Screening of *Ipomoea tuba* Leaf Extract for Identification of Bioactive Compounds and Evaluation of Its *in vitro* Antiproliferative Activity Against MCF-7 and HeLa Cells

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**SUMMARY**

Mangroves contain a wide range of bioactive compounds with pharmacological activities. In the present study, we analysed the separation and detection of phytoconstituents with the methanol extract of *Ipomoea tuba* leaf using gas chromatography-mass spectrometry (GC-MS) and tested its *in vitro* cytotoxicity effect against MCF-7 and HeLa cells. Phytochemical compounds such as docosanoic, octadecatrienoic and cis-9-octadecanoic acids, triterpenoid γ-sitosterol, and terpene alcohol in methanol extract of *I. tuba* leaf were identified. Furthermore, *in vitro* antiproliferative activity of the extract of *I. tuba* leaf was evaluated using MCF-7 and HeLa cells. The results indicated a reduction of cell viability of 37.43 and 41.89 % of MCF-7 and HeLa cells respectively. The methanol extract of *I. tuba* leaf proved to be effective in protecting the cells against oxidative stress. This is the first report on the *in vitro* cytotoxicity effect of *I. tuba* leaf extract on MCF-7 and HeLa cells.

**Key words:** mangrove species, *Ipomoea tuba*, bioactive compounds, antiproliferative activity

**INTRODUCTION**

Mangrove ecosystem plays a major role in the human life to protect us from natural disasters like tsunami, floods, high tides and soil erosion. Mangrove plants grow in water logging region and offer a shelter for wide ranges of endemic fauna and flora (1,2). They absorb and remove five times more carbon dioxide than normal terrestrial plants (3). The mangrove species, namely *Suaeda maritima*, commonly known as seablite is used in homemade foods such as salad, curry, soy sauce, and spicy soup in Thailand (4). In recent years, bioactive compounds produced from the plants have attracted the interest of pharmaceutical industries for formulation of drugs because the effectiveness of synthetic antibiotics against several pathogenic strains is slowly decreasing (5). The natural compounds and related drugs are used to treat different human diseases (6). The crude methanol extract of different medicinal plants contains many bioactive compounds having anticancer activity against several cancers like gastric, colon and breast cancer cell lines (7). Phytoconstituents and cytotoxicity of *I. tuba* have not been studied so far. Hence, the present study aims to evaluate the phytoconstituents of methanol extract of *I. tuba* leaf and their cytotoxicity effects on MCF-7 and HeLa cells.

**MATERIALS AND METHODS**

**Sample preparation**

*Ipomoea tuba* sample was collected from Nizampatnam mangroves, Guntur, Andhra Pradesh, India. The sample was prepared from leaves of *I. tuba* by soaking 50 g of powdered sample in 50 mL of absolute methanol for 72 h. The sample was filtered through Whatman No. 42 filter paper and then methanol was evaporated from the test sample by rotary vacuum evaporator (EV11; Equitron Medica Pvt Ltd, Mumbai, India). The final crude extract was dissolved in 100 % dimethyl sulfoxide (DMSO; Sigma-Aldrich Chemicals Pvt Ltd, Merck, Bangalore, India), made to final concentration of 100 mg/mL and used for antiproliferative studies. The concentration of DMSO maintained in the wells was less than 1 %, which is not toxic to the cell lines (8,9).
Identification of compounds by GC-MS analysis

Bioactive compounds in leaf extract of *Ipomoea tuba* were identified by GC-MS (6890 series; Agilent, Santa Clara, CA, USA). The following chromatographic conditions were maintained: initial column temperature 30 °C, heated up to 300 °C at 10 °C/min, flow rate 1.0 mL/min and helium was used as carrier gas in split mode. The bioactive compounds were identified based on retention times and quantified by integration of peak area. Similarity of compounds was compared with known compounds using NIST-based AMDIS software (10).

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay for cell viability

*Ipomoea tuba* leaf extract was screened for *in vitro* cytotoxicity activity on MCF-7 and HeLa cells (5 × 10⁴ cell/well) using MTT (Sigma-Aldrich, Merck, St. Louis, MO, USA) assay. The sample (100 µL diluted plant extract) was added to 100 µL of Dulbecco’s Modified Eagle’s medium (DMEM), then the cell lines were added to the 96-well microtiter plate, and incubated for 48 h at 37 °C. The MTT was added and allowed to incubate for 2 h until the purple precipitate was formed. Then, absorbance values were measured at 520 nm using UV-Vis spectrophotometer (Cary 60; Agilent Technologies, Selangor Darul Ehsan, Malaysia). The dose-response curve was plotted for evaluation of IC₅₀ values (11).

Statistical analysis

The experimental data of both cell lines were statistically analyzed using ANOVA method. The value p<0.05 is considered statistically significant for the analysis of the percentage of inhibition of cell viability.

