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Comparative Phyto-Pharmacognostic study of Field collected and Pharmacy sample of *Arjuna* (*Terminalia arjuna*) Bark

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ABSTRACT

Arjuna (*Terminalia arjuna*) is the most widely used drug in Ayurveda due to its potential therapeutic competence. Quality assurance is important issue nowadays due to poor collection and storage practices. So, it needs quality control both for small scale and large-scale preparations of *Arjuna* formulations. Hence present study is planned to compare both field collected and pharmacy sample of *Arjuna* bark by analytical testing. Macroscopic, microscopic, physicochemical and high performance thin layer chromatography (HPTLC) by using Arjunic acid as a marker is carried out. Macroscopic and microscopic characteristics observed as per Ayurveda Pharmacopeia (API) except color of field collected bark. In Physicochemical parameters, Ash value was higher in pharmacy sample but within limit whereas percentage of water soluble and alcohol-soluble extractives was high in field collected *Arjuna* as compare to pharmacy sample. Percentage of extractive values was not as per limits of API. Tannin percentage was three times more in field collected *Arjuna* bark. In HPTLC study, percentage of *Arjunic* acid was much higher in pharmacy sample (0.072%) than field collected sample (0.054%). Also, the spots observed were more in this sample. 0.60 was the R_f Value for *Arjunic* acid and one common unknown spot was noticed in both samples. Present study set preliminary data for percentage of Tannins and quantification of *Arjunic* acid in *Arjuna* Bark by HPTLC study which is not found in monograph of *Arjuna* hence can be used as reference for further study.

Key words: *Arjuna, Terminalia arjuna, Pharmacognostic study, Ayurveda, Physicochemical parameters.*

INTRODUCTION

Arjuna (*Terminalia arjuna*) is well-known drug in Ayurveda which is widely used for many therapeutic purposes. It consists of the stem bark of *Terminalia arjuna* W. & A. (Fam. Combretaceae); a large deciduous tree, commonly found throughout the greater parts of the country.[1] It is widely used for therapeutic purposes in Ayurveda in various forms such as *Kshirpaka, Siddha Gruta, Arishta* etc. It is indicated in *Medoroga* (Obesity), *Vrana* (Wounds), *Hridroga* (heart diseases), *Kshatashaya* (Debility), *Prameha* (Diabetes), *Vyanga* (Chloasma) etc. In Ayurveda, bark is collected under good collection practice, when the potency of drug is optimum at that particular season.[2] Although bark is widely available in market, it is hardly self-collected for pharmaceutical purposes. The market for herbal drugs has grown at an impressive rate due to a global resurgence in traditional and alternative healthcare systems, and therefore medicinal plants have great economic importance. However, loss of biodiversity, over-exploitation and unscientific use of

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medicinal plants, industrialization, bio piracy, together with lack of regulation and infrastructure are the major impediments to the growth of herbal medicine. In most countries, herbal products are launched into the market without proper scientific evaluation, and without any mandatory safety and toxicological studies. There is no effective machinery to regulate manufacturing practices and quality standards.[3] However, Standards of raw drugs in Pharmaceutical companies are maintained to some extend with in-house quality control laboratory. Also, storage condition may impact the quality specially before being supplied to pharmacy. The post- harvesting process of medicinal plants has the great importance in the production chain, because of its direct influence on quality and quantity of active principles in the product sold.[4] Due to large scale demand there need to assure quality of Pharmacy sample. Also, self- collected samples need quality check by different analytical testing. So, in present study, comparative study has been planned between Pharmacy and Self collected sample of *Arjuna* bark to ensure quality by pharmacognostic evaluation.

Methods and Materials

Collection and Authentication of drug

Arjuna is collected from Forest region of Bhimashanker, near Pune which is authenticated from Botanical survey of India. Whereas pharmacy sample of *Arjuna* is taken from GMP certified Ayurvedic pharmacy that was authenticated from in-house Quality control department.

Phyto-Pharmacognostic study

Macroscopic, Microscopic study, Physicochemical study and High performance thin layer chromatography (HPTLC) was carried out at Vasu Research Laboratory, Vadodara, Gujrat

Macroscopic and Microscopic study

Macroscopic Study[5]

Macroscopic study was carried out as per standard operating procedures in mentioned in pharmacognosy. Various Morphological characters and organoleptic characterization such as size, shape, color, odor, taste, external markings, fracture, were observed. The diameter of bark was measured with the help of vernier caliper.

