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In-vitro propagation, organic farming and comparative phytochemical evaluation of *Curculigo Orchioides* Gaertn. (*Musali*)

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ABSTRACT

Medicinal plants belong to the oldest known health care products that have been used by mankind all over the world in the form of folklore medicine, traditional medicines or ethnic medicines. But due to the over dependence of herbal drug industry on the plant population, wild source became depleted. Several drugs became endangered while some others had been in the verge of extinction. *Musali* (*Curculigo orchioides*) is such a plant which is facing a threat of extinction. These plants can be produced by different methods. The main 2 methods for the propagation of plants will come under the following headings viz; Conventional way of propagation and Non-conventional way of propagation (*In Vitro* propagation). If a small change in climate and temperature is affecting the chemical composition of plants, then obviously there may be chances for change in the same while propagating it through different techniques. In this study the preliminary analytical profile of the three samples were done in order to know whether the organic and *In Vitro* variety can be replaced with the wild. As *Musali* (*Curculigo orchioides* Gaertn.) is a drug used in several formulations and *Musali Khadiradi Kashayam* is one among the important one used for the reproductive problems. So the formulation also needs to be analysed in order to know whether the variation in cultivation techniques will affect the physicochemical and chromatographic parameters.

Key words: *Musali*, Herbal Drugs, Extinction, *In Vitro* Propagation, Physico-chemical.

INTRODUCTION

Medicinal plants play an important role in the health care of developing countries. Currently, near about 80% of the world population use herbal medicine and World Health Organization (WHO) also encourages,

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recommends and promotes the inclusion of herbal drugs in national health care programmes because such drugs are easily available at a price within the reach of common man and such are time tested and thus considered to be safer than modern synthetic drug.^[1]

About two-third of the drugs of the modern system of medicine have been developed from natural resources mainly from plants. Indian health care industry is rapidly growing and expected to reach over 280 billion USD by 2020.^[2] Most of the people are now depending on them for their health care needs. The demand of the raw materials and derivatives of plant for the indigenous drug industries is mainly satisfied from the wild source, depleting the natural population.^[3]

Musali is one of such drug with several clinical uses, especially for its aphrodisiac and rejuvenation

property. Even though the requirement flares up, no alternate cultivation techniques are adopted. This placed the drug into "Lower Risk near threatened" category. As moist climate is ideal for the growth of this plant the scanty rainfall adversely affect the growth. Hence there is an urgent need for the alternate way of propagation.^[4]

The main 2 methods that we can adopt for the propagation of *Musali* will come under the following main headings viz;

1. Conventional way of propagation (Organic farming)
2. Non-conventional way of propagation (Micropropagation)

As there are chances for the differences in the phytochemical variants while propagating through organic farming and *In Vitro* propagation, a thorough analysis has to be done.

AIMS AND OBJECTIVES

Phytochemical comparison of raw sample and *Kashayam* of *Curculigo orchioides Gaertn. (Musali)* prepared out of *In Vitro* propagation, organic farming, and wild variety; there by analyzing the best alternate way of propagation.

Methods/ Procedures

1. **Conceptual study:** It includes drug review, review of techniques and *Kashaya Kalpana*. The drug review includes the literary study of the drug *Musali*, both Ayurvedic and modern aspects. Then the technique of tissue culture and organic farming has been reviewed.
2. **Ethno botanical Survey:** A survey had been carried out to study the present agronomical practices being adopted by collectors, growers, type of processing facilities and related infrastructure available. A questionnaire was prepared including the cost, collection methods, cultivation techniques adopted etc.
3. **Cultivation:** Two Way of propagation has been followed; Conventional way of propagation (organic farming) and Non-conventional way of propagation (*In Vitro* propagation). The organic

farming has been done in the locality of Thrissur and the *In Vitro* propagation has been done in JNTBGRI, Palode.

4. **Collection:** Collection of wild variety of *Musali* has been done from the locality of Thrissur.
5. **Pharmacognostical Study:** The macroscopical and microscopical studies of the drug have been done at CARE KERALAM, Thrissur.
6. **Analytical Study:** The Phytochemical, Physico-chemical and HPTLC analysis of three samples of *Musali* have been done at CARE KERALAM, Thrissur.

