Phytochemical Profiling And HPTLC Analysis Of *Cryptocoryne Spiralis Var.Cognatoides*(Blatt.&Mc Cann.) S.R. Yadav, K.S.Patil & Bogner (Araceae).

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Abstract

Cryptocoryne spiralis var.cognatoides is a vulnerable species of *Cryptocoryne* of Araceae. Rhizome and leaves of *C.spiralis var. cognatoides* have been selected for phytochemical screening to identify different classes of metabolite and quantitative estimation of phenolics, flavonoids and starch. HPLTC fingerprint of leaves and rhizome extract has been done for fingerprint profile of plant extracts. Phytochemical screening revealed the presence of carbohydrates, proteins, phenolics, flavonoids, saponins, glycosides, alkaloids, fixed oils and fats. Quantitative phytochemical screening in terms of total phenolics, flavonoids and starch were found to be HPTLC finger print of rhizome showed seven peaks in methanol extract and eleven peaks in hexane extract and twelve peaks in methanol extract and eleven peaks in rhizome extracts of leaves with different chemical constituents.

Key Words: *Cryptocoryne spiralis var.cognatoides*, phytochemical screening, quantitative estimation, HPTLC.

Introduction

Cryptocoryne is an aquatic or amphibious genus with in the family Araceae. It is native to South East Asia extending from Mainland India and Indo – China through Indonesia to Papua New Guinea. The genus *Cryptocoryne* was established by Fischer in 1829 based on a species in India called *Cryptocoryne spiralis* (Jacobson 1979)[1]. More than 60 species of *Cryptocoryne* have since been described. Many of these are endemic to particular regions, a situation which is contrary to its wide distribution (Jacobson 1977)[2].The genus is characterized by its linear to lanceolate leaves and inconspicuous spadix inflorescence. The nature and characters of inflorescence and leaves are the main attributes used for the differentiation of species. They are widely used as aquarium plants (Mansor and Mansadi 1994)[3] and some have some medicinal properties. Rhizomes *of Cryptocoryne spiralis* were used for the treatment of diarrhea, fever, jaundice, burns and boils (Kamble *et al*., 2010)[4] and Divaka *et al*., 2013)[5]. HPLC profiling of *Cryptocoryne spiralis* were done by Adams *et al* 2013[6]. Phytochemical screening and antibacterial activity of *Cryptocoryne retrospiralis* were done by Wadkar *etal.,* (2017)[7]. Antioxidant activity and cytotoxic potential of *Cryptocoryne ciliata* were done by Nahar and Lina (2013) [8].

Cryptocoryne spiralis var. cognatoides is a vulnerable aroid species, endemic to South Western Ghats, grows along stream beds in forest areas and plateus in high altitude. Rhizome erect, roots thick and contractile, cylindrical. Leaves 5 – 12 per plant Leaves 15 – 30 cm long. Petiole 4 – 8 cm long, 0.3 – 0.5 cm wide, sheathing at the base up to 6 – 8 cm, colour varies from green to purple. Lamina linear to narrow lanceolate 10 – 20 cm long and 1.6 – 2.8 cm wide, green tip acute; midrib very prominent, secondary veins 4 – 6; leaf margin entire and sometimes undulate Spadix; pedunculate; peduncle 0.7 – 1.5 cm long, 0.4 – 0.6 cm wide, spathe24 – 30 cm long, kettle 2.8 – 4.2 cm long and 0.7 – 0.9 cm wide, constricted just above the middle, white, purplish both inside and outside towards the top, a septum covered at the opening with a small oval opening in the middle. Upper tube absent. Limb14 – 29 cm long, basally 0.7 – 1.4 cm wide,

margins toothed, basal part upright, apical part some times 1 – 2 time spirally twisted, pinkish green outside, yellowish in the upper part and reddish purple in the lower part within, rarely completely purple, with horizontal ridges at the base and smooth at the top. Collar absent. Spadix 2.8 – 3.8 cm long , 0.5 – 0.7 cm long basal female portion with 4 – 5 female flowers; style very short , each pistil having 3 – 7 ovules, sub basal to axile placentation, 5 – 10 olfactory bodies situated above the female flowers, 1.8 – 2.6 cm long sterile interstice. Upper 0.2 – 0.4 cm long male portion with 46 – 75 male flowers, very short sterile appendix with tapering end. Fruit globular 0.6 – 1.1 cm long 25 – 29 seeds. Seeds 0.65 – 1.2 cm long and 0.2 – 0.4 cm wide, brownish, slightly curved. Flowering usually occurs during September to November and fruiting November to January

Materials and methods

i. Collection and extraction of plant materials

Fresh leaves and rhizomes of *C.spiralis var.cognatoides* are collected from the mountain plateau of Gaganbouda, Kohlapur district of Maharashtra. Rhizomes and leaves were dried under shade and pulverized to powder using mechanical grinder. Powdered rhizome and leaves were extracted with hexane, chloroform, methanol and water by soxhlet apparatus at 60 – 70° C for Six hours. These extract were concentrated at 40-45^{0}C using a rotary evaporator to 50ml and used for phytochemical screening and HPTLC.

