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Research Article

Medicinal Potential of *Curcuma caesia* Roxb.: Phytochemical Composition and TLC Profile of Hydroalcoholic Rhizome Extract

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Abstract

Curcuma caesia (black turmeric) is a medicinal plant traditionally used in Southeast Asia for the treatment of various ailments, including inflammation and cancer. This study aimed to evaluate the phytochemical profile and chromatographic characteristics of its hydroalcoholic rhizome extract to explore its anticancer potential. The rhizome extract was subjected to preliminary phytochemical screening to identify major classes of bioactive compounds. Thin Layer Chromatography (TLC) was performed using six different solvent systems of varying polarity to achieve comprehensive phytochemical profiling. Chromatograms were visualized under UV light (365 nm), and Rf values were calculated to assess compound diversity and migration behavior. Phytochemical screening revealed the presence of flavonoids, phenolics, alkaloids, terpenoids, saponins, and glycosides. TLC profiling showed distinct separation patterns across all solvent systems, with the most effective resolution observed in Methanol:Ethyl Acetate (2:8) and Toluene:Ethyl Acetate (2:8) systems. A total of 69 phytoconstituent bands were detected across all chromatograms, with Rf values ranging from 0.08 to 0.94, indicating high chemical diversity. Bright fluorescent spots under UV light suggested the presence of conjugated aromatic compounds typical of phenolics and flavonoids. The study highlights the rich phytochemical composition and chromatographic complexity of *Curcuma caesia* rhizome extract, supporting its potential as a natural source of anticancer agents. The presence of biologically active compounds, especially flavonoids and terpenoids.

Keywords: *Curcuma caesia*, phytochemical screening, TLC profile, rhizome extract.

1. INTRODUCTION

Curcuma caesia Roxb., commonly referred to as black turmeric, is an ethnomedicinal herb native to India and Southeast Asia, extensively utilized in traditional healing systems such as Ayurveda and Siddha. Its rhizomes have been employed for the management of various health conditions, including asthma, epilepsy, wounds, arthritis, skin diseases, fever, and menstrual disorders [1,2]. The plant is characterized by its distinctive bluish-black rhizomes, which are rich in a diverse array of phytoconstituents contributing to its broad pharmacological profile. Phytochemical investigations have revealed that the rhizomes of *C. caesia* are abundant in flavonoids, phenolic compounds, terpenoids, alkaloids, and essential oils such as camphor, curcumenol, and xanthorrhizol [3-5,10]. These bioactive constituents have demonstrated a wide range of biological activities. Notably, antioxidant properties have been reported, involving the scavenging of reactive oxygen species (ROS) and the enhancement of endogenous antioxidant enzymes [4]. Anti-inflammatory activity has been observed through the downregulation of pro-inflammatory mediators, including COX-2, TNF-α, and IL-6 [6]. Additionally, *C. caesia* exhibits antimicrobial efficacy against various bacterial and fungal strains via mechanisms such as microbial cell walls disruption and growth inhibition [10]. Neuropharmacological studies further indicate analgesic, anticonvulsant, and antidepressant effects, suggesting central nervous system-modulating potential [9]. Recent research has increasingly focused on the plant's anticancer potential, attributed to its phytochemical constituents capable of inducing apoptosis, modulating the cell cycle, and interfering with critical signaling pathways in cancer cells. Essential oil components—such as germacrone, camphor, and ar-turmerone—have demonstrated selective cytotoxicity against several cancer cell lines, including cervical, hepatic, and breast cancer cells [6,8]. These effects are believed to be mediated through mechanisms such as inhibition of the PI3K/Akt and NF-κB signaling pathways, disruption of mitochondrial membrane potential, and induction of oxidative stress [7]. In the present study, ethanol was selected as the extraction solvent due to its intermediate polarity and effectiveness in dissolving a broad range of phytochemicals, including both polar and non-polar constituents. This property makes ethanol particularly suitable for extracting bioactive classes such as flavonoids, phenolics, and volatile oils [11]. The aim of this study is to perform a comprehensive phytochemical screening and thin-layer chromatography (TLC) profiling of the hydroalcoholic extract of *Curcuma caesia* rhizome. These analytical approaches are essential for the standardization of plant materials, providing visual chemical fingerprints and aiding in the preliminary identification of key bioactive compounds. The findings of this study are expected to contribute to the phytochemical foundation of *C. caesia* and support its therapeutic relevance as a source of antioxidant, antimicrobial, anti-inflammatory, neuroprotective, and anticancer agents.

