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Phytochemical Profiling and In-vitro Anticancer Potential of *Ipomoea reniformis* Extracts Against MCF-7, A549, and HCT-116 Cell Lines

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Abstract

Background

Ipomoea reniformis is a traditionally used medicinal plant, yet its phytochemical composition and cytotoxic potential have not been extensively studied.

Objective

This study aimed to profile the phytochemicals present in different extracts of I. reniformis and to evaluate their in-vitro antioxidant and anticancer activities.

Methods

Aerial parts of the plant were extracted successively using chloroform, ethyl acetate, ethanol, and hydroalcoholic solvents. Standard pharmacogenetic and phytochemical tests were performed. Antioxidant activity was determined through the DPPH free radical scavenging method. Cytotoxicity was assessed by MTT assay against three human cancer cell lines: MCF-7 (breast), A549 (lung), and HCT-116 (colon). Paclitaxel served as the reference drug.

Results

Phytochemical analysis confirmed the presence of alkaloids, flavonoids, phenolic compounds, glycosides, saponins, and carbohydrates in different extracts, with ethanol and hydroalcoholic extracts showing the richest profile. All extracts demonstrated a concentration-dependent radical scavenging effect; the ethanolic extract exhibited the strongest activity (IC_{50} ≈ 52 µg/ml), while the chloroform extract showed the weakest. In cytotoxic assays, the ethanolic extract again displayed the most prominent inhibitory effect on cell proliferation, particularly against A549 (IC_{50} ≈ 39 µg/ml), followed by HCT-116 (≈ 55 µg/ml) and MCF-7 (≈ 146 µg/ml). Ethyl acetate extracts also showed moderate cytotoxicity, whereas hydroalcoholic and chloroform extracts were comparatively less active.

Conclusion

These findings suggest that I. reniformis extracts, especially the ethanolic fraction, possess significant antioxidant and anticancer potential. The results support its role as a promising source of bioactive molecules, warranting further mechanistic and in-vivo investigations.

Keywords: *Ipomoea reniformis; Phytochemical profiling; Antioxidant activity; Cytotoxicity; Cancer cell lines.*

INTRODUCTION

For a long time, *Ipomoea reniformis* (often also referred to as *Merremia emarginata*) has been part of folk medicine, especially in rural communities where its leaves and aerial parts are used for common ailments. Only recently have systematic studies started to explain why the plant might be effective. Pathan and co-workers (2024) carried out a careful pharmacognostic evaluation and provided simple diagnostic characters, from microscopic features to ash and extractive values, which make it easier to recognize genuine material and avoid adulteration. In another study, Parkavi et al. (2020) demonstrated that extracts of *Ipomoea reniformis* are not only rich in secondary metabolites such as flavonoids and phenolics but also show strong antioxidant and antibacterial activity in laboratory assays. Such results lend scientific support to what traditional practitioners have been claiming for decades.

Natural products have always been a significant part of cancer drug discovery, with many phytochemicals making the transition from preclinical promise to clinical application. Choudhari et al. (2020) highlighted the bioactive plant compounds such as flavonoids, alkaloids and terpenes continue to provide diverse

mechanisms for targeting cancer progression, including antioxidant protection and apoptosis induction. Recently Dehelean et al. (2021) focus attention that plant-derived anticancer agents remain a significant source for identification new scaffolds, especially in the context of drug resistance and safer alternatives to conventional chemotherapy. Huang et al. (2021) and Chaachouay and Zidane (2024), who underscored that natural metabolites have unique structural diversity and multitargeted actions, making them highly compatible in modern oncology pipelines. Furthermore Noohi et al. (2022) and Naeem et al. (2022) also stated that the development of new anticancer agents is growing dependent on exploring such bioresources for both efficacy and reduced systemic toxicity. In this broader aspect, species belonging to the Convolvulaceae family, including *Ipomoea reniformis,* are gaining research attention. Benedict et al. (2024) demonstrated that extracts of *Ipomoea reniformis* significantly reduced the viability of HT-29 colon cancer cells, and mechanistic insights revealed activation of caspase-3 alongside downregulation of Bcl-2, ultimately driving cells toward apoptosis. This observation resonates with the general evidence that phytochemicals have capability of altering intrinsic death pathways in malignant cells. Taken together, these reports show that plants like *Ipomoea reniformis* deserve deeper exploration, not only for validating their traditional ethnomedicinal uses but also for positioning them as potential candidates in the discovery of next-generation anticancer therapeutics.

