic features

Feature	Guduchi Kashaya	Aqueous extract of Guduchi Kashaya
Colour	Dark brown	Orangish brown
Consistency	Semi thick liquid	Powder
Odour	Characteristic	Characteristic
Taste	Intense bitter	Intense bitter

2. Aqueous extract of Guduchi Kashaya

Table 2: Shows Physico-Chemical analysis of Aqueous extract of Guduchi Kashaya

Sl. No.	Physico chemical test	Results	Normal range
1.	LOD	5.29%	75%
2.	Ash value	14%	Not more than 16%
3.	Acid insoluble ash	2.55%	Not more than 3%
4.	Aqueous extract	12%	Not less than 11%
5.	Water soluble ash	68.15%	-

Phytochemical analysis

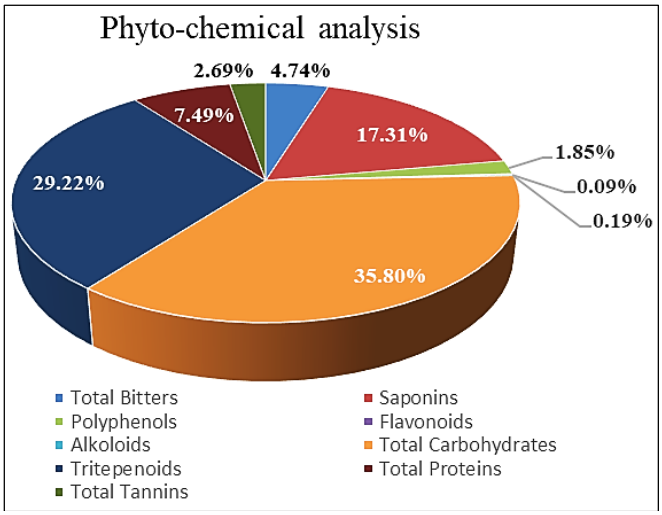

Fig 1: Shows phytochemical analysis of Guduchi extract

TLC findings of *Guduchi Kashaya* dry extract

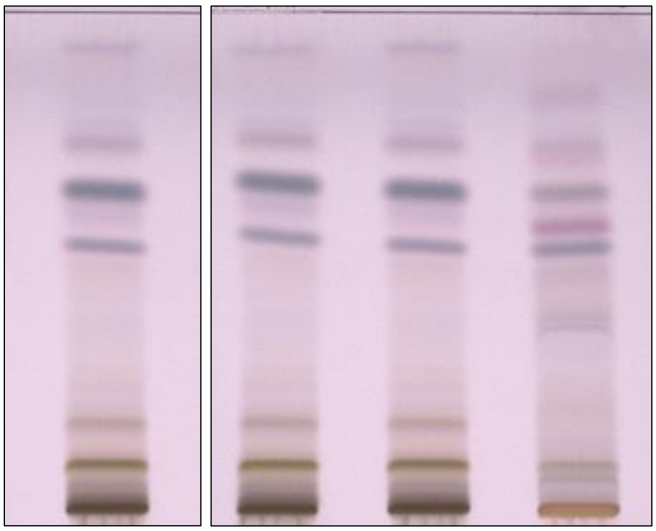

Fig 2: Showing TLC of *Guduchi*

Results

It was observed that 20-hydroxyecdysone was not detected in the tested plasma samples due to several reasons and one among them could be a low concentration in the Kashaya as well as molecule exhibits very poor bioavailability.

Further, we had screened the same plasma samples in Single Ion Monitoring (SIM) mode with selected parent molecular ion at m/z 481.1 and 361.1 for 20-Hydroxyecdysone and Prednisolone (IS), respectively. Furthermore, a study was conducted and reported by L. Dinan *et al*. Says that the active metabolites of 20-hydroxyecdysone metabolites viz., Poststerone, 14-deooxypoststerone, 14-deoxy-20-hydroxyecdysone, 2-deoxy-20hydroxyecdysone were detected in Wistar rats' plasma after oral gavage of 1g 20E/kg. As an opportunity to determine the bioavailability fate of Guduchi Kashaya, we performed the SIM mode scan to identify these reported active metabolites in this study samples. In conclusion, since 20E concentration is already low in the Guduchi Kashaya thereby neither 20E nor its metabolites were detected in the Guduchi Kashaya treated plasma samples. This study cumulative data provides a precise milieu information for future investigation on Guduchi Kashaya.

Discussion

The given drug-Guduchi Kashaya was not traced in the blood sample because of the following possible reasons:

- The Guduchi Kashaya was prepared as per the classics and subjected to LCMS and the analysis at 254nm has shown the major ten peaks, among which a peak retention time (RT) at 35.98 min with good intensity of area percentage and rest of the peaks had less area percentage. The ESI-MS of Kashaya had revealed that the protonated molecular ion mass at RT 35.98 min exhibited the mass of 20β-Hydroxy ecdysone at m/z 481.2 [M+H] + which appears to be strong and hence this principal compound was chosen for the bioavailability study.
- Drug standardization: Physical and Chemical parameters follow within a normal range as mentioned by API standards. Also, the HPLC analysis of Guduchi Kashaya traced the chemical constituent present in Guduchi which states that the taken drug was *Tinospora cordifolia* and not Tinospora crispa.
- And the fragmented ion at m/z 463.0, 445.2, 427.2, 409.3, 371.2, 301.4 confirmed the 20β-Hydroxy ecdysone compounds like Tinocordioside, Cordifolioside-A, Syringin.
- Furthermore, a study was conducted and reported by L. Dinan *et al*. Says that the active metabolites of 20-hydroxyecdysone metabolites viz., Poststerone, 14deooxypoststerone, 14-deoxy-20-hydroxyecdysone, 2-deoxy-20-hydroxyecdysone were detected in conclusion, Sin Wistar rats' plasma after oral gavage of 1g 20E/kg. hence an opportunity to determine the bioavailability fate of Guduchi Kashaya, SIM mode scan was performed to identify these reported active metabolites Poststerone 14-deooxypoststerone, 14-deoxy-20-hydroxyecdysone, 2-deoxy-2hydroxyecdysone in this study samples but was not detected.
- Probably we could have collected plasma at 30th min, 45th min, 60th min which was a possibility of tracing the compound in blood plasma since it was eluted in wistar rats at that time.
- Also, when compared the rate of metabolism in the rats and the gastric emptying time and that with human varies. So, considering this we drawn blood at 2, 4, 6 and 12th hour but could have drawn the sample at 30th min.
- Also, first pass metabolism differs from animals to humans it might have interfered with metabolism of guduchi hence bio active component not found in plasma.
- Also, when compared the rate of metabolism in the rats and the gastric emptying time is said to be 30 mins but that of human is longer. So, considering this we could have drawn the sample at 30th min.
- To list out few more reasons, it could also be because of the single dosing which might have been insufficient to be traced in the LCMS.
- Since 20 hydroxy-ecdysone is a steroid component the Probability of excretion might have occurred hence Urine excretion sample could have been screened within 2 hours after administration of Kashaya.
- Since 20 hydroxy-ecdysone concentration is already low in the Guduchi Kashaya thereby neither 20 hydroxy-ecdysone nor its metabolites were detected in the Guduchi Kashaya treated plasma samples. This study cumulative data provides a precise milieu information for future investigation on Guduchi Kashaya.

Conclusion

- This present study does not prove the Bioavailability of *Tinospora cordifolia* with reference to 20 hydroxy ecdysone and its metabolites. This possibly needs alternative/another component to study the bioavailability.
- This present study becomes a base for future study on Bioavailability of the drug.

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