Pharmacokinetic Studies on Rabbits as a Tool to Evaluate the Quality of *Phyllanthus Amarus* and *Ricinus Communis* by HPLC Method

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Abstract
Pharmacokinetics is a study of movement of a drug in our body. Usually, a modern drug consists of the ingredients and excipients. It is relatively easy to trace the pharmacokinetics of the modern drug. In case of herbal medicines, usually most of the over the counter products are polyherbal. There is no component clearly said to be the active principle responsible for the dedicated medicinal effect. Hence one needs to establish some phytochemical markers for the identification of the herb(s) in the blood, which can be used to trace the fate of the drug in the living system. This will help us to establish the absorption elimination process of the herb. The markers thus obtained need not be the active principle or constituent of the drug. This will help to decide the dosage regime and the therapeutic window of the drug. The present work was undertaken with an objective to establish a phytochemical marker for the detection of Phyllanthus amaras and Ricinus communis in the blood of rabbits and to establish the pharmacokinetics of the herb, we were able to identify two components from Phylanthusamarus and Ricinus communis (one each) in the plasma of the rabbits fed with the whole plant powder of Phyllanthus amaras and Ricinus communis as a combined dose, these two phytochemicals could be detected by using HPLC at Rt 10.70 minutes for Phyllanthus amaras and Rt 5.60 minutes for Ricinus communis. These phytochemicals were used to establish the pharmacokinetics of Phyllanthus amarusand Ricinus communis.
Keywords: *Phyllanthus amarus, Ricinus communis, HPLC, Pharmacokinetics.*

Introduction

The plants used in the pharmacokinetic study were *Ricinus communis* L. of family Euphorbiaceae, commonly called as Eranda or Gandharvahasta. It is commonly used in traditional medicine such as abdominal disorders and also show antidiabetic property. The plant is found throughout the hotter parts of India. Mostly growing wild on waste land and also cultivated. The other plant is a small shrub, *Phyllanthusamarus* of family Euphorbiaceae, commonly called as Bhuiavla. It is useful in the treatment of gastric disorders and against jaundice. It also has hepatoprotective, nephroprotective and diuretic properties.

Material and Method

Leaves of *Ricinus communis* and *Phyllanthus amarus* the whole plant were collected from Thane district of Maharashtra. The plant materials were thoroughly washed with water to remove dust and extraneous matter. The excess of water was absorbed by spreading the plant material over filter paper for three days in shade, away from sunlight. The filter paper was replaced daily. Herbaria were prepared and were sent for authentication to NBRI Lucknow. The plant material was then placed in preset oven and incubated at 45 ± 5^{0}. The plant material was allowed to dry for four days. Immediately after drying the plant material was powdered using an electric mixer grinder and sieved through a BSS mesh No 85 sieve. The sieved powdered plant material was stored commercially available airtight polythene containers with date and time of collection. This powdered plant material was used in the present study.

Animal Model

Animals used for the present study were New Zealand strain Albino rabbits, Weights of the animals ranged between 2.0 ± 0.2 kg.

Development of markers from the plant powder using HPTLC and HPLC methods.

0.5 g each of *Ricinus communis* leaf powder and *Phyllanthus amarus* were added separately with 10 ml of

chloroform in a clean, stoppered test tubes. The third test tube was added with the combination of these two plant powders.After using various combinations of polar, mid polar and non-polar solvents, a combination of Chloroform: methanol: Glacial Acetic Acid (8: 0.3: 0.1:: V/V) was found out to be ideal for the simultaneous detection of markers of *Ricinus communis* and *Phyllanthus amarus* by HPTLC method.Spectra of *Ricinus communis* leaf marker and that of *Phyllanthus amarus* plant powder by HPLC method were matching.

Pharmacokinetic study was carried out by using HPLC method. HPLC system used in the present study was JASCO PU-1580 with JASCO MD -1410 PDA detector. The column used in the present study was COSMOSIL 5C-18-MS, SIZE 4.6X150 mm. Manufacture No K01016.

Various mobile phases were tried like Methanol, Acetonitrile, and Distilled water. However best separation of marker of *Phyllanthus amarus* and *Ricinus communis* leaf marker was found in ACN: D/W (0.5:95) + (100 mg of Hexone sulfonic acid/ 100 ml of mobile phase). The Rt of marker was 5.707 for *Ricinus communis* and 10.783 min for *Phyllanthus amarus.*