2. MATERIAL AND METHODS

2.1 Plant Material Collection and Authentication

Mature rhizomes of *Curcuma caesia* Roxb. were collected during the post-monsoon season (October–November) from a naturally occurring population in the forested region of Haryana, India—an area known for its rich ethnobotanical diversity. The plant material was taxonomically identified and authenticated by Mr. Vinay Ranjan, Scientist-E and Head of Office, Botanical Survey of India, Central Regional Centre, Allahabad. Authentication was carried out under the auspices of the Ministry of Environment, Forest and Climate Change, Government of India.

2.2 Preparation of Plant Material and hydroalcoholic Extraction

The rhizomes of *Curcuma caesia* Roxb. were thoroughly washed with distilled water to remove residual soil, debris, and microbial contaminants. The cleaned samples were then shade-dried under ambient laboratory conditions (25–30 °C) for 10 days to preserve thermolabile phytoconstituents such as curcuminoids and volatile oils. Once completely dried, the rhizomes were ground into a coarse powder using a laboratory-grade mechanical grinder. The powdered material was stored in airtight, amber-colored containers to prevent moisture absorption and degradation of light-sensitive compounds [12-16]. For extraction, 200 g of the powdered rhizome was loaded into a Soxhlet apparatus using a cellulose extraction thimble shown in Figure 1. Extraction was carried out using 95% ethanol, selected for its moderate polarity and proven efficacy in solubilizing a wide spectrum of bioactive constituents, particularly flavonoids, phenolics, and essential oils [17,18]. The extraction was performed continuously for 6–8 hours at ethanol's boiling point (78.5 °C), until the solvent in the siphon tube turned colorless, indicating exhaustive extraction of phytochemicals. The resultant dark-orange hydroalcoholic extract was filtered using Whatman No. 1 filter paper and subsequently concentrated under reduced pressure using a rotary vacuum evaporator set at 40 °C. The crude, semi-solid extract obtained was transferred into amber-colored glass bottles and stored at 4 °C until further use in phytochemical screening and chromatographic profiling [19,20].