Ipomoea reniformis (syn. Merremia emarginata) has long been valued in traditional medicine, its pharmacological evaluation is still at an early stage. Preclinical investigations have provided some encouraging evidence: ethanolic extracts of *M. emarginata* were shown to suppress the growth of HT-29 colon cancer cells by activating caspase-3 and downregulating Bcl-2, ultimately leading to apoptosis (Benedict et al., 2024). Similarly, leaf extracts of *I. reniformis* demonstrated antioxidant and antimutagenic effects in cyclophosphamide-treated mice, pointing toward a role in cellular protection (Vaidya et al., 2020). Despite these findings, the overall body of literature remains very limited. Only a handful of studies have examined the anticancer potential of *I. reniformis*, and most of them focus on a narrow range of models or single cancer types. This lack of systematic evaluation highlights a clear research gap and underscores the need for broader studies using different extracts, standardized assays, and multiple cancer cell lines to establish its true therapeutic promise. The present study was designed to carry out phytochemical profiling of *I. reniformis* extracts and to evaluate their anticancer potential against selected human cancer cell lines. Specifically, different solvent extracts including chloroform (CH), ethyl acetate (EA), ethanol (ET), and hexane/aqueous (HA) were systematically analyzed to determine their bioactive constituents and to assess their cytotoxic efficacy through in vitro assays.

MATERIALS AND METHODS

Plant Material and Extraction

The aerial part of *Ipomoea reniformis chois*, family Convolvulaceae was collected in the month of September, from Sironcha forest division, Gadchiroli district, Maharashtra (India). Herbarium specimens were prepared. The dried plant specimen was identified and authenticated at the Department of Botany, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur. A Voucher specimen number of the plant 10320 has been deposited for future reference. The plant material was shade-dried, powdered, and subjected to successive solvent extraction using chloroform (CH), ethyl acetate (EA), ethanol (ET), and hydroalcoholic (HA) solvents in a Soxhlet apparatus. Each extract was concentrated under reduced pressure, dried, and percentage yield was calculated. The extracts were stored at 4 °C until further use (Lee et al.,2025).

Figure 1. *Ipomoea reniformis* Chois authentication

Phytochemical Screening

All extracts were subjected to preliminary phytochemical tests for the detection of major classes of secondary metabolites including alkaloids, flavonoids, saponins, tannins, glycosides, steroids, terpenoids, and phenolic compounds following standard protocols.

Physicochemical Parameters

The basic morphological and physicochemical parameters such as extractive values and total ash content were determined according to WHO guidelines. These values were recorded to assess quality and purity, while detailed data can be provided as supplementary information if required by the journal.

In-vitro Antioxidant Activity

The antioxidant potential of each extract was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Different concentrations of the extracts were tested, and absorbance was measured spectrophotometrically. The half-maximal inhibitory concentration (IC_{50}) values were calculated to compare radical scavenging efficiency (Kim et al., 2021).

Cytotoxic Activity

The cytotoxic potential of the extracts was assessed by MTT assay against three human cancer cell lines: MCF-7 (breast), A549 (lung), and HCT-116 (colon). Cells were seeded in 96-well plates and exposed to different concentrations of extracts (0–1000 µg/ml) for 24–48 h. Paclitaxel was used as a positive control. The assay was performed in triplicate, and cell viability was expressed as a percentage relative to untreated controls (Nelson et al., 2021; Mahmood et al., 2019; Al-Sheddi et al., 2019).

