RESEARCH PAPER

PHYTOCHEMICAL INVESTIGATION AND IN VITRO BIOLOGICAL ACTIVITIES OF CO-SOLVENT-ASSISTED SUPERCRITICAL CO_2 EXTRACTS OF *GARCINIA TALBOTII*

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Abstract

Background: *Garcinia talbotii Raizada ex Santapau* is a medicinal plant from the Western Ghats of India with potential phytochemical and biological properties. This study investigated the phytochemical composition and in vitro biological activities of extracts obtained using different co-solvents during supercritical fluid extraction (SCFE).

Methods: Aerial parts of *Garcinia talbotii* were extracted using supercritical CO_2 with ethanol, ethyl acetate, and chloroform separately used as co-solvents at 5%. The extracts were evaluated for extraction yield, preliminary phytochemical composition, total phenolic content (TPC), total flavonoid content (TFC), DPPH radical-scavenging activity, antibacterial activity against Staphylococcus aureus and Escherichia coli, antifungal activity against Candida albicans, and pepsin inhibition.

Results: The ethanol extract showed the highest extraction yield (33.17%), TPC (155.38 ± 8.75 mg GAE/g), and TFC (189.13 ± 9.01 mg QE/g), followed by ethyl acetate (152.45 ± 1.91 mg GAE/g and 177.46 ± 11.61 mg QE/g, respectively). Chloroform showed lower TPC (27.88 ± 2.50 mg GAE/g) and TFC (25.79 ± 7.64 mg QE/g). Ethanol exhibited the strongest DPPH radical-scavenging activity (IC_{50}: 553.84 ± 46.18 µg/mL), followed by chloroform (593.32 ± 39.22 µg/mL) and ethyl acetate (665.70 ± 60.07 µg/mL). It also showed the greatest antibacterial activity against both bacterial strains, although all extracts were less active than streptomycin. Antifungal activity was limited. Ethyl acetate showed the highest pepsin inhibition at 100 µg/mL (58.12 ± 1.64%), followed by ethanol (49.66 ± 1.91%) and chloroform (41.64 ± 0.23%), compared with 75.24 ± 1.39% for pepstatin.

Conclusion: The findings show that the measured phytochemical characteristics and biological activities of *Garcinia talbotii* extracts varied with the co-solvent used. The ethanol extract showed the strongest antioxidant and antibacterial responses, whereas the ethyl acetate extract exhibited greater pepsin-inhibitory activity. Antifungal activity was limited under the tested conditions. Further studies are needed to identify the active constituents and establish their biological relevance and safety. Pepsin inhibition alone does not demonstrate direct HIV-1 protease inhibition or anti-HIV activity.

Keywords: *Garcinia talbotii*, supercritical fluid extraction, antioxidant, antibacterial, antifungal, pepsin inhibition.

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Introduction

Medicinal plants have long been used in traditional healthcare and continue to attract scientific interest as sources of biologically active compounds. The search for plant-derived antioxidants and antimicrobial agents has become increasingly important because of oxidative stress and the growing problem of antimicrobial resistance. Many of the biological properties of medicinal plants are associated with secondary metabolites such as phenolics, flavonoids, tannins, xanthones, benzophenones, and terpenoids.[1] The genus Garcinia L. (Clusiaceae) is of particular interest because several species have been reported to possess antioxidant, antibacterial, antifungal, and other biological activities. However, the composition and activity of plant extracts can vary with the plant species, the part used, and the extraction method. Therefore, evaluating extracts prepared under different extraction conditions is important for understanding their biological potential.[2]

Garcinia talbotii Raizada ex Santapau, locally known as Tavir in Maharashtra, is a tree species found in the Western Ghats of India. Different parts of this plant have been used in traditional medicine

for conditions including digestive complaints, fever, rheumatism, and ulcers. Previous studies have reported the presence of several phytochemical groups in *Garcinia talbotii*, including phenolics, flavonoids, tannins, terpenoids, steroids, saponins, alkaloids, and glycosides.[3] Antioxidant activity has also been reported in its leaf extracts.[4] Despite these findings, further investigation is needed to understand the biological activities of extracts obtained from the aerial parts of this species, particularly when different extraction media are used.

