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In Vitro Cytotoxicity Assessment of HepG2 Cells of *Pulicaria jaubertii* Potential via MTT Assay

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Abstract

The search for novel anticancer agents from natural sources continues to be a promising avenue in cancer research. Pulicaria jaubertii, a medicinal plant with traditional therapeutic applications, has shown potential bioactive properties warranting investigation for anticancer activity. This study aimed to evaluate the in vitro cytotoxicity of Pulicaria jaubertii extracts against HepG2 hepatocellular carcinoma cells using the MTT assay to assess its anticancer potential. The plant material was extracted using methanol, petroleum ether, and aqueous solvents. Cytotoxicity was assessed using the MTT assay. The results demonstrated that the methanol extract exhibited the most potent cytotoxic activity with an IC_{50} value of 87.48 µg/mL. In contrast, petroleum ether and aqueous extracts showed significantly lower activity, with IC_{50} values of 283.3 µg/mL and 287.5 µg/mL, respectively. These findings suggest that P. jaubertii contains bioactive compounds, particularly in the methanolic fraction, warranting further investigation for anticancer drug development.

Keywords: *Pulicaria jaubertii, cytotoxicity, HepG2 cells, MTT assay, anticancer, hepatocellular carcinoma*

Introduction

Hepatocellular carcinoma (HCC) is the most common primary malignancy of the liver and represents a major global health burden. It is the sixth most frequently diagnosed cancer and the fourth leading cause of cancer-related mortality worldwide, accounting for a significant proportion of cancer deaths each year (Bray et al., 2018; Sung et al., 2021). The high mortality rate associated with HCC is largely attributed to late diagnosis, rapid disease progression, and limited therapeutic options. Although surgical resection, liver transplantation, and targeted therapies have improved patient outcomes, chemotherapy remains a key treatment modality for advanced-stage HCC. However, its clinical application is often constrained by severe adverse effects, systemic toxicity, and the development of multidrug resistance (Fotakis & Timbrell, 2006; Llovet et al., 2021).

The limitations of conventional anticancer therapies have driven increasing interest in alternative and complementary treatment strategies, particularly those based on natural products. Natural compounds are known for their structural diversity, biological specificity, and comparatively lower toxicity, making them attractive candidates for anticancer drug discovery (Newman & Cragg, 2020). Notably, approximately 60% of currently approved anticancer drugs are derived from natural sources or their synthetic analogues, underscoring the critical role of medicinal plants

in modern oncology (Atanasov et al., 2015; Newman & Cragg, 2016). Medicinal plants have been utilized in traditional systems of medicine for centuries and continue to provide a rich reservoir of bioactive secondary metabolites with therapeutic potential. These compounds, including flavonoids, phenolic acids, terpenoids, and alkaloids, have been shown to exert anticancer effects through multiple mechanisms such as induction of apoptosis, inhibition of cell proliferation, modulation of oxidative stress, and interference with cell signaling pathways (Greenwell & Rahman, 2015; Cragg & Pezzuto, 2016). Among medicinal plant genera, *Pulicaria* (family Asteraceae) has attracted attention due to its broad range of pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, and cytotoxic properties (Al-Fatimi et al., 2007; Al-Musayeib et al., 2011).

Pulicaria jaubertii is a traditionally used medicinal plant that has been reported to contain diverse bioactive constituents such as flavonoids, terpenoids, and phenolic compounds (Bouriche et al., 2011). These phytochemicals are widely recognized for their role in cancer prevention and therapy, particularly through their ability to regulate oxidative damage and promote programmed cell death in cancer cells (Khan et al., 2012; Zhang et al., 2015). Despite the documented biological activities of various *Pulicaria* species, systematic studies evaluating the anticancer potential of *P. jaubertii*, especially against hepatocellular carcinoma, remain limited. In vitro cytotoxicity assays play a crucial role in the preliminary screening of potential anticancer agents. The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay is one of the most widely used and reliable methods for assessing cell viability and cytotoxicity. This assay is based on the reduction of MTT to insoluble formazan crystals by mitochondrial dehydrogenases in metabolically active cells, providing a quantitative measure of cell survival and proliferation (Mosmann, 1983; van Meerloo et al., 2011).

