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# HISTOCHEMICAL STUDIES OF *CURCUMA NEILGHERRENSIS* – AN ANTIDIABETIC HERB

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## ABSTRACT

Histochemical analysis provides a best tool for the botanical identification and standardisation of crude drugs. The present study deals with the histochemical analysis of leaf and rhizome of the *C. neilgherrensis.* The study reveals the identification and location of the phytochemicals like alkaloids, saponins, tannins, oils, starch grains etc in various regions of leaf and rhizome of *C. neilgherrensis.* Free hand sections were taken and treated with respective reagents to localise the various cellular components. The observations could be of great use in chemotaxonomy and checking the drug adulteration.

**Key words:** *C. neilgherrensis*, Rhizome, Leaf, Histochemistry.

## INTRODUCTION

Nature has bestowed human beings with innumerable resources. India has been known for its rich repository of medicinal plants. Modern people being health conscious prefer herbal medicines over other ones because of the severe side effects of the later one. The forests in India are the main principal repository of the medicinal plants, which are largely considered as raw materials for manufacture of drugs and perfumery products. The knowledge about the use of medicinal plants has been acquired through centuries and such plants are still valued today. Medico scientist practicing allopathy and research minded vaidyas, hakims have contributed valuable knowledge regarding efficacy of reputed medicinal plants indigenous to

India [1]. Establishment of herbal forms in well selected localities will exercise scientific control over the cultivation of medicinal herbs [2].

*Curcuma neilgherrensis* belongs to the family Zingiberaceae and is known as Kattukalvazhai in Tamil Nadu. It is a herb with conical rhizomes, internally whitish in colour, ending in fusiform root tubers. Leaves are green, lanceolate/oblong, 25 cm in length and 8 cm width. Flowers usually appear in the season of February-March and the leaves are not visible at the time of flowering. The plant is endemic to the Nilgiri hills of Tamil Nadu. *C. neilgherrensis* is a folklore medicine widely used by the tribes of the Western Ghats for the management of diabetes mellitus. The traditional medicinal practitioners of Kodagu district of Karnataka have identified its usage in diabetes mellitus and the pillaiyar tribes of Tamil Nadu are using its tuber for edible purposes [3]. It is also used as anti inflammatory, Cholagogue, hepatoprotective, blood purifier, antioxidant, taoxifier, antiasthmatic, antitumor, carminative and regenerative of liver tissues [4]. It is also used for chronic hepatitis, antiarthritis, antiseptic and menstrual disorders [5]. According to traditional data from the herbalists and from the Yanadi tribes of Seshachalam hill ranges the rhizomes of this plant were used to treat cuts, boils, wounds, skin diseases, pimples, bone fractures, common cold and ulcers [6].

## MATERIAL AND METHODS

*Curcuma neilgherrensis* plant was collected from the Nilgiri Hills of Tamil Nadu, India during the month of Oct-Nov 2012 and is being maintained in the medicinal plant garden of the Department of studies in Botany, University of Mysore, Mysore, Karnataka. For Histochemical studies, free hand sections of organs to be studied were taken and treated with respective reagents to localize the cellular components, viz. saponins, alkaloids, fat, tannins, starch etc.

**Histological and histochemical staining techniques:** Staining of the sections is the most fascinating part in the preparation of specimens for microscopy. In general, most biological tissues have very little contrast, and cellular details are hard to discern with the ordinary light microscope. Stains can enhance and improve the visibility of the specimen. In addition, different stains have different affinities for various organelles and macromolecules. Therefore, the careful selection and utilization of stains can also suggest the chemical nature of the substances within the cell.

**I) Test for saponins:** Sections were placed directly in one drop of conc. sulphuric acid onto the slide, which gives a characteristic sequence of colour reactions, beginning immediately

with yellow, changing to red within 30 minutes and finally becoming violet or blue green in a short time. To determine localization of the saponins, sections were put in saturated barium hydroxide solution for about 24 hours. Sections were washed with calcium chloride, and then placed in potassium dichromate [7].

**II) Test for alkaloid**

Alkaloid presence was confirmed by using Wagner's Reagent (1gm iodine and 2 gms of potassium iodide were dissolved in 50ml of distilled water). Sections were stained for 1-2 minutes and then observed under microscope [7].

