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Phytochemical study, antioxidant and anti-inflammatory activity of the *Cryptocoryne tortuosa*

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Abstract
In present study, the leaves and roots of Cryptocoryne tortuosa plant were used for phytochemical analysis and biological activities such as antioxidants and anti-inflammatory activities. *Cryptocoryne tortuosa* is a rare plant with good activity that has been used to treat a wide range of diseases. In Phytochemical analysis, flavonoids, terpenes, phenolic nucleus, terpenes sterols, tannins, anthracene, saponins, reducing sugars, alkaloids, proteins, coumarins, saponosides were present in leaves extract of *Cryptocoryne tortuosa*and sterols, tannins, reducing sugars, were present in roots extract of Cryptocorynetortuosa. The phytochemical analysis followed a well-established protocol, and for evaluation of antioxidant and anti-inflammatory activities well known techniques were used.Both the leaves and roots extracts of Cryptocorynetortuosashown significant antioxidant and anti-inflammatory activities. Anti-inflammatory activity is maximum in ethanol extract for leaves and root extracts of Cryptocorynetortuosa.

Keywords: antioxidant, anti-inflammatory, cryptocoryne tortuosa, phytochemicals

Introduction
Phytochemicals or phytonutrients are biologically active, naturally occurring chemical compounds found in plants that have protective or disease preventive properties. Phytochemicals act as a natural defense mechanism in their host plants. They also provide pigments to the plants. Nowadays, pollution is a biggest problem in the world, due to which human faces variety of diseases and infections. Medicinal plants play a significant role to preserve our health. Medicinal Plants contains alkaloids, steroids, flavonoids, phytosterols, various reducing sugar, various fats, and proteins. There are approximately 60 known Cryptocoryne species and all belongs to the Araceae family of aquatic plants. These plants are all native to Asia but they can be found in a variety of different countries including Indonesia, Malaysia and New Guinea [1]. These plants prefer streams and rivers with low flow and can be found under varying light conditions. They are also found in seasonal areas that are affected by tides and rainfall, like forest pools and river banks. In the wild, Cryptocorynes can grow fully submerged underwater. Antioxidants are man-made or natural substances that protect body against toxic effect of free radicals and prevent cell damage. Inhibit oxidation and protect cell from damage caused by unstable molecule generating process of oxidation during metabolism in body such as free radicals [1]. Free radicals lead to chain reaction and start damaging cells. When level of free radical in body become high, it causes oxidative stress [2], which leads to various diseases like cardiovascular problems, cancers, diabetes. Lipid's, proteins, nucleic acids are major targets of free radicals [2]. The protective mechanism of antioxidants serves to scavenge free radical and prevent initiation of chain reaction. For proper physiological functions balance between antioxidants and free radicals is necessary [3]. Inflammation is critical protective response of body infection, irritation injury, Swelling, heat, redness,pain are some symptoms of inflammation [6]. Cell death, tissue injury, ischemia, cancer isalso responsible for inflammation. Inflammation can be acute or chronic. Anti- inflammatory compounds are usedto treat symptoms of inflammation in body [5]. In the present study, leaves and roots extracts of Cryptocoryne tortuosa plant were used various phytochemicals detection and analysis of antioxidant and anti-inflammatory activities were carried with standard protocol.

Fig 1: *Cryptocoryne tortuosa* Plant

Materials and Methods
The leaves and rootof *Cryptocoryne tortuosa* plant were collected from Lonavala, Maharashtra. The specimen of Leaves and Root washed with distilled water then specimens are dried. After completion of drying, then pulverized the specimens and powered of Leaves and Roots *Cryptocoryne tortuosa* plant stored in air tight bottles The roots and leavesextracts of the *Cryptocoryne tortuosa* plant was named as CT-R and CT-L respectively

Phytochemical Analysis
Phytochemical testing carried using estimated protocol for CT-R and CT-L extract [6].

Sterols
An equal volume of acetic anhydride was added to test tube and gently stirred. After that, 1 ml of concentrated H_2SO_4 was poured down the tube's side [7]. The presence of sterols is indicated by the formation of a brownish-red ring at the contact zone of the two liquids and a greenish tint in the separation layer. Test is Positive for both CT-R and CT-L

Tannins
Ferric chloride test was performed for detection of tannins[8]. In this test, appearance of a blue changed to olive green as additional ferric chloride was added. Ferric chloride test is positive for both CT-R and CT-L.

Anthracene
5 mL chloroform was added to the powder of leaves and roots in a test tube and agitated for 5 minutes. The mixture was filtered, and the filtrate was agitated with a 10% ammonia solution in an equal volume. when the aqueous layer is agitated, it turns pink, red, or violet, indicates the presence of free anthraquinone [9]. Anthracene detection testwas positivefor the CT-Land negative for CT-R.