The extraction method strongly influences the compounds recovered from plant material. Supercritical fluid extraction (SFE) is an alternative technique that uses a fluid under controlled pressure and temperature to extract selected constituents from a plant matrix. Carbon dioxide is commonly used for this purpose because of its relatively low critical temperature and ease of removal after extraction. However, its limited polarity may restrict the recovery of some polar compounds. Adding a co-solvent can modify the properties of the extraction medium and influence the range of compounds recovered.[5] Ethanol, ethyl acetate, and chloroform have different solvent characteristics and may therefore produce extracts with different chemical compositions. Ethanol can favour the extraction of many polar and moderately polar compounds, ethyl acetate may recover compounds of intermediate polarity, and chloroform can facilitate the extraction of relatively lipophilic constituents. The actual recovery depends on the extraction conditions and the chemical characteristics of the plant material. Comparing extracts prepared with these co-solvents may help explain differences in their phytochemical characteristics and biological activities.[6]

The biological evaluation of plant extracts provides an initial understanding of their potential applications. Antioxidant activity can be assessed using the DPPH radical-scavenging assay, while total phenolic and flavonoid content measurements provide information about the abundance of these phytochemical groups.[7] Antibacterial and antifungal assays can determine whether the extracts inhibit selected microorganisms. Protease inhibition is another area of interest because certain proteolytic enzymes are relevant targets in biomedical research. A pepsin inhibition assay can provide preliminary evidence of activity against an aspartyl protease, although it does not establish antiviral activity or inhibition of other proteases. These assays are useful for screening plant extracts, but their results must be interpreted within the limitations of the experimental methods.[8]

Although previous studies have investigated the phytochemical composition and antioxidant activity of *Garcinia talbotii*[9] comparative information on extracts obtained from its aerial parts using different co-solvents in SFE remains limited. In particular, it is important to determine whether the choice of co-solvent is associated with differences in extraction yield, phytochemical characteristics, and biological activity. Evaluating these properties together provides a more comprehensive preliminary assessment than examining antioxidant activity alone.

In the present study, the aerial parts of *Garcinia talbotii* were extracted using supercritical fluid extraction with ethanol, ethyl acetate, and chloroform as co-solvents. The resulting extracts were evaluated for extraction yield, preliminary phytochemical composition, total phenolic and flavonoid contents, and antioxidant activity using the DPPH assay. Their antibacterial activity was examined against Staphylococcus aureus and Escherichia coli, while antifungal activity was assessed against Candida albicans using the agar-well diffusion method. Preliminary protease-inhibitory activity was also investigated using a pepsin inhibition assay. The study aimed to compare the phytochemical characteristics and in vitro biological activities of the three extracts and determine how the choice of co-solvent was associated with their measured properties. The findings provide an initial comparative assessment of *Garcinia talbotii* aerial-part extracts and help establish a basis for further investigation of their biological activity.

MATERIAL AND METHODS

Collection and authentication of plant material

Aerial parts, including leaves, young stems, flowering buds, and fruiting buds of *Garcinia talbotii Raizada ex Santapau*, locally known as Tavir, were collected from the Vishalgad Fort area, Shahuwadi taluka, Kolhapur district, Maharashtra, India, during the flowering and fruiting season. The plant material was authenticated by the Botanical Survey of India, Regional Office, Pune, Maharashtra. A voucher specimen was deposited in the institutional herbarium for future reference.