Microscopy study[6]

Microscopic identification was carried out according to the Ayurveda pharmacopeia of India (Appendix - 2.1.1)

Physicochemical analysis

Physicochemical analysis namely pH value[7], Foreign matter[8], Ash Value[9], Acid insoluble ash[10], Water soluble extractives[11],and Alcohol soluble extractives[12], were carried out according to the Ayurvedic Pharmacopeia of India (API).

Tannin[13] - Tannin is detected by treating with ferric ammonium sulphate solution (blue-black or green black color shows the presence of Tannin) or with potassium-bichromate solution (brown color indicates the presence of Tannin).

HPTLC analysis of *Arjuna* (Identification and Quantification)[14]

Preparation of Test solution

1 g of *Arjuna* sample was accurately weighed in a conical flask. It was refluxed for 30 minutes with 15 mL of Methanol consecutively for 3 times. Then filtered with the help of Whatman filter paper No. 1 and concentrated the combined extracts in an evaporating dish on water bath. Thereafter, made up the volume up to 10 mL with Methanol. Test solution thus obtained was used for HPTLC fingerprinting.

Preparation of Standard solution

Accurately weighed 11.7 mg of standard Arjunic acid was taken into 2 mL volumetric flask and dissolved in Methanol to make up the volume up to 2 mL with Methanol. From this stock solution, 0.1 mL of solution was pipetted into 2 mL volumetric flask and made up the volume up to 2 mL with Methanol. The Standard solution thus obtained was used for HPTLC fingerprinting.

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OBSERVATIONS

Table 1: HPTLC Chromatographic Conditions

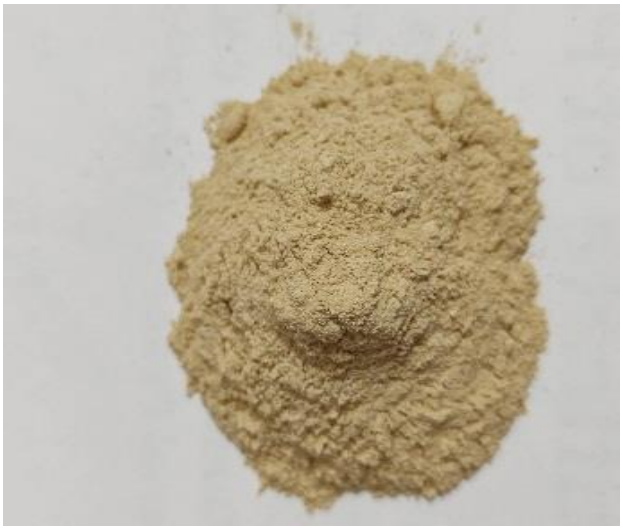
Chromatographic Conditions	
Application Mode	CAMAG Linomat 5 - Applicator
Filtering System	Whatman filter paper No. 1
Stationary Phase	MERCK - TLC / HPTLC Silica gel 60 F254 on Aluminum sheets
Application (Y axis) Start Position	10 mm
Development End Position	80 mm from plate base
Band Length	8 mm
Distance Between Tracks	15 mm
Sample Application Volume	12.0 µL
Standard Application Volume	12.0 µL
Development Mode	CAMAG TLC Twin Trough Chamber
Chamber Saturation Time	30 minutes
Mobile Phase (MP)	Chloroform : Methanol (9 : 1 v/v)
Visualization	@ 254 nm
Quantification Wavelength	205

1) Images of Field Collected *T. Arjuna* (Image I-IV, VII)

I. *T. Arjuna* tree

II. Wet bark of *T. Arjuna*

III. Dry bark of *T. Arjuna*



IV. Powder of *T. Arjuna*

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2) Images of Pharmacy Collected sample of *T. Arjuna* (V-VI,VIII)

