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The phytochemical screening, total cucurbitacin content, and in vitro anti-breast cancer activity of *Leucopaxillus gentianeus* mushroom

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Abstract

Background The popular Leucopaxillus gentianeus mushroom contains very high nutrients and bioactive compounds with good anti-breast cancer activity. The til oil extract seems to be the most active in preparation. This study aims to find the best extract using different solvents for extraction, to measure the total cucurbitacin content and anti-breast cancer activity in vitro of til oil extract of leucopaxillus gentianeus.

Result The dry mushroom material was extracted using continuous hot extraction with til oil, petroleum ether, ethyl acetate, chloroform, methanol, and water of leucopaxillus gentianeus which were used for phytochemical analysis, HPLC method was used for no of phytochemical and anti-breast cancer activity in vitro. The total cucurbitacin content was found based on the HPTLC method. The anti-breast cancer activity was carried out using progesterone and estrogen activity. The number of pecks found during HPLC it is indicated that the list of phytochemical presents in a different extract, also good yield found with til oil extract was 6.8 gm. Progesterone and estrogen inhibited high with til oil extract and cucurbitacin content was found to be 264.00 ng.

Conclusion The significance of the biotherapeutic effects increases with the number of bioactive components in the preparation. Leucopaxillus gentianeus til oil extract has high cucurbitacin content and strong anti-breast cancer properties.

Keywords Bitter false funnel cap, Breast cancer, Cucurbitacins, Tricholomataceae, HPTLC, HPLC

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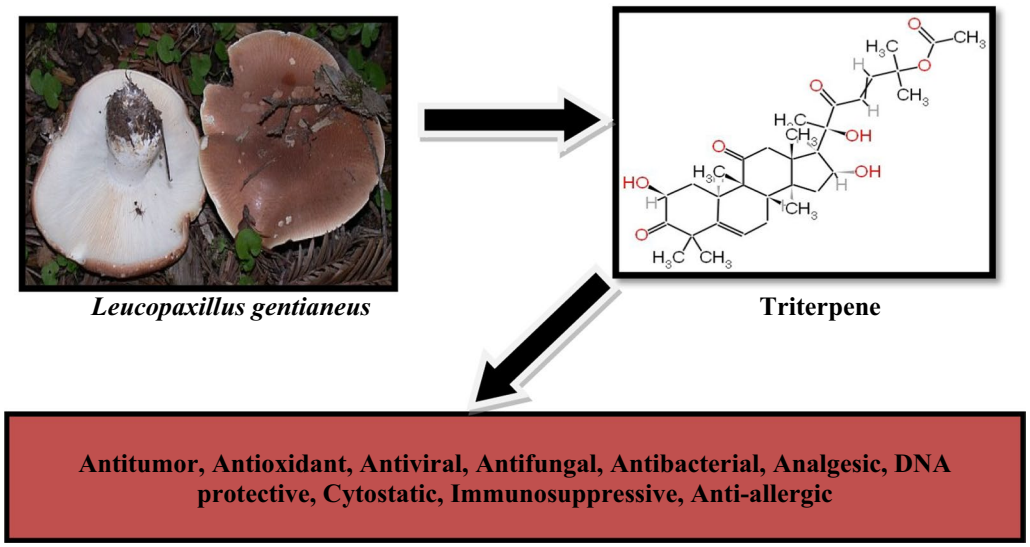
Graphical abstract



Background

The lining cells (epithelium) of the ducts (85%) or lobules (15%) in the glandular tissue of the breast are where breast cancer begins to develop. The malignant development is initially contained within the duct or lobule ("in situ"), where it often exhibits no symptoms and has a low risk of spreading (metastasis) [1].

These in situ (stage 0) tumors may develop over time and infect the breast tissue around them (invasive breast cancer), then disseminate to neighboring lymph nodes (regional metastasis), or other body organs (distant metastasis). Widespread metastasis is the cause of breast cancer deaths in women [1].

Scope of the problem [1]

In 2020, 685 000 people worldwide died and 2.3 million women were diagnosed with breast cancer. Breast cancer will be the most common cancer in the world by the end of 2020 when 7.8 million women will still be living who had received a diagnosis within the previous five years. In comparison to other cancer types, breast cancer causes more women to lose disability-adjusted life years (DALYs). In every nation in the world, women can develop breast cancer at any age after puberty, but rates rise as people age [1]. If used over a long period of time with minimal side effects, my research's novel approach to mushrooms lowers the likelihood that cancer will occur again.

Mushrooms have been regarded as gourmet cuisine across the globe since antiquity for their unique taste and subtle flavor. Recently, it has been discovered that many mushroom species are miniature pharmaceutical factories producing hundreds of novel constituents with miraculous biological properties. They have a long history of use in Oriental medicine, but their legendary effects on the promotion of good health and vitality are being supported by contemporary studies only. Of late, mushrooms have emerged as a wonderful source of nutraceuticals, antioxidants, anticancer, prebiotic, immunomodulating, anti-inflammatory, cardiovascular, antimicrobial, and anti-diabetic [1-4].

Mushrooms are the fruiting bodies of some members of a lower group of plants known as fungi. The fungi are characterized by the absence of chlorophyll and undifferentiated bodies except for the spore-bearing structures. The fruiting bodies, of mushrooms, are fleshy spore-bearing structures of the fungi. They contain numerous spores, functionally similar to seeds of the higher plants for the propagation of fungi. Mushrooms appearing after rains in various shapes, sizes, and colors have fascinated human beings since time immemorial and were sure to draw the attention of humans even when they were living as hunters and gatherers. Even though man started agriculture 10,000 years ago, the cultivation of mushrooms is a relatively new phenomenon and has picked up across the globe only in the last century which has witnessed newer innovations and applications. The

Chinese were reportedly the first to artificially cultivate tropical and subtropical mushrooms thousands of years back but commercial production started in Europe with button mushrooms in caves during the sixteenth and seventeenth centuries. The mushroom cultivation then made its way to the USA. The economic importance of mushrooms lies primarily in their use as food for human consumption. The exotic flavor, taste, and fleshiness of mushrooms have made them an important delicacy in the human diet. Mushroom is considered to be a complete, healthy food and suitable for all age groups. Though the nutritional value is determined by the type, stage of development, and other environmental conditions, mushrooms are rich in proteins, dietary fiber, vitamins, and minerals. They have insignificant lipid levels and a high proportion of polyunsaturated fatty acids resulting in low calorific value. The protein content, though varies greatly in different mushrooms, is usually high. Mushrooms are an excellent source of vitamins especially C and B (Folic acid, Riboflavin, Niacin, and Thiamine), and minerals like potassium, sodium, and phosphorus. It also contains other essential minerals like Cu, Zn, and Mg in traces. Mushrooms are also known to have medicinal values as these have been shown to promote immune function, boost health, lower the risk of cancer-inhibiting tumor growth, and support the body's detoxification mechanism. Mushroom thus has great potential for the production of quality food [5].

The productivity of mushrooms is higher than any other crop. Food, nutritional and medicinal values apart, mushroom growing can be an efficient means of waste disposal mainly agricultural wastes since it can use the wastes as a medium of growth thus mushroom cultivation is considered eco-friendly. Further, mushroom cultivation is highly labor-intensive which can help gainful employment for unemployed youths in rural and semi-urban areas. The activity also requires comparatively less land area [5].

The potential of the mushroom crop is rated high on account of the following reasons:-

- Availability of cheap and abundant raw materials and labor [5].
- Appropriate technology for small and Hi-Tech growers [5].
- Demand–supply gap in the world trade of mushrooms [5].
- Fall in production in many European countries due to high labor costs [5].
- Proximity to the Middle East, where the market is growing [5].
- Domestic market is also growing which can absorb the non-exportable surplus [5].

Fig. 1 *Leucopaxillus Gentianeus* is a medium-sized, striated, dull reddish-brown fungus that is inedible. As it creates rings and arcs beneath conifers, the environment resembles that of the Monterey cypress. The length and thickness of the stripes are 4 to 8 cm and 1 to 2.5 cm, respectively. It typically tastes nasty and has a very bad odor

Leucopaxillus is an inedible medium-sized mushroom, with a dull reddish-brown, striated cap. The habitat resembles that of the Monterey cypress, as it forms rings and arcs under conifers. The stripe height ranges from 4–8 cm in length and 1–2.5 cm in thickness. It usually has a very unpleasant odor and a bitter taste. The spores are nearly round and warted and their size ranges from 3.55 -to 4.56 µm [6] (Fig. 1).