Preparation of plant material

The collected aerial parts were washed thoroughly with running tap water to remove dust and adhering impurities. The material was shade-dried at room temperature until a constant weight was obtained and then coarsely pulverised using an electric grinder. The resulting powder was stored in an airtight container, protected from light and moisture, until extraction.[10]

Supercritical fluid extraction

The powdered aerial parts of *Garcinia talbotii* (500 g) were extracted using supercritical fluid extraction (SCFE), with carbon dioxide (CO_2) as the primary extraction fluid. Ethanol (E), ethyl acetate (EA), and chloroform (C) were used separately as co-solvents. Extractions were carried out at 300 bar and 40°C, with a CO_2 flow rate of 20 g/min for 180 min. Each co-solvent was introduced at a concentration of 5% through a high-pressure pump during the extraction

phase. At the end of extraction, the CO_2–co-solvent mixture was depressurised through a micrometric valve into a collection vessel. The collected extracts were concentrated using a rotary evaporator under reduced pressure at 40°C. The recovered extracts were weighed and stored in airtight amber containers until further analysis.[5,11]

The extraction yield was calculated as follows:

$$\text{Percentage yield} = \frac{\text{Weight of dried extract (g)}}{\text{Weight of dried plant powder(g)}} \times 100$$

Preliminary phytochemical screening

The extracts were screened for major classes of phytochemicals, including carbohydrates, proteins, amino acids, steroids, glycosides, flavonoids, alkaloids, phenolic compounds, tannins, and saponins. The tests were based on established colour reactions and precipitation methods.

Carbohydrates were examined using Molisch's, Fehling's, and Benedict's tests, while proteins and amino acids were assessed using Biuret, Millon's, and ninhydrin tests. Steroids were tested using the Liebermann–Burchard reaction, and flavonoids were screened using the sulphuric acid test. Alkaloids were examined using Dragendorff's, Mayer's, Hager's, and Wagner's reagents. Phenolic compounds and tannins were assessed using ferric chloride, lead acetate, and bromine water tests. Appropriate qualitative tests were also performed for glycosides and saponins. The results were recorded as positive or negative based on the reactions observed. [12,13]

Total phenolic content

Total phenolic content (TPC) was determined using the Folin–Ciocalteu colourimetric method, with gallic acid as the reference standard. A gallic acid stock solution (5 mg/mL) was prepared, and serial dilutions were used to construct the calibration curve. An aliquot of 80 µL of each standard or extract solution was mixed with 400 µL of Folin–Ciocalteu reagent and 320 µL of 7.5% sodium carbonate solution. The mixture was incubated in the dark at 45 °C for 30 min, and absorbance was measured at 765 nm using a UV–Visible spectrophotometer. TPC was calculated from the gallic acid calibration curve and expressed as milligrams of gallic acid equivalents per gram of dried extract (mg GAE/g). Each sample was analysed in triplicate, and the results were expressed as mean ± SEM.[14,15]

Total flavonoid content

Total flavonoid content (TFC) was determined using the aluminium chloride colourimetric method, with quercetin as the reference standard. Serial dilutions of quercetin were used to prepare the calibration curve. An aliquot of 0.5 mL of each standard or extract solution was mixed with 1.5 mL of ethanol, 0.1 mL of 10% aluminium chloride solution, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water. The mixture was incubated at room temperature for 30 min, and absorbance was measured at 415 nm. The blank was prepared by replacing the aluminium chloride solution with distilled water. TFC was calculated from the quercetin calibration curve and expressed as milligrams of quercetin equivalents per gram of dried extract (mg QE/g). All measurements were performed in triplicate, and the results were expressed as mean ± SEM.[16,17]

DPPH radical-scavenging assay

The antioxidant activity of the extracts was assessed using the DPPH radical-scavenging assay. Different concentrations of each extract were prepared in the appropriate solvent. An aliquot of 100 µL of each extract solution was mixed with 100 µL of 0.1% methanolic DPPH solution in a 96-well microplate. The plate was incubated in the dark at room temperature for 30 min, and absorbance was measured at 517 nm. Ascorbic acid was used as the positive control, while DPPH solution without extract served as the control. Appropriate sample blanks were used to correct for background absorbance where necessary. All measurements were performed in triplicate.[18,19] The percentage of DPPH inhibition was calculated using the following equation:

$$\%\ \text{inhibition} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100$$