**III) Test for fats and oils**

Sections were stained for 20 minutes using Sudan-IV (0.5g of Sudan- IV in 100 ml of chloroform) and rinsed quickly with 50% alcohol and mounted in glycerine for observation [1].

**IV) Test for tannin, lignin and pectin- toluidine blue O stain.**

Sections were stained with an aqueous solution of 0.1% TBO for one minute. Excess stain was gently removed using blotter paper followed by washing with water till there is no excess stain around the sections. A drop of clean water was dropped on the section followed by a cover glass. The slides were then observed under microscope [8].

**V) Test for cellulose**

A drop of 0.1% aqueous methylene blue was put on the specimen and allowed to stain for 15-20 min. The stain was replaced with water and the specimen was then observed under microscope [9].

VI) **Test for carbohydrate**

Sections were oxidized in 0.5% to 1% periodic acid for 2-3 minutes followed by treatment with Schiff's reagent for 5 minutes (Schiff's reagent is prepared by the following procedure. 1g basic fuchsin is added to 200 ml boiling distilled water and cooled to $50^0$C then 30ml of 1N HCl is added followed by the addition of 3g $K_2S_2O_5$. The solution is stored in dark for 24-48 hrs, during this time the red colour should disappear. To the decolorized solution, 0.5g of activated charcoal is added followed by shaking for 1 min. The mixture is filtered and stored at $5^0$C in dark for use.) Sections were then stained for 20 min and observed under microscope [10].

**VII) Test for starch.**

Sections were transferred onto the slide followed by a drop of IKI solution directly on the specimen (The IKI solution is prepared by first dissolving 2 g of KI in 100 ml of water followed by addition of 0.2 g of iodine into the KI solution. Solution is stored in dark glass bottle and capped tightly as exposure to light and air degrades the solution's usefulness). After few minutes, excess IKI solution was removed by putting cover glass and then examined under the microscope [11].

**RESULTS**

Histochemical colour reactions of *C. neilgherrensis* leaf and rhizome were carried out by taking free hand sections of leaf and rhizome followed by treatment with various reagents and observation under microscope. The results revealed the presence of starch, oils and fats, saponins, alkaloids, carbohydrates, tannins, lignin, pectin and cellulose. The results are presented in Table-1.

The presence of saponin in free hand sections of leaf (Fig.1a) and rhizome (Fig.1b) was revealed by the presence of yellow coloured cells after treating the sections with potassium dichromate solution. The saponins were located in the parenchymatous regions of the leaf tissue and in parenchyma and cortex regions of rhizome tissues. The confirmation of alkaloids in leaf sections (Fig.1c) and rhizome sections (Fig.1d) was revealed by the presence of golden yellow coloured cells after treating the sections with Wagner's reagent. The alkaloids were located in upper and lower epidermises and hypodermal regions of leaf tissues while in case of rhizome, it is in parenchymatous region of tissues. The occurrence of oils/fats in leaf (Fig.1e.) and rhizome sections (Fig.1f) was revealed by the presence of red and pink coloured spots after treating the sections with Sudan- IV stain.

The oils and fats were located in upper and lower epidermises and hypodermal regions of leaf tissues while in case of rhizome they were mainly found in parenchyma and cortex regions. The confirmation of tannins in leaf (Fig.1g) and rhizome sections (Fig.1.h.) was revealed by the presence of bluish green coloured cells after treating the sections with toluidine blue solution. The tannins were located in the vascular bundle regions of leaf tissues while in case of rhizome tannins were found in epidermis and parenchymatous regions. The presence of lignin in leaf (Fig.1i) and rhizome sections (Fig.1j) was confirmed by the presence of bluish green coloured cells after treating the sections with toluidine blue solution. The lignin was located in the vascular bundle (Sclerenchymatous) regions of the leaf tissues while in case of

rhizome it was found in the epidermis. The presence of pectin in leaf (Fig.1k) and rhizome sections (Fig.1l) was confirmed by the presence of pink to purple coloured cells after treating the sections with toluidine blue stain. The pectin was located in the parenchymatous region of leaf tissue while in case of rhizome it was found in endodermis and cortical regions.

