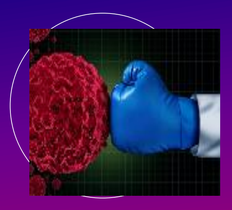

# Anticancer activity of Annona Squamosa with its phytochemical analysis by Liquid chromatography mass spectroscopy

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## OBJECTIVE

Traditionally, *A. squamosa* is also utilized for the remedy of jaundice and other liver related and cancer diseases.

In view of previously established activity profile of leaf extract and the fact that sometimes seeds show better activity than other parts of the plants we envisage to evaluate the seed extract of Annona *squamosa* for hepato-protective and anticancer activity particularly against totally unexplored ovarian cancer cell lines i.e SiHa cervix and ovcar-5 ovary cancer cell lines. More over LCMS analysis of has not been done previously, which will be the part of this study regarding phytochemical analysis.

## EXPERIMENTAL

The seeds of *A Squamosa* was obtained from Natural Remedies Pvt. Ltd., Banglore (sample invoice No. DF249) and were authenticated by, Department of Botany, Dr. H. S. Gour University Sagar (M.P.). The herbarium number was Bot/H/12114/20.

The instrument,Mass Hunter SG11351102 (Agilent Technologies)was conducted in both polarities (positive and negative) of Electron spray ionization (ESI) mode where better in positive ESI. Fragmentor voltage was set at 135 V. The flow rate was optimized to 0.5 ml/min.

Two different solvents were used, Solvent A: water +a 0.1% formic acid and Solvent B: Acetonitrile.

Column used for this study was Chromolith - 50X4.6mm.

Total run time was for 30 min The dried coarsely powdered of *A Squamosa* seed was subjected to extraction using 50% ethanol eThe anticancer activity of hydroalcoholic extract of *A. Squamosa* Linn. seeds was performed on five cancer cell lines. The cancer cell line was obtained by Indian Institute of Integrative medicine, Jammu. The cancer cell lines on which anticancer activity was performed are:The human breast adenicarcinoma (MCF-7), Liver (HepG2), Colon (HT-29), Ovary cancer cell line (ovcar-5), The human cervix (SiHa)

The human tumor cytotoxicity was determined by the protocols established by NCI (Monks *et al.*, 1991). The sulphorhodamine B (SRB) assay was used in this study to assess growth inhibition.

The colorimetric assay estimated cell number indirectly by staining total cellular protein with the dye SRB. Single-cell suspensions were prepared by the treatment of cells with 0.5-1 ml of 0.1% trypsin-EDTA (Sigma Chemical Co.).

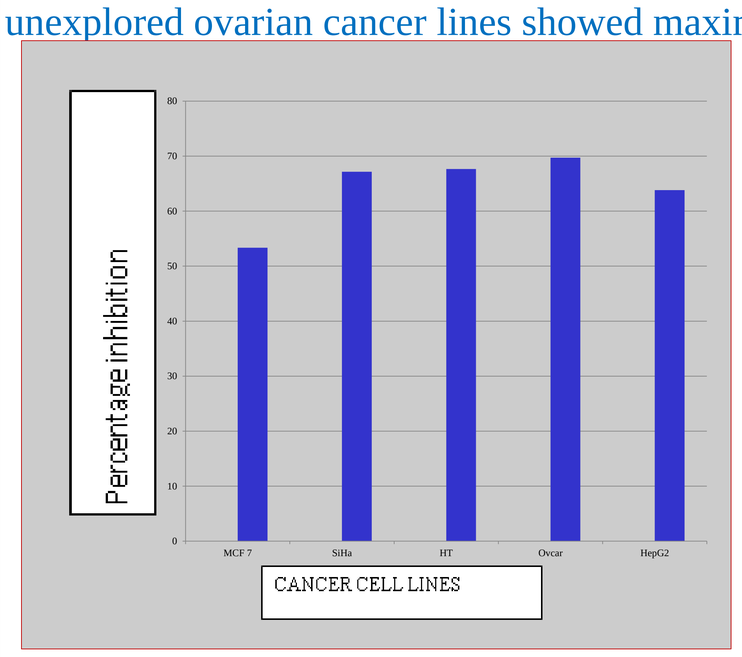
The viable cells were counted using a Coulter counter and diluted with RPMI medium and final densities of $100 \times 10^4$ cells/ml were obtained.