V. Bark of *T. Arjuna*

VI. Powder of *T. Arjuna*

VII. Bark of *T. Arjuna* (Field collected)

VIII. Bark of *T. Arjuna* (Pharmacy sample)

Table 2: Macroscopic study of T. *Arjuna* Bark

Sample Parameters	Macroscopy of *Arjuna* Field Collected Sample (FCS)	Macroscopy of *Arjuna* Pharmacy Sample (PCS)
Stem Bark	Stem bark pieces flat or slightly curved	Stem bark pieces flat and slightly curved
Outer surface	Smooth and greenish brown in colour;	Smooth and light brown in colour
Inner surface	Brown and longitudinally striated;	Brown and longitudinally striated;

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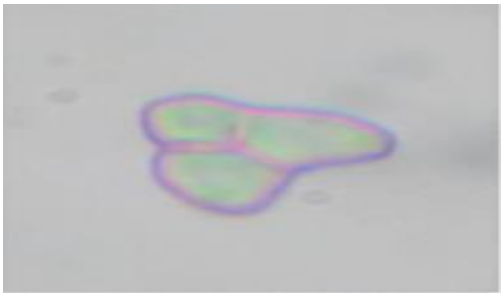
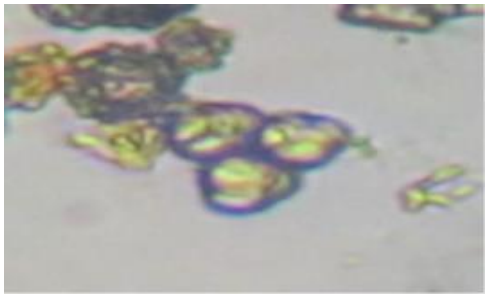
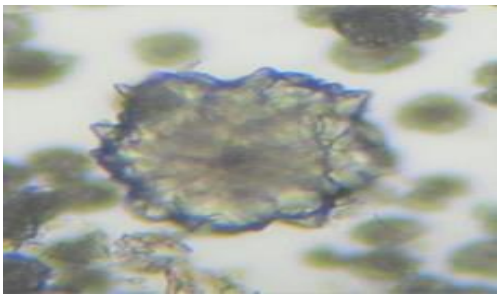
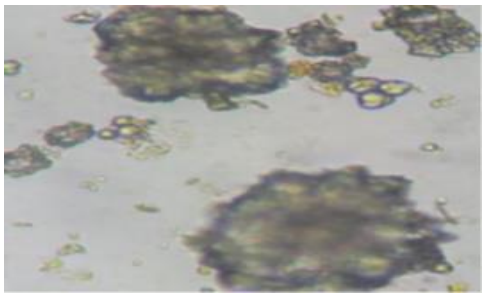
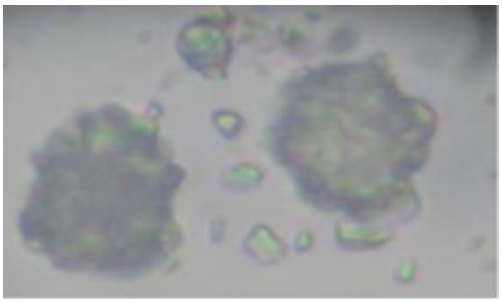
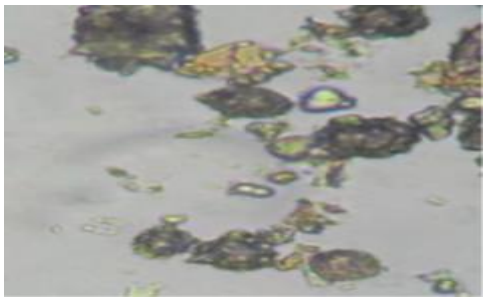
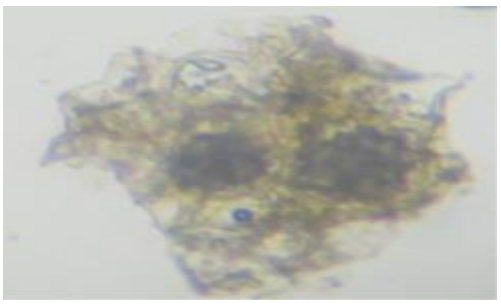
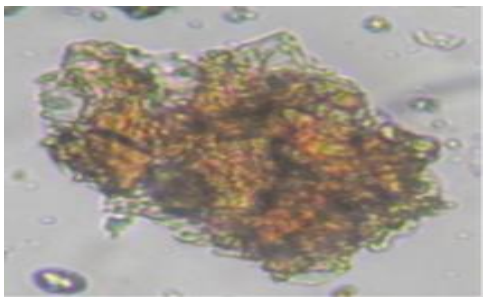
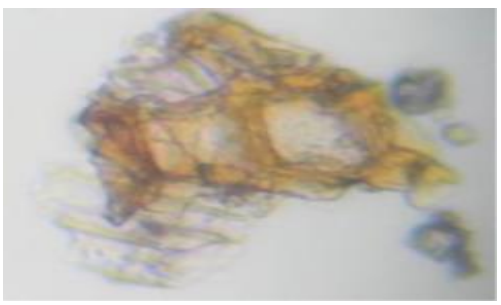
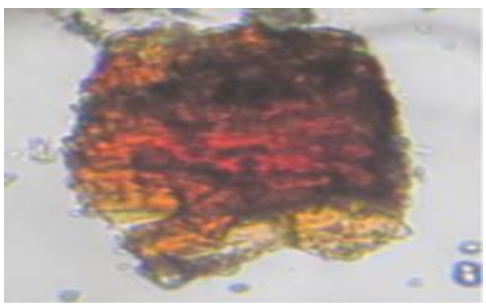
Table 3: Microscopy study of T. *Arjuna* Bark