In the future, this property can be a potential source for new drugs to treat various bacterial infections. Therefore, the present study can serve as an upcoming avenue for the pharmaceutical industry [6].

Overall, this article aims to provide an upgrade on the *Leucopaxillus gentianeus*, as well as Phytochemical Composition, phytochemical screening, pharmacological effects, and Modern and Ayurvedic, Uses for breast cancer.

Methods

Mushroom materials

Procurement and authentication of mushroom material

The fruiting body of Leucopaxillus gentianeus was obtained in October 2021 from a rural location in Maharashtra's Sangli District and verified by Dr. M. V. Kale, the head of the botany department at Jaysingpur College in Jaysingpur, Maharashtra, India.

Collection and processing of mushroom material

The harvested mushroom material was naturally dried in the shade and put through an electrical grinder to reduce its size. The resulting powder was sieved before being employed in a further extraction procedure.

Chromatographic and chemical used

Shimadzu HPLC is comprised of one pump, ultraviolet detector, and LC software to analyze the sample. A mixture of Methanol and water (80:20) was used as a mobile phase. This solution was degassed in a sonicator during the pre-examination phase. The used column for analysis was the C18 column and C8, with the protective column. The flow rate was adjusted at 0.8 ml/min, injecting 10 µl of samples by auto-sampler. All extract samples were detected by a UV detector at 230 nm wavelength. The room temperature was below 25 °C.

Ethanol, Petroleum ether, Chloroform, and Ethyl acetate were obtained from Merck, and progesterone and estrogen were obtained from Sigma Chemical Co. (Sigma-Aldrich). All other chemicals and solvents were in analytical grade.

Extraction

Preparation of mushroom extract

The continuous hot Soxhelation method was used for successive extraction of the fruiting body of *Leucopaxillus gentianeus* with Til oil, Pet. Ether, Chloroform, Ethyl Acetate, Methanol, and Water, according to their polarity.

Extraction of the fruiting body of Leucopaxillus gentianeus by using Til oil

100 g powder of dried fruiting body of *Leucopaxillus gentianeus* was packed in a Soxhlet extractor. 1000 ml of til oil was added to the round bottom flask and the maintained temperature was 90–100 °C by using a heating mantle. Extraction was carried out with til oil for several cycles till a drop of solvent from the syphon tube did not leave a greasy spot on the filter paper after evaporation. It took approx. 15 cycles and it requires approx. 4 days. After completion of the process, the marc was taken out from the extractor and was spread as a bed on a clean filter paper and naturally dried till the whole solvent got evaporated. 93 g of dried marc was obtained and used for further extraction with petroleum ether solvent.

Extraction of the fruiting body of Leucopaxillus gentianeus by using Petroleum ether

93 g powder of dried fruiting body of *Leucopaxillus gentianeus* was packed in a Soxhlet extractor. 1000 ml of petroleum ether was added to the round bottom flask and the maintained temperature was 90–100 °C by using a heating mantle. Extraction was carried out with petroleum ether for several cycles till a drop of solvent from the syphon tube did not leave a greasy spot on the filter paper after evaporation. It took approx. 8 cycles and it requires approx. 35 h. After completion of the process, the marc was taken out from the extractor and was spread as a bed on a clean filter paper and naturally dried till the whole solvent got evaporated. 88 g of dried marc was obtained and used for further extraction with chloroform solvent.

Extraction of the fruiting body of Leucopaxillus gentianeus by using Chloroform

88 g powder of dried fruiting body of *Leucopaxillus gentianeus* was packed in a Soxhlet extractor. 1000 ml of chloroform was added to the round bottom flask and the maintained temperature was 90–100 °C by using a heating mantle. Extraction was carried out with chloroform for several cycles till a drop of solvent from the syphon tube did not leave a greasy spot on the filter paper after evaporation. It took approx. 10 cycles and it requires approx. 41 h. After completion of the process, the marc was taken out from the extractor and was spread as a bed on a clean filter paper and naturally dried till the whole solvent got evaporated. 83 g of dried marc was obtained and used for further extraction with ethyl acetate solvent.

Extraction of the fruiting body of Leucopaxillus gentianeus by using Ethyl acetate

83 g powder of dried fruiting body of *Leucopaxillus gentianeus* was packed in Soxhlet extractor. 1000 ml of ethyl acetate was added to the round bottom flask and the maintained temperature was 90–100 °C by using a heating mantle. Extraction was carried out with ethyl acetate for several cycles till a drop of solvent from the syphon tube did not leave a greasy spot on the filter paper after evaporation. It took approx. 10 cycles and it requires approx. 41 h. After completion of the process, the marc was taken out from the extractor and was spread as a bed on a clean filter paper and naturally dried till the whole solvent got evaporated. 79 g of dried marc was obtained and used for further extraction with methanol solvent.

Extraction of the fruiting body of Leucopaxillus gentianeus by using Methanol

79 g powder of dried fruiting body of *Leucopaxillus gentianeus* was packed in a Soxhlet extractor. 1000 ml of methanol was added to the round bottom flask and the maintained temperature was 90–100 °C by using a heating mantle. Extraction was carried out with methanol for several cycles till a drop of solvent from the syphon tube did not leave a greasy spot on the filter paper after evaporation. It took approx. 13 cycles and it requires approx. 54 h. After completion of the process, the marc was taken out from the extractor and was spread as a bed on a clean filter paper and naturally dried till the whole solvent got evaporated. 74 g of dried marc was obtained and used for further extraction with water solvent.

Extraction of the fruiting body of Leucopaxillus gentianeus by using Water

74 g powder of dried fruiting body of *Leucopaxillus gentianeus* was packed in Soxhlet extractor. 1000 ml of water was added to the round bottom flask and the maintained temperature was 90–100 °C by using a heating mantle. Extraction was carried out with water for several cycles till a drop of solvent from the syphon tube did not leave a greasy spot on the filter paper after evaporation. It took approx. 12 cycles and it requires approx. 48 h. After completion of the process, the marc was taken out from the extractor and was spread as a bed on a clean filter paper and naturally dried till the whole solvent got evaporated. 66 g of dried marc was obtained.

Distillation of mushroom Extract

After completion of the extraction process solvent was removed by distillation and the concentrated extract obtained was dried naturally.

Determination of anti-breast cancer activity

HPLC method for mushroom extract

Using HPLC to check extracts purity and efficacy for cancer activity, 1 mg of each extracted sample dissolve in respective solvent extract and run in HPLC.

Pharmacological Activity of mushroom extract

Progesterone and estrogen activity with each mushroom extract Progesterone and estrogen activity was studied as per different review literature, briefly; 1 mg/ml extract mix with 1 mg/ml concentration of progesterone and estrogen solvent. The reaction was done using the sonication method and absorbance was measured at 254 nm. The control was without any extract used as a positive control. Progesterone and estrogen activity was checked based on the change in peak height.

Screening of cucurbitacin content using TLC and HPTLC

TLC

Analyses using thin-layer chromatography (TLC) for both samples Til Oil extract were examined using TLC. Using the dimensional ascending approach, the TLC analysis was performed. Scissors were used to cut a 20×20 cm TLC plate covered with silica gel 60G F254 (Merk, India) into a 14×3 cm form. After that the plate was lightly chalked with a pencil 1.5 cm away from the bottom and top.

The sample was placed on the TLC plate using glass capillaries at the bottom line that had been pencil-marked. The sample was then loaded once more until a black spot was formed, after which the plate was dried in the fume hood. Then, 20 ml of the solvent (81:11:8) ethyl acetate, methanol, and water was added to the chamber. The plate was inserted into the top of the chamber's liner. Plates were utilized to find the spots after being dried in the fume hood following the run.

Detection of the spot:

After drying each plate, spots were found using UV light at 254 and 289 nm. The retention factor (R f) represented how the active chemical moved.

R_f = Distance traveled by solute/Distance traveled by the solvent

HPTLC

Preparation of standard solution 10 mg of precisely weighed cucurbitacin B was dissolved in 10 ml of methanol to create a stock solution of cucurbitacin B (1 mg/ml). A standard solution of cucurbitacin B (40 µg/ml) was created by further diluting the stock solution with methanol. The HPTLC method's working standard was this concentration.