Cell suspensions (100 µl/well) was seeded in 96-well microtiter plate containing 1ml of media and incubated for cell attachment.

## RESULT

### Anticancer activity of hydroalcoholic extract of *A. squamosa* seeds

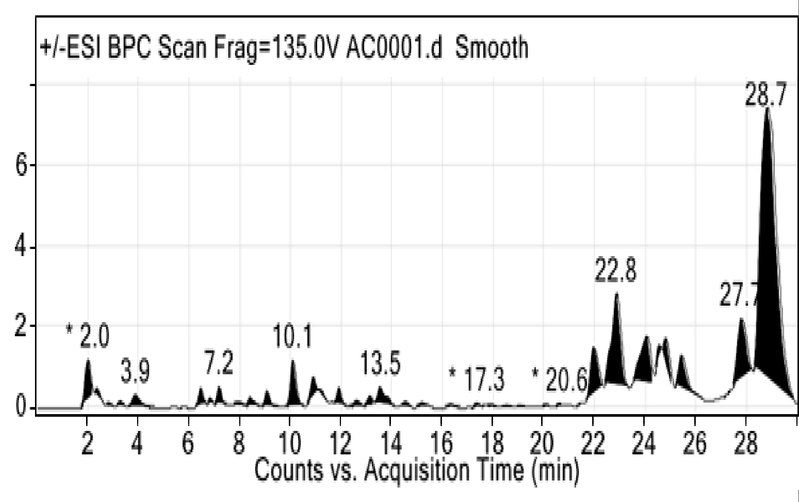
The anticancer activity of the hydroalcoholic extract was performed using sulphorhodamine B assay method on various cancer cell lines i.e. MCF-7, SiHa, HT, Ovcar-5, and HepG2. Comparing various cancer lines, the maximum anticancer activity was observed on ovary cancer lines and minimum anticancer activity was observed on adenocarcinoma. The unexplored ovarian cancer lines showed maximum anticancer activity.




| Type of cell line | % inhibition |
|---|---|
| | AS |
| MCF 7 | 53.34 |
| SiHa | 67.15 |
| HT | 67.66 |
| Ovcar-5 | 69.72 |
| HepG2 | 63.82 |

### LCMS analysis of *A. Squamosa* seed extract

| S. No. | Retention time range (min) | Molecular weight (m/z) |
|---|---|---|
| 1. | 2.0–2.5 | 181.10 |
| 2. | 6.3–6.6 | 3.6 |
| 3. | 7.0–7.3 | 3.7 |
| 4. | 8.2–8.7 | 6.9 |
| 5. | 9.9–10.5 | 4.9 |
| 6. | 12.9–13.2 | 8.7.30 |
| 7. | 13.0–13.4 | 3.9.10 |
| 8. | 16.1–16.5 | 269.10 |
| 9. | 19.4–19.7 | 6.7.40 |
| 10. | 18.9–19.2 | 6.6.10 |
| 11. | 19.9–20.1 | 6.4.40 |
| 12. | 11.7–12.1 | 8.2.40 |
| 13. | 21.7–22.2 | 6.8.10 |
| 14. | 24.1–24.6 | 6.8.10 |
| 15. | 25.2–25.7 | 6.8.10 |




The mass spectrum of extract by LC-MS showed various peak of different peak of 15 compounds of which molecular ion peak at m/z 316 which resembles the molecular weight of the Isorhamnetin.

The pharmacological activities of Isorhamnetin include antimicrobial, anticancer, nerurological, hepatoprotective, antioxidant, anti-inflammatory and anti-obesity.

## CONCLUSION

The phytochemical characterization was done using LCMS method which showed 15 different molecular weight compounds. The extract showed an average in vitro anticancer activity at a concentration of 100 µg/ml against all cancer cell lines. The best activity was observed against Ovcar-5 cell line (69.72) and was also significant against HT and SiHa cell lines. The phytochemical analysis showed the wide range of phenols and flavonoid which are showing potent anticancer activity of AS seeds.

## REFERENCES

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