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Investigation of the Phytochemical Composition and Cytotoxic Properties of *Antidesma menasu* (Tul.) Mull.Arg. Extract in Human Pancreatic Cancer Cells

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KEYWORDS

Antidesma menasu, ethanolic extract, Anticancer, MTT assay, phytochemicals.

ABSTRACT:
Antidesma menasu (Tul.) Mull.Arg. belongs to the family Phyllanthaceae. The plant is known for its anti-inflammatory and analgesic properties. Traditionally, the plant is used for the treatment and management of pain associated with arthritis and lower back pain. It is also used for the treatment of inflammatory swellings. In the present study, phytochemical analysis and assessment of cytotoxic activity of the ethanolic extract of A. menasu were carried out. The ethanolic extract was prepared by the cold extraction method and subjected to qualitative phytochemical analysis. The phytochemical investigations confirmed the presence of flavonoids, alkaloids, glycosides, steroids and terpenoids. The cytotoxic activity of the extract was evaluated by MTT assay against the human MiaPaCa-2 cell line. The ethanolic extract of A. menasu exhibited a significant cytotoxic activity against the human MiaPaCa-2 cell line with an IC50 value of 285.97 ± 1.78 µg/ml. Further studies are underway to explore the molecular mechanisms and identify the active phytochemical agents.

Introduction

Cancer is a complex disease characterized by diverse biological mechanisms with diverse contributing factors. Cancer arises due to a series of alterations that occur at the genomic or molecular levels in a cell. These changes lead to uncontrolled growth and proliferation of cells. It causes a rapid expansion in tissue volume, disrupting normal structural integrity and functional dynamics. Among noncommunicable diseases, cancer holds the position of the second-highest mortality rate globally, after heart disease (Anand et. al., 2022). Daily, 52,900 new cases of cancer and more than 27,000 deaths are reported due to cancer. The global cancer burden, as reported in 2022, included around 19.98 million new cases and 9.7 million fatalities. Currently, India holds the third rank after China and the United States of America. It is estimated that India will account for 2.08 million cases of cancer by 2040, and a 57.5 % rise in the incidence of cancer will occur from 2020 to 2040 (Sathishkumar et. al., 2022; Sung et. al., 2021).

Antidesma menasu, commonly known as *A. monatum* Blume. belongs to the family Phyllanthaceae. It is a shrub or small tree that grows to a height of 6-8 m; branchlets tomentose; leaves are elliptic-lanceolate, oblong or oblong-lanceolate, glabrous; flowers are terminal or axillary in position, arranged in paniculate racemes, greenish white in color; fruits are ellipsoid, red, black when ripe. It is commonly distributed in Karnataka, Tamil Nadu, Kerala, Andhra Pradesh, Odisha, Goa and Andaman & Nicobar Islands (Rao et. al., 2025).

Fig. 1: Pictures showing aerial parts of *Antidesma meanasu*

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The leaves of *A. menasu* are used in traditional medicine by folklore practitioners for the treatment of joint pain, lower back pain, inflammatory swelling, skin diseases and ulcers. Scientific research has confirmed that the plant possesses antimicrobial, anti-inflammatory, antioxidant and analgesic properties (Sithara et. al., 2013). The literature review has revealed a significant gap in research concerning the phytochemical constituents and anticancer efficacy of *A. menasu*. Consequently, this study was undertaken to investigate its phytochemical profile and evaluate its anticancer potential.

Materials and Methods

Chemicals and reagents: The study was conducted using cell culture/analytical-grade reagents, chemicals, and solvents. Ethanol, Dimethyl Sulfoxide, Dulbecco's Modified Eagle Medium, Fetal Bovine Serum, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide and Trypsin were procured from HiMedia Laboratories Pvt. Ltd., India. Chemicals and reagents used for qualitative analysis were purchased from SD Fine Chemicals Ltd., India.

Collection and processing of Plant Material: Leaves of *A. menasu* were collected from the Western Ghats region near Agumbe, located in the Shimoga District of Karnataka. The collected specimens were authenticated, and a voucher herbarium sample has been deposited in the departmental herbarium for reference. The leaf samples were washed with distilled water to remove the dust and soil particles. Samples were dried in the shade, powdered coarsely using an electrical pulverizer, and stored in an air-tight container for further use (Nagappan, 2012).

Preparation of the extracts: The leaf ethanolic extract was prepared using the method described by Utispan et al. (2020). Briefly, 100 g of the powdered material was suspended in 400 mL of ethanol in a conical flask. The mouth of the conical flask was tightly covered with aluminium foil. The flask was placed on a rotary shaker and the extraction was carried out for 48 hrs at 120 rpm under ambient conditions. Then, the mixture was filtered using a muslin cloth, followed by filtration using Whatman No. 1 filter paper. The extract was concentrated under reduced pressure using the Rotary flash evaporator (Buchi, Switzerland). The extract was stored in an air-tight container in the dark.

Phytochemical analysis of the extract: The ethanolic extract of *A. menasu* was subjected to preliminary phytochemical screening to identify key bioactive constituents, including alkaloids, saponins, flavonoids, tannins, steroids, and terpenoids. Standard qualitative protocols were employed with minor procedural adjustments based on Harborne (1988).

Alkaloid Detection: The presence of alkaloids was assessed using Mayer's and Wagner's reagents. Upon addition of a few drops to 0.5 ml of the extract, the formation of a reddish-brown precipitate or turbidity indicated a positive result.

Saponin Test: A few drops of sodium bicarbonate were added to 0.5 ml of the extract and vigorously shaken to induce frothing. The appearance of stable foam exceeding 1 cm in height after 15–20 minutes confirmed the presence of saponins.