OBSERVATIONS AND RESULTS

Ethno botanical Survey

Ethno botanical survey which was conducted among 25 growers and cultivators in the locality of Kechery, Thrissur was completed successfully. Most of the persons who participated in the study had years of experience in the field of cultivation, collection and supplying the goods especially the medicinal plants. Most of the plants were collected from the wild; while very few were cultivated. Most of the Persons always collect the drugs from the same person for years. But usually the customers vary each time, except some regular customers.

The samples of *Musali* were always collected from the wild. Unfortunately, the drug is no where under cultivation. They always depend on wild population. Out of the 25 participants, only 5 were having *Musali* growing wildly in their house. Usually they charge 350/kg for *Musali*, while they buy it for 300/kg. The sale was adequate for the drug. Generally the drug had been used in case of infertility, general debility, menstrual problems, cosmetic uses etc.

Pharmacognostical Study

Macroscopic Evaluation of *Curculigo orchioides Gaertn. Rhizome*

1. Size : Length ranges from 5-20cm long and 1-1.5cm in diameter.
2. Shape : Stout, more or less cylindrical
3. Colour : i) Outer Surface -Brown

ii) Inner Surface -White

4. Odour : Characteristic Odour

5. Taste : Slightly bitter

Microscopic Evaluation

Microscopic Evaluation of three samples of *Curculigo orchioides* Gaertn; wild variety, organic farming and invitro variety had been done.

Description of T.S. of rhizome of *Curculigo orchioides* Gaertn.

Diagrammatic T.S. of the rhizome is circular in outline with irregularly running in peripheral margin of the cork, outer wide zone of cortex and inner stellar region, transverse with vascular bundles, arranged in circular fashion underneath the endodermis mucilage cells and starch grains.

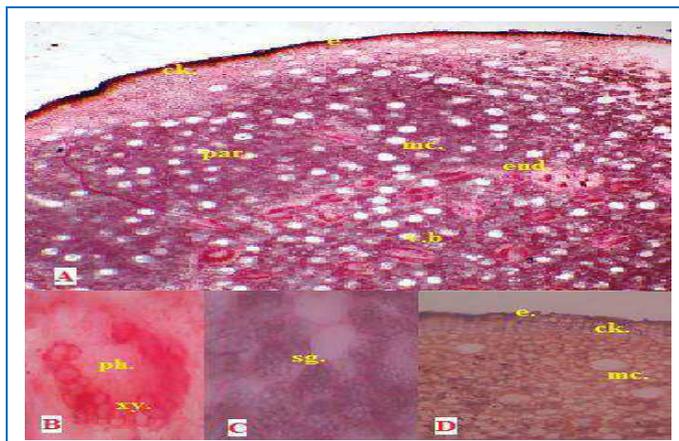


Fig. 1c: T. S. of rhizome of Tissue culture of *Curculigo orchioides* Gaertn.

e - epidermis, ck - cork, par - parenchyma cells, mc - mucilage cells, end - endodermis, v.b. - vascular bundles, ph - phloem, xy - xylem, sg - starch grains.

Detailed T.S. shows outer 5-8 rows of cork with peripheral irregularly running dark brown cells of epidermis followed by 5-6 rows of thin walled tangentially running cells of collenchyma, wide parenchymatous zone of cortex densely packed with compound starch grains and scattered idioblasts embedded with bundles of long acicular crystals of calcium oxalate, very few occasional prismatic crystals mucilage cells and few amphivasal and collateral vascular bundles; endodermis is distinct, parenchymatous stellar tissue is almost identical to the cortical zone but here the vascular bundles are more in number and mucilage cells are very few. Vascular bundles lying underneath the endodermis are arranged in rows and are well developed; xylem vessels are lignified and devoid of fibres.^[5]

Table 1: Analysis of Physico chemical Parameters of Rhizome of *Curculigo orchioides* Gaertn.