The occurrence of cellulose in leaf (Fig.1m) and rhizome sections (Fig.1n) was revealed by the presence of blue coloured cells after treating the sections with methylene blue stain. The cellulose was located in the upper and lower epidermises of leaf while in case of rhizome it was found in upper epidermis. The presence of carbohydrates in leaf (Fig.1o) and rhizome sections (Fig.1p) was confirmed by the presence of magenta coloured cells after treating the sections with Schiff's reagent. The carbohydrates were located in the parenchymatous regions of the leaf and rhizome tissues. The presence of starch in leaf (Fig.1q) and rhizome sections (Fig.1r) was confirmed by the presence of blue coloured spots after treating the sections with IKI solution. The starch grains were located in the parenchymatous regions of leaf tissues while in case of rhizome it was found in upper epidermis, cortex and endodermal regions.

**Table 1.Histochemical tests for fresh section of leaf and rhizome of *C.neilgherrensis***

| Sl. no | Ergastic contents | Colour | Localization | |
|---|---|---|---|---|
| | | | Leaves | Rhizome |
| 1 | Saponin | Yellow | Parenchymatous regions of the tissue | Cortex regions of the tissue |
| 2 | Alkaloids | Golden yellow | Upper and lower epidermises and hypodermal regions of the tissue | Parenchyma regions of the tissue |
| 3 | Oils and fats | Red and pink | Upper and lower epidermises and hypodermal regions of the tissue | Cortex regions of the tissue |
| 4 | Tannin | Bluish green | Vascular bundle regions of the tissue | Epidermis and parenchymatous regions of the tissue |
| 5 | Lignin | Green | Vascular bundle (scleranchyamatous) regions of the tissue | Upper epidermal regions of the tissue |
| 6 | Pectin | Pink to purple | Parenchymatous regions of the tissue | Endodermis and cortical regions of the tissue |
| 7 | Cellulose | Blue | Upper and lower epidermises and parenchymatous regions of the tissue | Upper epidermis and parenchymatous regions of the tissue |
| 8 | Carbohydrates | Dark pink (or) Magenta | Parenchymatous regions of the tissue | Parenchymatous regions of the tissue |
| 9 | Starch | Blue | Parenchymatous regions of the tissue | Upper epidermis, cortex and endodermal regions of the tissue |