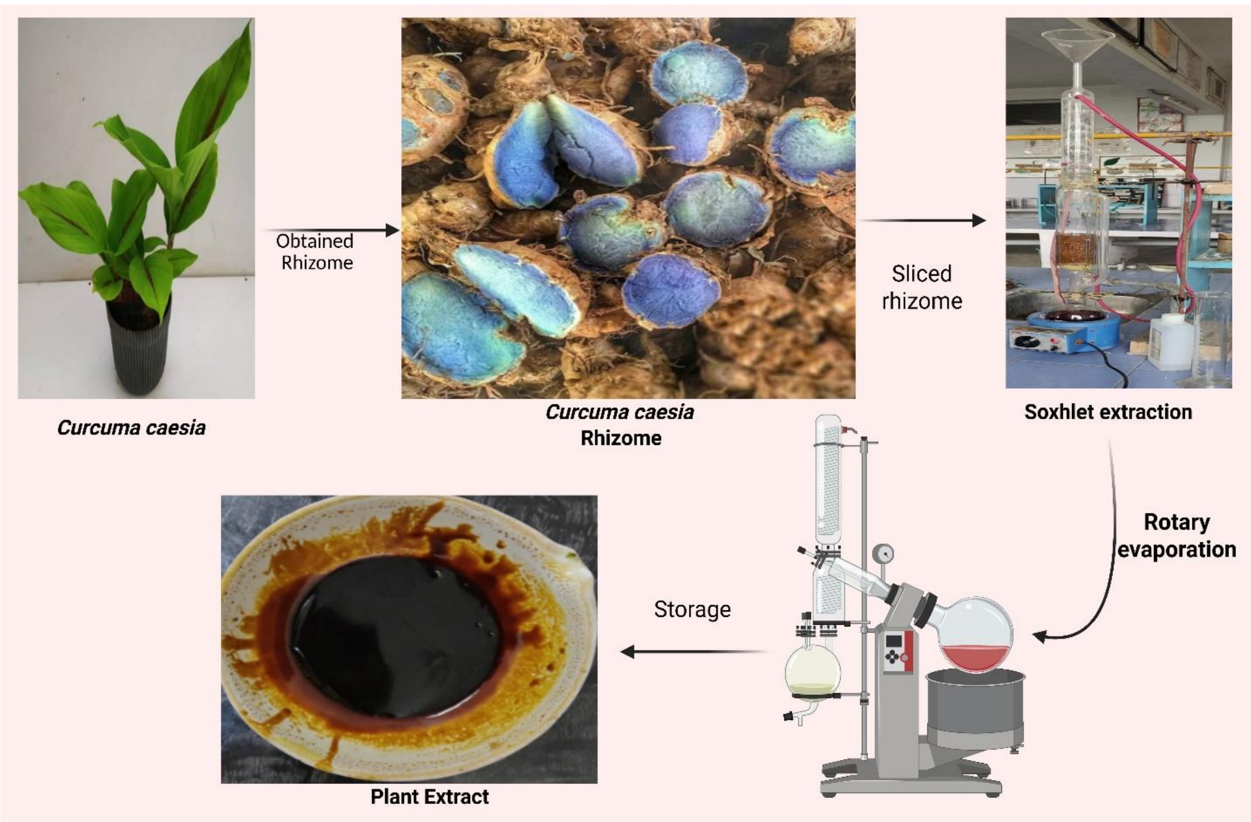

Figure 1: *Extraction process of Curcuma caesia* Roxb. *rhizome extract.*

2.3 Physicochemical Parameters

The physicochemical properties of *Curcuma caesia* rhizome powder were evaluated following standard pharmacopeial methods to assess its quality and purity. Total ash content was determined by incinerating the sample to measure total inorganic matter, while acid-insoluble and water-soluble ash values were obtained to estimate the siliceous and water-soluble mineral content, respectively. Alcohol-soluble and water-soluble extractive values were measured by macerating the powdered sample in 90% ethanol and distilled water, respectively, to assess the presence of polar and semi-polar phytoconstituents. These parameters aid in the standardization and authentication of the plant material [18,19].

2.4 Preliminary Phytochemical Screening

A preliminary qualitative phytochemical analysis of the hydroalcoholic extract of Curcuma caesia Roxb. rhizome was conducted to detect the presence of key secondary metabolites, including alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenoids, steroids, and glycosides. The screening was carried out using standard phytochemical testing protocols as described by Khandelwal [18] and Kokate [19]. These assays provided qualitative evidence for the presence of bioactive phytoconstituents that may underlie the plant's therapeutic potential [24].

2.5 Thin Layer Chromatography (TLC)

Thin Layer Chromatography (TLC) was employed to generate a chemical fingerprint of the hydroalcoholic extract of *Curcuma caesia* Roxb. rhizome and to qualitatively identify various classes of phytochemicals. TLC was selected as a cost-effective, reproducible, and straightforward technique for the separation and visualization of phytoconstituents of differing polarity and chemical nature.

2.5.1 Plate Preparation and Sample Application

TLC analysis was carried out using pre-coated silica gel 60 F254 aluminum plates (Merck, Germany). A working solution of the extract was prepared at a concentration of 1 mg/mL in ethanol. Using a fine capillary tube, 1–2 µL of the extract was applied 1 cm above the lower edge of the TLC plate. Multiple plates were used in parallel to test different solvent systems, allowing comparative evaluation of their efficacy in separating phytoconstituent groups [22].

2.5.2 Mobile Phase Systems

To achieve optimal resolution of both polar and non-polar constituents, several solvent systems were employed, including Methanol:Ethyl acetate (2:8), Toluene:Ethyl acetate (2:8, 4:6, 6:4, and 8:2), and Chloroform:Ethyl acetate (1:9). These mobile phases were selected to provide a range of polarity gradients, facilitating effective separation of diverse phytochemicals such as flavonoids, phenolics, terpenoids, and essential oils.

2.5.3 Development and Visualization

TLC plates were developed in airtight glass chambers pre-saturated with the respective mobile phases for 20 minutes to ensure uniform solvent front migration. The solvent was allowed to ascend to a distance of approximately 8 cm from the baseline. After development, the plates were removed, air-dried, and visualized under ultraviolet (UV) light at wavelengths of 254 nm and 365 nm. Multiple fluorescent and non-fluorescent spots were observed, indicative of diverse secondary metabolites [20-22].