Therefore, the present study aims to evaluate the in vitro cytotoxic effects of different solvent extracts of *Pulicaria jaubertii* against HepG2 hepatocellular carcinoma cells using the MTT assay. The findings of this study are expected to contribute to the understanding of the anticancer potential of *P. jaubertii* and support its possible development as a natural therapeutic agent for the management of liver cancer.

Materials and Methods

Plant Material Collection and Extract Preparation

Fresh plant material of *Pulicaria jaubertii* was Collected from southern Yemen at the end of summer 2023. Collected plant material was thoroughly washed with distilled water to remove dust and debris, shade-dried at room temperature, and then ground into a fine powder using a mechanical grinder.

Extraction was carried out using three different solvents of varying polarity to obtain a broad spectrum of phytochemicals. For the methanolic extract, the powdered plant material was subjected to maceration with methanol (99.9% purity) for 48–72 hours with occasional stirring to facilitate maximum extraction of polar compounds. The petroleum ether extract was prepared using petroleum ether to selectively extract non-polar constituents such as lipids and terpenoids. For the aqueous extract, plant powder was extracted using distilled water to obtain watersoluble phytoconstituents. All extracts were filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator. The concentrated extracts were then lyophilized

to obtain dry residues, which were stored at −20 °C until further use. Stock solutions of each extract were freshly prepared in dimethyl sulfoxide (DMSO) prior to biological assays, ensuring that the final concentration of DMSO in the culture medium did not exceed 0.5% (Harborne, 1998; Newman & Cragg, 2020). Qualitative and quantitative assessments were conducted using Gas Chromatography-Mass Spectrometry (GCMS) and Fourier Transform Infrared Spectroscopy (FTIR) to identify and characterize bioactive compounds.

Cell Culture

The human hepatocellular carcinoma cell line HepG2 was obtained from a certified cell repository. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin. The cultures were maintained at 37 °C in a humidified incubator with 5% CO_2 atmosphere. Cells were regularly monitored for morphology and confluency, and those in the exponential growth phase were used for all experiments to ensure reproducibility and accuracy of results (Freshney, 2016).

MTT Cytotoxicity Assay

The cytotoxic activity of *Pulicaria jaubertii* extracts against HepG2 cells was evaluated using the MTT assay, following the method described by Mosmann (1983) with slight modifications. Briefly, HepG2 cells were seeded into 96-well culture plates at a density of 1×10^4 cells per well and allowed to adhere for 24 hours.

After incubation, cells were treated with different concentrations of methanolic, petroleum ether, and aqueous extracts (0, 62.5, 125, 250, 500, and 1000 µg/mL). Untreated cells served as the negative control. Following 24 hours of treatment, 20 µL of MTT solution (5 mg/mL prepared in phosphate-buffered saline) was added to each well, and the plates were further incubated for 2 hours at 37 °C. During this period, metabolically active cells reduced the MTT reagent to insoluble purple formazan crystals via mitochondrial dehydrogenase activity. Subsequently, the culture medium was carefully removed, and 150 µL of DMSO was added to each well to dissolve the formazan crystals. The absorbance was measured at 570 nm using a microplate reader. The intensity of the color formed was directly proportional to the number of viable cells (Mosmann, 1983; van Meerloo et al., 2011).

Statistical analysis

Cell viability was calculated as a percentage relative to untreated control cells using the following equation:

$$\text{Cell viability (\%)} = \left(\frac{\text{Absorbance of treated cells}}{\text{Absorbance of control cells}}\right) \times 100$$

The half-maximal inhibitory concentration (IC_{50}), defined as the concentration of extract required to inhibit 50% of cell viability, was determined using non-linear regression analysis. All experiments were performed in triplicate, and results are expressed as mean ± standard deviation (SD). Data analysis and graphical representation were carried out using SPSS statistical software.

Results and Discussion

Extraction yields of plant extracts.