SN	Pharmacognostic Features of Powder	Field collected Sample (FCS)	Pharmacy Sample (PCS)
1.	Compound starch crystals		
2.	Rosette Crystals of Calcium Oxalate		
3.	Cluster Crystals of Calcium Oxalate		
4.	Parenchyma Containing Cluster Crystal of calcium oxalate		
5.	Parenchyma Containing Tannin		

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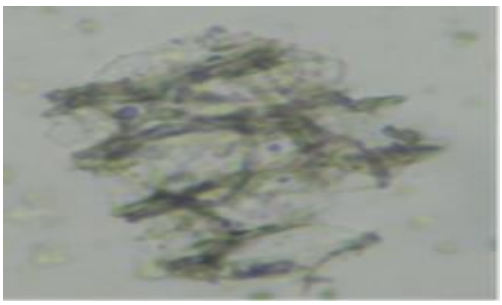
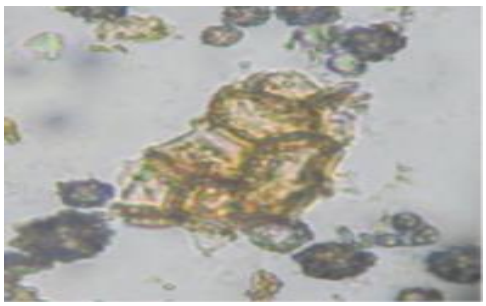
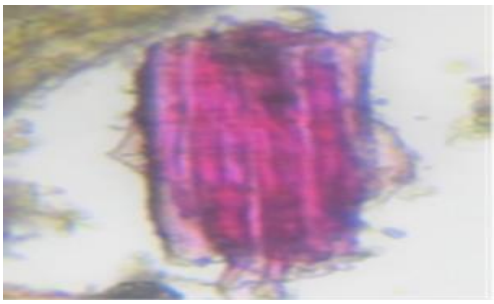
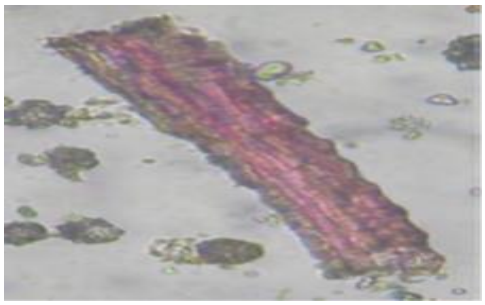
6.	Cork Fragment		
7.	Lignified Phloem Fibers		

Table 4: Organoleptic Characteristics of *Arjuna*

SN	Parameters	*Arjuna* field Collected (FCS)	*Arjuna* Pharmacy collected (PCS)
1.	Fracture (*Shabda*)	Medium	Medium
2.	Touch	Rough	Rough
3.	*Rupa* (color)	Smooth and greenish brown	Smooth and light brown
4.	*Rasa* (Taste)	Astringent	Astringent
5.	*Gandha* (Smell)	Characteristic	Characteristic

