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Investigation of phytochemical constituent antioxidant and anti-inflammatory activity of plant Canscora concanensis

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Abstract
The Gentianaceae family includes the plant *Canscora concanensis*. This is a rare, active herb that has traditionally been used to treat a wide range of diseases. The present study, the leaves and roots of the *Canscora concanensis* plant for phytochemical analyses as well as biological activities such as antioxidants and anti-inflammatory effects. Canscora concanensis plant leaves and root extract contained flavonoids, terpenes, phenolic nucleus, terpenes, sterols, tannins, anthracene, saponins, reducing sugars, alkaloids, proteins, coumarins, and saponosides. Techniques for determining antioxidant and anti-inflammatory activities that are well-known. Both the leaves and root extract of *Canscora concanensis* have substantial antioxidant and anti-inflammatory properties. The ethanol extract of Canscora concanensis leaves and roots has the strongest anti-inflammatory activity.

Keywords: canscora concanensis, phytochemicals, antioxidant, anti-inflammatory

Introduction
Canscora concanensis is a species of gentian that was described by CB Clark. Canscora concanensis is a member of the Canscora genus and a member of the gentian keluarga. Its haslower leaves are elliptic and petiolate and the top leaves are elliptical, sessile, 3-ribbed, and glabrous.Pink to pale flowers in terminal or axillary panicles. FruitSeeds are numerous, spherical or angular, and minutely rugose, in an oblong-compressed capsule.
A large number of free radicals which act as oxidizing agents are produced due to pollution [1]. This free radical causes harmful effects in the human body due to these human faces various diseases therefore a balance between free radicals and antioxidants is required for proper physiological activities [1, 2]. Antioxidants are chemicals that protect the body from free radicals damaging effects. Inhibit oxidation and protect cells from free radical damage, which is caused by unstable molecules formed during the oxidation process in the body [3, 4]. Free radicals set off a chain reaction that damages cells. A spike in free radical levels in the body causes oxidative stress [1]. Several diseases, including heart disease, cancer, and diabetes, are connected to oxidative stresss [5]. Lipid proteins and nucleic acids are both targets for free radicals. The protective mechanism of antioxidants helps to scavenge free radicals and prevent chain reactions from commencing [1, 6]. According to the mechanism of antioxidant action, antioxidants can be divided into three groups. Primary antioxidants function as free radical terminators, Secondary antioxidants are important preventive antioxidants which are function by retarding chain initiation and tertiary antioxidants are concerned with the repair of damaged biomolecules [7-9]. Inflammation is the body's natural defense against infection, discomfort, and injury. Swelling, heat, redness, and discomfort are all symptoms of inflammation [6]. Cell loss, tissue injury, ischemia, and cancer all induce inflammation. Inflammation can be acute or persistent. Chronic inflammation might last for a long time. Anti-inflammatory agents block certain substances in the body that causes inflammation [10]. Anti-inflammatory substances are employed in the body to alleviate inflammation-related symptoms [5]. In this study, several phytochemicals were studied on the leaves and roots of the *Canscora concanensis*plant using traditional methods. Various extracts of *Canscora concanensis*plant leaves and roots are being evaluated for antioxidant and anti-inflammatory effects.

Fig 1: *Canscora concanensis* Plant

Materials and Methods
*Canscora concanensis*leaves and roots were taken in Lonavala, Maharashtra.The leaves and roots specimens are washed with distilled water and then dried. After drying, the specimens were pulverized and the leaves and roots of the

*Canscora concanensis*plant were powered and stored in airtight bottles. The *Canscora concanensis*plant's leaves and roots extracts were given the names CC-L and CC-R, respectively.

Analytical Phytochemistry
Phytochemical testing was done on the CC-L and CC-R extracts using an approximated protocol [11].

Tannins
Tannins were detected using a ferric chloride test [12]. The appearance of a blue in this test changed to olive green as more ferric chloride was applied. For CC-L and CC-R, a ferric chloride test is positive.