Saponins
The powder ofleaves or rootswere put to a test tube with 10 mL distilled water and vigorously shaken for 30 seconds. Afterwards, it was left to stand for 30 minutes. The presence of saponins is indicates by the production of honeycomb foam [3]. Saponins's detection test was Positive for CT-Land negative for CT-R.

Flavonoids
Acetone was used to totally retain two grams of powder of leaves or roots. After evaporating the acetone over a water bath, the residue was removed with warm water. After filtering the mixture while it was still hot, the filtrate was allowed to cool before being used for the next test: Shinoda's experiment, in 3 ml of an aqueous solution, a few magnesium chips were added, and 2 drops of weak HCl were added and warmed. The presence of flavonoids is indicates by a pink or red tint [11]. Flavonoid's detection test was Positive for CT-L and negative for CT-R.

Phenolic nucleus
Sodium hydroxide test is used for detection of phenolic nucleus [12]. Phenolic nucleus's detection test was Positive for CT-L and negative for CT-R.

Terpenes
The Liebermannreagent test aids in the identification of terpene, resulting in the production of a blue greencolour that shows the presence of a terpene, whereas no pink colour shows the absence of terpenes [13]. Terpene's detection test was Positive for CT-L and negative for CT-R.

Reducing Sugars
The Fehling reagent was used to identify reducing sugars, which was then validated by the Tollens reagent test [11]. Reducing Sugar's detection test was Positive for CT-L as well as for CT-R.

Alkaloids
Bouchardat reagent and (reagent iodo-iodized) Bouchardat reagent were used to characterize alkaloids [14]. Alkaloid's detection test was Positive for CT-L and negative for CT-R.

Proteins
The biuret reaction was used to detection the proteins. Add 2 -3 drops of an aqueous portion of CuSO4 to 2% to a small volume of extract diluted in 2 mL of 20% aqueous NaOH in a test tube. Purple colour formations indicates the presence of protein [15]. Proteins's detection test was Positive for CT-L andnegative for CT-R.

Coumarins
2 mL ethanolic solution produced from each residue during extraction in two test tubes.Heats both test tubes in a water bath until boiling, then add 0.5 mL of 10% NaOH to one of the test tubes. 4 mL distilled water in each test tube to cool it down. If the liquid from the test tube in which the alkaline solution was added is transparent or more translucent than the liquid from the control test tube (without the alkaline solution), a faint yellow solution indicates the presence of coumarin [16]. Coumarin's detection test waspositive for CT-L and negative for CT-R.

Saponosides
8-10 mL aqueous complete extract in a test tube to discover saponosides. The tube was shaken for 10-15 seconds before being left alone for 12-15 minutes. Saponosides, Saponins are detects when the height of persistent foam was greater than 1 to 2 cm [12]. Saponoside's test was positive for CT-L andnegative for CT-R.

Antioxidant Activity Determination
DPPH Scavenging Test:The percentage of the antioxidant present in the sample was determined using the typical protocol of the DPPH scavenging test. This test was carried out using the specific protocol. This test was carried out by preparing the various extracts of the plant material [17].

Study of anti-inflammatory activity (In-vitro models)
The anti-inflammatory activity of the different extracts was carried out using a slight modification of Mizushima and Kobayashi protocol with doses. The albumin test method was used [18].

Results and Discussions

Phytochemical Analysis

Based on the present study it can be concluded that the ethanolic extract from leaves and roots Cryptocorynetortuosa showed the presence of various Phytochemical. In Phytochemical analysis, sterols, tannins, anthracene, saponins, flavonoids, terpenes, phenolic nucleus, terpenes, reducing sugars, alkaloids, proteins, coumarins, saponosides were present in leaves extract of Cryptocoryne tortuosa whereas sterols, tannins and reducing sugars were present in roots extract of Cryptocoryne tortuosa.

Table 1: Qualitative analysis of phytochemicals of CT-L and CT-R

Sr. No	Plant Constituents	CT-L	CT-R
1	Sterols	+	+

2	Tannins	+	+
3	Anthracene	+	-
4	Saponins	+	-
5	Flavonoids	+	-
6	Terpenes	+	-
7	Reducing sugars	+	+
8	Alkoloids	+	-
9	Proteins	+	-
10	Coumarins	+	-
11	Saponosides	+	-
12	Phenolic nucleus	+	-

*+ = Positive test, - =Negative test

Antioxidant Activity Determination

The antioxidant activities of the organic solvents of leaves and roots extract of *Cryptocoryne tortuosa* tabulated in table 2 and table 3 for BHT(Butylated hydroxytoluene), C_2H_5OH(Ethanol), $CHCl_3$(Chloroform) and CCl_4(Carbon tetrachloride) extract. The graphical performance of BHT, C_2H_5OH, $CHCl_3$ and CCl_4 for CT-L displayed in Fig. 2 (a). It shown that C_2H_5OH and $CHCl_3$have better antioxidants performance than CCl_4.