Table 5: Physico Chemical Analysis of *Arjuna*

SN	Parameters	*Arjuna* field Collected	*Arjuna* Pharmacy collected
1.	Foreign matter	Nil	Nil
2.	Total Ash	9.72%	10.48 %
3.	Acid insoluble Ash	Nil	Nil
4.	Water Soluble extractives	18.40%	16.09%
5.	Alcohol Soluble Extractives	10.71%	8.17%
6.	Total Tannin	12.10%	4.04%

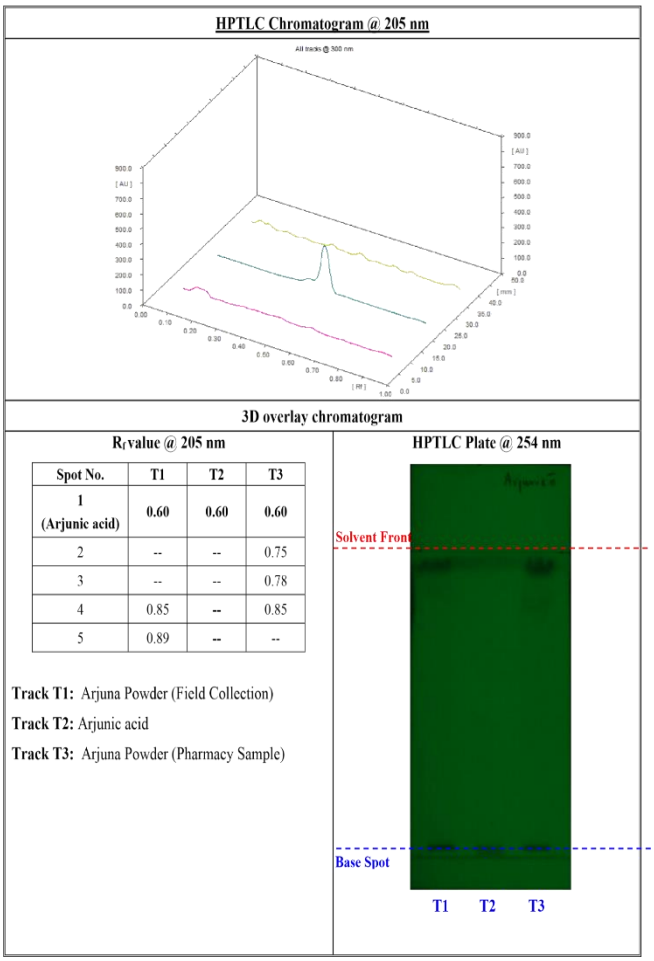


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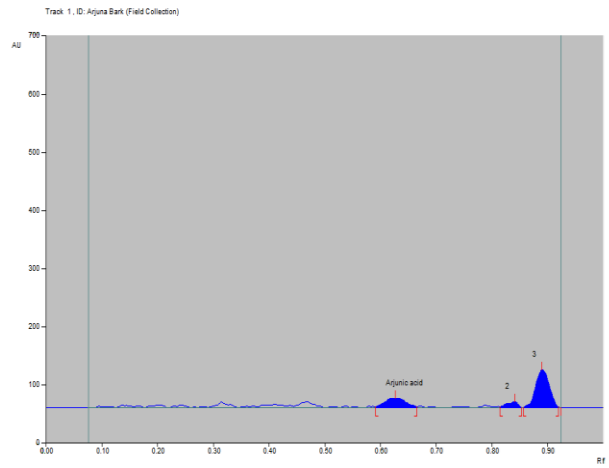
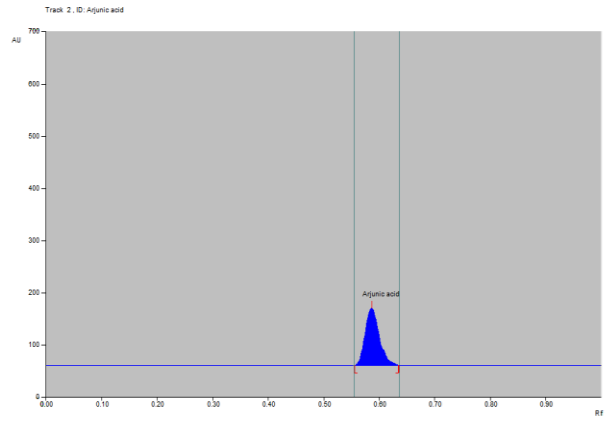
Image 3: HPTLC 3D overlay chromatogram@205nm of *Arjuna* Samples.