Sterols
An identical volume of acetic anhydride was added to a test tube and stirred slowly. 1 ml concentrated H_2SO_4 was then poured into the tube's side. 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 hue in the separation layer [5]. The CC-L and CC-R test both come back positive.

Tannins
Tannins were detected by a ferric chloride test [13]. As more ferric chloride was applied, the blue colour of the test changed to olive green. A ferric chloride test is positive for CC-L and CC-R

Anthracene
5 mL chloroform was added to leaves or roots powder in a test tube and swirled for 5 minutes. The mixture was filtered, and the filtrate was agitated with a 10% ammonia solution in an equal amount. When the aqueous layer is stirred, it turns scarlet, crimson, or violet, indicating the presence of free anthraquinones [12]. The CC-L tested positive for anthracene, whereas the CC-R tested negative.

Saponins
In a test tube with 10 mL distilled water, the powdered leaves or roots were shaken briskly for 30 seconds. After then, it was left to stand for 30 minutes. Saponins are required for the formation of honeycomb foam [14]. For CC-L was found positive in Saponins' detection test, however, CC-R was found negative.

Flavonoids
To retain 2 gm of leaves or roots powder, acetone was used. After the acetone was evaporated over a water bath, the residue was removed with warm water. After sifting the mixture while it was still hot, the filtrate was allowed to cool before being used for the next test: A few magnesium chips were added to 3 mL of an aqueous solution, then 2 drops of mild HCl were added and warmed in Shinoda's experiment. The presence of flavonoids is indicated by a pink or scarlet color [14, 15]. The flavonoid detection test was positive for CC-L but negative for CC-R.

Phenolic nucleus
The phenolic nucleus is detected using a sodium hydroxide test [16]. The detection test for phenolic nucleus was positive for CC-L but negative for CC-R.

Terpenes
The Liebermann reagent test aids in terpene identification by producing a blue-green colour that shows terpene presence, whereas no pink colour indicates terpene absence [17]. Terpene was found to be positive for CC-L but negative for CC-R in a detection test.

Sugar Reduction
The Tollens reagent test was used to validate the Fehling reagent, which was used to identify reducing sugars [15]. The detection test for CC-L and CC-R in Reducing Sugar proved positive.

Alkaloids
Bouchardat reagent and (reagent iodo-iodized) Bouchardat reagent were used to identify alkaloids. The alkaloid detection test for CC-L was positive, but not for CC-R.

Proteins
The biuret reaction was used to detect the proteins. Add 2 -3 drops of aqueous $CuSO_4$ diluted to 2% to a small volume of extract diluted in 2 mL of 20% aqueous NaOH in a test tube. Purple colour formations suggest the presence of protein [18]. The biuret detection test found positive for CC-L but negative for CC-R.

Coumarins
Each residue yielded a 2 mL ethanolic solution in two test tubes during extraction. In a water bath, heat both test tubes until they are both boiling, then add 0.5 mL of 10% NaOH to one of them. Add 4 mL distilled water to each test tube to chill it down. If the liquid from the test tube in which the alkaline solution was added is clear or more translucent than the liquid from the control test tube (without the alkaline solution), coumarin is present [11, 19]. Coumarin tested positive for CC-L but negative for CC-R in a detection test.

Saponosides
Fill a test tube with 8-10 mL of aqueous complete extract to identify saponosides. The tube was shaken for 10-15 seconds before being left alone for 12-15 minutes. saponosides When the persistent foam reaches a height of 1 to 2 cm, saponins are detected [16]. The saponosides test for CC-L was positive, but not for CC-R.

Determination of Antioxidant Activity
DPPH Scavenging Test: A conventional DPPH scavenging test protocol was used to determine the proportion of antioxidants in the sample. This test was conducted under established procedures [9, 20, 21]. The various extracts of the plant material were prepared for DPPH scavenging test.