Fig. 2 (b) displayed that DPPH radical activity of CT-Rand C_2H_5OH and CCl_4 exhibited better performance than $CHCl_3$ extract.

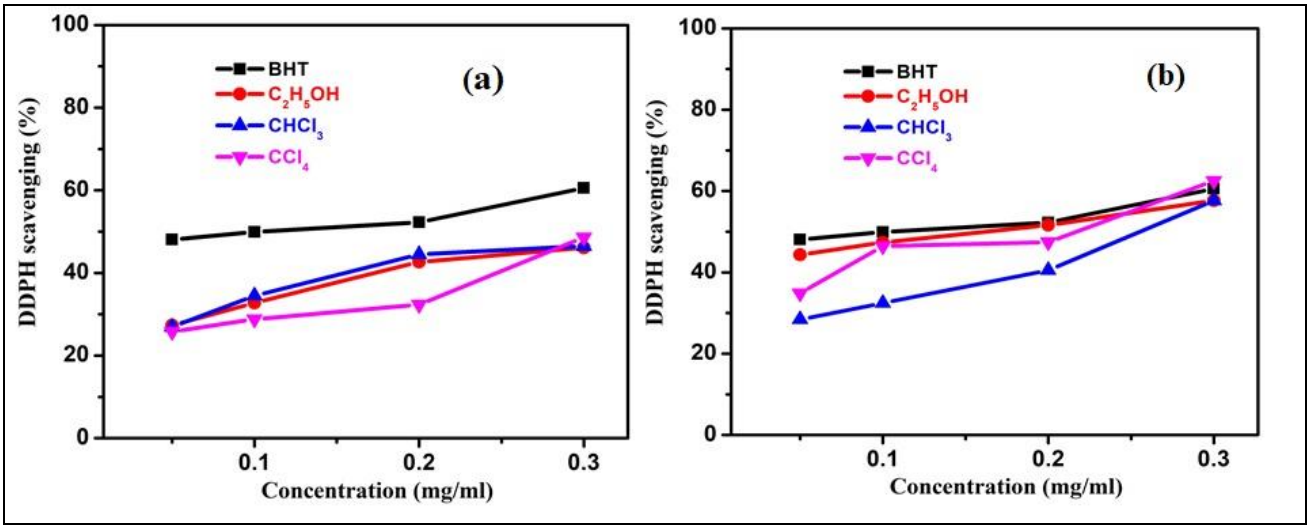

Fig 2: Antioxidant activity of CT-Land CT-Ra) DPPH radical activity of CT-Lb) DPPH radical activity of CT-R

Table 2: Antioxidant activity of CT-L

Extract Conc. Mg/ml	BHT	Ethanol	$CHCl_3$	CCl_4
0.05	48.1	27.30	26.90	25.80
0.1	49.91	32.77	34.50	28.79
0.2	52.24	42.66	44.50	32.32
0.3	60.57	46.12	46.50	48.60

Table 3: Antioxidant activity of CT-R

Extract Conc. Mg/ml	BHT	Ethanol	$CHCl_3$	CCl_4
0.05	48.1	44.34	28.44	34.87
0.1	49.91	47.34	32.44	46.45
0.2	52.24	51.63	40.53	47.44
0.3	60.57	57.66	57.67	62.50

Determination of Anti-inflammatory Activity

Anti-inflammatory activity (In-vitro models) studied for *Cryptocoryne tortuosa* plant's roots and leaves extract tabulated in table 4 and table 5 respectively for standard (Ibuprofen), petroleum ether, chloroform, ethyl acetate, n-Butanol and ethanol. Fig. 3 exhibited percent inhibition of CT-R and CT-L for standard, Petroleum Ether, Chloroform, Ethyl acetate, n-Butanol and Ethanol. Fig. 3 shown that anti-inflammatory activity is maximum in ethanol extract for both CT-R and CT-L than other extract. Mostly Ethanol extract of the leaves and rootof *Cryptocoryne tortuosa* possess*in-vitro* anti-inflammatory activity which might be attributed to the presence of various Phytochemical in the extract. The chloroformextract found less performance in CT-L while ethyl acetate extract found less performance in CT-R