**Fig.1.a**

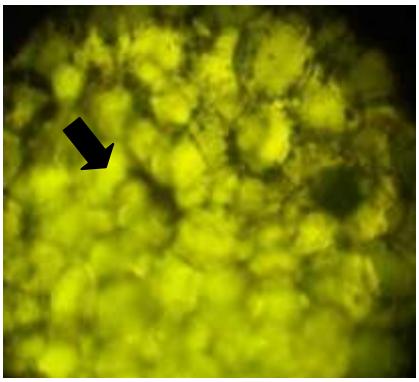


**Fig.1.b**

Saponins

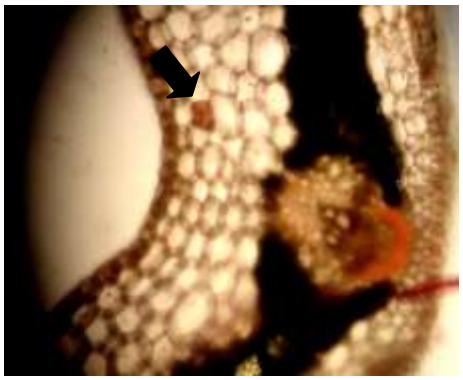


**Fig.1.c**

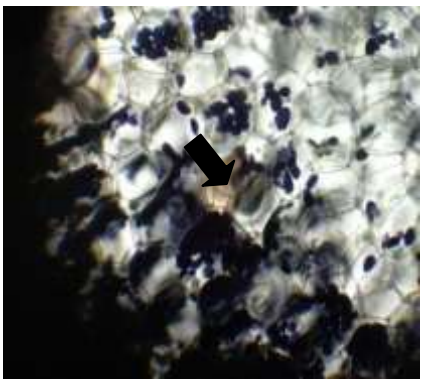


**Fig.1.d**

**A**lkaloids

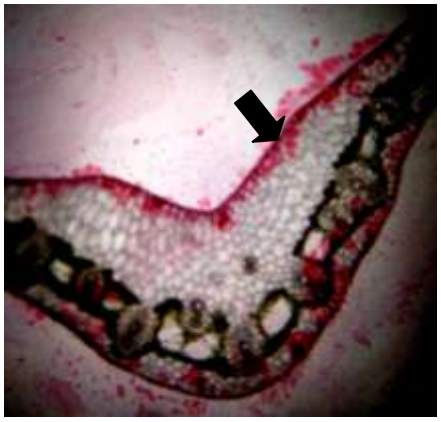


**Fig.1.e**

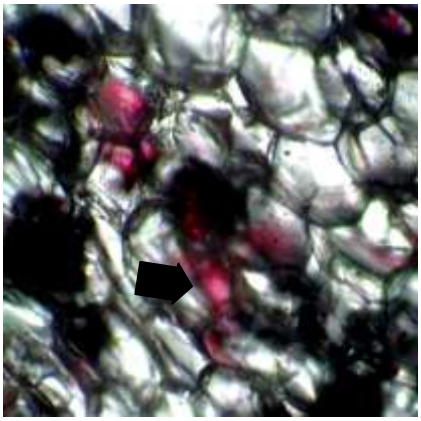


**Fig.1.f .**

Oils/Fat

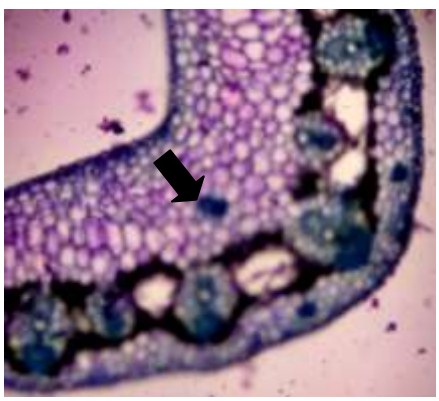


**Fig.1.g**

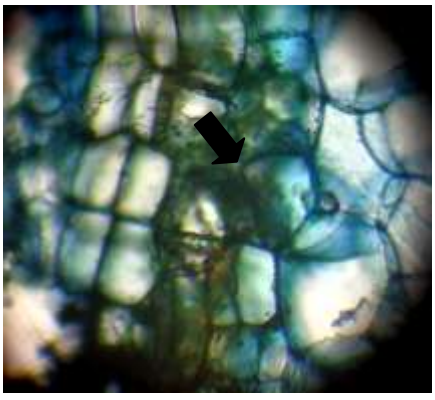


**Fig.1.h**

Tannins

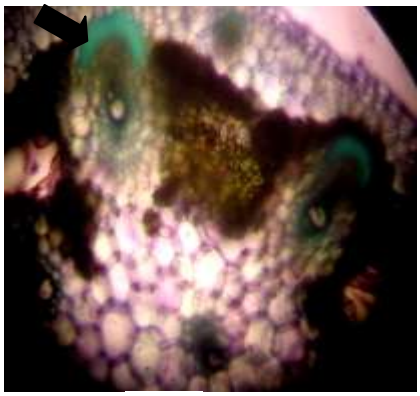


Fig.1i

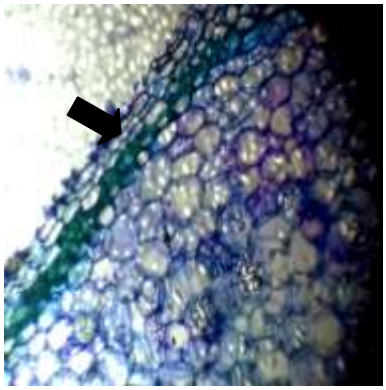


Fig.1j

Lignin

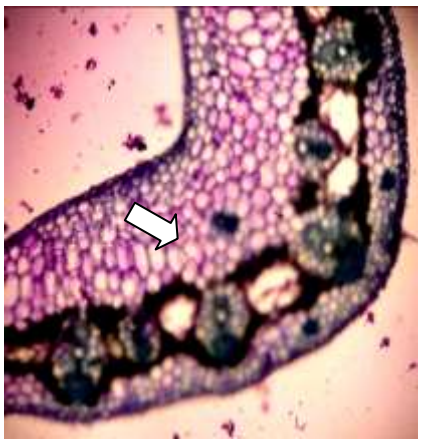


Fig.1k

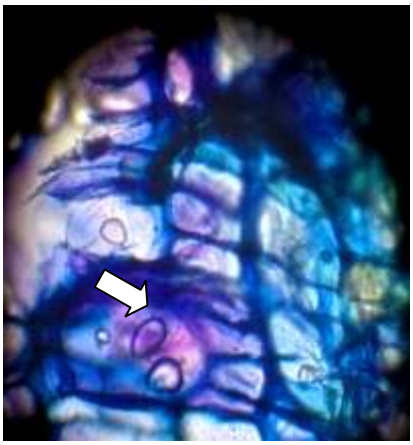


Fig.1l

Pectin

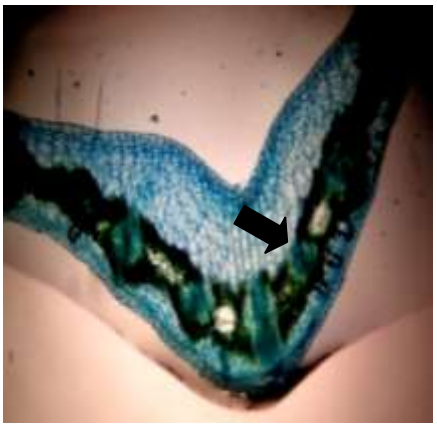


Fig.1m