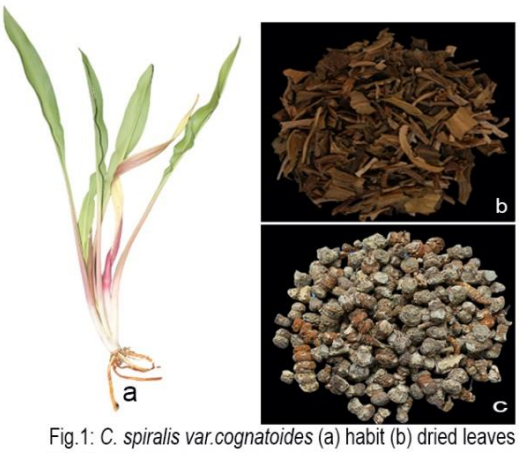


Fig.1: *C. spiralis var.cognatoides* (a) habit (b) dried leaves (c) dried rhizomes

ii. Preliminary phytochemical screening

Preliminary phytochemical analysis was performed by using standard procedure by Kokate [9] and Raaman [10].

iii. Quantitative Estimation of Phytochemicals.

iii. (1). Estimation of total phenolic content

The total phenolic content was determined by the method proposed by Singleton *et al*., 1965[11] and the results were presented in table 2.

iii. (2). Estimation of total flavonoid content

Total flavonoid content was measured by aluminium chloride calorimetric assay [12].). The result of total flavonoid content and the ratio of total flavonoid / total phenolics in the studied plant extracts were presented in table 2.

iii. (3). Estimation of total starch

Total starch content was estimated by method developed by Daniel and Haris [13] and the results were presented in table 2

iv. HPTLC Fingerprint Profiling

HPTLC analysis was performed by Camag HPTLC system (Switzerland) samples were applied using Camag ATS-IV on aluminium backed pre-coated silica gel 60F_{254} TLC plate (Merck India). Mobile phase was standardized as for methanol extract and for hexane extract. The chromatogram was developed in a saturated Twin Trough Chromatographic chamber (Camag, Switzerland). The developed plate was visualized under UV at 254nm, 366nm and visible light (550nm) after derivatizing with anisaldehyde sulfuric acid reagent followed by heating at 105°C for 5 minu

Statistical analysis

The statistical analysis was done by using Microsoft excel. Each set of data is an average of triplicates. The data represent a mean ± standard deviation.

Results and Discussion

i. Preliminary Phytochemical Screening

Preliminary phytochemical screening gives a brief account about the qualitative nature of bioactive phytochemical constituents present in plant extract , which will helps the future investigators regarding the selection of particular plant extracts for further investigation(Mishra et al., 2010)[14]. The results of preliminary phytochemical screening of water, methanol, hexane and chloroform extracts of rhizome and leaf of *C.spiralis var. cognatoides* were presented in table 1. Carbohydrate, proteins, glycosides, alkaloids, sterols, phenolics, tannins, flavonoids, fixed oils and fats were the major phytochemicals found in the leaves and rhizome of *C.spiralis var. cognatoides.* The aqueous extract contains carbohydrate, proteins, phenolics, tannins, glycosides, saponins, alkaloids and flavonoids. Methanol extract contains carbohydrate, proteins, alkaloids, phenolics, tannins, flavonoids, glycosides, saponons and sterols. Hexane and chloroform extracts contains fixed oils and fats. The present results were supported by Prasad et al., 2012[15], who reported carbohydrates, proteins, alkaloids, stereoids, phenolics, flavonoids and glycosides were present in the rhizome extracts of *Cryptocoryne spiralis*

ii. Quantitative Estimation of Phytochemicals

Table 1. Preliminary phytochemical screening of leaf and rhizome of *C.spiralis var.cognatoides*
WE=Water extract, ME=Methanol extract, HE=Hexane extract CE=Chloroform extract,+= Presence,- = Absece