Anti-inflammatory activity research (In-vitro models)
A slightly modified Mizushima and Kobayashi dosing regimen was used to investigate the anti-inflammatory effects of the different extracts [17, 22].

Results and Discussions
Analytical Phytochemistry
The ethanolic extract of *Canscora concanensis*leaves and roots contained a variety of phytochemicals, according to the findings of this study. According to a phytochemical study, the leaves extract of *Canscora concanensis*included sterols, tannins, anthracene, saponins, flavonoids, terpenes,

phenolic nuclei, reducing sugars, alkaloids, proteins, coumarins, and saponosides. Sterols, tannins and reducing sugars, were found in the extract of the root of *Canscora concanensis*

Determination of Antioxidant Activity

The antioxidant activity of organic solvents in Canscora concanensis leaves and roots extract for BHT, C_2H_5OH, $CHCl_3$, and CCl_4 extract is shown in Tables 1 and 2. The graphical performance of BHT, C_2H_5OH, $CHCl_3$, and CCl_4 for CC-L is shown in Figure 2. C_2H_5OH, $CHCl_3$andCCl_4have been found significant antioxidants activity. Demonstrated in Fig. 3, the DPPH radical activity of CC-R, $CHCl_3$, and CCl_4 was higher than that of C_2H_5OH extract.

Table 1: Antioxidant activity of CC-L

Extract Conc. Mg/ml	BHT	Ethanol	$CHCl_3$	CCl_4
0.05	48.1	30.20	24.80	24.60
0.1	49.91	32.70	36.59	36.70
0.2	52.24	40.60	46.55	42.30
0.3	60.57	44.12	48.50	49.50

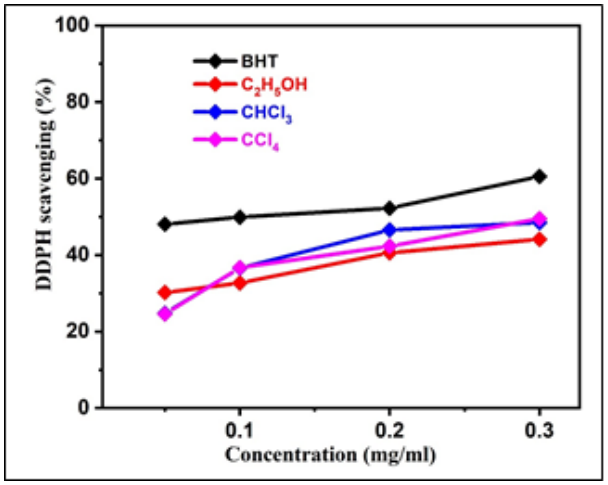

Fig 2: Antioxidant activity of DPPH radical activity of CC-L

Table 2: Antioxidant activity of CC-R

Extract Conc. Mg/ml	BHT	Ethanol	$CHCl_3$	CCl_4
0.05	48.1	40.30	25.42	34.86
0.1	49.91	45.30	32.42	48.47
0.2	52.24	49.60	48.53	49.48
0.3	60.57	55.60	52.60	62.58