Sample preparation 100 mg of Luffa echinata and Leucopaxillus gentianeus dry fruit and fruiting body powder extracted with til oil was precisely weighed into a volumetric flask of 50 ml. It was sonicated into suspension in 10 ml of methanol. This solution was pipette into a 10 ml volumetric flask in a volume of 2 ml, and 8 ml more methanol was added to make a total volume of 10 ml. This led to the preparation of the sample's stock solution, which had a concentration of 0.4 mg/ml (0.4 g/l). The measurement of cucurbitacin B from the dried fruit powder of the plant material was taken using this concentration. A 0.22 µ membrane filter from Millipore was used to filter all samples.

Instrumentation and chromatographic conditions In this investigation, the stationary phase was made up of HPTLC aluminum plates that had been pre-coated with silica gel F60 254 and measured 20×10 cm with a 200 m thickness (E. Merck, Germany). 254 Before chromatography, the plates were pre-washed with methanol and activated at 110 C for 10 min. Using a Camag Linomat V (Switzerland) sample applicator and a Camag 100 µl syringe, the samples were spotted in the shape of 8 mm bands. The two bands were separated by 12 mm at a constant application rate of 100 nl s°. With a scanning speed of 20 mm/second and a data resolution of 100 m/step, the slit dimension was retained at 6 mm 0.45 mm. The composition of the mobile phase was: ethyl acetate: methanol: and water. A twin-trough glass chamber filled with the mobile phase and used to conduct the linear ascending development. Optimization of chamber satu-

ration. The mobile phase lasted for 30 min at 25 2 °C (room temperature). The chromatogram runs measured 80 mm in length. The plate was then let to air dry at ambient temperature. On the HPTLC plates, the separated bands were scanned from 200 to 400 nm in wavelength. The tungsten lamp was used as the radiation source. At 289 nm, the greatest absorption was discovered. The pictures were taken with the CAMAG Linomat 5 "Linomat5_171103" S/N 171103 (1.00.12) software.

Calibration curve of cucurbitacin B Cucurbitacin B (0.4 g/l) was produced as a stock solution in methanol. To obtain concentrations of 40, 60, 100, 120, 160, 200, and 240 ng of cucurbitacin per spot, different volumes of standard solution were spotted on the HPTLC plate. Least-square regression was applied to the data of the peak areas plotted against the corresponding concentrations.

Specificity The standard medication and extract were examined to determine the method's specificity. By contrasting the Rf values, the presence of cucurbitacin B in the sample was confirmed. Comparing the spot's measurements and spectrum to the standard. By comparing the spectra at three different levels, namely the peak start (S), peak apex (M), and peak end (E) positions of the spot, the peak purity of the cucurbitacin was determined.

Statistical analysis

All data on anti-progesterone and anti-estrogen activity tests were the average of triplicate analyses. Data were recorded as mean ± standard deviation. Significant differences between means were determined by the Student's test, *p* values.

Result

Based on a thorough literature assessment of published pharmacognostic and phytochemical characteristics, the mushroom was carefully chosen. The material from the mushrooms was gathered and verified. Both a qualitative and quantitative estimation of the active ingredient was made. An approximation of the amount of a certain constituent present in the sample may frequently be made using the amount of extractive, drug yield to a particular solvent. The nature and quantity of a crude drug's ingredients are revealed by its extractive values (Table 1).

Table 1 Successive solvent extractive value of mushroom

Plant	Solvent used	Color and Consistency	% Yield w/w
Luffa echinata	Til Oil	Light Brown mass	6.8 gm
	Petroleum ether	Yellows Brown mass	2 gm
	Chloroform	Light Brown mass	2.2 gm
	Ethyl acetate	Brown mass	3.2 gm
	Methanol	Dark brown mass	3.9 gm
	Water	Dark brown mass	4 gm

RP-HPLC and graph of extracts

Since it enables the purification of the majority of compounds, including those present in diverse herbal products, RP-HPLC is frequently the most advantageous option for assessing, attempting to separate, and identifying chemicals from a complicated combination (Fig. 2, Tables 2, 3, 4, 5, 6).

Progesterone and estrogen activity

If breast cancer cells are removed during a biopsy or surgery, the presence of specific proteins that are estrogen or progesterone receptors will be examined. The hormones progesterone and estrogen promote the growth of cancer when they bind to these receptors. Consequently, it was discovered that leucopaxillus gentianeus inhibits progesterone and estrogen activities (Figs. 3, 4, Tables 7, 8).

TLC

Development of the optimum mobile phase

Using TLC methods, the extract's mobile phase was produced. Standard and test samples were spotted on the TLC plates using several solvent solutions to get a good separation. The standard had an excellent resolution of 0.70 when it was employed, which was a mixture of ethyl acetate, methanol, and water in a ratio of 81:11:8. Well-defined spots were created when the chamber was saturated with the mobile phase for 30 min in the room temperature.

The R f values of the standard and the plant extract were compared. The HPTLC plate images and the selected solvent system are shown a little later in the text (Table 9).

HPTLC

The existence of cucurbitacin metabolites in varying quantities was confirmed by the HPTLC fingerprint results for the Til Oil extract of Leucopaxillus gentianeus (Table 10).

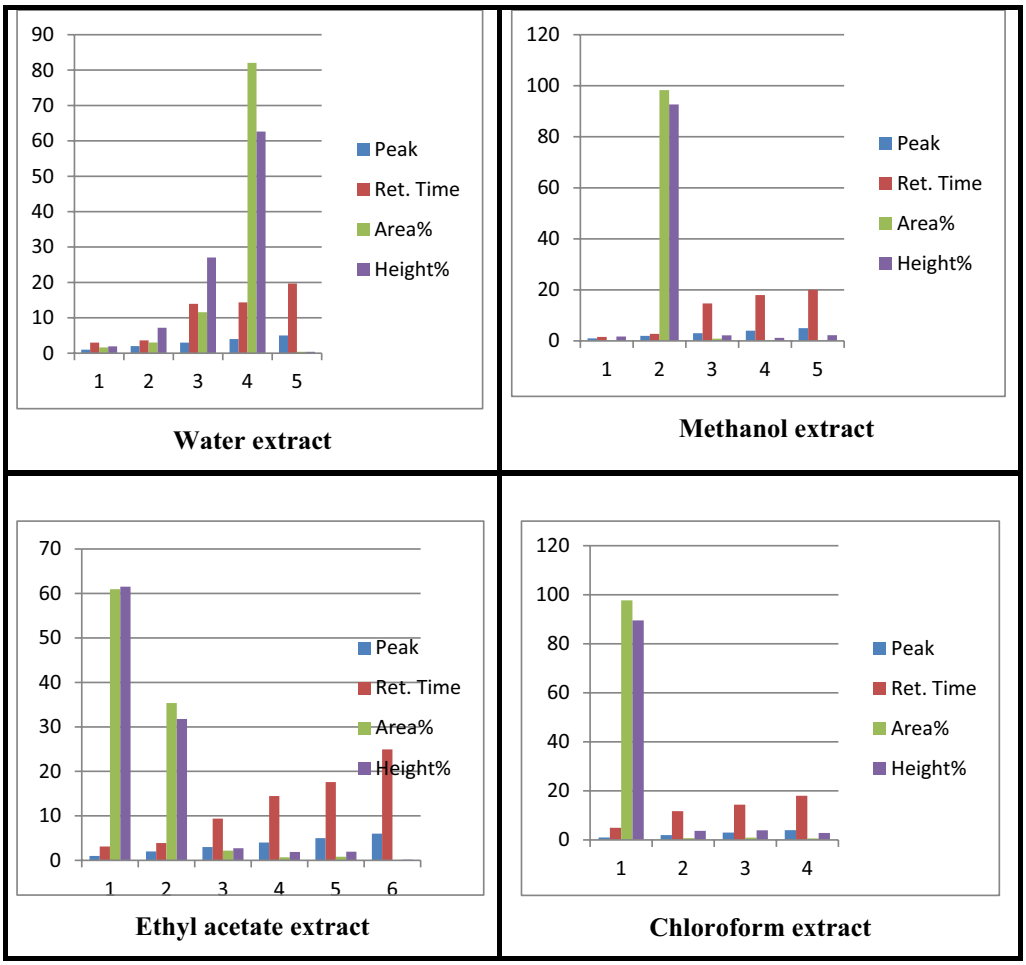


Fig. 2 Comparison of different extracts of *Leucopaxillus gentianeus*

Table 2 No of a constituent in Petroleum ether extract

Peak	Ret. Time	Area%	Height%
1	1.793	18.069	18.341
2	2.778	29.095	29.641
3	4.506	20.749	19.219
4	12.204	17.041	14.620
5	14.503	15.046	18.180