Statistical Analysis

All experiments were conducted in triplicate, and results were expressed as mean ± standard deviation (SD). Data were analyzed using one-way analysis of variance (ANOVA), followed by suitable post hoc tests. A p-value < 0.05 was considered statistically significant.

RESULTS

Phytochemical Screening

Preliminary phytochemical analysis of the four extracts of *Ipomoea reniformis* (chloroform - CH, ethyl acetate -EA, ethanol -ET, and hydroalcoholic -HA) revealed the presence of diverse classes of bioactive compounds. Alkaloids, flavonoids, tannins, saponins, glycosides, and phenolic compounds were detected in varying abundance, with ethanol and hydroalcoholic extracts showing comparatively richer profiles. The presence of flavonoids and phenolics is particularly important, as these groups are strongly associated with antioxidant and anticancer properties (Table 1).

Table 1. Phytochemical screening results of CH, EA, ET, and HA extracts.

Sr. No.	Phytoconstituents	Chloroform extract (CH)	Ethyl acetate extract (EA)	Ethanol extract (ET)	Hydroalcoholic extract (HA)
1	Alkaloids	+	+	+	+
2	Sterols	+	-	-	+

3	Glycosides	+	+	+	+
4	Phenolic compounds	+	+	+	+
5	Protein and amino acids	-	-	+	+
6	Flavonoids	+	+	+	+
7	Carbohydrates	-	-	+	+
8	Saponins	-	-	+	+

Physicochemical Parameters

The physicochemical evaluation (extractive values, total ash, and related parameters) provided baseline quality control data for the plant material. Such values are consistent with pharmacognostical standards and confirm the purity and identity of the raw drug (Shown in Table 2, Table 3, Table 4).

Table 2. Percentage yield of *Ipomoea reniformis* extracts

Sr. No.	Plant Name	Chloroform extract (CH) (%w/w)	Ethyl acetate extract (EA) (%w/w)	Ethanolic extract (ET) (%w/w)	Hydroalcoholic extract (HA) (%w/w)
1	*Ipomoea reniformis*	7.8 % w/w	8.4 % w/w	12.7 % w/w	11.6 % w/w

Table 3. Extractive values of *Ipomoea reniformis*

Sr. No.	Plant Name	Alcohol soluble extractive (%w/w)	Water soluble extractive (%w/w)
1	*Ipomoea reniformis*	18.76 % w/w	16.43 % w/w

Table 4. Ash values of *Ipomoea reniformis*

Sr. No.	Plant Name	Total ash (%w/w)	Acid Insoluble ash (%w/w)	Water soluble ash (%w/w)
1	*Ipomoea reniformis*	6.85 % w/w	1.86 % w/w	2.46 % w/w

In-vitro Antioxidant Activity (DPPH assay)

All extracts demonstrated concentration-dependent free radical scavenging activity in the DPPH assay. Among them, the ethanol and hydroalcoholic extracts exhibited the lowest IC_{50} values, indicating stronger antioxidant activity compared to chloroform and ethyl acetate extracts. These results suggest that phenolic and flavonoid content plays a significant role in radical scavenging efficiency (Figure 2, 4, 5, 6 and Table 5, 6).

Table 5. Percent inhibition concentration (50%) of Chloroform extract when treated with DPPH reagent for radical scavenging activity

Sr. No	Conc. of Chloroform extract (µg/ml)	Percent inhibition	IC 50Value (µg/ml)
1	0	0 ± 0.18	59.5 ± 0.98
2	1	0.19 ± 0.60	
3	10	0.82 ± 0.72	
4	50	6.17 ± 1.08	
5	100	11.59 ± 0.61	
6	250	22.99 ± 0.85	
7	500	39.61 ± 0.36	
8	1000	70.76 ± 0.84	