Rf Value Determination and Data Interpretation:

Retention factor (Rf) values were calculated for each observed spot using the formula

$$RF = \frac{\text{Distance travelled by compound}}{Distance \text{ travelled by solvent front}}$$

Each spot was documented based on its color, fluorescence intensity, location, and corresponding Rf value. These chromatographic profiles served as a reproducible chemical signature for *C. caesia*, supporting the identification of phytochemical classes and providing insight into chemical diversity and batch-to-batch consistency. Furthermore, the TLC data may facilitate preliminary identification of compounds based on comparison with existing phytochemical literature (Lawand & Gandhi, 2013; Asante et al., 2016). The results confirmed the presence of multiple classes of secondary metabolites and established a visual fingerprint useful for chemotaxonomic studies and quality control assessments.

3. RESULTS

3.1 Physicochemical Characteristics of *Curcuma caesia* Rhizome

The physicochemical parameters of *Curcuma caesia* Roxb. rhizome were determined to assess its purity, quality, and the presence of both inorganic and extractable organic constituents. The results are summarized in Table 1. The total ash content was found to be 5.75% w/w, indicating a moderate level of total inorganic matter, which includes both physiological ash derived from the plant tissue and non-physiological ash such as environmental contaminants. The acid-insoluble ash value, which represents the siliceous matter mainly from soil contamination, was measured at 2.30% w/w, suggesting a relatively low presence of siliceous impurities. The water-soluble ash content was 3.10% w/w, signifying the presence of readily soluble inorganic salts. The extractive values further support the phytochemical richness of the rhizome. The alcohol-soluble extractive value was 15.53 w/w, indicating the abundance of semi-polar phytoconstituents such as flavonoids, terpenoids, and certain essential oils. In contrast, the water-soluble extractive value was 11.78% w/w, reflecting the presence of highly polar constituents including glycosides, phenolics, and tannins. These values are within acceptable pharmacopoeial limits and comply with quality standards prescribed by the Indian Pharmacopoeia and World Health Organization guidelines [16].

Table 1: Physicochemical Parameters of *Curcuma caesia* Rhizome

S. No	Parameter	Value (% w/w)
1.	Total Ash	5.75
2.	Acid-Insoluble Ash	2.30
3.	Water-Soluble Ash	3.10
4.	Alcohol-Soluble Extractive	15.53
5.	Water-Soluble Extractive	11.78

3.2 Phytochemical Screening of *Curcuma caesia* Rhizome Extract

Preliminary phytochemical screening of the hydroalcoholic extract of *Curcuma caesia* Roxb. rhizome revealed the presence of multiple classes of secondary metabolites, as summarized in **Table 2.** The extract tested positive for alkaloids, flavonoids, phenolic compounds, tannins, terpenoids, steroids, glycosides and saponins. The detected phytoconstituents are widely recognized for their therapeutic relevance. Flavonoids and phenolic compounds are particularly noted for their potent antioxidant activity, attributable to their free radical-scavenging and metal-chelating properties. Alkaloids and terpenoids have been associated with anti-inflammatory and antimicrobial actions, whereas steroids and glycosides may contribute to cytotoxic and anticancer effects through modulation of cell signaling and apoptosis pathways [2]. The phytochemical profile obtained in this study is consistent with prior reports on *C. caesia* and supports its traditional use in the treatment of inflammatory, infectious, and neoplastic conditions [22].

Table 2. Qualitative Phytochemical Screening of *Curcuma caesia* hydroalcoholic Extract.

S. N.	Phytochemical Class	Presence (+) / Absence (–)
1.	Alkaloids	+
2.	Flavonoids	+
3.	Phenolic compounds	+
4.	Tannins	+
5.	Terpenoids	+
6.	Steroids	+
7.	Glycosides	+
8.	Saponins	+

3.3 Thin Layer Chromatography (TLC) Profiling

Thin Layer Chromatography (TLC) analysis of the hydroalcoholic extract of *Curcuma caesia* rhizome was performed using six solvent systems with varying polarity to chemically fingerprint its phytochemical profile shown in **Figure 2.** The TLC plates revealed multiple well-defined bands under UV light (365 nm), indicating the presence of a wide spectrum of bioactive constituents. The observation of fluorescent spots further validated the presence of compound classes such as flavonoids, phenolics, terpenoids, and glycosides, consistent with prior phytochemical screenings [19].