Table 3 No of a constituent in Chloroform extract

Peak	Ret. Time	Area%	Height%
1	4.928	97.680	89.529
2	11.742	0.725	3.734
3	14.394	0.956	3.904
4	17.995	0.640	2.833

Table 4 No of a constituent in Ethyl acetate extract

Peak	Ret. Time	Area%	Height%
1	3.116	60.952	61.537
2	3.899	35.371	31.773
3	9.381	2.172	2.709
4	14.454	0.678	1.876
5	17.602	0.797	1.949
6	24.946	0.030	0.155

Table 5 No of a constituent in Methanol extract

Peak	Ret. Time	Area%	Height%
1	1.543	0.218	1.693
2	2.770	98.294	92.681
3	14.691	0.872	2.203
4	17.954	0.234	1.192
5	19.983	0.382	2.232

Table 6 No of a constituent in water extract

Peak	Ret. Time	Area%	Height%
1	2.974	1.651	1.952
2	3.639	3.021	7.192
3	13.935	11.578	27.034
4	14.360	82.027	62.649
5	19.676	0.383	0.410

On silica gel, the HPTLC fingerprinting of the standard cucurbitacin fractions was produced. Using anisaldehyde sulfuric acid reagents, the standard sample fraction had the greatest amount of UV active chemicals. It was subsequently identified at 289 nm, following visualization (Fig. 5a–g; Table 11). Table 11 displays solvent systems utilized for cucurbitacin fingerprinting, the number of spots present in each, and their associated values.

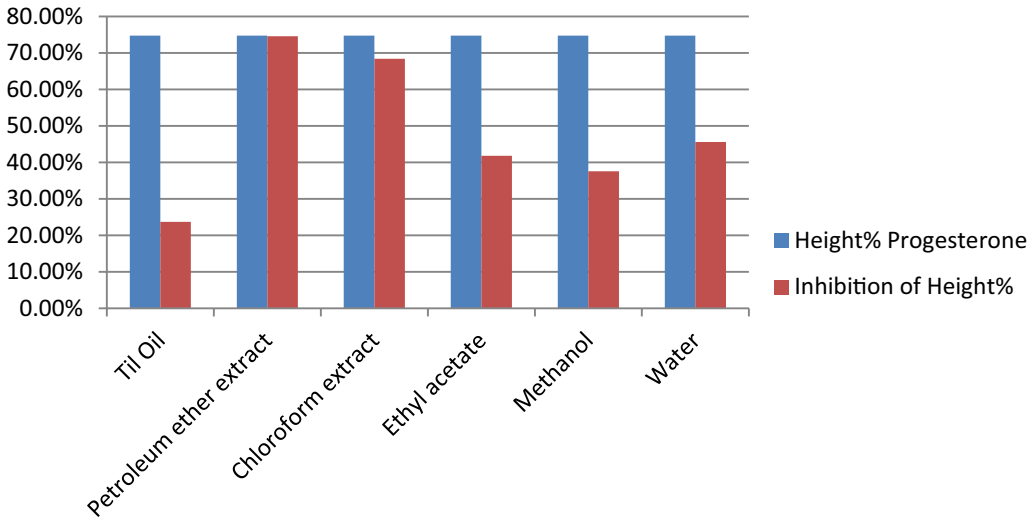


Fig. 3 Progesterone activity by leucopaxillus gentianeus

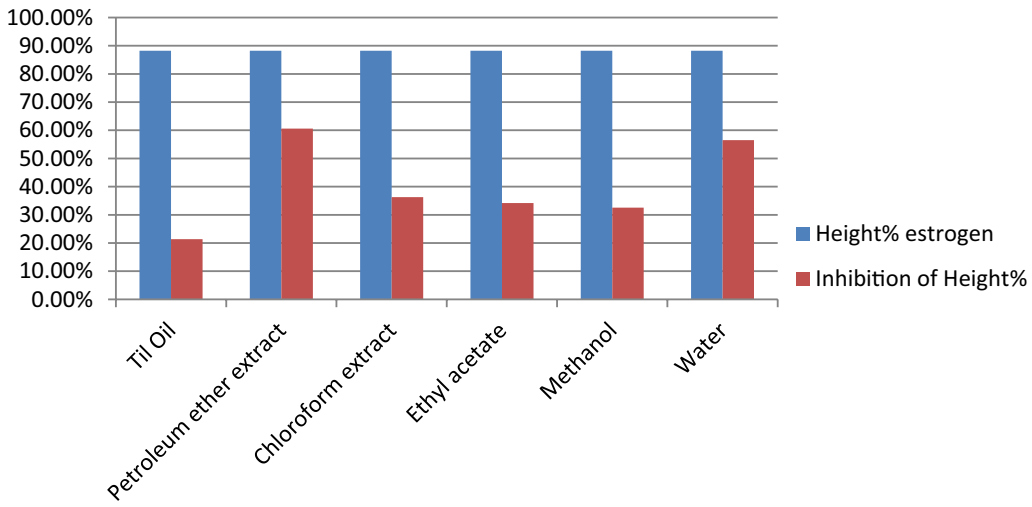


Fig. 4 Estrogen activity by Leucopaxillus gentianeus

Table 7 Pharmacological activities of *Leucopaxillus gentianeus* extract on progesterone

Extract Name	Height% Progesterone	Inhibition of Height%
Til Oil	74.735	23.689
Petroleum ether extract	74.735	74.582
Chloroform extract	74.735	68.399
Ethyl acetate	74.735	41.818
Methanol	74.735	37.582
Water	74.735	45.582

Table 8 Pharmacological activities of *Leucopaxillus gentianeus* extract on estrogen

Extract Name	Height% estrogen	Inhibition of Height%
Til Oil	88.237	21.384
Petroleum ether extract	88.237	60.582
Chloroform extract	88.237	36.278
Ethyl acetate	88.237	34.159
Methanol	88.237	32.561
Water	88.237	56.498

Table 9 R f values of TLC solvent system for Til Oil extract of *both species*

Sample	Plant species	No. Of spot detected	Rf
2	*Leucopaxillus gentianeus*	5	0.86

On silica gel, the HPTLC fingerprinting of the til oil extract was produced. Using anisaldehyde sulfuric acid reagents, the til oil extract had the greatest amount of UV active chemicals. It was subsequently identified at 289 nm, following visualization (Fig. 6a–c; Table 11). Table 12 displays solvent systems utilized for extract fingerprinting, the number of spots present in each, and their associated values (Fig. 7).

Calibration curves of cucurbitacin B

The calibration curve for cucurbitacin was produced at a specific Rf value. A calibration curve for cucurbitacin in the concentration range of 40–240 ng/spot has been shown by the current HPTLC approach. Regression analysis for cucurbitacin found a significant linear relationship with $r > 0.96565$. The intercept's standard deviation is less than 2%. The slopes of the standard curves have not changed noticeably (Table 13).

Calibration for test sample

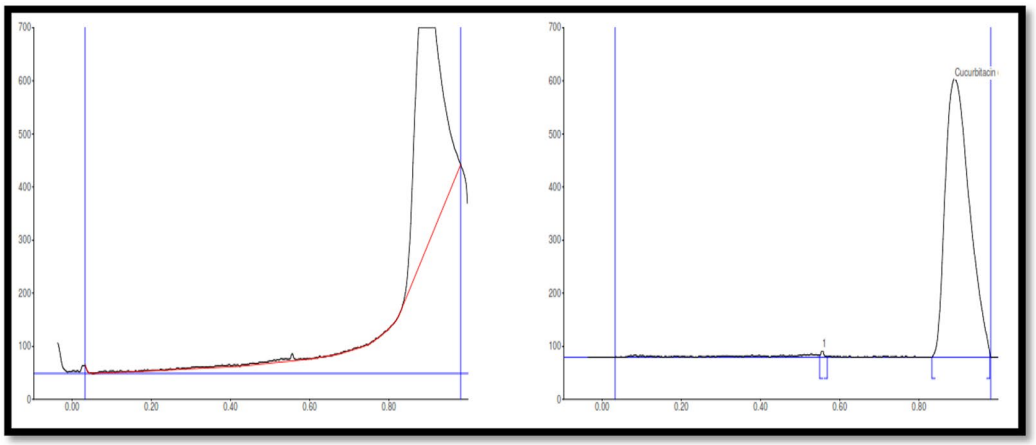
Substance: Cucurbitacin @ 289 nm

Regression via height: Linear $Y = 506.3 + 0.955 * X$ $r = 0.94792$ SDV = 4.03
Regression via area: Linear $Y = 2.732e + 004 + 57.19 * X$ $r = 0.96565$ SDV = 3.51