S N	Parameter	Wild variety	Organic Farming	Tissue culture
1	Loss in drying	7.67%w/w	8.13%w/w	8.32w/w
2	Total ash	4.15%w/w	10.88%w/w	7.18%w/w

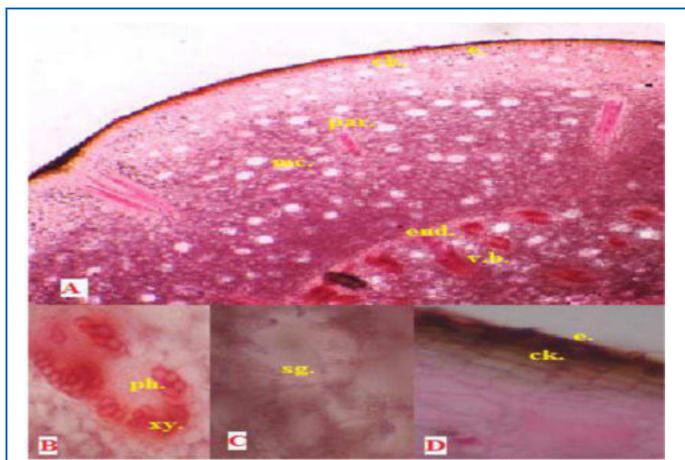


Fig. 1a: T. S. of rhizome of Wild variety of *Curculigo orchioides* Gaertn.

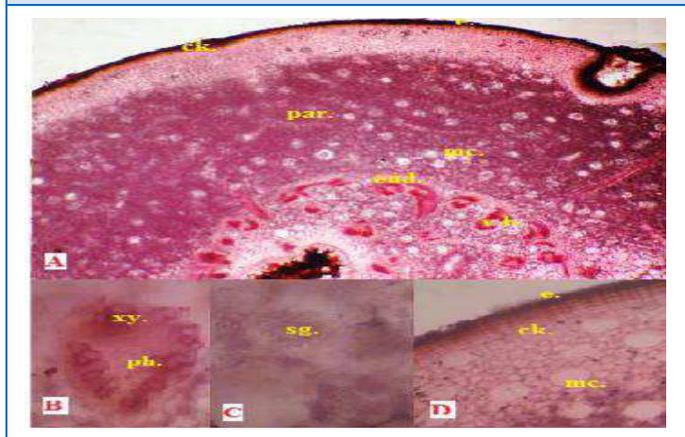


Fig. 1b: T. S. of rhizome of Organic variety of *Curculigo orchioides* Gaertn.

3	Acid insoluble ash	0.08%w/w	0.15%w/w	0.12%w/w
4	Water Soluble ash	1.38%w/w	2.37%w/w	1.54%w/w
5	Alcohol soluble extract	9.79%w/w	29.94%w/w	19.56%w/w
6	Water soluble extract	15.87%w/w	31.18%w/w	24.92%w/w

				s powder	
2	20.5mm	5.0µl	2	Organic farming <i>Curculigo orchioide</i> s powder	Yes
3	31.0mm	5.0µl	3	Tissue culture <i>Curculigo orchioide</i> s powder	Yes

Table 2: Comparative Phytochemical evaluation of rhizome of Wild, Organic and *In Vitro* variety of *Curculigo orchioides Gaertn.*

SN	Parameters	Wild variety	Organic Farming	Tissue culture
1	Alkaloids	-	-	-
2	Flavanoids	-	-	+
3	Phenol	-	-	-
4	Saponins	+	+	+
5	Sterols	-	-	-
6	Tannins	+	+	+
7	Triterpenoids	-	-	-
8	Glycosides	-	+	+
9	Carbohydrate	+	-	+

Table 3: HPTLC pathway of *Curculigo orchioides Gaertn.*

S	Application Position	Application Volume	Vial #	Sample ID	Active
1	10.0mm	5.0µl	1	Wild variety <i>Curculigo orchioide</i>	Yes