Antibacterial activity

Antibacterial activity was evaluated against *Staphylococcus aureus* and *Escherichia coli* using the agar-well diffusion method. Bacterial suspensions were adjusted to a turbidity equivalent to a 0.5 McFarland standard and spread uniformly over nutrient agar plates. Wells of uniform diameter were prepared using a sterile cork borer. Each extract was dissolved in dimethyl sulfoxide (DMSO), and 100 µL of the test solution was added to each well. DMSO served as the negative control, and streptomycin was used as the positive control. The plates were incubated at 37 °C for 24 h. The inhibition zones were then measured in millimetres. Each test was performed in triplicate, and the results were expressed as mean ± SEM.[20,21]

Antifungal activity

The antifungal activity of the extracts was tested against *Candida albicans* using the agar-well diffusion method. Sabouraud dextrose agar was prepared according to the manufacturer's instructions, sterilised at 121°C for 15 min, and poured into sterile Petri plates. The fungal inoculum was prepared according to the procedure used in the laboratory and spread uniformly over the agar surface. Wells of uniform diameter were prepared using a sterile cork borer. Each extract was dissolved in DMSO at a concentration of 5 mg/mL, and 100 µL was added to each well. Fluconazole served as the positive control, while DMSO served as the negative control. The plates were incubated at 35°C for 24–48 h, after which the inhibition zones were

measured in millimetres. All tests were performed in triplicate, and the results were expressed as mean ± SEM.[22,23]

Preliminary pepsin inhibition assay

Pepsin inhibition was evaluated as a preliminary measure of the protease-inhibitory potential of the extracts. Pepsin was used as a model aspartyl protease, with haemoglobin as the substrate and pepstatin as the reference inhibitor. The reaction mixture contained 500 µL of pepsin solution, 800 µg of haemoglobin substrate, and the test extract at the required concentration. The mixture was incubated at 37°C for 30 min. The reaction was stopped by adding 700 µL of 5% trichloroacetic acid (TCA), followed by centrifugation for 5 min. The absorbance of the supernatant was measured at 280 nm. Pepstatin served as the positive inhibition control, while the enzyme–substrate was used for the negative control. Pepsin inhibition was evaluated as a preliminary screening method to investigate the protease-inhibitory potential of the *Garcinia talbotii* extracts. Pepsin, an aspartic protease, was used as a model enzyme because of its shared catalytic features with HIV-1 protease. However, inhibition of pepsin does not necessarily indicate inhibition of HIV-1 protease.[8]

Preliminary phytochemical screening

Table 1: Phytochemical screening of *Garcinia talbotii* plant extracts

Test for	Test	SCFE Extracts		
		E	EA	C
Carbohydrates	Molisch test	+++	+	-
	Fehling's solution test	++	-	-
	Benedict's test	+++	-	-
Proteins	Biuret test	++	-	-
	Millon's test	++	-	-
Amino acid	Ninhydrin test	++	+	-
Steroid	Liebermann-Burchard test	+++	++	++
Glycosides	Keller-Kiliani test	+++	-	-
	Foam test	++	+	-
	Guignard reaction & sodium picrate test	-	-	-
Flavonoids	Sulphuric acid test	+++	++	+
Alkaloids	Dragendorff's test	+++	+	+
	Mayer's test	++	+	+
	Hager test	+++	+	+
	Wagner's Test	++	-	+
Tannins and phenolics	Ferric Chloride Test	+++	++	-

(The intensity of phytochemical reactions was semi-quantitatively graded as − = absent, + = weak, ++ = moderate, and +++ = strong. E-ethanol, EA-ethyl acetate and C-chloroform)

RESULTS AND DISCUSSION

Extraction

Supercritical fluid extraction with co-solvents of *Garcinia talbotii* aerial parts yielded the highest amount with ethanol (33.17%), followed by ethyl acetate (27.80%) and chloroform (6.24%). All extracts were semi-solid and dark greenish in colour. The higher ethanol yield suggests efficient extraction of polar constituents such as phenolics, flavonoids, tannins, and glycosides. In contrast, the lower chloroform yield indicates a comparatively lower abundance of moderately nonpolar constituents, including terpenoids, steroids, and chloroform-soluble xanthones.