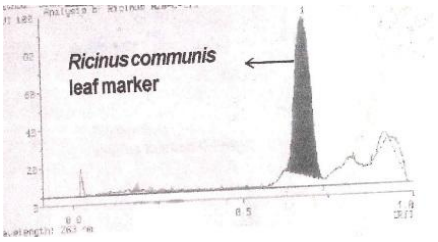
Oral dose of leaf powder of *Ricinus communis* and *Phyllanthus amarus* whole plant powder at 1g/kg body weight was given to the same rabbit. 3cm^3 of blood was removed in sterile, heparinised appendorff tube at the intervals of 0.50, 1.00, 2.0, 4.0, 12.0, 24.0 hours of post dose. The appendorff tubes were centrifuged at 4500 rpm for 15 minutes, and 0.5cm^3 of plasma was separated in 10.00cm^3 clean dry stoppered test tubes. 10.00 cm^3 of dichloromethane (DCM) was added to every test tube and the test tubes were shaken for 10 minutes. The test tubes were centrifuged for 10 minutes at 400 rpm. Supernatant aqueous layer was removed carefully, using hypodermic syringe. 8.00 cm^3 of Dichloromethane methane was transferred to a low volume evaporating tubes. Tubes were transferred to a water bath, preset at 50^{o}C for the evaporation of the organic layer. Rapid evaporation was done under nitrogen flow. After evaporation, the residue was reconstituted in 500µL of mobile phase. 20µl of reconstituted extract was injected in HPLC system to compare the spectra developed by using HPTLC method.

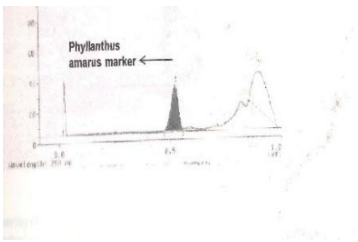
Main Study

Three male New Zealand albino rabbits weighing between 1.5 to 2.0 kg were used for the simultaneous detection. The rabbits were starved for 18 hrs prior to the oral administration of the plant material. Water was given ad libitum. Blank sample (0.00hrs) was collected from one of the rabbit's peripheral ear vein. 5.00cm^2 of blood was collected in a clean, dry heparinised appendorff tube. The tubes were centrifuged at 4500 rpm for 15 minutes. The plasma was speared and was used for spiking the plant material. Standards of Ricinus communis leaf powder and Phyllanthus amarus plant powder were prepared so as to get the concentrations of 50,100,250 and 500-µ/ml

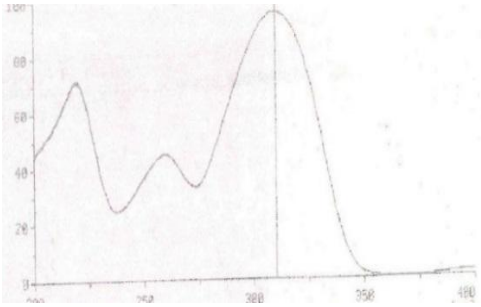
Observations

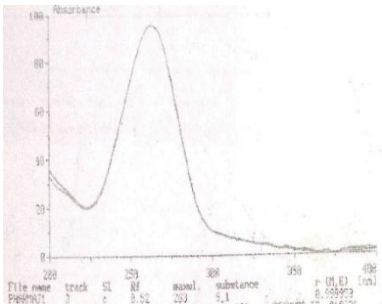
- Marker of *Ricinus communis* (310 nm) was detected in the plasma at 0, 25 hrs. of an oral dose. However the marker of *Phyllanthus amarus* (265nm) was detected in the plasma at 2.50 hrs. Post dose. The retention time (RT) of these markers were 6.17 and 10.783 mins respectively.



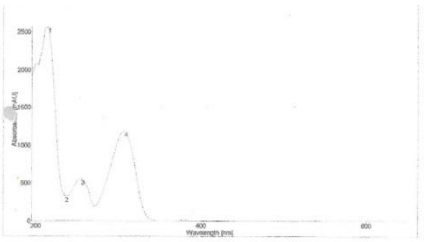


Marker of *Ricinus communis* (310nm) by HPTLC by HPTLC Marker of *Phyllanthus amarus* (265nm) by HPTLC

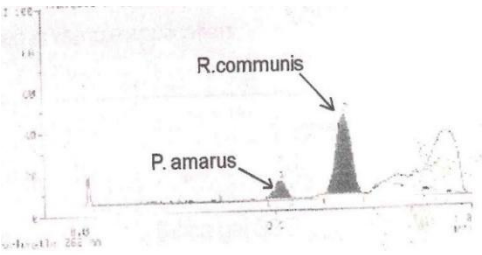


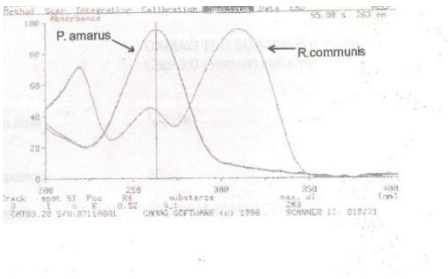


Spectrum of *Ricinus communis* leaf marker (310nm) Spectrum of *Phylanthus amarus* marker (260nm)