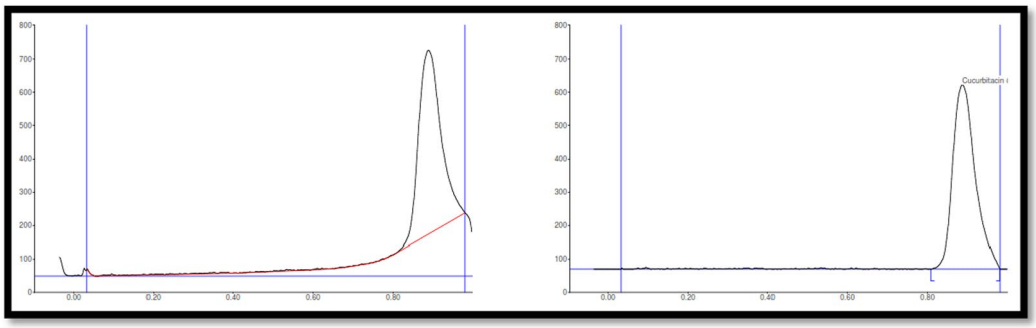
For the examination of cucurbitacins of Leucopaxillus gentianeus the recently established and validated HPTLC method was used. The regression equation derived from the calibration plot was used to analyze the peak regions of the three replicate samples. Table 14 reports the content found good content of cucurbitacin.

Table 10 Tracks representing sample amount and sample ID

Track	Track type	Vial	Sample ID	Apply position (mm)	Amount (µl)
1	Standard 1	1	Cucurbitacin	15.00	4.0
2	Standard 2	1	Cucurbitacin	29.1	6.0
3	Standard 3	1	Cucurbitacin	43.2	10.0
4	Standard 4	1	Cucurbitacin	57.3	14.0
5	Standard 5	1	Cucurbitacin	71.4	16.0
6	Standard 6	1	Cucurbitacin	85.5	20.0
7	Standard 7	1	Cucurbitacin	99.6	24.0
8	Sample	3	Til oil extract of Leucopaxillus gentianeus	156.0	10.0
9	Sample	3	Til oil extract of Leucopaxillus gentianeus	170.1	10.0
10	Sample	3	Til oil extract of Leucopaxillus gentianeus	184.2	10.0



(a)



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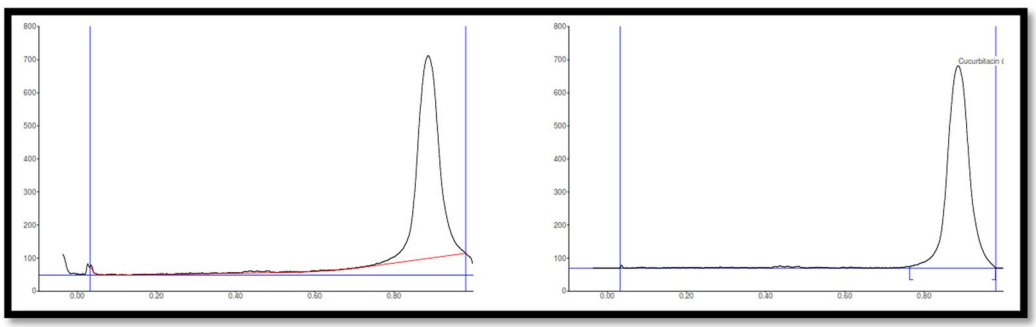

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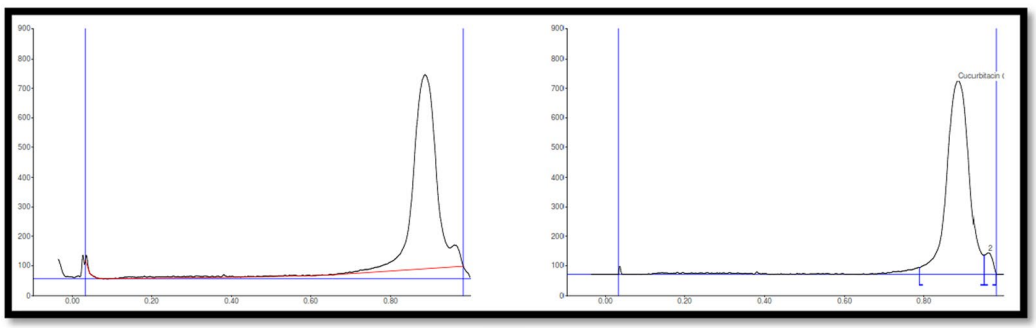
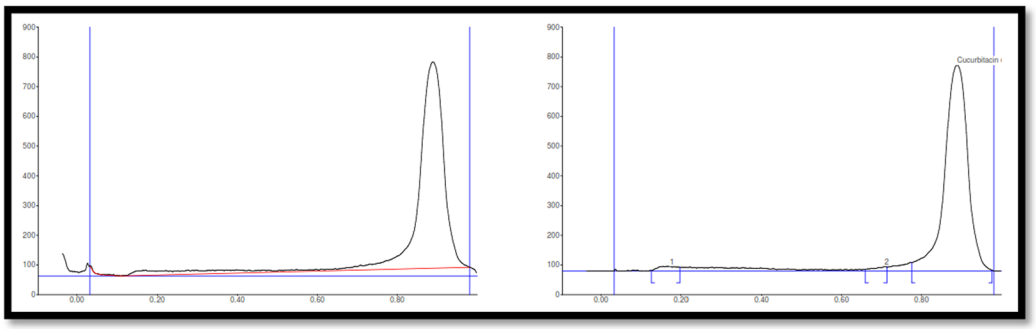
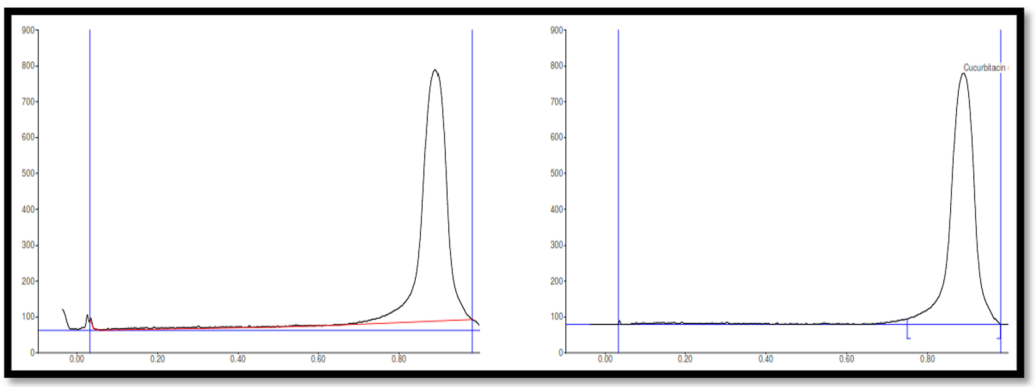


(d)

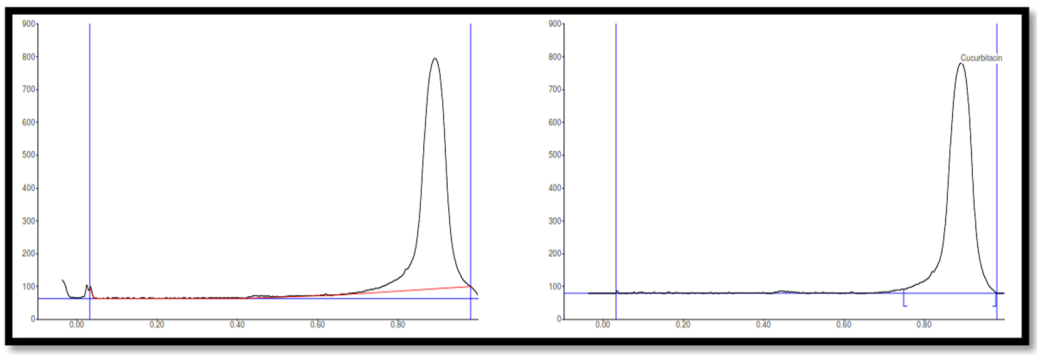
Fig. 5 The standard cucurbitacin spectrum, stratified from a to g, showed various known concentrations that could be compared with test samples



(e)



(f)



(g)