Phytochemical screening of *Garcinia talbotii* extracts (Table 1) showed that the ethanol extract had the broadest range of positive reactions, particularly for carbohydrates, flavonoids, alkaloids, and phenolic compounds. The ethyl acetate extract showed moderate reactions for steroids and flavonoids, whereas the chloroform extract was positive mainly for steroids and alkaloids. These differences may reflect the influence of co-solvent properties on the recovery of plant constituents. The Guignard (sodium picrate) test was negative in all three extracts (Table 1). Overall, the ethanol extract showed the strongest preliminary phytochemical response, although these qualitative results do not establish the identity or concentration of individual compounds.

Total phenolic and flavonoid content

The ethanol extract of *Garcinia talbotii* showed the highest total phenolic content (155.38 ± 8.75 mg GAE/g) and total flavonoid content (189.13 ± 9.01 mg QE/g), followed by the ethyl acetate extract, with 152.45 ± 1.91 mg GAE/g and 177.46 ± 11.61 mg QE/g, respectively. In contrast, the chloroform extract showed considerably lower values of 27.88 ± 2.50 mg GAE/g for TPC and 25.79 ± 7.64 mg QE/g for TFC. The calibration curves showed good

linearity for gallic acid and Quercetin; gallic acid: $Y = 0.0008X + 0.0167$, ($R^2 = 0.9931$); quercetin: $Y = 0.0008X + 0.0237$, ($R^2 = 0.9891$). The higher phenolic and flavonoid contents in the ethanol and ethyl acetate extracts suggest that these co-solvents recovered more of the compounds detected by these assays than chloroform. Ethanol gave the highest values in both tests, although its phenolic content was close to that of ethyl acetate. These findings provide a basis for examining whether the differences in phenolic and flavonoid content are reflected in the antioxidant activity of the extracts.

DPPH radical-scavenging activity

Table 2: Effects of Different Extracts E, EA, C Against DPPH

Sample	Conc. (µg/ml)	Absorbance (OD)			% Inhibition			IC_{50} Value (µg/ml)
		I	II	III	I	II	III	
Control	--	0.751	0.781	0.834	--	--	--	--
Ascorbic acid	200	0.462	0.47	0.472	38.488	39.882	43.441	331.94 ± 44.34
	400	0.368	0.36	0.361	51.000	53.911	56.711	
	600	0.32	0.315	0.304	57.399	59.677	63.555	
	800	0.224	0.232	0.228	70.177	70.29	72.666	
	1000	0.195	0.198	0.192	74.003	74.655	76.698	
E	200	0.595	0.59	0.585	20.777	24.466	29.866	553.84 ± 46.18
	400	0.475	0.472	0.473	36.755	39.566	43.299	
	600	0.375	0.37	0.372	50.007	52.662	55.540	
	800	0.295	0.291	0.293	60.772	62.744	64.887	
	1000	0.224	0.23	0.235	70.177	70.555	71.882	
EA	200	0.625	0.634	0.64	16.788	18.822	23.266	665.70 ± 60.07
	400	0.5335	0.542	0.532	28.766	30.600	36.211	
	600	0.422	0.416	0.413	43.811	46.733	50.488	
	800	0.345	0.355	0.348	54.406	54.555	58.277	
	1000	0.278	0.285	0.288	62.998	63.511	66.433	
C	200	0.622	0.614	0.617	17.188	21.388	26.022	593.32 ± 39.22
	400	0.497	0.5	0.493	33.822	35.988	40.889	
	600	0.385	0.395	0.394	48.744	49.422	52.766	
	800	0.311	0.322	0.316	58.772	58.777	62.111	
	1000	0.242	0.248	0.246	67.788	68.255	70.550	