Fig. 2: HPTLC Pathway of *Curculigo orchioides Gaertn.*

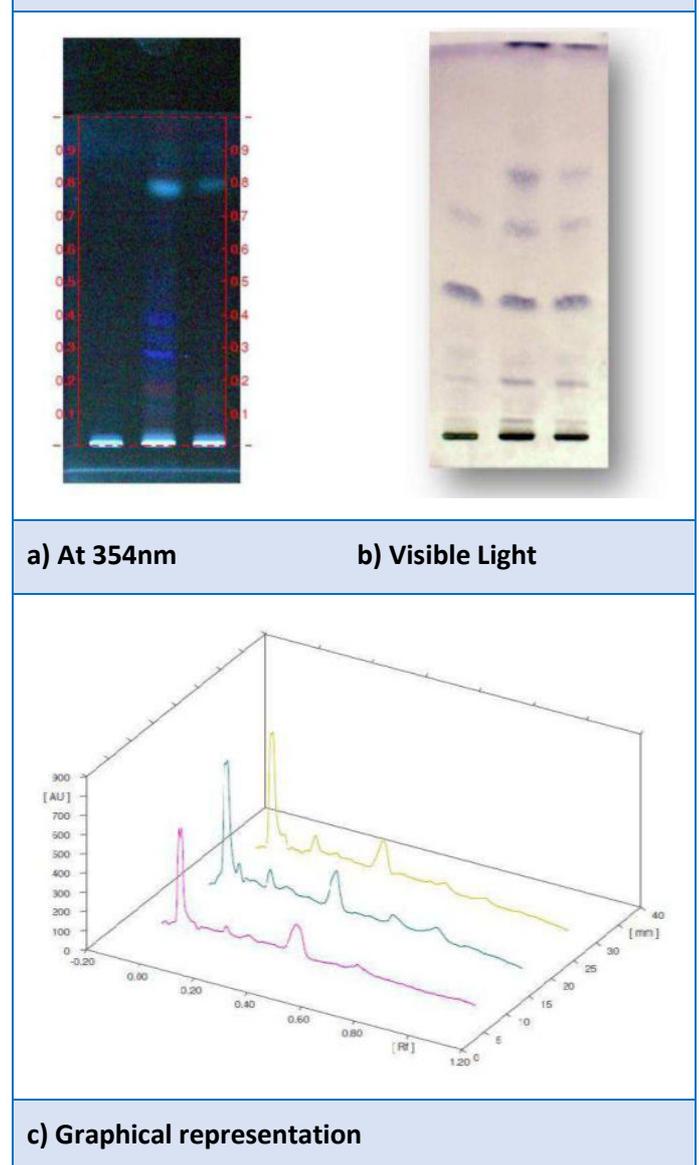


Fig. 3a: HPTLC of Wild variety of *Curculigo orchioides gaertn.*

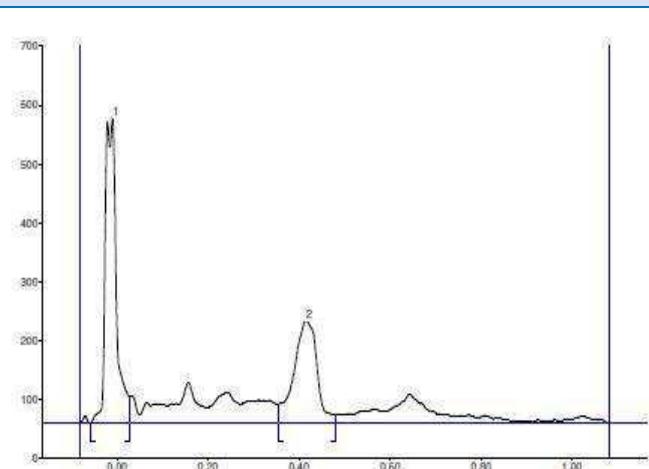


Fig. 3b: HPTLC of Organic farming of *Curculigo orchioides gaertn.*

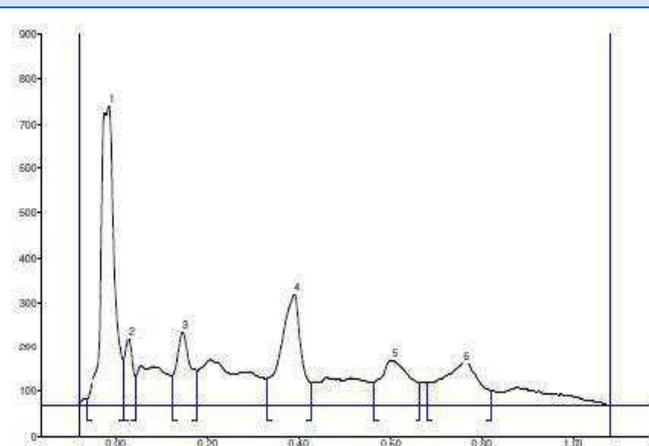


Fig 3c: HPTLC of Tissue culture of *Curculigo orchioides gaertn.*

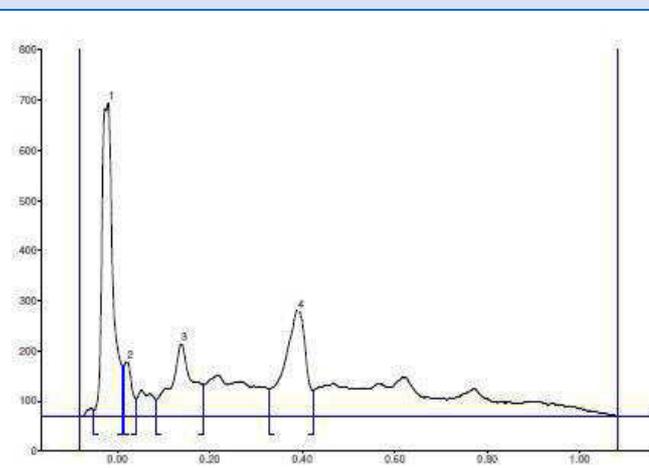


Table 4: HPTLC peaks of wild variety of *Curculigo orchioides Gaertn.*

Pe ak	St art Rf	Sta rt hei ght	M ax Rf	Ma x hei ght	Ma x %	En d Rf	End hei ght	Are a	Ar ea %
1	- 0.06	0.3	- 0.01	519 .1	74. 85	0. 03	45. 4	889 0.9	59. 10
2	0.35	32. 8	0. 42	174 .4	25. 15	0. 48	14. 0	615 2.8	40. 90

Table 5: HPTLC peaks of Organic farming of *Curculigo orchioides Gaertn.*

Pe ak	St art Rf	Sta rt hei ght	M ax Rf	Ma x hei ght	Ma x %	En d Rf	End hei ght	Area	Ar ea %