Fig. 5 continued

Table 11 HPTLC-Cucurbitacin profile of the standard sample

Amount	Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
40 ng	1	0.55	3.9	0.56	13.6	2.53	0.57	1.6	97.7	0.32	Unknown*
	2	0.83	0.5	0.89	523.9	97.47	0.98	9.1	30,386.9	99.68	Cucurbitacin
60 ng	1	0.81	0.3	0.89	552.7	100.0	0.98	2.5	29,701.9	100.00	Cucurbitacin
100 ng	1	0.76	5.3	0.89	612.7	100.0	0.98	1.5	32,354.5	100.00	Cucurbitacin
140 ng	1	0.79	23.9	0.89	656.7	89.87	0.95	65.6	34,849.4	96.22	Cucurbitacin
	2	0.95	65.6	0.96	74.0	10.13	0.98	4.7	1367.4	3.78	Unknown*
160 ng	1	0.13	1.8	0.17	16.7	2.30	0.20	12.8	761.6	1.93	Unknown*
	2	0.66	5.9	0.71	16.0	2.21	0.71	13.7	478.7	1.21	Unknown*
	3	0.78	29.1	0.89	693.5	95.49	0.98	3.3	38,313.2	96.86	Cucurbitacin
200 ng	1	0.75	15.0	0.89	700.4	100.0	0.98	0.9	39,450.0	100.00	Cucurbitacin
240 ng	1	0.75	11.2	0.89	689.5	100.0	0.98	2.8	39,929.5	100.0	Cucurbitacin

*indicates that this is a different peak identified in the standard

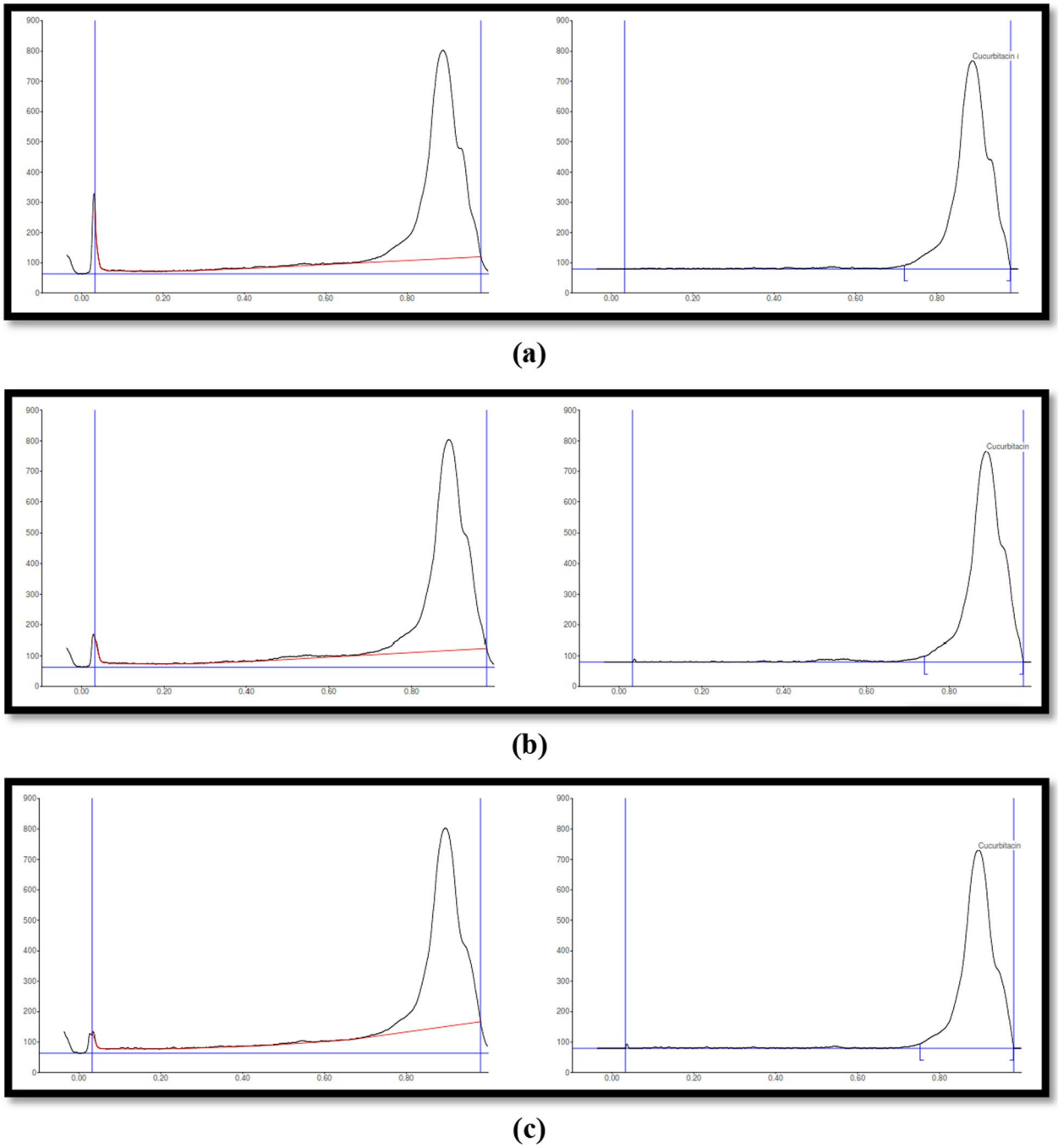


Fig. 6 Til oil extract spectra displayed a good peak with Rf value

Table 12 HPTLC-Cucurbitacin profile of the Til Oil extract of Leucopaxillus gentianeus

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.72	11.2	0.89	689.5	100.00	0.98	7.6	52,561.0	100.00	Cucurbitacin
1	0.74	19.6	0.89	686.1	100.00	0.98	8.4	51,395.3	100.00	Cucurbitacin
1	0.75	14.9	0.90	651.6	100.00	0.98	10.7	4509.2	100.00	Cucurbitacin