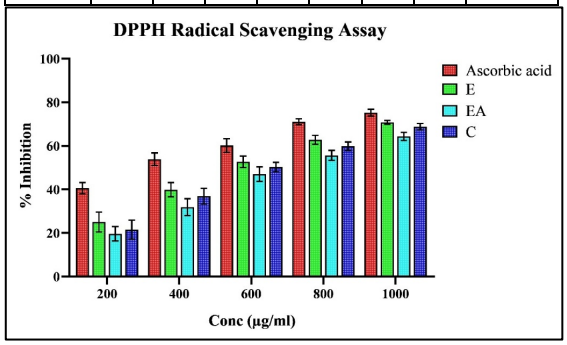
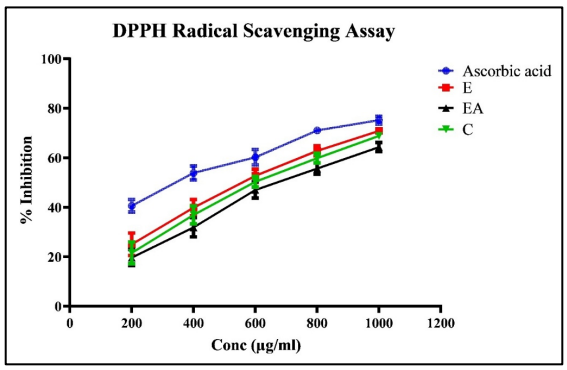


Figure 1: Effect of Different Extracts E, EA, C Against DPPH

Error bars in the figure represent ± SEM (n = 3). Statistical analysis was carried out using GraphPad Prism 8.0.



The DPPH radical-scavenging assay showed a concentration-dependent increase in inhibition for all three extracts (E, EA, and C) and the reference antioxidant, ascorbic acid, over the concentration range of 200–1000 µg/mL. Ascorbic acid exhibited the highest activity, with inhibition increasing from 38.48–43.41% at 200 µg/mL to 74.03–76.98% at 1000 µg/mL. Among the extracts, E showed the highest activity, increasing from 20.77–29.86% to 70.17–71.82%, followed by C, which increased from 17.18–26.02% to 67.78–70.50%. Extract EA showed comparatively lower activity, with

Figure 2: Dose-Response Effect of E, EA, C on DPPH Radical Scavenging

Error bars in the figure represent ± SEM (n = 3). Statistical analysis was carried out using GraphPad Prism 8.0

inhibition increasing from 16.78–23.26% at 200 µg/mL to 62.98–66.43% at 1000 µg/mL (Table 2). The IC_{50} values further supported these findings, with ascorbic acid showing the lowest value (331.94 ± 44.34 µg/mL), followed by E (553.84 ± 46.18 µg/mL), C (593.32 ± 39.22 µg/mL), and EA (665.70 ± 60.07 µg/mL). The lower IC_{50} of extract E indicates greater radical-scavenging potency than extracts C and EA (Figures 1 and 2). Overall, the results suggest that extract E had the strongest antioxidant activity among the tested extracts, although all three showed lower potency than ascorbic acid.

Antibacterial activity

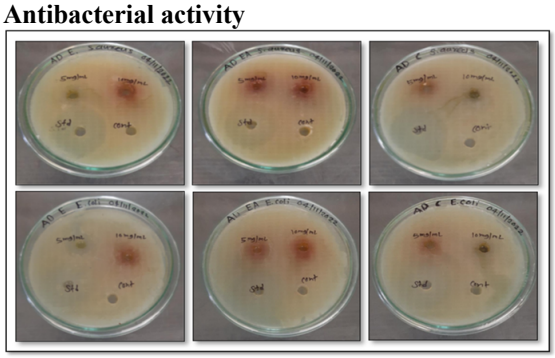


Figure 3: Bacterial Zone Inhibition by E, EA And C Extracts

Table 3: Bacterial Zone Inhibition by Different Extracts E, EA and C

Treatment	Conc. mg/ml	Zone Diameter (mm)	
		Stap. Aureus	***E. Coli***
Control	0	--	--
Std. Streptomycin	1	21 ± 0.57	24.33 ± 0.88
E	5	10 ± 1	12.33 ± 0.33
	10	16.33 ± 0.88	16 ± 0.57
EA	5	7 ± 1.15	7.66 ± 0.33
	10	12.33 ± 1.20	12.33 ± 0.88
C	5	4.66 ± 0.88	10 ± 0.57
	10	7.66 ± 0.33	12 ± 0.57