1	- 0.06	13. 5	- 0.01	672 .2	46. 83	0. 02	100 .0	1345 3.5	37. 00
2	0. 02	102 .8	0. 03	154 .0	10. 73	0. 04	63. 1	1815 .4	4.9 6
3	0. 12	64. 9	0. 14	165 .6	11. 53	0. 18	77. 6	3584 .2	9.7 9
4	0. 33	58. 7	0. 39	250 .6	17. 46	0. 43	50. 6	7709 .4	21. 06
5	0. 56	50. 6	0. 61	99. 6	6.9 4	0. 67	49. 9	4560 .4	12. 46
6	0. 68	48. 6	0. 76	93. 4	6.5 1	0. 82	31. 0	5390 .3	14. 73

Table 6: HPTLC peaks of *In Vitro* culture of *Curculigo orchioides Gaertn.*

Pe ak	St art Rf	Sta rt hei ght	M ax Rf	Ma x hei ght	Ma x %	En d Rf	End hei ght	Area	Ar ea %
1	- 0.05	12. 4	- 0.02	626 .3	57. 22	0. 01	99. 6	1042 2.9	11. 59
2	0. 01	99. 6	0. 02	110 .9	10. 14	0. 04	35. 3	1331 .8	5.7 0

3	0.08	33.1	0.14	145.0	13.25	0.19	62.7	4742.6	20.29
4	0.33	55.3	0.39	212.3	19.39	0.42	51.8	6875.6	29.42

Analysis of Kashayam

Table 7: Physicochemical evaluation of Wild, Organic and In Vitro variety of (*Curculigo orchioides Gaertn.*) Musali Kashayam.