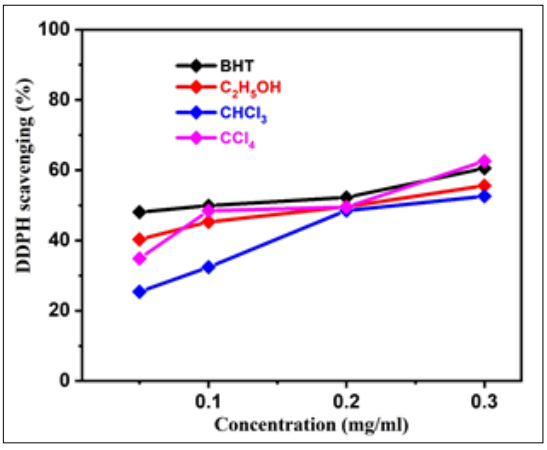

Fig 3: Antioxidant activity of DPPH radical activity of CC-R

Determination of Anti-inflammatory Activity

Standard (Ibuprofen), petroleum ether, chloroform, ethyl acetate, n-Butanol, and ethanol anti-inflammatory activity (in-vitro models) for Canscora concanensis plant's leaves and root extract tabulated in tables 3 and 4 respectively for standard (Ibuprofen), petroleum ether, chloroform, ethyl acetate, n-Butanol, and ethanol. Figure 3 shows percent inhibition of CC-L and CC-R for standard, Petroleum Ether, Chloroform, Ethyl acetate, n-Butanol, and Ethanol. In comparison to other extracts, ethanol extract has the strongest anti-inflammatory efficacy for both CC-L and CC-R, as shown in Figure 3. Ethanol extracts of Canscora concanensisleaves and roots showed anti-inflammatory activity in vitro, which could be due to the presence of several phytochemicals in the extract.

Table 3: Anti-inflammatory activity of CC-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	0.15	71.40
Chloroform extract	200mg/kg	0.14	64.80
Ethyl acetate extract	200mg/kg	0.12	48.20
n-Butanol+/*	200mg/kg	0.16	52.30
Ethanol	200mg/kg	0.17	88.44

Table 4: Anti-inflammatory activity of CC-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	0.12	68.30
Chloroform extract	200mg/kg	0.13	34.23
Ethyl acetate extract	200mg/kg	0.12	54.19
n-Butanol	200mg/kg	0.14	57.78
Ethanol	200mg/kg	0.12	87.21

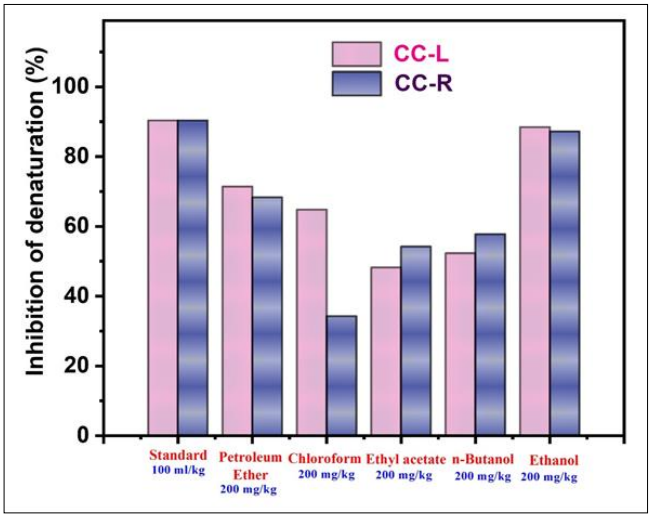

Fig 4: % Inhibition of CC-L and CC-Rfor standard, Petroleum Ether, Chloroform, Ethyl acetate, n-Butanol, Ethanol

Conclusions

Sterols, tannins, anthracene, saponins, flavonoids, terpenes, phenolic nuclei, terpenes, reducing sugars, alkaloids, proteins, coumarins, and saponosides were present in the leaves extract of *Canscora concanensis*, according to phytochemical studies. Sterols, tannins, and reducing sugars, were all present in the extract of the root of

Canscora concanensis. The highest anti-inflammatory activity was found in ethanol extracts of both leaves and roots extracts. *Canscora concanensis*leaves and roots have strong antioxidant activity, as evidenced by their ability to scavenge free radicals, making them a potent antioxidant that can protect against oxidative stress, which has been linked to a variety of diseases including aging, diabetes, cancer, cardiovascular disease, and rheumatoid arthritis. Overall, it's a natural antioxidant that can aid in the prevention of illness and overall wellness. Its ethnomedicinal claims were correct, according to the above-mentioned trial findings. This supports the idea that the herb has previously been utilized to treat inflammation. The findings of the study suggest that Canscora concanensis leaves and roots be used to ensure effective plant resource protection and long-term use. By fusing traditional knowledge with scientific results, local community awareness should be strengthened.

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