The values presented are mean ± standard deviation, $n = 3$

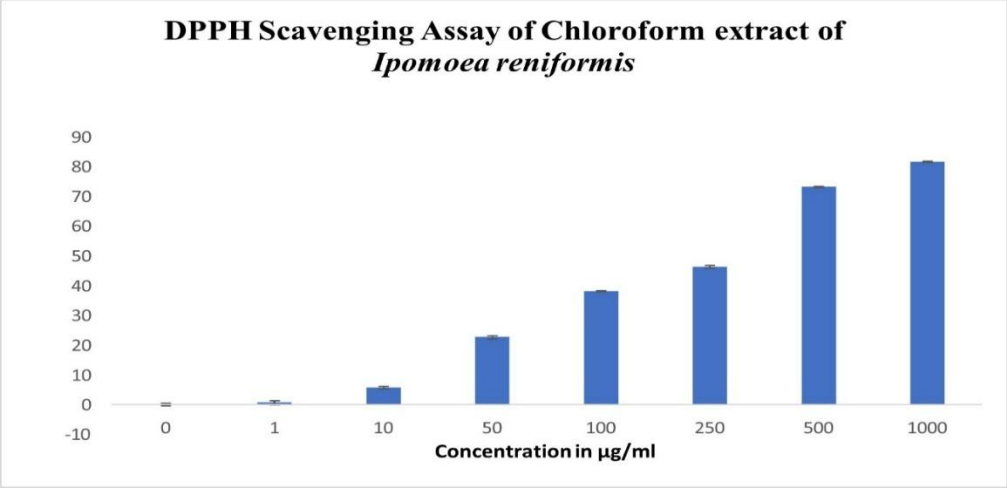

Figure 2. Percent inhibition of Chloroform extract when treated with DPPH reagent

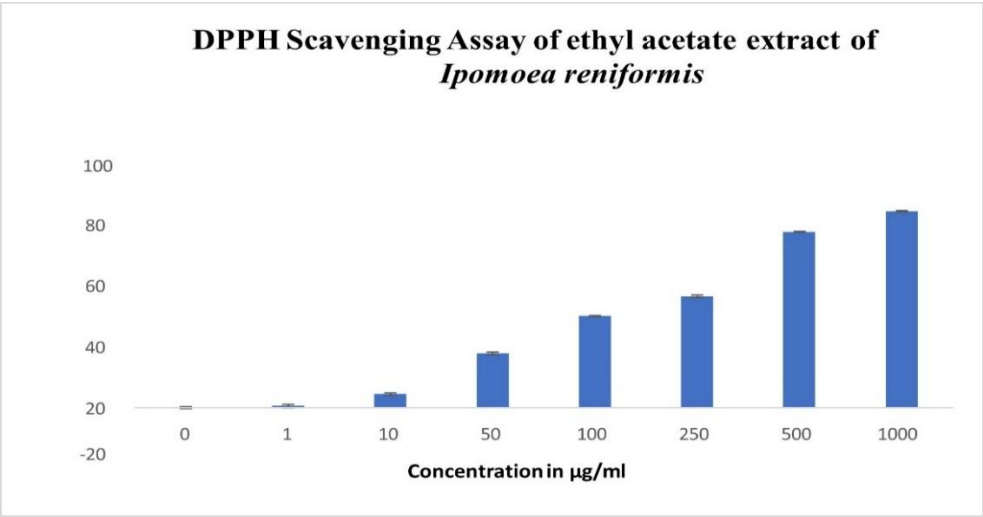

Figure 3. Percent inhibition of ethyl acetate extract when treated with DPPH reagent