Table 4: Anti-inflammatory activity of CT-R

In-Vitro Anti – inflammatory activity	Dose (mg / kg)	Absorbance value (Mean + SE)	Inhibition of denaturation (%)
Control	5ml / kg	0.098	----
Standard (Ibuprofen)	100mg/kg	0.18	90.32
Petroleum ether extract	200mg/kg	0.15	77.40
Chloroform extract	200mg/kg	0.14	66.28
Ethyl acetate extract	200mg/kg	0.12	43.26
n-Butanol	200mg/kg	0.16	44.39
Ethanol	200mg/kg	0.17	88.47

Table 5: Anti-inflammatory activity of CT-L

In-Vitro Anti – inflammatory activity	Dose (mg / kg)	Absorbance value (Mean + SE)	Inhibition of denaturation (%)
Control	5ml / kg	0.098	----
Standard (Ibuprofen)	100mg/kg	0.18	90.32
Petroleum ether extract	200mg/kg	0.12	78.19
Chloroform extract	200mg/kg	0.13	24.28
Ethyl acetate extract	200mg/kg	0.12	44.18
n-Butanol	200mg/kg	0.14	67.75
Ethanol	200mg/kg	0.12	86.19

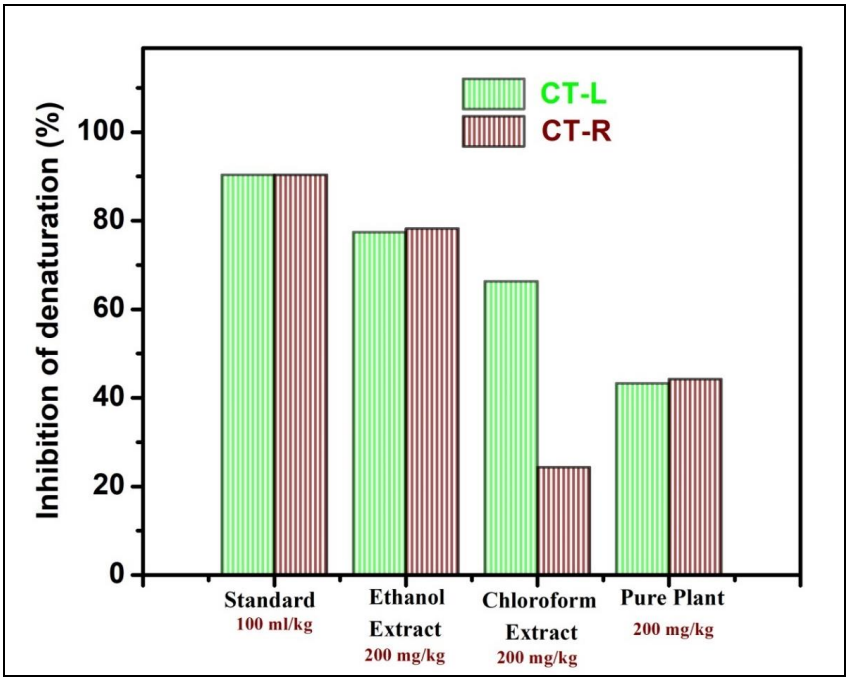

Fig 3: % Inhibition of CT-R and CT-L for standard, Petroleum Ether, Chloroform, Ethyl acetate, n-Butanol, Ethanol

Conclusions

From the result of Phytochemical analysis, concluded that Cryptocoryne tortuosa plant were found to contain phytoconstituents like sterols, tannins, anthracene, saponins, flavonoids, terpenes, phenolic nucleus, terpenes, reducing sugars, alkaloids, proteins, coumarins, saponosides were present in leaves extract of Cryptocoryne tortuosa. In roots extract of Cryptocoryne tortuosasterols, tannins and reducing sugars, were present. Anti-inflammatory activity is maximum in ethanol extract for both leaves and roots extract. Cryptocoryne tortuosa shown strong antioxidant activity as evidenced by the free radical scavenging property, Results suggest that due to the presence of phenolic and flavonoid content in leaves of Cryptocoryne tortuosa has an excellent ability to protect against oxidative damage that is found to be an important path physiological event in a variety of diseases including aging, diabetes, cancer, cardiovascular disorders, and rheumatoid arthritis. Overall, it is a source of natural antioxidant that can be play significant role in various disease prevention and health preservation. Therefore, it's ethno medical claims was true according to the above experimental results. This gives support to the claim for the traditional use of the plant in the treatment of inflammation. The result of these study has seen to provide support for the use leaves and rootsof Cryptocorynetortuosato promote proper conservation sustainable use of such plant resources, awareness of local communities should be enhanced incorporating the traditional knowledge with scientific findings.

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