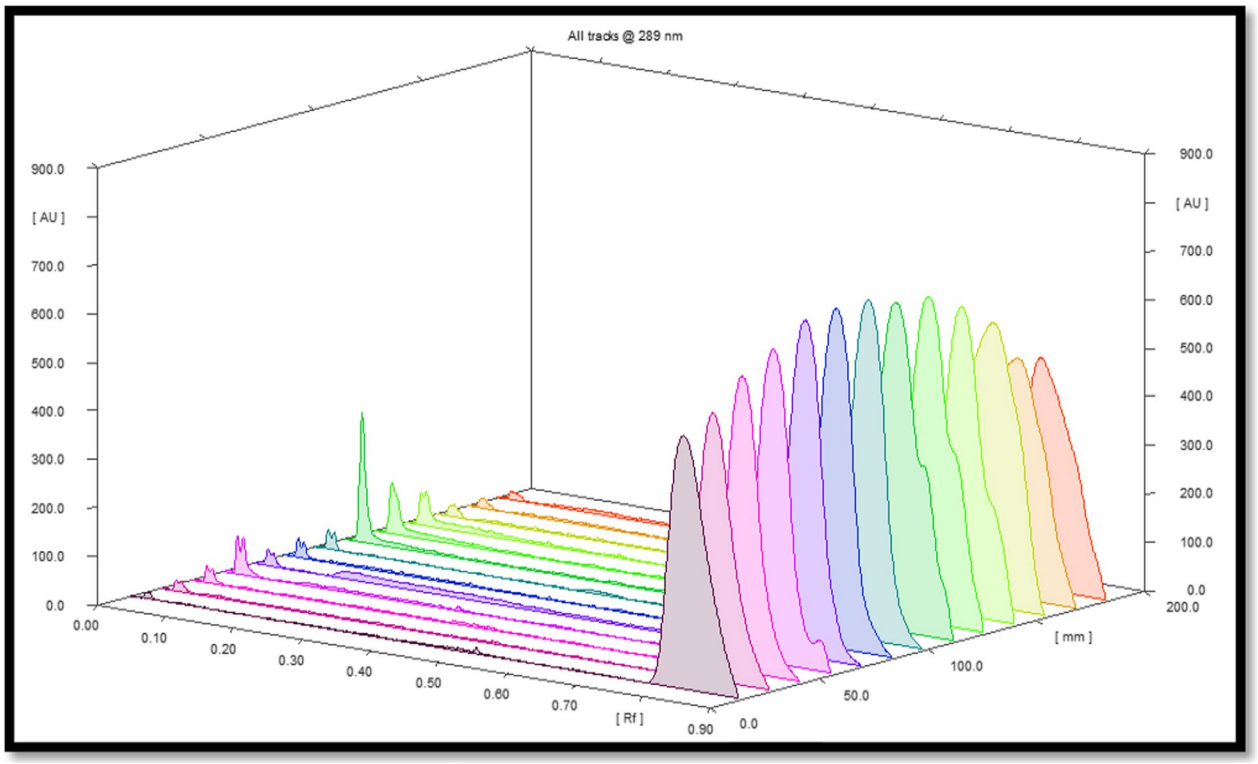


Fig. 7 3D Graph of Cucurbitacin standard and test samples

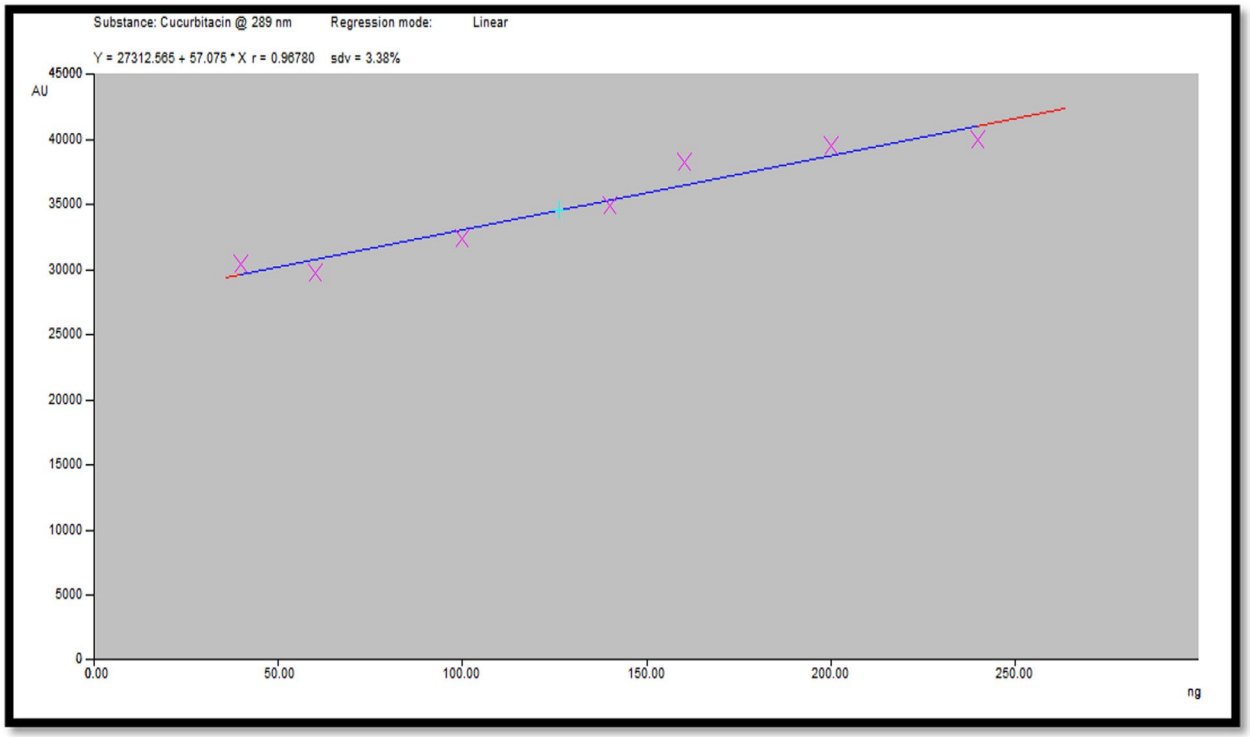
Table 13 Calibration results

Sample	Rf	Amount	Height	*X* (calc)	Area	X (calc)
STD	0.89	40.00 ng	523.86		30,386.91	
STD	0.89	60.00 ng	552.67		29,701.87	
STD	0.89	100.00 ng	612.67		32,354.47	
STD	0.89	140.00 ng	656.66		34,849.38	
STD	0.89	160.00 ng	693.48		38,313.16	
STD	0.89	200.00 ng	700.40		39,450.51	
STD	0.89	240.00 ng	701.76		39,929.49	
A	0.90		591.03	> 191.86 ng	50,914.41	> 264.00 ng
A	0.89		502.15	> 118.27 ng	45,487.97	> 264.00 ng
A	0.88		480.68	> 152.17 ng	35,517.45	> 264.00 ng

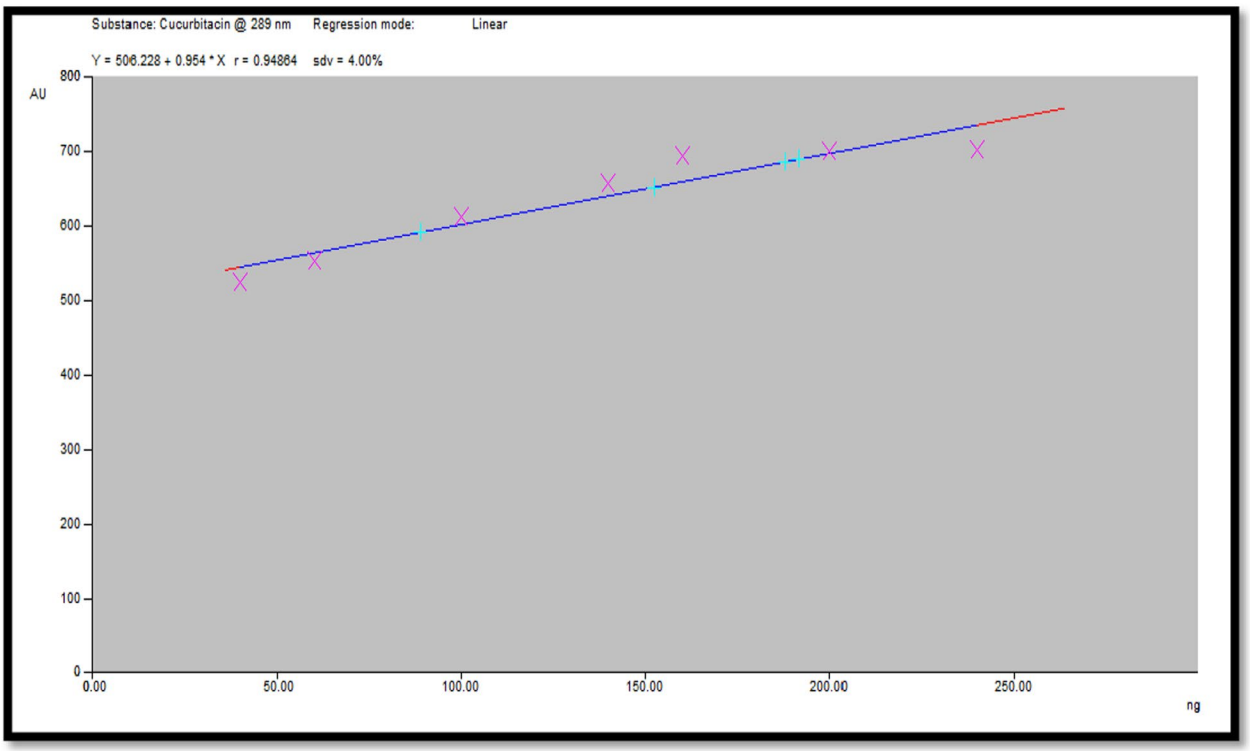
STD: Standard, A: Leucopaxillus Gentianeus

