Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 7 [11] October 2018 : 103-109

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.876
Universal Impact Factor 0.9804
NAAS Rating 4.95

ORIGINAL ARTICLE **OPEN ACCESS**

Isolation and Characterization of Phytochemicals From N-Butanol Fraction of *Chlorophytum Tuberosum* baker

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ABSTRACT
India is the largest producer of medicinal plants and is rightly called the "Botanical garden of the World". Medical information referred in the old Indian literatures includes several medicinal herbs, which have been in the use for thousands of years, in one form or the other, under the indigenous system of medicine. In India, 45,000 plant species have been identified, out of which about 15-20 thousand plants are of good medicinal value. Only few medicinal plants have attracted the interest of scientists, to investigate them for a remedy for tumour. Since chemotherapy, radiation, etc. cause severe toxicity, herbal plants have become popular throughout the world nowadays, and are also used as a therapy for tumours or cancer. The antitumour (antineoplastic) activities of medicinal plants need to be explored.
Key words: *Chlorophytum tuberosum, n-butanol, ethyl acetate, chromatography etc.*

Received 28.07.2018 Revised 01.09.2018 Accepted 19.09.2018

INTRODUCTION

A number of herbs belonging to the specie *Chlorophytum* are noted for their medicinal benefits in Ayurvedic, and Unani system of medicine. A lot of medicinally important attributes have been assigned to the plants of this specie. The genus *Chlorophytum* belonging to the family Liliaceae is widely distributed in the pantropical regions. There are almost 215 species that have been reported in the genus *Chlorophytum*. They are perennial rhizomatous herbs. Rhizomes are often short and inconspicuous while roots are usually thicker or slightly fleshy [1]. *Chlorophytum tuberosum* Baker belongs to family Liliaceae, commonly referred as'Safed Musli' is been widely used in the Indian traditional systems of medicine for rejuvenation and instant energy as a 'Rasayana' drug. Traditionally it is used as general tonic, in treating rheumatism apart from having immunomodulating property. The tubers of *Chlorophytum tuberosum* are used as a medicinal expectorant and are used in fever. It is also used in leucorrhoea and also as aphrodisiac [2].
Present investigation reveals the presence of phytochemicals with its isolation and characterization.

MATERIAL AND MATERIALS [3-5]

The present material were collected, identified and extract out by using suitable solvent. The n butanol fraction was taken for the isolation of phytoconstituents and further characterization. The solvents required were n- hexane, Benzene, n-butanol, column for chromatography, TLC plates etc.

Methods

3.1 Thin layer chromatography (TLC) of n-butanol fraction of *C. tuberosum*-[6]
Thin layer chromatography was performed to select mobile phase for separation of phytoconstituents by column chromatography and the chromatographic conditions were as-

Chromatographic conditions: **[7]**

- Stationary phase : TLC aluminum sheets coated with silica gel-G
- Mobile phase : Different combination of Benzene, n-butanol, Methanol
- Length of run : 6.2 cm
- Chamber saturation : 30 min

- Visualizing agent : UV (254nm, 365nm) and Vanillin-H_2SO_4reagent

Column chromatography of n-butanol fraction of *C. tuberosum* [8]

Fractions from the n-butanol fraction of *C. tuberosum* were collected by column chromatography. Height and diameter of column was 21, 2.0 cm respectively with the stationary phase Silica for column chromatography (#60-120). The solvent system used for the same was n-hexane: Benzene: n-butanol. Each fraction was about 10mL with Flow rate: 8-10 drops/Min. No. of fractions collected were 66 in nos.

Table1: Fractions Obtained from Column Chromatography of n-butanol Fraction of *C. tuberosum*

Sr. No	Mobile Phase	Fraction	TLC pattern with anillin-H_2SO_4
1	Benzene	1-5	No spot
2	Benzene	6-13	Mixture of 2 spots
3	Benzene : n-butanol(9:1)	14-20	1 spot
4	Benzene : n-butanol (8:3)	21-26	Mixture of 3 spots
5	Benzene : n-butanol (1:1)	27-36	Mixture of 2 spots
6	Benzene : n-butanol (2:8)	37-45	Mixture of 2 spots
7	n-butanol	46-51	Mixture of 4spots
8	n-butanol: Methanol (8:2)	52-55	Mixture of 2 spots
9	n-butanol: Methanol (1:1)	56-61	Mixture of 3 spots
10	Methanol	61-66	

Further, Compound of fraction 14-20 was purified, designated as **CTB-1.** Again Fraction 27-36 collected which shows 2 spot, further purified with column chromatography.