The extraction yields obtained in this study demonstrated significant variations across different solvents and plant species, reflecting the differential solubility of bioactive compounds in various extraction media. *Pulicaria jaubertii* showed preferential extraction with water (23.8%), achieving the highest water yield among all three species, with substantially lower yields in methanol (11.33%) and petroleum ether (2.47%).

The extraction yields obtained demonstrate significant solvent-dependent variations that reflect the differential solubility profiles of bioactive compounds in *Pulicaria jaubertii*, providing crucial insights into the phytochemical distribution and optimal extraction strategies. The remarkably high aqueous extraction yield (23.8%) compared to methanol (11.33%) and petroleum ether (2.47%) indicates that *P. jaubertii* contains a substantial proportion of highly polar, water-soluble constituents including polysaccharides, glycosides, phenolic compounds, and inorganic salts (Harborne, 1998). This aqueous extraction efficiency aligns with traditional preparation methods where water-based decoctions and infusions have been predominantly used for medicinal applications, suggesting that indigenous knowledge systems have empirically optimized extraction procedures to maximize therapeutic compound recovery (Heinrich et al., 2004).

The moderate methanolic yield (11.33%) reflects the extraction of semi-polar bioactive compounds including flavonoids, alkaloids, saponins, and terpenoids, which are recognized as primary contributors to pharmaceutical activities in medicinal plants (Cos et al., 2006). This yield is consistent with findings in other *Pulicaria* species where methanol has demonstrated effectiveness in extracting bioactive constituents with antimicrobial, antioxidant, and cytotoxic properties (AlMusayeib et al., 2011). The significantly lower petroleum ether yield (2.47%) indicates limited content of non-polar constituents such as essential oils, fatty acids, and lipophilic terpenoids, though these compounds often possess potent biological activities despite their lower abundance (Mothana et al., 2014).

GC–MS analysis

GC–MS analysis of *Pulicaria jaubertii* extracts showed a clear retention time– dependent distribution of metabolites. In the methanol and aqueous extracts, early retention times (RT 8.6–13.6 min) were characterized by monoterpenes and oxygenated terpenoids such as cyclohexanone derivatives, alcohols, and terpenoid ketones, followed at mid-retention times (RT 15.2–15.5 min) by diterpenes including neophytadiene and phytol. The fatty acid region (RT 16.0–16.9 min) was dominated by palmitic acid (n-hexadecanoic acid) and linoleic acid (9,12octadecadienoic acid), while later retention times (RT 18.4–21.8 min) revealed long-chain fatty alcohols and sterol derivatives such as heptatriacotanol and sterol esters. In contrast, the petroleum ether extract exhibited predominantly non-polar metabolites, with saturated fatty acids and methyl esters (myristic acid, palmitic acid, methyl palmitate) at RT 14.7–16.0 min, diterpenes (neophytadiene, phytol derivatives) at RT 15.2–15.5 min, polyunsaturated fatty acids (linoleic acid) at RT ~16.9 min, and wax hydrocarbons and ether lipids (heptacosane, octadecyl vinyl ether) at RT 18.6–20.1 min. Collectively, these profiles demonstrate a systematic transition from volatile terpenoids to fatty acids and complex lipids, underscoring the phytochemical diversity and medicinal relevance

of *P. jaubertii*. (Fig:1,2,3) The GC-MS analysis of *Pulicaria jaubertii* extracts demonstrates a comprehensive phytochemical profile with systematic elution patterns transitioning from volatile terpenoids to complex lipids, reflecting the chemical diversity underlying its therapeutic potential. Early retention times (RT 8.6–13.6 min) revealed monoterpenes and oxygenated terpenoids including cyclohexanone derivatives with established antimicrobial and antioxidant properties similar to other *Pulicaria* species (Al-Musayeib et al., 2011), while mid-retention times (RT 15.2–15.5 min) identified crucial diterpenes neophytadiene and phytol possessing antimicrobial, antioxidant, anti-inflammatory, and cytotoxic activities (Islam et al., 2018; Ravi & Krishnan, 2017). The fatty acid composition dominated by palmitic and linoleic acids (RT 16.0–16.9 min) contributes significantly to biological activity through anti-inflammatory and antimicrobial mechanisms (Das, 2020), while long-chain fatty alcohols and sterol derivatives including heptatriacotanol (RT 18.4–21.8 min) enhance therapeutic profiles with cholesterol-lowering and immunomodulatory effects (Moreau et al., 2002). The petroleum ether extract's distinct non-polar metabolite profile, featuring wax hydrocarbons like heptacosane with potential antimicrobial properties (Rajab et al., 1998), demonstrates solvent-dependent extraction efficiency and provides scientific validation for the plant's ethnomedicinal applications across diverse therapeutic domains.