2D chromatogram of Arjuna Powder (Field Collection) @ 205 nm



2D chromatogram of Arjunic Acid @ 205 nm



2D chromatogram of Arjuna Powder (Pharmacy Sample) @ 205nm

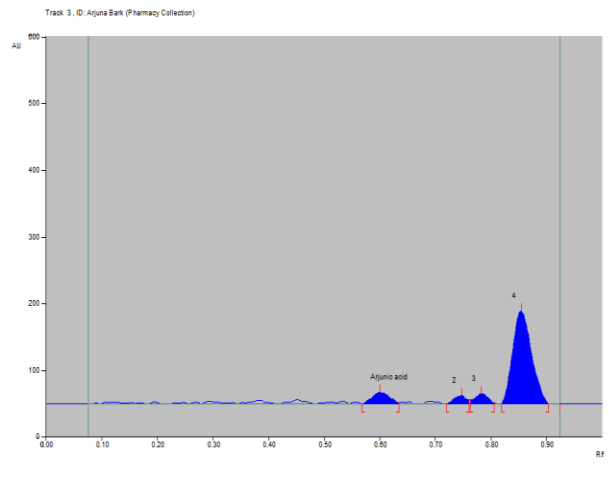


Table 6: HPTLC study of *Arjuna* Samples

Sample	Arjunic Acid	*Arjuna* Powder (Field Collection)	*Arjuna* Powder (Pharmacy Sample)
Weight per 10 mL	2.9 mg	1013 mg	1130 mg
Area	2636.6	510.7	759.6
% Arjunic Acid	--	**0.054 %**	**0.072 %**

DISCUSSION

Termanalia arjuna is widely available all over India. While collecting any drug its authenticity needs to be taken into account as many species of single plants are available in the field. Studied sample of *Arjuna* was noticeably collected from field by good collection practices which showed distinctive characteristics of T. *Arjuna* in organoleptic study, whereas pharmacy collected sample (PCS) was already authenticated by quality control department. While comparing both sample of *Arjuna* Bark, it was observed that field collected sample (FCS) was quite greenish white in colour whereas pharmacy sample was pinkish brown in colour. There also occurred marked difference in powders of both samples in color i.e., field and pharmacy sample which was cream white (Image 1: I, II, III, IV) and faint brown (Image 2: V, VI) respectively. Pinkish brown is the original character of *Arjuna* stem and Reddish – brown is the color of *Arjuna* powder. So, this dissimilarity in color may be due to difference in maturity of plant or due to long storage. (Table 2) Microscopic study of both samples shown compound starch crystals, Rosette Crystals of Calcium Oxalate, Cluster Crystals of Calcium Oxalate, and Parenchyma Containing Cluster Crystal of calcium oxalate and tannins. It also observed cork fragment and Lignified Phloem Fibers. (Table 3) Physicochemical analysis have shown more percentage of ash value (10.48%) in PCS but not more than 25% as specified in API. Water soluble and alcohol soluble extractive % is seen higher in FCS than PCS but less than 20% as per API. Tannin is the most important constituent of *Arjuna* but its

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standards are not mentioned in API. (Table: 5) Percentage of tannin in FCS was seen three times more than PCS. However, in HPTLC study % of Arjunic acid is seen more in PCS than that of FCS. Also, the number of spots which indicates unknown phyto-constituents are more in PCS (03) than FCS (02) other than Arjunic acid spot (0.60). Presence of common spot (0.85) in both samples which suggest potent unknown phyto-constituent of *Arjuna*. In this study single samples were compared on the basis of quality parameters which are not sufficient to specify the standards, however identification and quantification of Arjunic acid by HPTLC study is the important parameter which is not mentioned in the API.

Conclusion

Field collected sample is qualitatively best in physicochemical parameters than pharmacy sample. Whereas in HPTLC study, pharmacy sample has higher percentage of Arjunic Acid and more spots. This study used single sample for quality comparison which is not sufficient for substantial conclusion. Hence their needs more number of samples for significant inferences. However, Percentage of Tannins and quantification of Arjunic acid by HPTLC is not specified in the monograph of *Arjuna* which can be used as a preliminary data for further study.

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