Table 14 Calibration results per analysis

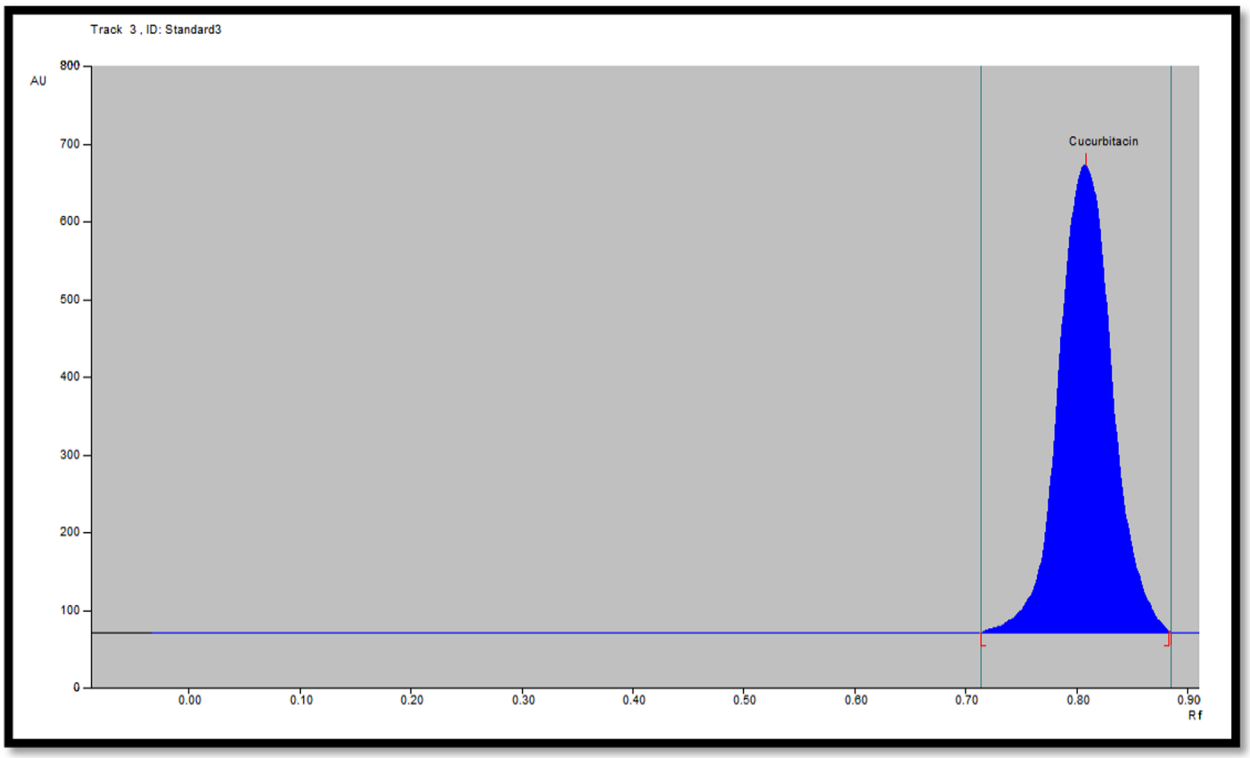
Name of Species	Result via	Substance	Rf	Average	CV%	*n*	Regression Remark
Leucopaxillus Gentianeus	Height	Cucurbitacin	0.89	177.43 ng	12.371	3	Linear
	Area	Cucurbitacin	0.89	0.00 ng	0.000	3	Linear



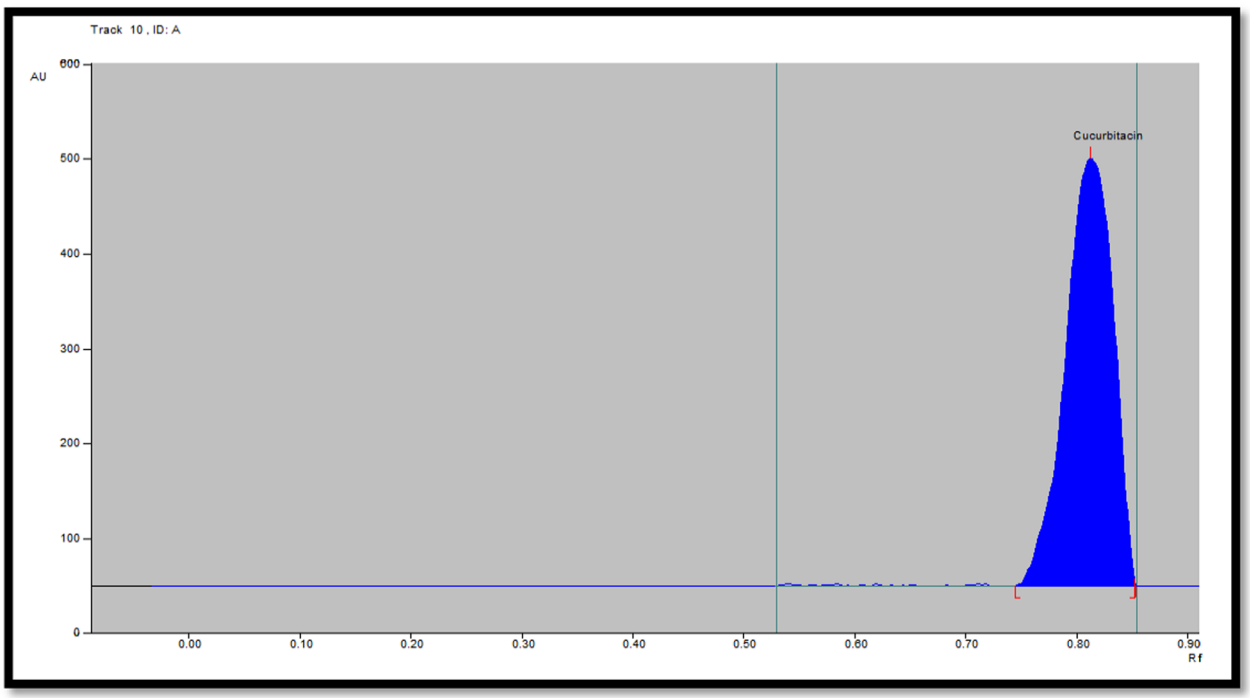
Graph Area



Graph Height



HPTLC chromatogram of standard Cucurbitacin



HPTLC chromatogram of Cucurbitacin from Leucopaxillus gentianeus

Discussion

Medicinal mushroom contains many chemical compounds or active substances that have therapeutic properties. Depending on the plant species, when and how they are picked, how they are grown, and how they are dried, different therapeutic mushrooms have different amounts of active chemicals. Numerous active metabolites found in the medicinal fungus Leucopaxillus gentianeus have been used as anticancer, anti-inflammatory, antioxidant, and cholesterol-lowering agents. One of the bitter false funnel mushroom treatments under development is a distinct extract of Leucopaxillus gentianeus. Drug interactions, several toxicities related to long-term anti-breast cancer drug use, and cost-effective therapy are all downsides of commercial anti-breast cancer drugs. The mushroom extract has demonstrated remarkable anti-breast cancer capabilities against progesterone and estrogen, making it a potentially effective substitute [7–12].

An HPTLC method was developed and validated to determine cucurbitacin B concurrently. The method was found to be easy to follow, quick, precise, concentrated, and reliable for the analysis of cucurbitacin B in Leucopaxillus gentianeus. Any laboratory could use this method to check the quality of unfinished drugs and formulations that contain cucurbitacin B as active markers or Leucopaxillus gentianeus as an ingredient.

The quality of herbal remedies, such as the til oil extraction of Leucopaxillus gentianeus, is greatly influenced by the drying process, the choice of solvents used, and the percentage of solvents and this solved ones. Ethanol is widely used in the creation of extractions since it is a universal solvent that can dissolve both polar and nonpolar substances. The ability of ethanol to attract polar active compounds can be improved by altering the makeup of the water in the substances so that the fraction of ethanol declines. This study compares how various solvents are used. Ethanol can dissolve a wide range of active substances, including tannins, phenols, saponins, reducing sugars, flavonoids, terpenoids, and glycosides. In comparison to other chemicals, water and Methanol solvents contain the most steroids. One of the solvents discovered to provide the best action for breast cancer was oil, according to this study. The study's results showed that the yield (6.8 gm) of the til oil extract was higher than that of other solvents. Leucopaxillus gentianeus oil extract demonstrated strong progesterone inhibition activity (23.689%) and estrogen inhibition activity (21.384%) level suppression during anti-breast cancer activity, which is crucial for the treatment of breast cancer. During RT-HPLC it is shown that the number of peaks indicates the number of phytochemicals present in the extract. In this study, cucurbitacin content was tested for anti-breast cancer activity using the HPTLC method. According to the study, the average concentration of cucurbitacin in Leucopaxillus gentianeus was 177.43 12.37 ng. Each value is noteworthy (p 0.05).

Conclusion

The findings of this investigation made it abundantly evident that a significant amount of cucurbitacin was present in the leucopaxillus gentianeus til oil extract, along with a variety of other chemical components. The hormones progesterone and estrogen, which are crucial for preventing breast cancer, are inhibited by the compound cucurbitacin. The next stage of our research will concentrate on developing a leucopaxillus gentianeus-based anti-breast cancer formulation that can be tested in vitro and in vivo.

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Author contributions
SK is the main contributor of the manuscript, writing and editing, and collecting data, editing, and submission/correspondence of the above research article. All authors read and approved the final manuscript.

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