RESULTS AND DISCUSSION

GC-MS analysis for compound identification

The chromatogram confirmed the presence of compounds such as fatty acids: docosanoic, octadecatrienoic and cis-9-octadecanoic acids, triterpenoid γ-sitosterol, and terpene alcohol in the leaf extract of mangrove plant *Ipomoea tuba*. The compounds were identified based on retention times. Table 1 gives the molecular mass and retention times of each compound. Angaye et al. (12) reported various bioactive compounds in the extracts of mangroves *Ipomoea tuba* in *in vitro* antiproliferative activity. Similar findings are reported for *Ipomoea tuba* leaf extract of 100 µg/mL (13). 

In *in vitro* antiproliferative activity of *Ipomoea tuba* leaf extract on MCF-7 cells

Extract of *Ipomoea tuba* leaves showed significant *in vitro* antiproliferative effect on MCF-7, and the viability of MCF-7 was reduced with the increase in the concentration of the sample. High reduction of MCF-7 cells was observed at the concentration of *Ipomoea tuba* leaf extract of 100 µg/mL (Table 2) and the IC₅₀ value against MCF-7 cells was found to be (40.4±0.1) µg/mL. During experiments, it was observed that the increase in sample concentration altered the morphology of MCF-7 cells, leading to cell death (Fig. 1). Similar findings are reported for *in vitro* cytotoxicity effect of *Avicennia marina* extracts on different cancerous cells (21-23). Patra and Thatoi (24) reported the antiproliferative activity of the methanol extract of *Heritiera fomes* leaves against melanoma cell lines and achieved 40% inhibition. The methanol extract of *Avicennia marina* leaf showed antitumor activity against MDA-MB 231 and MCF-7 cell (25).

| Peak no. | tᵣ/min | Compound name               | Formula         | M(g/mol) | CAS number |
|---------|---------|------------------------------|-----------------|----------|------------|
| 1       | 19.49   | docosanoic acid             | C₂₀H₃₄COOH      | 340.59   | 112-85-6   |
| 2       | 21.24   | 3,7,11,15-tetramethyl-2-hexadecene-1-ol | C₃₂H₅₄O       | 296.00   | 7541-49-3  |
| 3       | 27.86   | octadecatrienoic acid-ethyl ester | C₁₈H₃₀O        | 292.46   | 1191-41-9  |
| 4       | 31.07   | cis-9-octadecanoic acid     | C₁₈H₃₀O₂       | 282.00   | 112-80-1   |
| 5       | 32.80   | γ-sitosterol                 | C₂₈H₄₈O        | 414.71   | 83-47-6    |
Table 2. Inhibition of MCF-7 and HeLa cells using *Ipomoea tuba* leaf extract

| γ(extract)/(μg/mL) | Viability of MCF-7 cells/% | Viability of HeLa cells/% |
|-------------------|---------------------------|--------------------------|
| 100               | (37.4±0.1)″               | (41.4±0.1)″              |
| 75                | (42.0±0.2)″               | (44.7±0.2)″              |
| 50                | (45.4±0.6)″               | (48.3±1.0)″              |
| 25                | (53.5±0.8)″               | (51.4±0.4)″              |
| 10                | (55.8±0.5)″               | (53.9±0.3)″              |
| 5                 | (60.7±0.3)″               | (55.6±0.8)″              |

Values are expressed as mean±S.D. Values with different letters in superscript in the same column are significantly different (p<0.05) determined by ANOVA.

In vitro antiproliferative activity of *I. tuba* leaf extracts on HeLa cells

HeLa cell viability was decreased with the increased concentration of leaf extract and the maximum reduction in HeLa cells was observed at 100 µg/mL (Table 2), with the IC\textsubscript{50} value of (37.4±0.1) µg/mL. It was observed that after the treatment with *I. tuba* extract, the HeLa cells slowly detached from one another. Fig. 2 shows the change in morphology of HeLa cells. Khajure and Rathod (26) reported that the extract of *A. ilicifolius* had cytotoxic activity against HeLa and KB cells. Rajeswari et al. (27) also reported that the flavone molecule from *Excoecaria agallocha* has the cytotoxic activity against HeLa cells.

![Fig. 1. Morphology of MCF-7 cells after the treatment with the extract of *Ipomoea tuba* leaf: a) untreated MCF-7 cell lines, and b-g) treated with different concentrations (5, 10, 25, 50, 75 and 100 µg/mL respectively) of the leaf extract](image1)

![Fig. 2. Morphology of HeLa cells after the treatment with the extract of *Ipomoea tuba* leaf: a) untreated HeLa cell lines, and b-g) treated with different concentrations (5, 10, 25, 50, 75, and 100 µg/mL respectively) of the leaf extract](image2)
CONCLUSIONS

In conclusion, GC-MS analysis confirmed the presence of different phytoconstituents and the Ipomoea tuba extracts were proved to have antiproliferative effect on MCF-7 and HeLa cells. This is the first report of high antiproliferative activity of the extract of I. tuba leaf on MCF-7 and HeLa cells. Furthermore, these bioactive compounds could be used in functional food applications for health benefits.

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

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