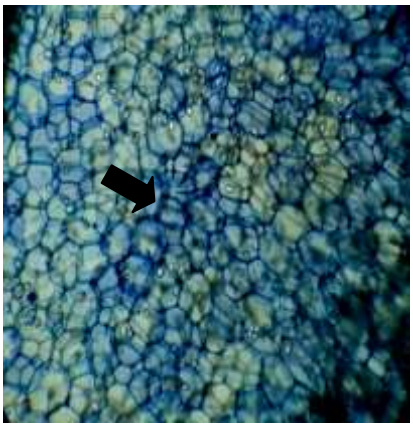


Fig.1n

Cellulose

Fig.1o

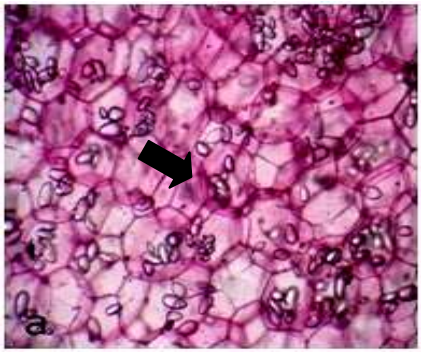


Fig.1p

Carbohydrate

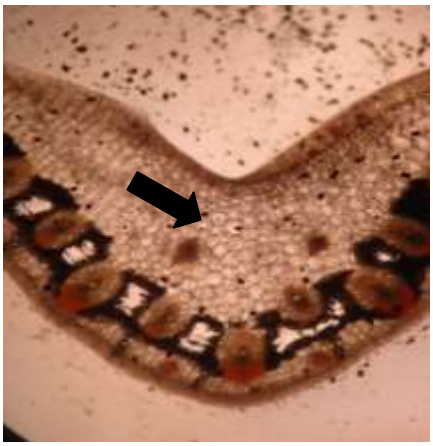
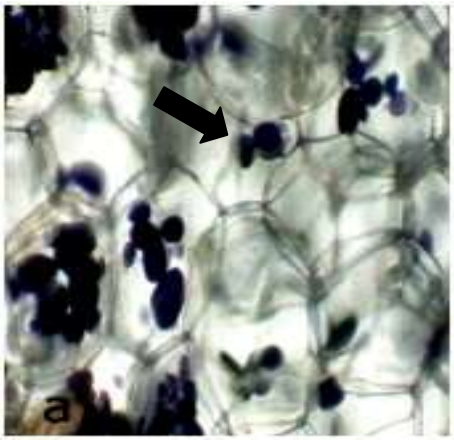


**Fig.1q**



Starch

**Fig.1r.**

## DISCUSSION

Histochemical analysis is one of the valuable standardization processes of quality control of crude drugs to locate the presence of cell contents in the histological zones of the plant organs. Free hand sections of the leaf and rhizome were taken and treated with the respective reagents to confirm the presence of different compounds Viz., saponins, alkaloids, fats and oils, tannins, lignins, pectins, cellulose, starch and carbohydrates in the tissues.