C.spiralis var.cognatoides			**Leaf**				**Rhizome**			
Sl.No	**Name of the compound**	**Name of the test**	**WE**	**ME**	**HE**	**CE**	**WE**	**ME**	**HE**	**CE**
1	**Carbohydrate**	Fehling's test	+	+	-	-	+	+	-	-
		Molisch's test	+	+	-	-	+	+	-	-
		Benedict's test	+	+	-	-	+	+	-	-
2	**Proteins and Amino acids**	Millions test	+	+	-	-	+	+	-	-
		Biuret test	+	+	-	-	+	+	-	-
		Ninhydrine test	+	+	-	-	+	+	-	-
3	**Alkaloids**	Mayer's test	+	+	-	-	+	+	-	-
		Drangendroff test	+	+	-	-	+	+	-	-
		Wagner's test	+	+	-	-	+	+	-	-
4	**Phenolics and tanins**	Ferric chloride test	+	+	-	-	+	+	-	-
		Alkaline reagent test	+	+	-	-	+	+	-	-
		Vanillin hydro chloride test	+	+	-	-	+	+	-	-
5	**Flavonoids**	Aqueous sodium hydroxide test	+	+	-	-	+	+	-	-
		Conc.sulphuric acid test	+	+	-	-	+	+	-	-
		Shinoda test	+	+	-	-	+	+	-	-
6	**Glycosides**	Borntrager's test	+	+	-	-	+	+	-	-
		Legal test	+	+	-	-	+	+	-	-
7	**Phytosterols**	Libermann-Buchard test	-	+	-	-	-	+	-	-
		Libermann sterol test	-	+	-	-	-	+	-	-
		Salkowski test	-	+	-	-	-	+	-	-
8	**Saponins**	Foam test	+	+	-	-	+	+	-	-
		Haemolysis test	+	+	-	-	+	+	-	-
9	**Fixed oils and fats**	Spot test	-	-	+	+	-	-	+	+
		Saponification test	-	-	+	+	-	-	+	+

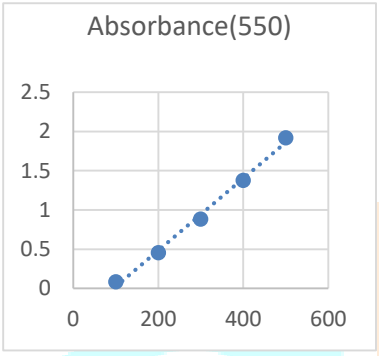
ii.(i). Total phenolic content

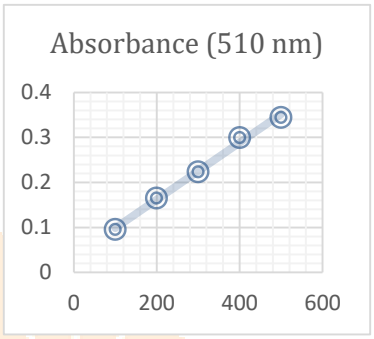
Standard curve used for the determination of total phenolic content was prepared by using different concentration of gallic acid equivalent (GAE)and it's optical density were shown in fig. 1

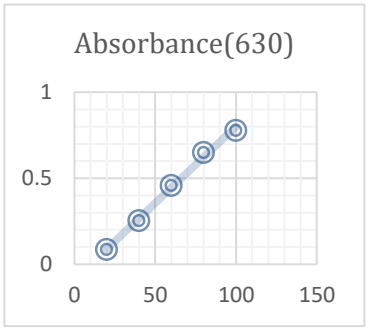
Total phenolic content from the methanol extracts of leaf and rhizome of *C.spiralis var. cognatoides* were shown in table 2

ii (ii.) Total flavonoid content

Standard curve used for the determination of total flavonoid content was prepared by using different concentration of quercetin equivalent (QUE) and it's optical density were shown in fig. 2. Total flavonoid content from the methanol extracts of leaf and rhizome of *C.spiralis var. cognatoides* were shown in table 2. Prasad *et al* (2012) found that 18.610 mg/g gallic acid equivalent phenolics and 1.102 mg/g rutin equivalents of flavonoids were found in the rhizome extracts of *C.spiralis*







Total Flavonoid content content mg QE/100g		Total Phenolic content mg GAE/100g		Ratio of TF/TP		Total starch content mg DE/100g	
Leaf	**Rhizome**	**Leaf**	**Rhizome**	**Leaf**	**Rhizome**	**Leaf**	**Rhizome**
45.08±1.15	1.15±0.06	46.17±0.92	22.76±0.06	0.97	0.05	121.80±0.90	861.42±0.89