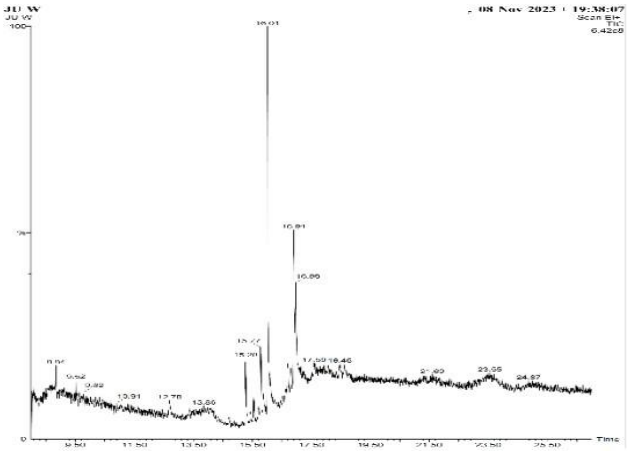

Fig 1: GC–MS analysis of (Ju-w) extract

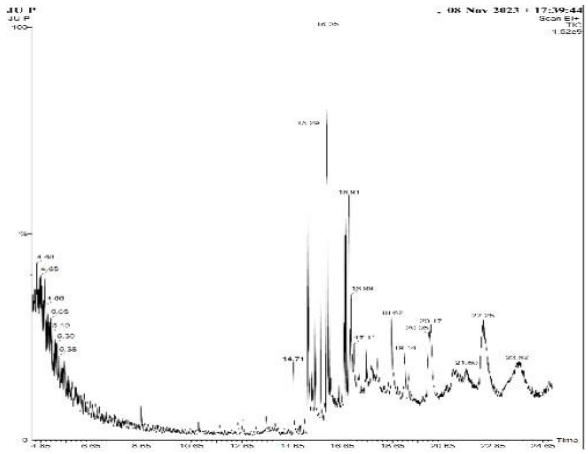

Fig 2: : GC–MS analysis of (JU-P) extract

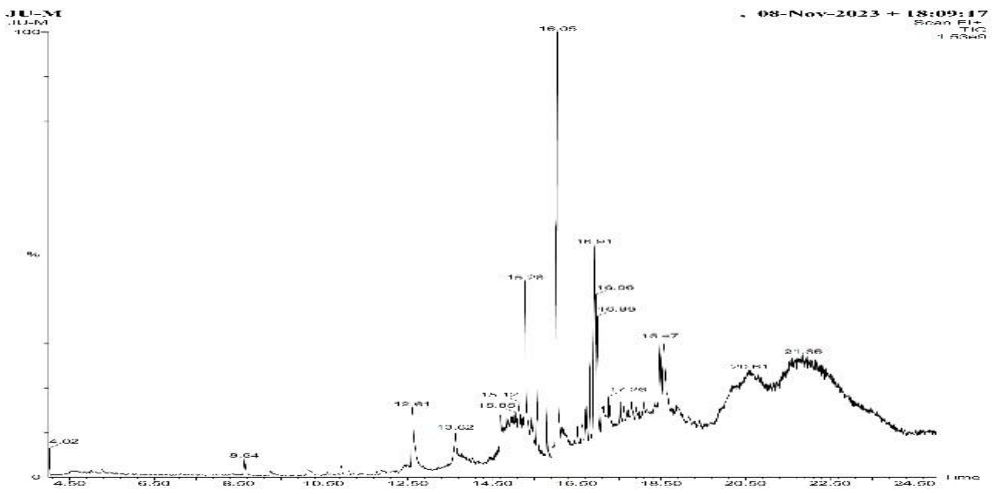

Fig 3: GC–MS analysis of methanol extract of (JU-M)