SN	Parameters	Wild Variety	Organic Farming	Tissue culture
1	pH	3.84	4.27	4.22
2	Total Solids	2.39%w/w	3.69%w/w	5.62%w/w
3	Specific gravity	1.0116	1.0218	1.0211
4	Brix Index	1.25%	2.0%	2.5%
5	Organoleptic characters	Brown coloured	Dark brown coloured	Brown coloured

Table 8: HPTLC pathway of Musali Kashayam.

S N	Application Position	Application Volume	Vial #	Sample ID	Active
1	10.0mm	5.0µl	1	Wild variety of <i>Curculigo orchioides</i> Kashayam	Yes
2	20.5mm	5.0µl	2	Organic farming of <i>Curculigo orchioides</i> Kashayam	Yes
3	31.0mm	5.0µl	3	Tissue culture of <i>Curculigo orchioides</i> Kashayam	Yes

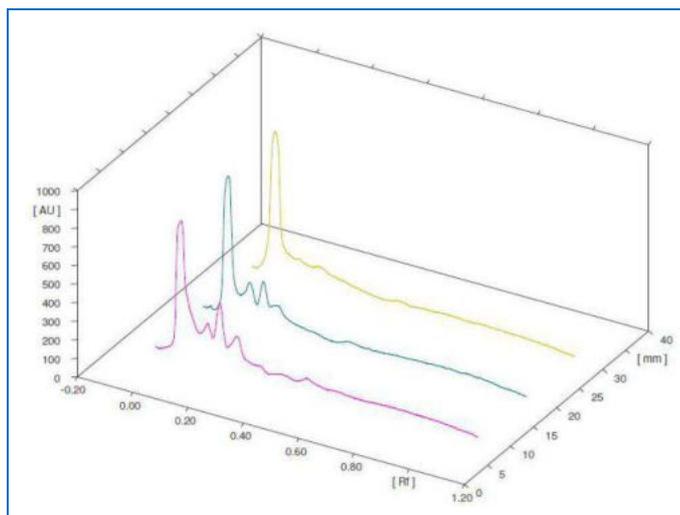


Fig. 4a: Graphical representation of HPTLC of Musali Kashayam

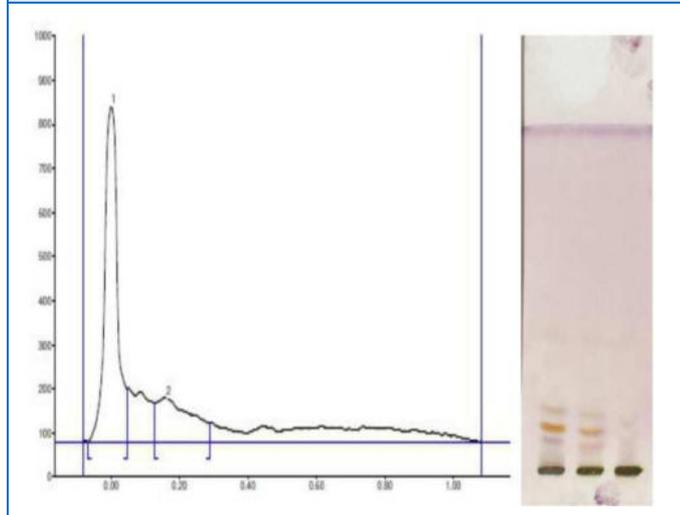


Fig. 4b&c: HPTLC of In Vitro Musali Kashayam & HPTLC of Musali Kashayam at 254nm, 366nm after derivatization.