Recolumn chromatography of fractions 27-36:

For the Recolumn chromatography of fractions 27-36the specifications of the column used are as -Height of column: 13 cm, Diameter of column: 1.2 cm, Stationary phase : Silica for column chromatography (#60-120). Mobile phase: Benzene: n-butanol (8:2) Elution: Isocratic elution, Fractions quantity: 10mL, Flow rate: 6-7 drops per minute

Table 2: Fractions of Recolumn Chromatography of Fraction 27-36

Sr. No.	Fraction	TLC pattern with Vanillin- H_2SO_4
1	1-3	No spot
2	4-10	Single spots
3	11-14	Mixture of 2 spots

Compound of fraction 4-10 was purified, designated as **CTB-2** and also separated from fraction 11-14 using Preparative TLC.

RESULTS

Characterization of CTB-1isolated from n-butanol fraction of *Chlorophytum tuberosum*

TLC Data of CTB-1

Table 3: TLC Data of CTB-1

Rf	Visually	Detection by UV (254nm)	Detection by Vanillin H_2SO_4 reagent
0.78	Colourless	Blue	Violet

The chromatographic specifications for TLC of CTB-1 are as- Stationary phase: Silica gel GF ^{254,} Mobile phase: Benzene: n-butanol (8:1),Length of run: 6.1cm,Time for Chamber saturation: 30min, Visualizing agent- : UV (254nm), Vanillin- H_2SO_4reagent.

Colour: White waxy powder Odor: Odorless

Solubility: Freely soluble in methanol and n-butanol Melting Point:164-166°

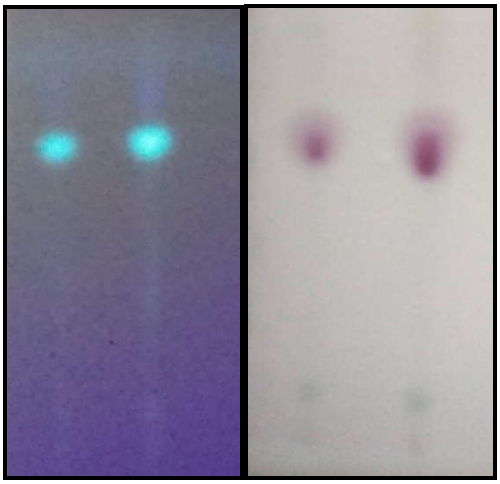


Figure 1: TLC of CTB-1

UV-Vis Spectrum of CTB-1 at257 nm

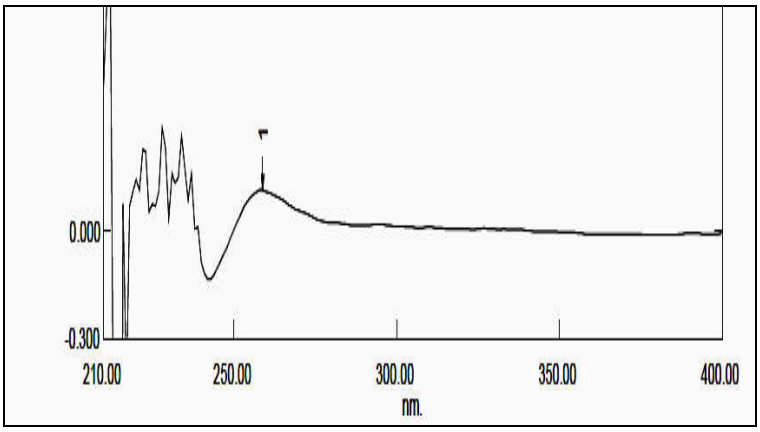


Figure 2: UV-Vis Spectrum of CTB-1

IR Spectra of CTB-1:

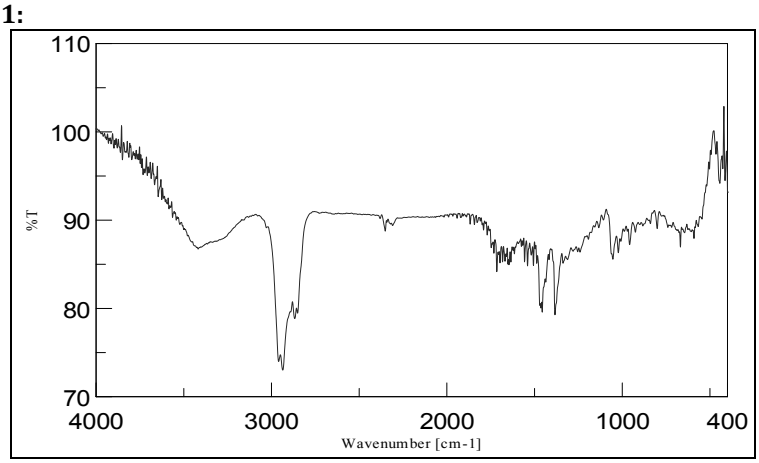


Figure 3: FTIR Spectra of CTB-1

Table 4: Interpretation of FTIR Spectra of CTB-1

Frequency(Cm^{-1})	Assignment
3480	O - H stretch
2976, 2855	C-H stretch alkane
1657	C = C stretch. Non-conjugated
1443	CH_3, C – H bending
1265	O – H bending
1230	C–O stretch
1179	C – C stretching

^{1}H NMR SpectraCTB-1

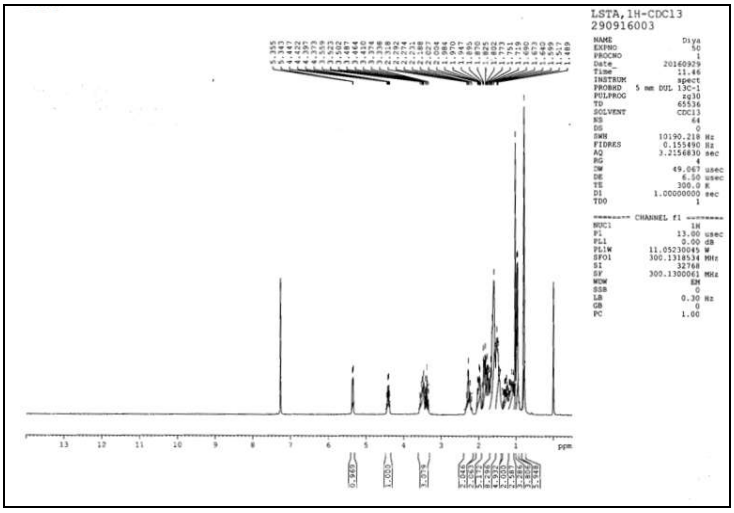


Figure 4: H^{1}NMR Spectra CTB-1

Mass Spectra of CTB-1

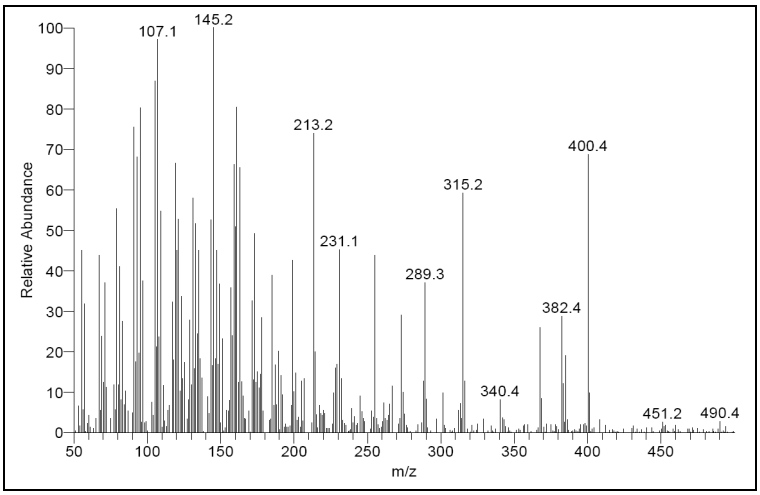


Figure 5: Mass Spectra of CTB-1 (Molecular ion peak: 400

Characterization of CTB-2isolated from n-butanol fraction of *Chlorophytum tuberosum*