Flavonoid Identification

Alkaline Reagent Test: Mixing 2 ml of 2% NaOH with the crude extract produced a bright yellow coloration, which disappeared upon acidification, indicating the presence of flavonoids.

Lead Acetate Test: The addition of lead acetate solution to the extract resulted in a yellow precipitate, further confirming the presence of flavonoids.

Tannin Detection (Ferric Chloride Test): A few drops of 5% ferric chloride were added to 0.5 ml of the extract. The emergence of a black or blue-green coloration signified the presence of tannins.

Steroids and Terpenoids (Salkowski Test): Equal volumes (2 ml) of extract and chloroform were combined, followed by the addition of 2 ml concentrated sulfuric acid. A red coloration in the lower chloroform layer indicated the presence of steroids and terpenoids.

Evaluation of *in vitro* Cytotoxic Activity by MTT Assay: The cytotoxic potential of the ethanolic extract was assessed in vitro using the MTT assay on human MiaPaCa-2 cell lines. Cells were plated at a density of 10,000 cells per well in a 96-well microplate and incubated for 18 to 24 hours. Following this, varying concentrations of the extract were administered, and the cells were incubated for another 18 to 24 hours. After a total of 24 hours, the MTT reagent was introduced to each well and incubated for 3 hours before being

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discarded. Subsequently, 100% dimethyl sulfoxide (DMSO) was added to solubilize the formazan crystals formed. The absorbance was then recorded at 540 nm using a microplate reader. The % cell viability was then calculated using the following relationship. The IC_{50} value was calculated using the linear regression equation.

% Cell viability = OD of the tested sample/ OD of the control x 100

Results and Discussion

Phytochemical analysis: Preliminary phytochemical analysis revealed the presence of flavonoids, alkaloids, glycosides, steroids and terpenoids (Table 1).

Table 1: Phytochemicals present in the ethanolic extract of *A. menasu*

Phytoconstituent	**Result**
Tannins	Negative
Flavonoids	Positive
Alkaloids	Positive
Saponins	Negative
Glycosides	Positive
Steroids	Positive
Terpenoids	Positive

MTT Assay: The *A. menasu* ethanolic extract demonstrated strong cytotoxic potential toward human MiaPaCa-2 cells. The IC_{50} value was found to be 285.97 ± 1.78 µg/ml. The cytotoxic activity of the ethanolic extract of *A. menasu* can be attributed to the phytochemicals such as flavonoids, alkaloids, and glycosides present in it. Several independent studies have also reported potent cytotoxic and anticancer activities in plants belonging to the *Antidesma* genus (Mili et al., 2024). Phytochemicals hold significant potential in both the prevention and treatment of cancer. Their diverse biological activities, including antioxidant, anti-inflammatory, and cytotoxic effects, contribute to reducing cancer risk by safeguarding cellular DNA, modulating inflammatory pathways, and promoting apoptosis in malignant cells (Ashong, 2024).

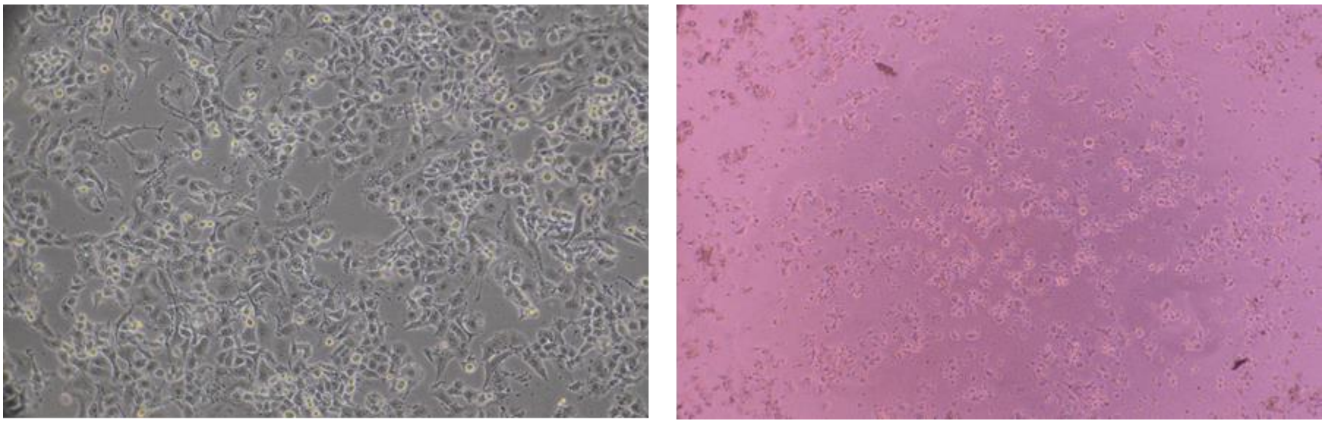

Fig. 2: Morphology of MiaPaCa-2 cell lines during MTT assay. a) Control cells after 24 h of incubation b) Cells treated with 400 µg/ml *A. menasu* ethanol extract for 24 h

Conclusion

Medicinal plants are rich sources of bioactive secondary metabolites that play a crucial role in the treatment of various diseases and disorders. These botanicals are widely accessible and offer a cost-effective alternative to synthetic drugs. Their chemical diversity, particularly the presence of structurally unique secondary metabolites, makes them promising candidates for novel therapeutic development. This study successfully reports the anticancer property of the plant *A. meanasu*. However, comprehensive research is required to uncover the molecular pathways and identify the bioactive compounds contributing to the plant's anticancer effects

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Conflict of Interest

The authors declare no conflicts of interest regarding this manuscript.

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