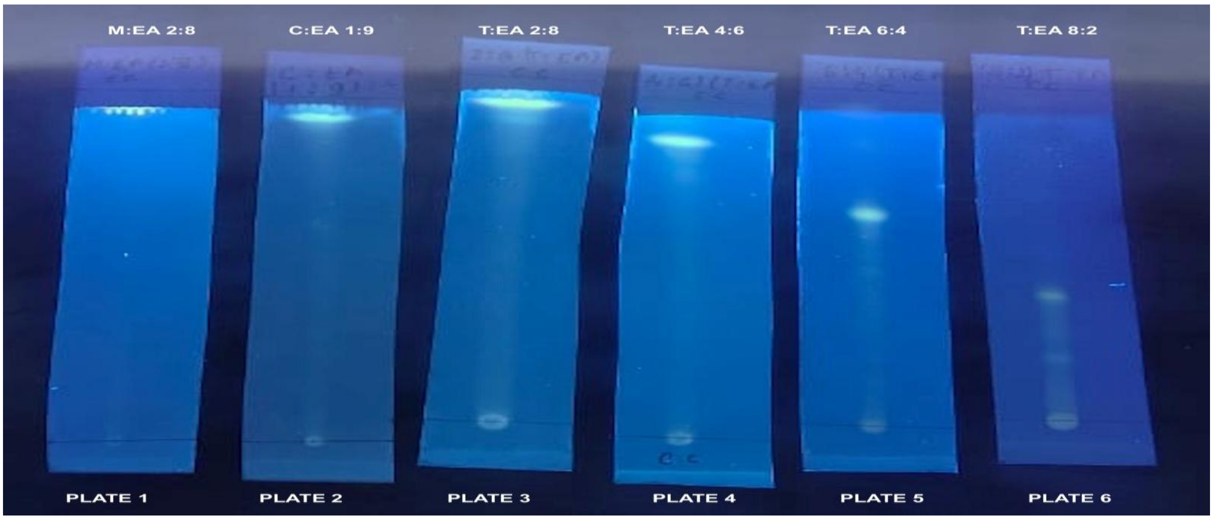

Figure 2: *Thin Layer Chromatography (TLC) profiling of hydroalcoholic extract of* Curcuma caesia *rhizome developed in six different solvent systems of varying polarity and visualized under UV light at 365 nm.* Plate 1: Methanol: Ethyl Acetate (2:8, v/v) – Polar system showing multiple upper fluorescent bands indicating efficient separation of phenolics and flavonoids. Plate 2: Chloroform: Ethyl Acetate (1:9, v/v) – Moderate polarity, showing fewer but intense spots suggestive of mid-polar compounds. Plate 3: Toluene: Ethyl Acetate (2:8, v/v) – Moderate polarity system demonstrating migration of several mid-polar phytochemicals. Plate 4: Toluene: Ethyl Acetate (4:6, v/v) – Medium polarity, showing bright bands indicative of terpenoids and flavonoids. Plate 5: Toluene: Ethyl Acetate (6:4, v/v) – Less polar system revealing a narrow range of bands, indicating non-polar to mid-polar constituents. Plate 6: Toluene: Ethyl Acetate (8:2, v/v) – Low polarity system with limited compound migration, showing retention of polar phytoconstituents near the baseline.

3.3.1 Solvent System 1: Methanol: Ethyl Acetate (2:8, v/v) – High Polarity (Hydrophilic Focus)

This highly polar solvent system exhibited strong resolving power, particularly for hydrophilic compounds. Several bright fluorescent bands were observed under UV light (365 nm), suggestive of phenolics, flavonoids, and glycosides. A total of seven distinct spots with Rf values ranging from 0.10 to 0.90 were recorded (Figure 2 and Table 2). Refer: Plate 1

Table 2: Rf values of phytoconstituent spots observed on TLC Plate 1 using Methanol: Ethyl Acetate (2:8, v/v) solvent system.