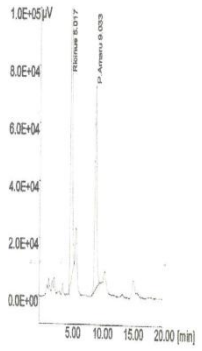


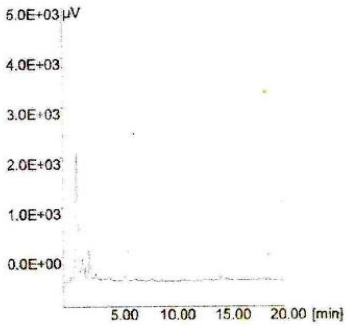
Spectrum of marker of *Ricinus communis* 310nm Spectrum of marker of *Phyllanthus amarua* 260 nm HPLC by HPLC method.

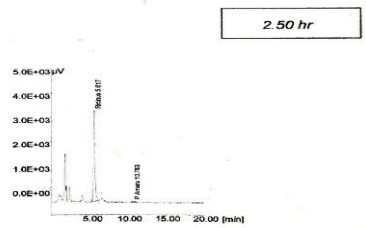




Markers of *Phyllanthus amarus* and Overlay of spectra of *Phyllanthus amarus* and *Ricinus communis* by HPTLC method *Ricinus communis*by HPTLC method





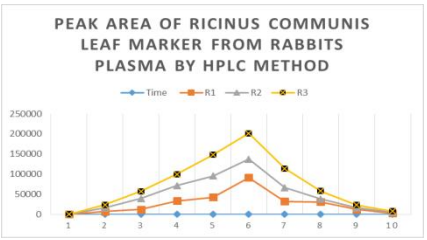


Plasma at 2.50 Hrs postdose

Simultaneous detection of markers of *Ricinus communis* Plasma at 0.00 Hrs (no markers detected.) and *Phylanthusamarus* By HPLC method

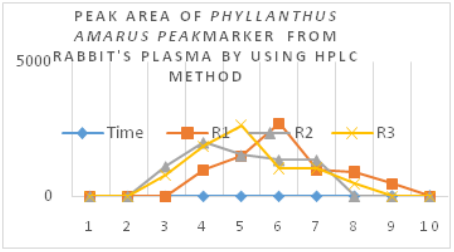
Peak area of *Phyllanthus amarus*

Time	R1	R2	R3
0.00	0	0	0
0.25	7011	8961	7921
0.50	12137	26840	18324
1.00	33177	37994	28563
2.00	42136	53279	51782
2.50	91512	45442	64263
4.00	31812	34551	47361
8.00	31089	6822	19726
10.00	11900	3804	7682

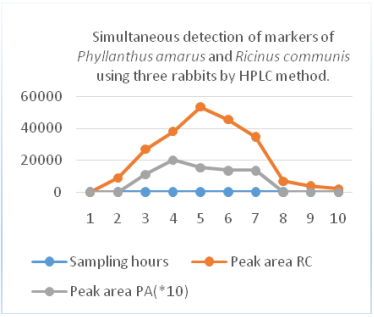


Peak area of *Phyllanthus amarus*

Time	R1	R2	R3	
	0	0	0	
0.25	0	0	0	
0.5	0	1106	786	
1	973	2008	1872	
2	1517	1525	2648	
2.5	2721	1351	1036	
4	994	1351	1036	
8	912	0	468	
10	471	0	0	
		0	0	0



Sampling hours	Peak area RC	Peak area PA(*10)
0	0	0
0.25	8961	0
0.5	26840	11060
1	37994	20080
2	53279	15250
2.5	45442	13500
4	34551	13510
8	6822	0
10	3804	0
24	1977	0



Observations

1. Marker of *Ricinus communis* (310 nm) was detected in the plasma at 0.25hrs post dose. However, the marker of *Phyllanthus amarus* (265nm) was detected in the plasma at 2,50hrs post dose. The retention time (RT) of these markers were 5.617 and 10.763 respectively after 2.50 hrs.post dose respectively.
2. Two rabbits showed$C_{max}$of *Ricinus communis* at 2.50 hrs after the oral dose administration.
3. However,Cmaxof *Phyllanthus amarus* was found to be fluctuating between 2.00 and 2.50 hrs of oral administration.
4. After reaching C max marker of *Ricinus communis* showed a decline, indicating a rapid process of elimination.
5. Marker of *Phyllanthus amarus* reached its C max at 2.00 hrs post dose and reached a plateau up to 4.00 hrs and then declined, reaching almost 0.00 after 8.00 hrs of an oral dose.

Conclusion

Markers of *Ricinus communis* and *Phyllanthus amarus* showed a typical absorption, elimination pattern. The study demonstrated the probability of developing methods to detect markers of other plants in biological matrix using similar approach.

References

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