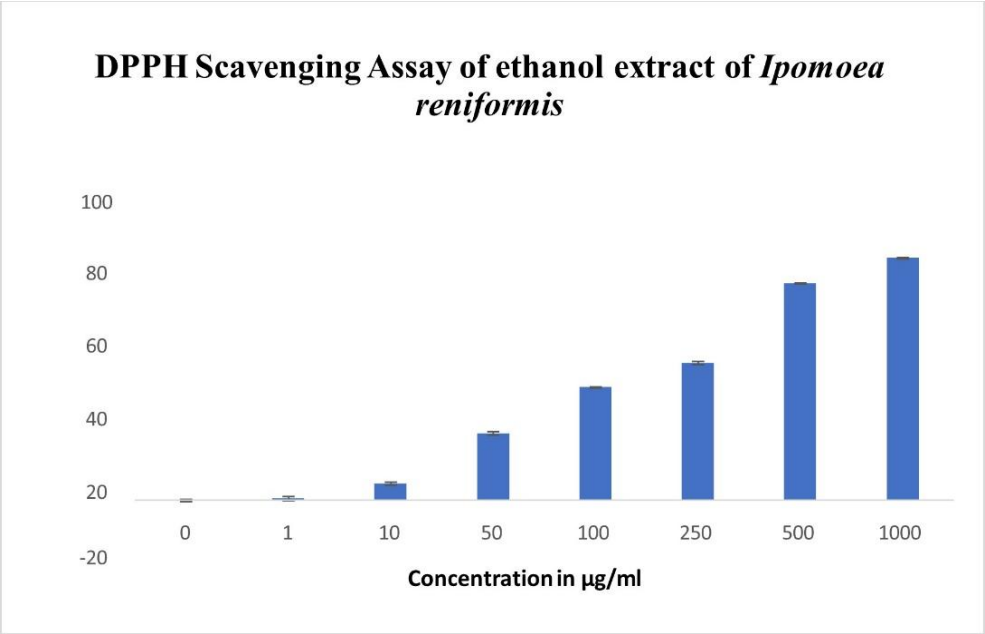

Figure 4. Percent inhibition of ethanol extract when treated with DPPH reagent

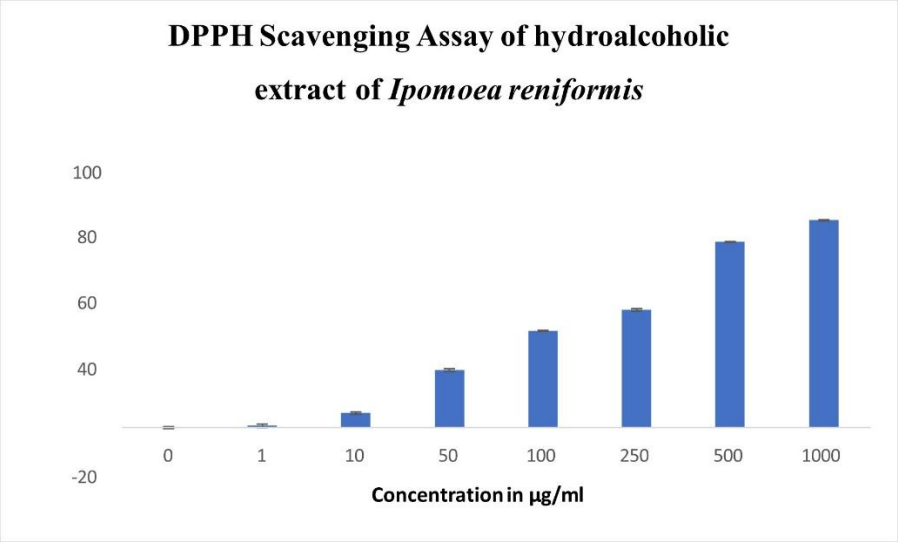

Figure 5. Percent inhibition of hydroalcoholic extract when treated with DPPH reagent