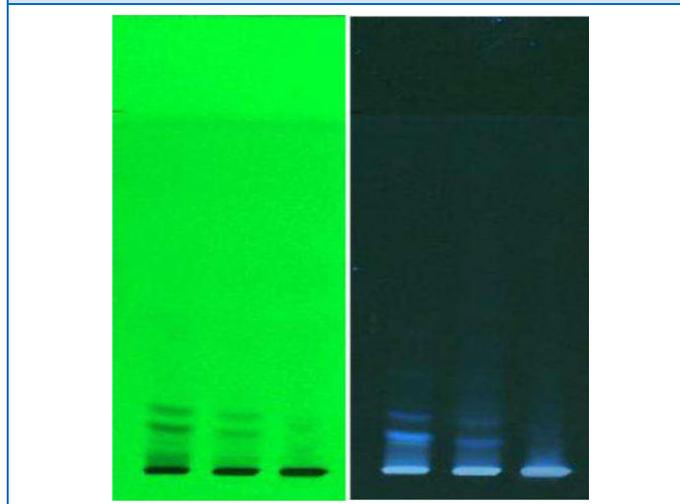


Fig. 4d: HPTLC of wild variety of Musali Kashayam

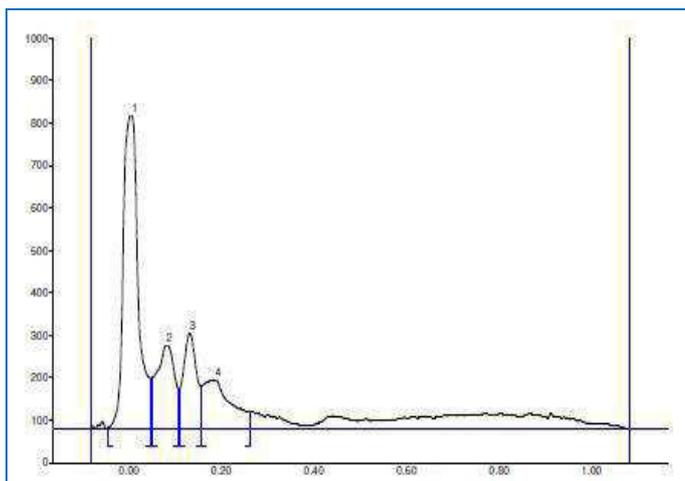


Fig. 4e: HPTLC of Organic farming of *Musali Kashayam*

Table 9: HPTLC Peaks of Wild variety of *Musali Kashayam*

Peak	Start Rf	Start height	Max Rf	Max height	Max %	End Rf	End height	Area	Area %
1	0.07	0.4	0.01	719.5	50.07	0.08	142.6	21248.0	53.95
2	0.08	142.6	0.10	204.9	14.26	0.12	137.0	4910.2	12.47
3	0.13	137.2	0.15	333.7	23.22	0.18	106.2	6965.6	17.69
4	0.18	107.7	0.21	178.9	12.45	0.27	52.7	6259.9	15.89

Table 10: HPTLC Peaks of Organic farming of *Musali Kashayam*

Peak	Start Rf	Start height	Max Rf	Max height	Max %	End Rf	End height	Area	Area %
1	0.05	3.1	0.00	740.3	57.69	0.05	120.4	16820.0	53.06
2	0.12	120	0.15	200	15.00	0.15	92.0	5407	17.00

	05	.7	08	.1	59	11	8	.4	06
3	0.11	94.7	0.13	226.3	17.63	0.16	101.9	4472.4	14.11
4	0.16	101.9	0.19	116.6	9.08	0.26	39.9	4998.4	15.77

Table 11: HPTLC Peaks of *In Vitro Musali Kashayam*

Peak	Start Rf	Start height	Max Rf	Max height	Max %	End Rf	End height	Area	Area %
1	0.07	0.9	0.00	762.2	88.31	0.05	122.4	19993.4	73.49
2	0.13	89.6	0.16	100.9	11.69	0.29	43.4	7211.0	26.51

DISCUSSION

The survey done with the local collectors and cultivators revealed that they depend only on the wild variety without initiating any solution for its extinction. Scanty rainfall is adversely affecting the growth of this plant. In the contemporary text book, data base on medicinal plants in Ayurveda, Siddha and Unani, the cost of the drug in market was only Rs.25/kg in 2001. But now due to the increase in demand and low availability, the cost flared upto Rs.350/kg. All these are warning signs of extinction of this species.