Spot No.	Distance Traveled by Compound (cm)	Rf Value
1	0.8	0.10
2	1.4	0.18
3	2.0	0.25
4	3.4	0.43
5	4.0	0.50
6	4.8	0.60
7	7.2	0.90

3.3.2 Solvent System 2: Chloroform: Ethyl Acetate (1:9, v/v) – Moderate Polarity

This moderately polar system facilitated efficient migration and separation of a wide range of mid-polar phytochemicals. A total of nine fluorescent bands with Rf values between 0.09 and 0.94 were recorded, indicating the presence of phenolics, sterols, and alkaloids (Table 3). *Refer: Plate 2*

Table 3. Rf values of phytoconstituent spots observed on TLC Plate 2 using Chloroform: Ethyl Acetate (1:9, v/v) solvent system.

Spot No.	Distance Traveled by Compound (cm)	Rf Value
1	0.7	0.09
2	1.3	0.16
3	2.0	0.25
4	2.8	0.35
5	3.6	0.45
6	4.5	0.56
7	5.3	0.66
8	7.0	0.88
9	7.5	0.94

3.3.3 Solvent System 3: Toluene: Ethyl Acetate (2:8, v/v) – Moderate Polarity

This polar system showed strong resolution of mid-polar and polar compounds. Nine distinct bands with Rf values ranging from 0.10 to 0.94 were observed, suggesting the presence of flavonoids, terpenoids, and glycosides (Table 4). Refer: *Plate 3*

Table 4: Rf values of phytoconstituent spots observed on TLC Plate 3 using Toluene: Ethyl Acetate (2:8, v/v) solvent system.

Spot No.	Distance Traveled by Compound (cm)	Rf Value
1	0.8	0.10
2	1.4	0.18
3	2.0	0.25
4	2.7	0.34
5	3.5	0.44
6	4.2	0.53
7	5.0	0.63
8	7.0	0.88
9	7.5	0.94

3.3.4 Solvent System 4: Toluene: Ethyl Acetate (4:6, v/v) – Medium Polarity

Eight well-separated spots were detected with Rf values ranging from 0.10 to 0.80, reflecting efficient separation of flavonoid and terpenoid-rich constituents (Table 5). Refer: *Plate 4*

Table 5: Rf values of phytoconstituent spots observed on TLC Plate 4 using Toluene: Ethyl Acetate (4:6, v/v) solvent system.

Spot No.	Distance Traveled by Compound (cm)	Rf Value
1	0.8	0.10
2	1.3	0.16
3	2.0	0.25
4	2.7	0.34
5	3.5	0.44
6	4.2	0.53
7	5.0	0.63
8	6.4	0.80

3.3.5 Solvent System 5: Toluene: Ethyl Acetate (6:4, v/v) – Low Polarity

This low-polarity solvent system allowed the migration of less polar compounds. A total of six bands were observed with Rf values ranging from 0.11 to 0.89 (Table 6), indicating the presence of lipophilic phytoconstituents including terpenoids. Refer: *Plate 5*

Table 6: Rf values of phytoconstituent spots observed on TLC Plate 5 using Toluene: Ethyl Acetate (6:4, v/v) solvent system.

Spot No.	Distance Traveled by Compound (cm)	Rf Value
1	0.9	0.11
2	1.6	0.20
3	2.2	0.28
4	3.0	0.38
5	4.6	0.58
6	7.1	0.89

3.3.6 Solvent System 6: Toluene: Ethyl Acetate (8:2, v/v) – Very Low Polarity

Six fluorescent spots with Rf values ranging from 0.11 to 0.85 were detected. The migration pattern suggests efficient movement of volatile oils and non-polar terpenoids (Table 7). *Refer: Plate 6*

Table 7: Rf values of phytoconstituent spots observed on TLC Plate 6 using Toluene: Ethyl Acetate (8:2, v/v) solvent system.