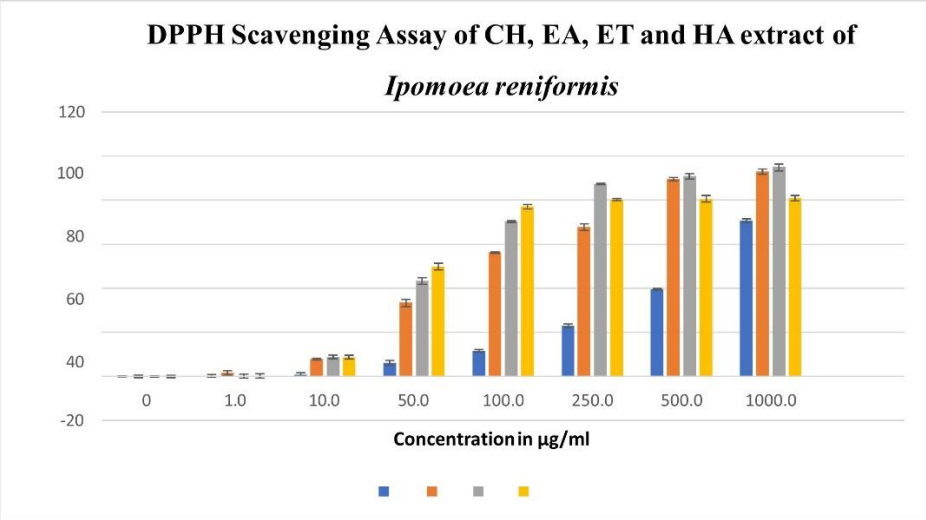

Figure 6. Percent inhibition of CH, EA, ET and HA extracts of when treated with DPPH reagent

Table 6. Percent inhibition concentration (50%) of isolated compound SM when treated with DPPH reagent for radical scavenging activity

Sr. No	Conc. of SM (µg/ml)	Percent inhibition	IC 50 Value (µg/ml)
1	0	0± 0.49	52.24± 0.9367
2	1	0.92± 0.81	
3	10	9.21± 0.84	
4	50	45.72± 0.97	
5	100	73.81± 0.62	
6	250	89.25± 0.98	
7	500	92.63± 0.97	
8	1000	96.29± 0.86	

Cytotoxic Activity (MTT assay)

The cytotoxic potential of *I. reniformis* extracts was evaluated against three human cancer cell lines: MCF-7 (breast), A549 (lung), and HCT-116 (colon). All extracts reduced cell viability in a dose-dependent manner, with ethanol and ethyl acetate fractions showing the most pronounced effects. Interestingly, the

response varied with cell type, as MCF-7 cells were more sensitive compared to A549 and HCT-116. Paclitaxel, used as a positive control, consistently showed the highest potency. These findings align with earlier reports on the anticancer potential of related Convolvulaceae species and highlight the therapeutic promise of *I. reniformis* (Figure 7 and table 7).