FTIR Spectroscopic analysis

FTIR spectroscopic analysis of *Pulicaria jaubertii* extracts confirmed solventspecific functional group profiles. The aqueous extract (JU-W) exhibited a broad O–H stretching band at 3326.01 cm^{-1} indicating alcohols and phenols, a weak band at 2117.36 cm^{-1} corresponding to triple-bonded functionalities (alkynes/nitriles), and a peak at 1636.48 cm^{-1} attributed to C=C stretching or O–H bending, reflecting polar bioactive constituents. The petroleum ether extract (JU-P) was dominated by non-polar components, showing very strong aliphatic C–H stretching bands at 2956, 2924, and 2858 cm^{-1}, a carbonyl (C=O) stretching band at 1711.90 cm^{-1}, and characteristic methylene bending and rocking vibrations at 1460.98, 1378.41, and 724.00 cm^{-1}, confirming long-chain saturated hydrocarbons. The methanol extract (JU-M) similarly displayed strong aliphatic C–H stretching bands at 2957, 2925, and 2868 cm^{-1}, a C=O stretching peak at 1697.20 cm^{-1} indicative of carboxylic acids or ketones, C–H bending vibrations at 1460.67 and 1378.92 cm^{-1}, a weak vinylidene (=C–H) band at 886.72 cm^{-1}, and a methylene rocking band at 724.31 cm^{-1}, suggesting the presence of long-chain hydrocarbons with unsaturated and oxygenated functional groups. (fig: 4,5,6)

The FTIR spectroscopic analysis of *Pulicaria jaubertii* extracts revealed distinct solvent-dependent functional group profiles that validate the phytochemical diversity observed in GC-MS analysis. The aqueous extract (JU-W) exhibited characteristic O–H stretching at 3326.01 cm^{-1} and C=C stretching at 1636.48 cm^{-1}, confirming the presence of phenolic compounds and aromatic systems typical of antioxidant-active constituents (Silverstein et al., 2005; Coates, 2000). The petroleum ether extract (JU-P) demonstrated predominant aliphatic characteristics with strong C–H stretching bands (2956, 2924, 2858 cm^{-1}) and carbonyl stretching at 1711.90 cm^{-1}, indicating long-chain hydrocarbons and ester functionalities consistent with fatty acid derivatives and terpenoids (Stuart, 2004; Pavia et al., 2009).

Similarly, the methanol extract (JU-M) displayed complex functional groups including C=O stretching at 1697.20 cm^{-1} and vinylidene bands at 886.72 cm^{-1}, suggesting carboxylic acids and

unsaturated compounds with established therapeutic properties (Nakamoto, 2009). These solvent-specific extraction patterns demonstrate that aqueous extraction favors polar phenolic compounds while organic solvents efficiently capture non-polar bioactive constituents, providing scientific validation for *P. jaubertii's* traditional medicinal applications and pharmaceutical potential.