The antibacterial activity of extracts E, EA, and C was evaluated against Staphylococcus aureus and Escherichia coli at concentrations of 5 and 10 mg/mL, using streptomycin as the standard antibiotic. All three extracts showed antibacterial activity, with inhibition zones generally increasing with concentration. Extract E exhibited the highest activity among the tested extracts, producing inhibition zones of 10 ± 1 mm and 16.33 ± 0.88 mm against S. aureus at 5 and 10 mg/mL, respectively, and 12.33 ± 0.33 mm and 16 ± 0.57 mm against E. coli. Extract EA showed moderate activity, with inhibition zones of 7 ± 1.15 mm and 12.33 ± 1.20 mm against S. aureus and 7.66 ± 0.33 mm and 12.33 ± 0.88 mm against E. coli at 5 and 10 mg/mL, respectively. Extract C showed the lowest activity against S. aureus, with zones of 4.66 ± 0.88 mm and 7.66 ± 0.33 mm, while its activity against E. coli increased from 10 ± 0.57 mm to 12 ± 0.57 mm at the corresponding concentrations. Streptomycin produced inhibition zones of 21 ± 0.57 mm against S. aureus and 24.33 ± 0.88 mm against E. coli,

indicating greater antibacterial activity than the tested extracts. No inhibition was observed in the control (Table 3; Figure 3).

Overall, extract E demonstrated the strongest antibacterial activity against both bacterial strains, followed by EA and C in most comparisons. The results suggest that the extracts contain constituents with potential antibacterial properties, although further studies are required to identify the active compounds and determine their effectiveness using additional antibacterial assays.

Antifungal activity

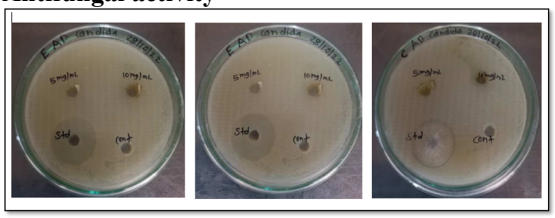


Figure 4: Fungal Zone Inhibition by Different Extracts E, EA And C

Table 4: Fungal Zone Inhibition by Different Extracts E, EA and C

Samples	Conc. (mg/ml)	Zone Diameter (mm)
		Against *Candida albicans*
Control	--	--
Standard ((Fluconazole)	1	24 ± 0.57
E	5	2 ± 0
	10	6.33 ± 0.33
EA	5	0
	10	0
C	5	4.33 ± 0.33
	10	5.66 ± 0.33

The antifungal activity of extracts E, EA, and C was tested against Candida albicans, with fluconazole used as the standard. Extract E showed an increase in inhibition from 2 ± 0 mm at 5 mg/mL to 6.33 ± 0.33 mm at 10 mg/mL. Extract C produced inhibition zones of 4.33 ± 0.33 mm and 5.66 ± 0.33 mm at the respective concentrations. In contrast, extract EA showed no inhibition at either concentration. Fluconazole exhibited the highest activity, with an inhibition zone of 24 ± 0.57 mm (Table 4; Figure 4). Overall, extracts E and C showed limited antifungal activity against C. albicans, while EA was ineffective under the tested conditions. Although extract E showed the largest inhibition zone at 10 mg/mL, further studies are needed to determine the compounds responsible for this activity and assess their antifungal potential.