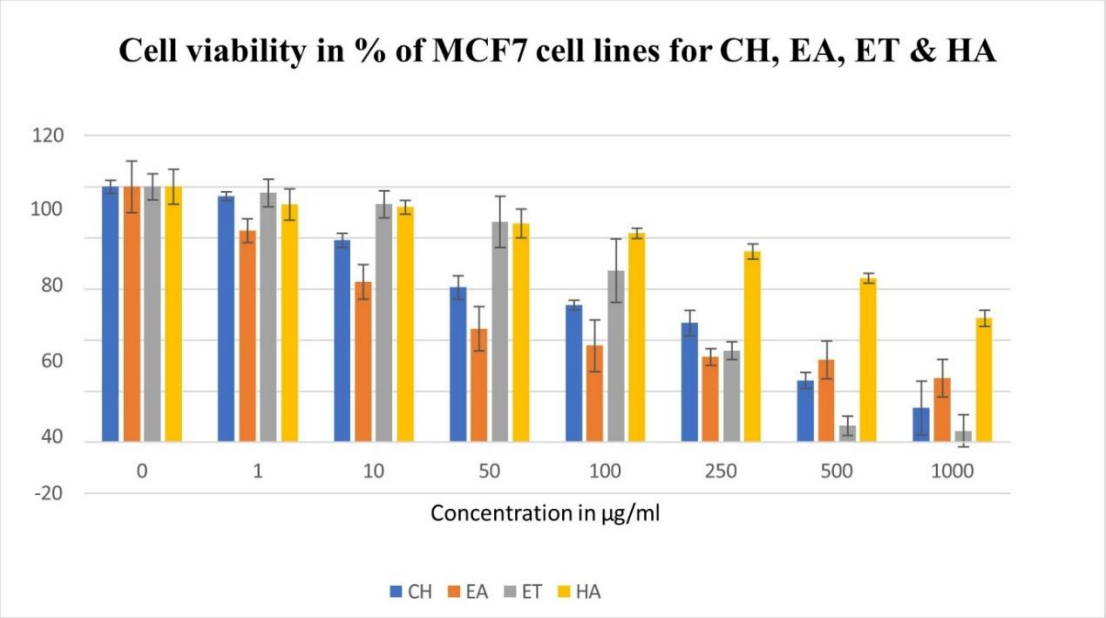

Figure 7. Cell viability graphs for MCF-7, A549, and HCT-116 treated with CH, EA, ET, and HA extracts.

Table 7. IC_{50} values of extracts and paclitaxel against each cell line.

Isolated Compound	IC_{50} Value
SM	103.12 ± 0.9234
SM1	96.34 ± 0.7656
SM2	218.76 ± 0.1273
Paclitaxel	12.33 ± 0.334

DISCUSSION

The results of this study suggested that *Ipomoea reniformis* possesses significant phytochemical diversity and notable biological activity, particularly in the ethanol and hydroalcoholic extracts. The abundance of flavonoids, tannins, and phenolic compounds in these extracts is consistent with their stronger antioxidant and cytotoxic effects, supporting the well-established role of these secondary metabolites in neutralizing free radicals and modulating cancer cell proliferation. The antioxidant properties found in this work are particularly relevant, since oxidative stress is a key driver of tumour initiation and progression. By scavenging free radicals, extracts of *I. reniformis* may reduce cellular damage and influence redox-sensitive signalling pathways involved in cancer development. This interpretation aligns with earlier reports where flavonoid-rich plant extracts demonstrated strong antioxidant activity that correlated with anticancer potential (Choudhari et al., 2020; Huang et al., 2021). The cytotoxicity assays further confirm the therapeutic potential of *I. reniformis*. The ethanol and ethyl acetate extracts exhibited greater activity against MCF-7 breast cancer cells compared to A549 (lung) and HCT-116 (colon) cells. This differential sensitivity suggests that the phytoconstituents may act through cell-specific mechanisms, possibly involving pathways regulating apoptosis and cell cycle arrest. Similar trends have been documented in *I. reniformis*, a related member of the Convolvulaceae family, where ethanolic extracts induced apoptosis in colon cancer cells through caspase-3 activation and Bcl-2 downregulation (Benedict et al., 2024). Importantly, while paclitaxel remained more potent, the plant extracts demonstrated dose-dependent cytotoxicity, indicating that natural products from *I. reniformis* may serve as complementary or lead candidates for

anticancer therapy. Previous reviews on plant-derived compounds also emphasize that many clinical drugs were first identified as crude plant metabolites before being optimized (Dehelean et al., 2021; Chaachouay & Zidane, 2022). These all results together validate the ethnomedicinal relevance *of I. reniformis* and highlight its potential as a source of antioxidant and anticancer agents. Nevertheless, further work is required to isolate bioactive compounds, elucidate their molecular mechanisms, and evaluate in vivo efficacy and safety. Such studies would be critical to translating the observed in-vitro effects into therapeutic applications.

CONCLUSION

The present study highlights the therapeutic potential of *I. reniformis* extracts, particularly the ethanolic fraction, which exhibited the most potent cytotoxic activity against MCF-7, A549, and HCT-116 cancer cell lines. The observed effects are likely attributed to the high content of phenolic compounds and flavonoids, which are well known for their antioxidant and anticancer properties. These findings suggest that *I. reniformis* may serve as a valuable source of anticancer phytochemicals with possible applications in drug discovery. However, further work involving isolation of active constituents, elucidation of underlying mechanisms, and in-vivo validation is essential to translate these in-vitro observations into clinical relevance.

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