Saponins were found in the parenchymatous regions of leaf and parenchyma and cortex regions of rhizome sections. From the colour reactions it is clearly reported that saponin content in rhizome cells is much higher than leaf cells, which could be attributed to the bitter taste of rhizome as reported earlier [12]. Thus they serve as anti-feed ants and protect the plant from microbes and fungi. Further research is needed to define the role of these natural products in their host organisms, which have been described as "poorly understood" to date [13]. The alkaloid content in plants is usually within a few percent and was located in the upper and lower epidermises and hypodermal regions of leaf and in rhizome it is in parenchymatous regions of tissues. From the colour reaction it was reported that alkaloid content in leaf is more than in rhizome cells. Furthermore, different tissues of the same plants may contain the different alkaloids [14]. Most of the known functions of alkaloids are related to protection. For example, aporphine, alkaloid liriodenine produced by the tulip tree (*Liriodendron tulipifera*) protects it from parasitic mushrooms. In addition, presence of alkaloids in the plant prevents insects and chordate animals from eating it.

Fats are widely distributed in the plant body, and they probably occur in small amounts in every plant cell. The oils and fats were located in upper and lower epidermises and hypodermal regions of leaf tissues while in case of rhizome they were mainly found in cortex regions. As protoplast inclusion, fats are common reserve material in seeds, spores and embryos in meristematic cells and occasionally in differentiated tissue of the vegetable body [15]. They occur as solid bodies or more frequently as fluid droplets of various sizes, either dispersed in the cytoplasm or aggregated in large masses.

The tannins were located in the vascular bundle regions of leaf tissues while in case of rhizome tannins were found in epidermis and parenchymatous regions of tissues. Tannins are particularly abundant in the xylem, in the testa of seeds and in pathological growth like galls. No tissue, however, appears to lack tannins entirely. The tannin compounds are widely distributed in many species of plants, where they play a role in protection from predation, and perhaps also as pesticides and in plant growth regulation. The lignin was located in the vascular bundle (Sclerenchymatous) regions of the leaf tissues while in case of rhizome it was found in the epidermis and parenchymatous regions of tissue. Lignin function is not yet clear but they effectively strengthen bonds to increase stability of water transport vessels and also keep them open to allow the passage of water, dissolved nutrients and waste products from the plant [16].The pectin was located in the parenchymatous region of leaf tissue while in case of rhizome it was found in endodermis and cortical regions of tissues. Pectin constitutes a compound collection of polysaccharides in plant cells and is found in the majority of major cell walls and especially in profusion in all non-woody terrestrial plants. Cellulose is a polymer of glucose monosaccharide that plants use as their primary building material. The cellulose was located in the upper and lower epidermis and parenchymatous regions of the tissues of leaf while in case of rhizome it was found in upper epidermis and parenchymatous regions of tissues. The most important function of cellulose is that it makes up the cell wall in a plant cell and helps the plant to remain rigid and stand upright.

Like all living organisms, plants require energy in chemical form so that they can grow and carry out basic life functions. Plants produce, store and burn carbohydrates in the form of sugar to provide themselves with energy. The carbohydrates were located in the parenchymatous regions of the leaf and rhizome tissues. Carbohydrates serve two main functions in plant, i) energy storage ii) structural components. Carbohydrates in the form of starch and insulin serve as energy storage in fruits and roots or in any other part of the plant

as cellulose which is a polymer of beta-D-glucose and is an important structural component of plant cells, making up a majority of plant cell wall. Starch is the principal ergastic substance of the protoplast. The starch grains were located in the parenchymatous regions of leaf tissues while in case of rhizome it was found in upper epidermis, cortex and endodermal regions of the tissue. The morphometric variation of starch grain is so extensive that they may be used taxonomically and pharmacognostically up to a limited extent. Starch deposition occurs widely in the plant body, but the particularly common places of its accumulation are seeds, the parenchyma of the secondary vascular tissue in stem and roots, tuber, rhizome and corms. The function of starch in plant cells is primarily the storage, and then the releasing of biochemical energy [17]. If a rhizome is separated into pieces, each piece may be able to give rise to a new plant. The plant uses the rhizome to store starch, proteins, and other nutrients. These nutrients become useful for the plant when new shoots are to be formed or after passing through the dormant phase to germinate later.

## CONCLUSION

Upon primary organic analysis of leaf and rhizome, presence of phytoconstituents was revealed. The present study could be useful to carry out further advanced studies to unfold the nature of different classes of phytochemicals present in leaf and rhizome of *C. neilgherrensis* and suitable methods could be adopted for their isolation and characterisation to scrutinise their biological activities.

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