TLC Data of CTB-2

Table 5: TLC Data of CTB-2

Rf	Visually	Detection by UV (254nm)	Detection by Vanillin H_2SO_4 reagent
0.32	Colourless	Blue	Violet

The chromatographic specifications for TLC of CTB-2 are as- Stationary phase : Silica gel GF ^{254,} Mobile phase: Benzene: n-butanol (4:6),Length of run: 6.2cm,Time for Chamber saturation: 30 min, Visualizing agent- : UV (254nm), Vanillin- H_2SO_4 reagent.

Colour: Cream amorphous powder Odor: Odorless

Solubility: Freely soluble in methanol and n-butanol Melting Point:232-234°C

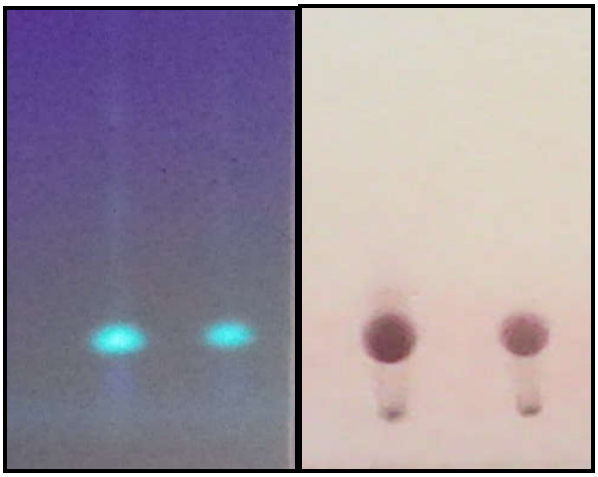


Figure 1: TLC of CTB-2

UV-Vis Spectrum of CTB-2 at257 nm

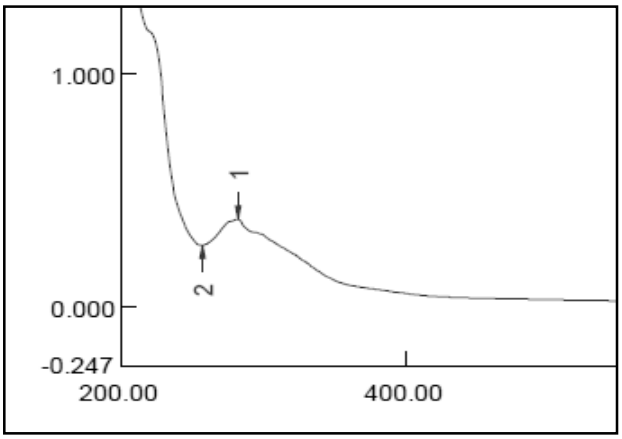


Figure 2: UV-Vis Spectrum of CTB-2

FTIR Spectra of CTB-2:

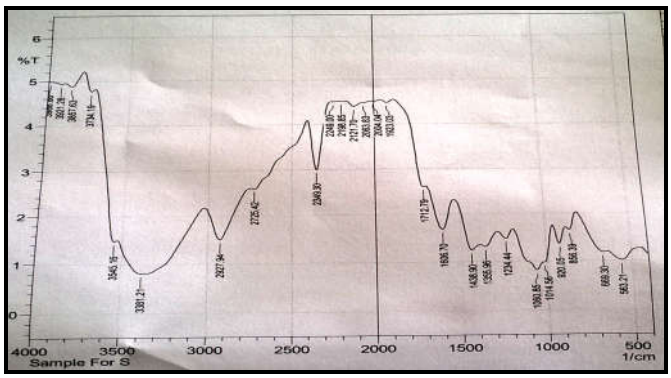


Figure 3: FTIR Spectra of CTB-2

Table 6: Interpretation of FTIR Spectra of CTB-2

Frequency(Cm^{-1})	Assignment
3381,3445	O - H stretch
2927,	C-H stretch alkane
1712, 1606	C = C stretch. Non-conjugated
1438, 1355	CH_3, C – H bending
1234	C–O stretch
1179	C – C stretching