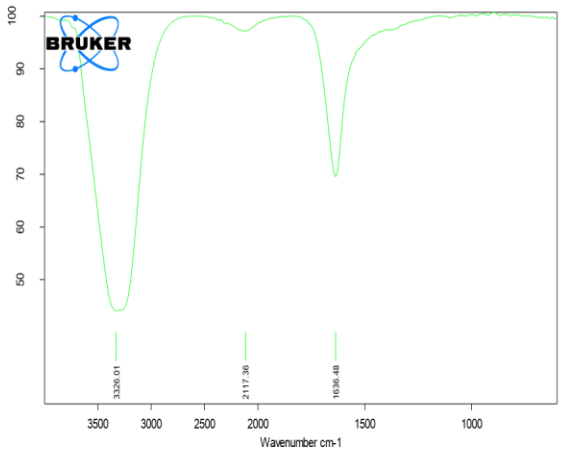

Fig 4: FTIR Spectroscopic analysis of *(JU-W) aqueous* extract

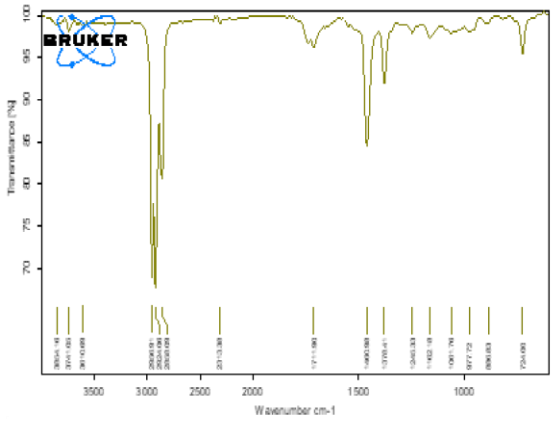

Fig 5: FTIR Spectroscopic analysis of JU-P extract

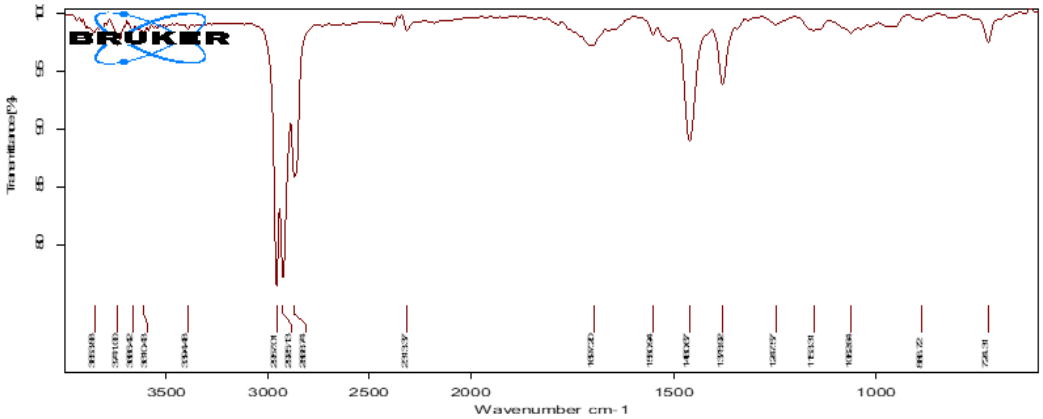

Fig 6: FTIR spectroscopic analysis of *JU-M* extract

Cytotoxic Activity of *Pulicaria jaubertii* Extracts

The cytotoxic potential of *Pulicaria jaubertii* extracts against HepG2 hepatocellular carcinoma cells was evaluated using the MTT assay after 24 hours of treatment. All tested extracts—methanolic, petroleum ether, and aqueous—exhibited a clear dosedependent reduction in cell viability, indicating their cytotoxic effects on HepG2 cells. At lower concentrations, the extracts showed moderate effects on cell viability; however, a progressive and significant decline in viable cells was observed with increasing extract concentrations (Fig. 7). Among the three extracts, the methanolic extract demonstrated the strongest cytotoxic response across all tested concentrations, whereas the petroleum ether and aqueous extracts exhibited comparatively weaker effects. These

findings suggest that the cytotoxic constituents of *P. jaubertii* are more efficiently extracted in polar solvents such as methanol.

The MTT assay results demonstrate significant cytotoxic potential of *Pulicaria jaubertii* extracts against HepG2 hepatocellular carcinoma cells, with all tested extracts exhibiting dose-dependent antiproliferative effects after 24 hours of treatment. This cytotoxic activity aligns with previous studies on *Pulicaria* species, where bioactive compounds have shown promising anticancer properties against various cell lines (Al-Rehaily et al., 2008).