Fig.2 Standard calibration curve for gallic acid

Fig.3 Standard calibration curve for quercetin

Fig.4 Standard calibration curve for dextrose

Table 2: Estimation of total phenolics, flavonoids and starch content in leaves and rhizomes of *C. spiralis var.cognatoides.*

ii. (iii). Total starch content

Standard curve used for the determination of total starch content was prepared by using different concentration of dextrose equivalent (DE) and it's optical density were shown in fig. 2. Total starch content from the methanol extracts of leaf and rhizome of *C.spiralis var. cognatoides* were shown in table 2.

iii. HPTLC Finger Print Profiling

HPTLC finger print profile of leaf and rhizome of *C.spiralis var. cognatoides* were developed by using methanol and hexane extracts .A literature review was done to develop the accurate solvent system and varied solvent combination were tried. Among them, toluene, toluene and ethyl acetate in 9 :1 ratio was the most suitable solvent combination for hexane extract. The densitogram for HPTLC finger print profile of leaf in hexane extracts at different wave length is shown in fig. 5-7 and the respective chromatogram were shown in fig. 8-10. The results showing number of peaks, R_f values and area percentage are presented in table 3. In the HPTLC densitometric scan the finger print profile of leaf in hexane extract under 254nm revealed 7 peaks, major peak at R_f 0.51 with

area percentage of 59.19, followed by peak at R_f 0.44 with area percentage of 12.97 and minor peak at R_f 0.02 with area percentage of 2.53, under 366nm, the major peak appeared at R_f 0.50 with area percentage of 62.52.96, followed by peak at R_f 0.44 with area percentage of 10.04 and minor peak at R_f 0.29 with area percentage of 25.27; under white light after derivatization the peak at R_f 0.17 with area percentage of 25.52came out as major peak followed by peak at 0.52 with area percentage 16.58 along with nine more minor peaks.

The densitogram for HPTLC finger print profile of rhizome in hexane extracts at different wave length is shown in fig.11-13and the respective chromatogram were shown in fig. 14-16. The results showing number of peaks, R_f values and area percentage are presented in table 6. 13 peaks were shown at 254 nm, with major peaks 0.08 with area percentage of 34.07 followed by 0.97 with area percentage of 9.24 and minor peak 0.65 with area percentage of 3.30 respectively. Only three peaks were shown at 366 nm and includes 0.34 with area percentage of 20.83, followed by0.64 with area percentage of 50.16 and 0.79 with area percentage of 29.02.13 peaks were available under 550 nm with major peaks 0.34 with area percentage of 60.02, followed by 0.38 with area percentage of 9.53.19 and nine more minor peak.

Table 3. HPTLC fingerprint profile of Hexane extract of leaves of *C.spiralis var.cognatoides* at different wave length

254 nm			366 nm			550 nm		
No of peaks	**R_f value**	**Area %**	**No of peaks**	**R_f value**		**No of peaks**	**R_f value**	**Area %**
1	**0.02**	**2.53**	**1**	**0.06**	**2.79**	**1**	**0.04**	**0.68**
2	**0.05**	**4.57**	**2**	**0.11**	**9.48**	**2**	**0.06**	**1.10**
3	**0.11**	**10.23**	**3**	**0.29**	**2.27**	**3**	**0.11**	**2.92**
4	**0.39**	**6.04**	**4**	**0.33**	**8.45**	**4**	**0.17**	**25.53**
5	**0.44**	**12.97**	**5**	**0.44**	**10.04**	**5**	**0.29**	**4.49**
6	**0.51**	**59.19**	**6**	**0.50**	**62.52**	**6**	**0.34**	**9.31**
7	**0.98**	**4.46**	**7**	**0.86**	**2.50**	**7**	**0.36**	**13.91**
			8	**0.92**	**1.96**	**8**	**0.46**	**14.66**
						9	**0.52**	**16.58**
						10	**0.66**	**3.59**
						11	**0.97**	**7.23**

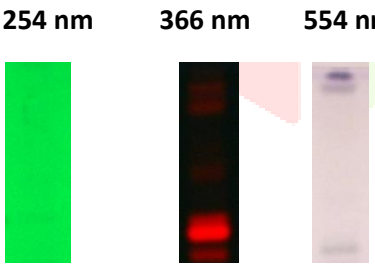


Fig.5 Fig.6 Fig.7

Fig.5-7 Densitogram of hexane extract of Leaves of *C.spiralis var.cognatoides* at different