H[1] NMR SpectraCTB-2:

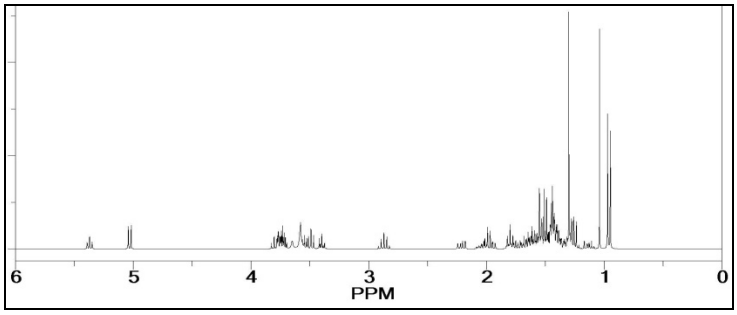


Figure 4: H[1]NMR Spectrum CTB-2

Mass spectra of CTB-2

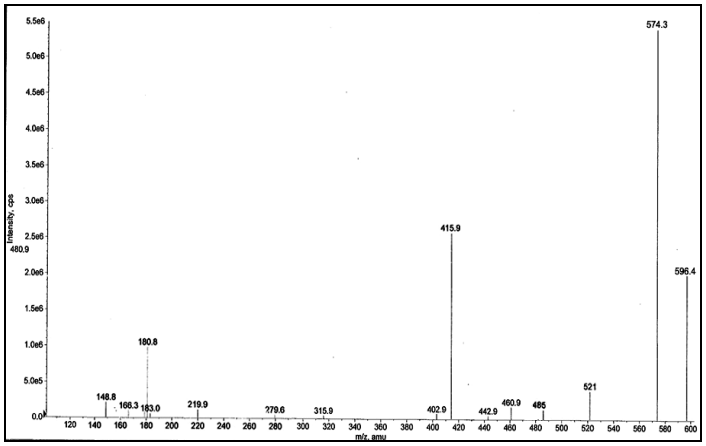
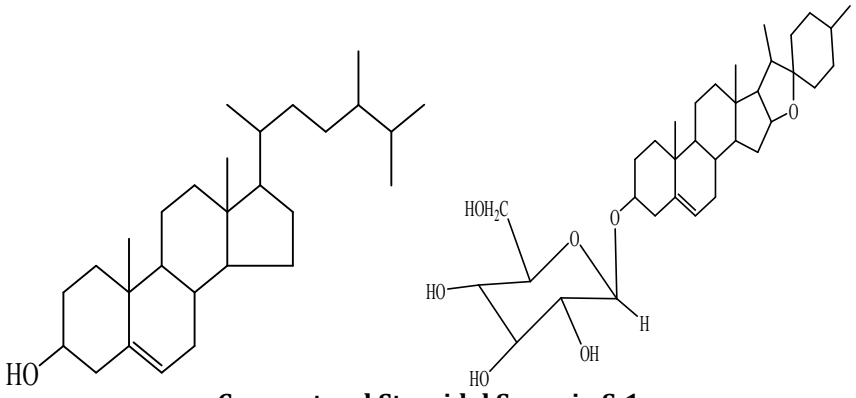


Figure 5: Mass Spectrum of CTB-2

m/z=596.4, 574.3, 521, 485, 460.9, 415.9, 219, 180, 148.

Proposed structure of isolated phytoconstituents from *Chlorophytum tuberosum*

A) CTB-1:B) CTB-2



Campesterol Steroidal Saponin S-1

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CITATION OF THIS ARTICLE

Chittam K. P., Deshmukh T. A.Isolation and Characterization of Phytochemicals From N-Butanol Fraction of C*hlorophytum Tuberosum* baker. Bull. Env. Pharmacol. Life Sci., Vol 7 [11] October 2018: 103-109