The superior cytotoxic performance of the methanolic extract compared to petroleum ether and aqueous extracts suggests that the primary anticancer constituents of *P. jaubertii* are moderately polar compounds efficiently extracted by methanol. This observation correlates with the phytochemical profile revealed through GC-MS and FTIR analyses, where the methanolic extract contained a diverse array of bioactive compounds including terpenoids, phenolic compounds, and oxygenated derivatives (Mothana et al., 2011). The enhanced cytotoxicity of methanolic extracts is consistent with findings in other medicinal plants, where polar and semi-polar metabolites, particularly flavonoids, phenolic acids, and terpenoids, contribute significantly to anticancer activity through multiple mechanisms including apoptosis induction, cell cycle arrest, and oxidative stress modulation (Newman & Cragg, 2016).
The dose-dependent cytotoxic response observed across all extracts indicates a concentration-threshold relationship typical of bioactive natural products, where therapeutic effects become pronounced at higher concentrations while maintaining selectivity against cancer cells (Cragg & Newman, 2013). The moderate effects at lower concentrations followed by significant cell viability reduction at higher doses suggest that *P. jaubertii* extracts may possess selective cytotoxicity, a desirable characteristic for potential anticancer therapeutics.

The weaker cytotoxic effects of petroleum ether and aqueous extracts, while still significant, indicate that both non-polar and highly polar constituents contribute to the overall anticancer activity, albeit to a lesser extent than the compounds extracted by methanol. This multi-extract activity profile suggests synergistic effects among different phytochemical classes, supporting the traditional use of whole plant extracts in ethnomedicine (Heinrich et al., 2020).These findings establish *P. jaubertii* as a promising candidate for anticancer drug development, with the methanolic extract showing particular potential for further investigation into specific bioactive compounds and their mechanisms of action against hepatocellular carcinoma.

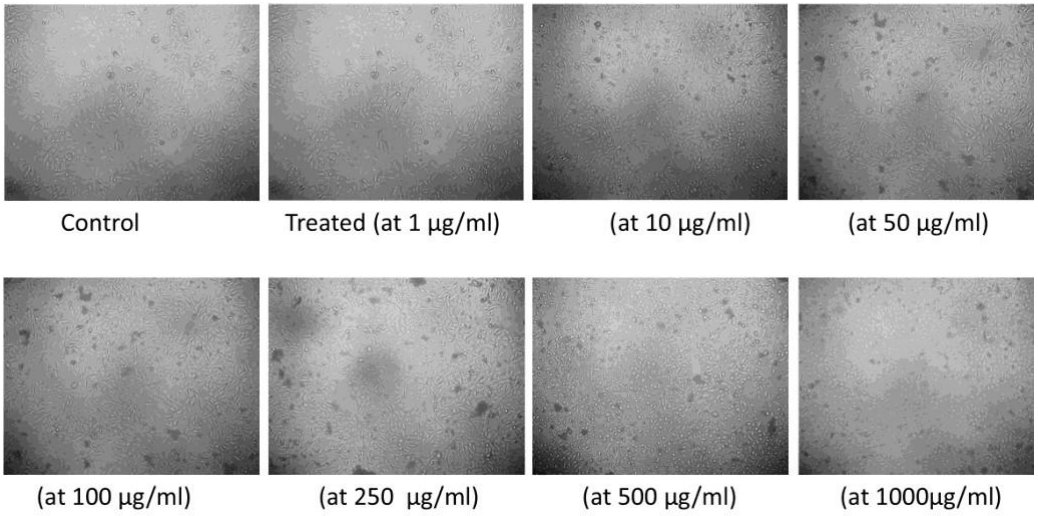

Fig 7: Treatment with the methanol extract on HepG2 cell viability

IC_{50} Determination

The half-maximal inhibitory concentration (IC_{50}) values were calculated from dose–response curves to quantitatively compare the cytotoxic potency of the different extracts. The IC_{50} values obtained after 24 hours of treatment were as: Methanol extract: 87.48 µg/mL, Petroleum ether extract: 283.3 µg/mL and Aqueous extract: 287.5 µg/mL. (Table 1 and fig 8).

The methanolic extract exhibited the lowest IC_{50} value, indicating the highest cytotoxic potency against HepG2 cells. Notably, the IC_{50} value of the methanol extract was approximately 3.2-fold lower than those of the petroleum ether and aqueous extracts, highlighting its superior anticancer efficacy. The relatively higher IC_{50} values of the petroleum ether and aqueous extracts indicate a lower concentration of active cytotoxic compounds in these fractions.