Spot No.	Distance Traveled by Compound (cm)	Rf Value
1	0.9	0.11
2	1.6	0.20
3	2.3	0.29
4	3.2	0.40
5	4.6	0.58
6	6.8	0.85

4. DISCUSSION

The present study provides compelling evidence supporting the diverse phytochemical constitution and therapeutic potential of the hydroalcoholic extract of *Curcuma caesia* rhizome. Preliminary phytochemical screening revealed the presence of key classes of secondary metabolites, including flavonoids, phenolics, alkaloids, terpenoids, saponins, and glycosides. These findings are consistent with previous ethnobotanical reports and pharmacological studies highlighting the traditional use of *C. caesia* in treating inflammatory, microbial, and neoplastic conditions [4,8]. Among the identified compounds, flavonoids and phenolics were especially abundant. Flavonoids, known for their strong antioxidant, anti-inflammatory, and anticancer properties, may contribute significantly to the pharmacological actions of the extract. Phenolic compounds, likewise, are well-documented for their ability to neutralize free radicals and mitigate oxidative stress—a process closely associated with cancer development and progression [11,13]. The presence of alkaloids and terpenoids, both recognized for their cytotoxic and antitumor activity, further adds to the anticancer prospects of the extract. Alkaloids can modulate cellular apoptosis and inhibit DNA synthesis, while terpenoids have demonstrated antiproliferative and antiangiogenic effects in various cancer models [10,14]. The TLC analysis reinforced the chemical diversity of the *C. caesia* extract by enabling the visualization and semi-quantitative assessment of phytoconstituents based on polarity. Six different solvent systems were employed to maximize separation efficiency across a range of compound polarities. Solvent systems with higher polarity, particularly Methanol:Ethyl Acetate (2:8, v/v) and Toluene:Ethyl Acetate (2:8, v/v), demonstrated superior resolution of polar compounds such as flavonoids, phenolics, and glycosides. These systems revealed multiple bright fluorescent spots under UV light at 365 nm, indicating the presence of strongly conjugated systems typical of polyphenolic structures [22-24]. Moderately polar systems such as Chloroform:Ethyl Acetate (1:9, v/v) and Toluene:Ethyl Acetate (4:6, v/v) offered efficient migration and resolution of both mid-polar and slightly non-polar compounds, capturing a broader chemical spectrum. In contrast, low-polarity systems (e.g., Toluene:Ethyl Acetate at 6:4 and 8:2, v/v) displayed limited migration of polar constituents but enabled better separation of lipophilic components, including essential oils and non-polar terpenoids—consistent with the aromatic and volatile nature of *C. caesia* rhizomes. The range of Rf values observed (0.08 to 0.94 across all plates) and the appearance of multiple distinct spots across different solvent systems underscore the extract's complex phytochemical nature. Such diversity is indicative of synergistic therapeutic potential, particularly in targeting multifactorial diseases such as cancer, where multiple signaling pathways are involved. Taken together, the integration of phytochemical screening and comprehensive TLC profiling offers a robust foundation for future bioassay-guided isolation of active constituents from *Curcuma caesia*. The rich presence of flavonoids, phenolics, and terpenoids—validated both qualitatively and chromatographically—positions this species as a promising source of natural compounds for anticancer research. Further investigations, including compound isolation, structural elucidation, and cytotoxic assays against relevant cancer cell lines (e.g., breast cancer), are warranted to establish pharmacological efficacy and mechanistic insights.

5. CONCLUSION

The findings of this study demonstrate that the hydroalcoholic extract of *Curcuma caesia* rhizome is rich in diverse phytochemicals, particularly flavonoids, phenolics, terpenoids, and alkaloids—compounds known for their potent antioxidant and anticancer properties. The preliminary phytochemical screening confirmed the presence of multiple bioactive groups, while Thin Layer Chromatography (TLC) profiling across six different solvent systems revealed a wide range of phytoconstituents with varying polarity. High-resolution separation achieved in polar and moderately polar solvent systems underscores the chemical complexity and therapeutic potential of the extract. Given the prominent presence of compounds associated with anti-proliferative, pro-apoptotic, and cytotoxic effects, *C. caesia* represents a promising natural candidate for further pharmacological investigations against cancer, particularly breast cancer. Future studies focusing on the isolation, structural characterization, and in vitro/in vivo evaluation of individual bioactive compounds will be instrumental in validating its therapeutic efficacy and elucidating mechanisms of action.

Declaration of Competing Interest: The authors declare no competing interest.

Ethics approval and consent to participate: NA

Consent for publication: The publication of the material in print, online or other media formats as determined by publisher.

Availability of data and material: All data and materials related to this work are available in the manuscript.

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