To establish the quality and reliability of the drugs, various Pharmacognostical and phytochemical screening measures were adopted. The procedures were carried out at CArE Kerala, Koratty. The macroscopic and microscopic features of the collected samples of three varieties of drug viz; wild, organic and *In Vitro* propagated was compared with that of the description of *Musali (Curculigo orchioides Gaertn.)* available in Ayurvedic Pharmacopoeia of India and Quality standards of medicinal plants for its authentication.

While considering the macroscopic features the drug grown in wild is comparatively larger in size, while that cultivated through *In Vitro* propagation was smaller. The colour of the drug grown in wild was dark brown in colour whereas the others were slight brown.

In microscopic features of *Musali*, the starch grains were numerous in the *In Vitro* variety compared to the wild one. The Xylem and Phloem were clearly seen in the wild variety. The numbers of mucilage cells were very high in the *In Vitro* variety which may be due to its interaction with the MS medium.

Most of the physico-chemical parameters of the drug also comply with API. The value of Total ash mentioned in API was not more than 9%w/w. But it was slightly higher in organic farming i.e., 10.88%w/w. The Acid insoluble ash of *Curculigo orchioides* was said to be not more than 2%w/w. But here all the three samples comply with the standards. The value of Alcohol soluble extract in API is not less than 3%w/w. But the value of organic farming was comparatively higher than the other two varieties. i.e., 29.94% where as in the *In Vitro* variety it was 19.56%w/w and in wild it is 9.79%. In case of water soluble extractive value, it had been mentioned as not less than 17%. But the wild variety, had the value below that i.e., 15.87%, where as in the organic farming the value is very high i.e., 31.18% and in the *In Vitro* variety it was 24.92%.

The analytical results of *Kashaya* of all the three samples are very similar except, the value of Total solids which is slightly higher in tissue cultured variety i.e., 5.62% and the pH of wild variety is slightly low i.e., 3.84. While the other two organic farming and *In Vitro* variety were having 4.27 and 4.22 respectively.

The phytochemical evaluations of all the three samples of *Curculigo orchioides* were done. Most of the results comply with that of API. Still some differences are present. The presence of Alkaloids mentioned in API, was not found in any of the three samples. At the same time Flavanoids which were not mentioned in API, were present in the *In Vitro* sample of *Curculigo orchioides*. Saponins and tannins

mentioned in API are present in all the three samples. Glycosides which are not mentioned in API are present in both the samples of organic and *In Vitro* variety of *Curculigo orchioides*. Likewise, carbohydrates which were not mentioned in API were present in wild and tissue cultured sample of *C.orchioides*.

HPTLC of three samples of fresh samples of *Curculigo orchioides* had been done. The similar bands produced on photo documentation at visible light shows the presence of similar compounds. But after derivatization at 366nm in the pathway of wild variety no bands were seen. By observing the peaks in the graphical representation. The numbers of peaks formed in all the three samples were different. The numbers of peaks present in wild, organic and *In Vitro* were two, six and four respectively. But the dominant peaks are only two which were similar in all the three samples at the Rf of 0.33. While observing the bands produced in HPTLC of *Musali Kashayam*, the band was completely absent in the *In Vitro* pathway at 254nm, but a slight band is observed at 366nm after derivatization and at visible light. The path of organic farming is also not clear at 254nm.

CONCLUSION

Based on the observations and analysis of results in the study, *In Vitro* propagation, Organic farming and comparative phytochemical evaluation of *Curculigo orchioides* Gaertn. (*Musali*). Organic and the *In Vitro* variety can be considered as an alternate to the wild variety of *Curculigo orchioides* Gaertn. (*Musali*). *In Vitro* propagation helps us in propagating a number of plants with a single meristem allowing conserving the plant and making it an unthreatened species. Organic farming helps in saving the plant even in different climatic conditions making it available throughout the season.

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