Extractions	**IC50 value (µg/ml)**
Methanol extract	87.48
Petroleum ether	283.3
Aqueous extracts	287.5

Table 1: IC_{50} Determination

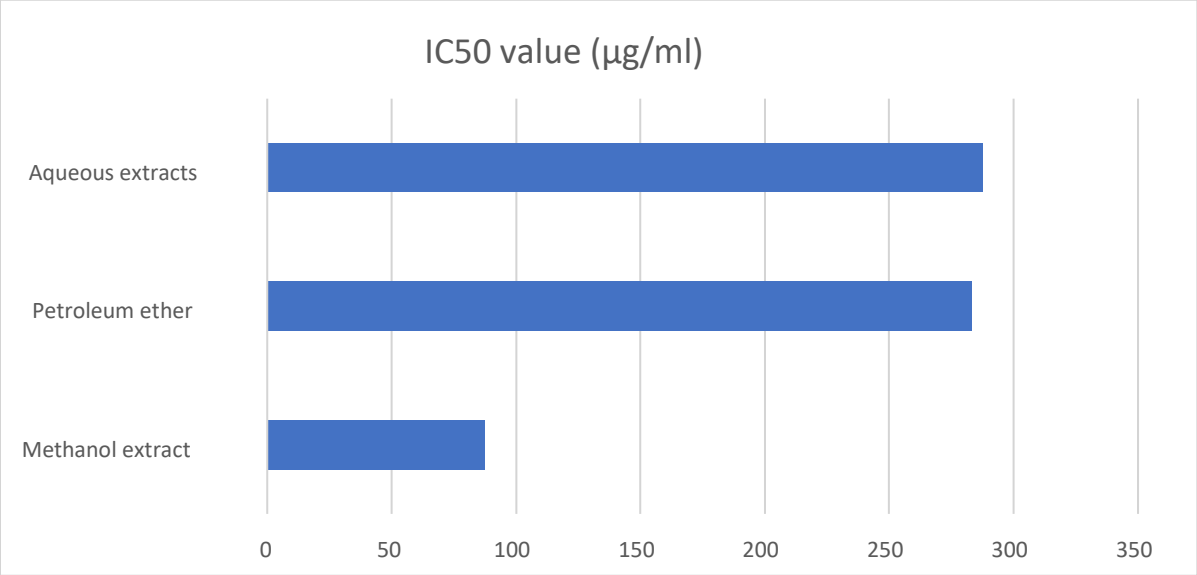

Fig 8: graphical representation of IC_{50} Determination

Comparative Analysis of Extract Efficacy

A comparative analysis of cell viability across different extracts further confirmed the enhanced cytotoxic activity of the methanolic extract. At the highest tested concentration (1000 µg/mL), treatment with the methanol extract reduced HepG2 cell viability to approximately **15% of control values**, demonstrating a strong inhibitory effect on cancer cell survival.

In contrast, cells treated with petroleum ether and aqueous extracts at the same concentration retained higher viability, with approximately **35% and 38%** viable cells, respectively. This marked difference in cytotoxic response underscores the influence of extraction solvent on the recovery of bioactive anticancer constituents from *P. jaubertii*.

Overall, the results indicate that the methanol extract of *Pulicaria jaubertii* exhibits significantly greater cytotoxic activity against HepG2 cells compared to petroleum ether and aqueous extracts, suggesting that polar phytochemicals may play a key role in mediating its anticancer effects.

Conclusion

The present study demonstrates the significant in vitro anticancer potential of *Pulicaria jaubertii* against HepG2 hepatocellular carcinoma cells. Among the evaluated extracts, the methanolic extract exhibited the most pronounced cytotoxic activity, with an IC_{50} value of 87.48 µg/mL, highlighting its superior efficacy compared to petroleum ether and aqueous extracts. These findings provide scientific support for the traditional medicinal use of *P. jaubertii* and underscore its potential as a promising source of bioactive compounds for liver cancer therapy. Further investigations involving mechanistic studies, selectivity assessment on normal cells, and in vivo validation are warranted to advance its development as a natural anticancer agent.

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