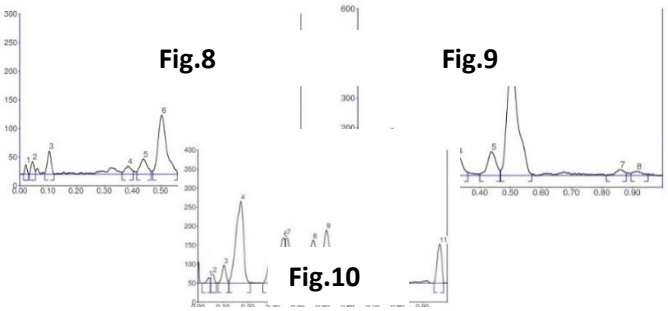


Fig.8-10 Chromatogram of hexane extract of leaves of *C.spiralis var*

Table 4. HPTLC fingerprint profile of Hexane extract of rhizome of *C.spiralis var.cognatoides* at different wave length

254 nm			366 nm			550 nm		
No of peaks	**R_f value**	**Area %**	**No of peaks**	**R_f value**	**Area %**	**No of peaks**	**R_f value**	**Area %**
1	**0.03**	**4.97**	**1**	**0.34**	**20.83**	**1**	**0.01**	**5.54**

2	**0.08**	**34.07**	**2**	**0.64**	**50.16**	**2**	**0.04**	**3.91**
3	**0.11**	**6.34**	**3**	**0.79**	**29.02**	**3**	**0.10**	**5.00**
4	**0.19**	**5.00**				**4**	**0.12**	**0.93**
5	**0.26**	**4.24**				**5**	**0.34**	**60.02**
6	**0.34**	**8.24**				**6**	**0.38**	**9.53**
7	**0.41**	**6.22**				**7**	**0.46**	**1.81**
8	**0.55**	**5.76**				**8**	**0.54**	**5.88**
9	**0.65**	**3.30**				**9**	**0.61**	**0.64**
10	**0.79**	**4.26**				**10**	**0.67**	**0.92**
11	**0.82**	**3.75**				**11**	**0.72**	**0.79**
12	**0.90**	**4.60**				**12**	**0.84**	**3.19**
13	**0.97**	**9.24**				**13**	**0.92**	**1.85**

254 nm **366 nm** **550 nm**

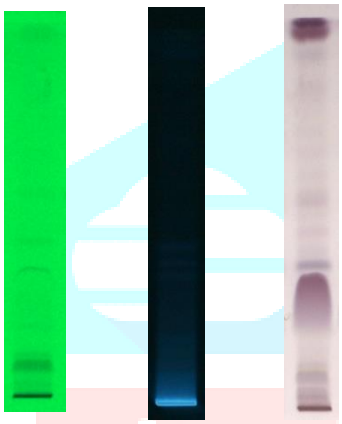
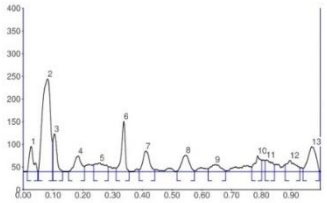


Fig.11 Fig.12 Fig.13

Fig.11-13. Densitogram of methanol Extract of rhizome of *C. spiralis var. cognatides* length



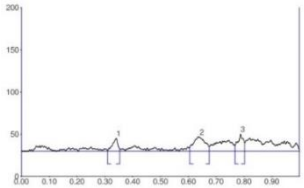


Fig.14

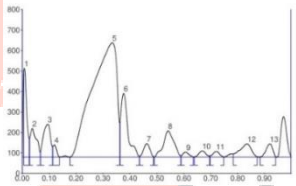


Fig.16

Fig.14-16. Chromatogram of hexane extract of *C.spiralis var.cognatoides* at different wave at different wave length length

Conclusion

Phytochemical screening of methanol and hexane extracts of *C. spiralis var. cognatoides* revealed the presence of carbohydrate, proteins, phenolics, alkaloids, saponins, glycosides, flavonoids, sterols fixed oils and fats. This result is in agreement with the findings of *C. spiralis* (Prasad et al., 2012). The information on the presence or absence of the type of phytochemical constituents especially the secondary metabolites are useful taxonomic key in identifying a particular species and distinguishing it from related species (Jonath and Tom, 2008) [16]. The leaves and rhizome of the plant have average phenolic and flavonoid and starch content. HPTLC finger printing of leaves and rhizomes ascertain a good metabolite profile, which could be used for standardization of plant extract. Improved techniques like LCMS profiling will needed to identify the exact chemical components of plant extracts. To our knowledge phytochemical screening, HPTLC finger print profiling were generated for the first time in various solvents.

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