Preliminary pepsin inhibition assay

Table 5: Effect of Different Extracts E, EA and C Against Protease Enzyme

Compound (100 µg/ml)	OD			% Inhibition			Mean % Inhibition
	I	II	III	I	II	III	
control	0.32	0.309	0.317	--	--	--	--
Pepstatin	0.075	0.081	0.078	76.5625	73.78641	75.39432	75.24 ± 1.39
E	0.154	0.159	0.163	51.875	48.54369	48.58044	49.66 ± 1.91
EA	0.128	0.133	0.135	60	56.95793	57.41325	58.12 ± 1.64
C	0.186	0.181	0.185	41.875	41.42395	41.64038	41.64 ± 0.23

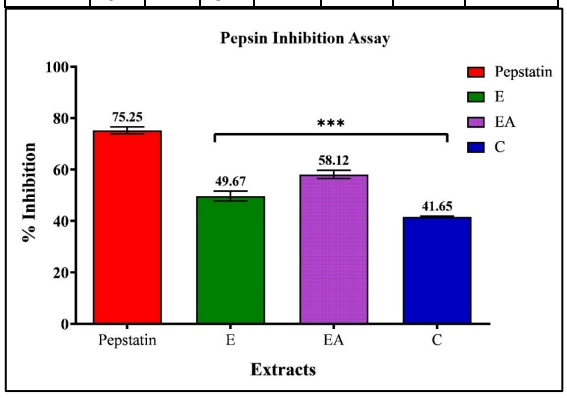


Figure 5: Effect of Different Extracts E, EA and C Against Protease Enzyme

Error bars in figures represent ± SEM (n = 3). The study utilised ANOVA followed by Dunnett's t-test to examine the data; ***<0.001 were considered statistically significant. Statistical analysis was carried out using GraphPad Prism 8.0.

Pepsin inhibition was evaluated as a preliminary method to assess the protease-inhibitory potential of

extracts E, EA, and C. Pepsin and HIV-1 protease belong to the aspartic protease family and share similarities in their catalytic mechanisms; therefore, pepsin was used as a model enzyme for preliminary screening. At 100 µg/mL, extract EA showed the highest inhibition (58.12 ± 1.64%), followed by E (49.66 ± 1.91%) and C (41.64 ± 0.23%). The reference inhibitor pepstatin exhibited 75.24 ± 1.39% inhibition, indicating greater activity than the tested extracts (Table 5). Statistical comparisons were performed using one-way ANOVA followed by Dunnett's multiple-comparisons test, with significance determined relative to the control. These findings suggest that EA had the greatest pepsin-inhibitory activity among the tested extracts under the experimental conditions (Figure 5). These findings suggest that EA has the strongest protease-inhibitory potential among the extracts under the tested conditions. However, because we used pepsin instead of HIV-1 protease, further studies are needed to confirm whether this activity translates into direct HIV-1 protease inhibition and antiviral effects.

CONCLUSION

This study highlights how co-solvent selection affects extraction yield, phytochemical composition, and biological activities of *Garcinia talbotii* aerial parts using supercritical fluid extraction. Among the three extracts, the ethanol extract showed the highest extraction yield, phenolic and flavonoid contents, along with the strongest antioxidant and antibacterial activities. In contrast, the ethyl acetate extract exhibited the highest pepsin-inhibitory activity, suggesting that different extraction media may selectively recover compounds responsible for specific biological effects. Pepsin inhibition alone does not demonstrate direct HIV-1 protease inhibition or anti-HIV activity. The chloroform extract also demonstrated measurable biological activity, particularly against Candida albicans, despite its lower phenolic and flavonoid contents. Overall, these findings indicate that the biological potential of *Garcinia talbotii* is closely associated with the choice of extraction co-solvent and that no single extract showed superiority across all evaluated activities. This comparative investigation provides a basis for further exploration of *Garcinia talbotii* as a potential source of bioactive natural compounds. Further chemical characterisation, isolation of active constituents, and mechanistic and in vivo studies are necessary to understand the compounds responsible for these activities and establish their potential applications.

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AUTHOR CONTRIBUTIONS

Abhishek V. Desai: conceptualisation, methodology, analysis, data collection, writing, review, and editing. Visualisation, reference search, and data interpretation. Jameel Ahmed S. Mulla: Supervision and administration of the project**.**

CONFLICT OF INTEREST

The authors declare no conflict of interest in this research.

AI USE STATEMENT

The authors used Grammarly for grammar correction, language refinement, and improving sentence clarity during manuscript preparation. The authors reviewed and approved all suggested changes and take full responsibility for the final content of the manuscript.

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