Phytochemical Profiling and Cytotoxic Assessment of a Traditional Asava from Epiphyllum oxypetalum.

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***Abstract*—The present study investigated the chemical composition and pharmacological properties of Epiphyllum oxypetalum Asava, with emphasis on ethanol content, total solids, phytochemical profiling, and in vitro anticancer activity. The ethanol content, determined via distillation followed by alcoholometry, was 7.5% v/v within the Ayurvedic Pharmacopeia's acceptable range (5–10%) indicating effective self-fermentation and preservation, likely aided by Woodfordia fruticosa flowers. Total solids were quantified at 32.8 mg/mL, suggesting a substantial presence of bioactive phytoconstituents, sugars, minerals, and dissolved matter contributing to therapeutic efficacy.**

Phytochemical analysis through HPTLC fingerprinting confirmed the presence of 4-hydroxy-2-methylacetophenone, a phenolic acetophenone derivative, by matching Rf values of the standard and methanolic extract. UV–Visible spectrophotometry further validated this finding, displaying a broad absorbance plateau (250–350 nm) characteristic of aromatic and phenolic structures, with high absorbance (~5.0 AU) indicating significant UV-active compound content.

The MTT assay assessed anticancer potential against MCF-7 (breast adenocarcinoma) and Colo-205 (colon cancer) cell lines. The methanolic extract (Sample-RK) exhibited dose-dependent cytotoxicity, achieving 40.41% and 38.59% cell viability at 100 µg/mL for MCF-7 and Colo-205, respectively. IC_{50} values were 52.82 µg/mL (MCF-7) and 64.71 µg/mL (Colo-205), reflecting moderate activity compared to the standard chemotherapeutic 5-fluorouracil.

Overall, the study demonstrates that E. oxypetalum Asava possesses notable ethanol content, key phytochemicals, and moderate in vitro anticancer activity, supporting its traditional medicinal use and warranting further pharmacological and clinical evaluation as a potential natural anticancer agent.

***Index Terms*—Epiphyllum oxypetalum, Asava, Ayurvedic fermentation, 4-hydroxy-2-methylacetophenone, anticancer activity, MCF-7, Colo-205.**

I. INTRODUCTION

Herbal remedies, phytonutrients, and nutraceuticals have been used for centuries across diverse healthcare systems, and their global usage continues to expand rapidly. Over the past decade, both industrialised and developing nations have witnessed a surge in public acceptance of natural medicines, now available not only in pharmacies but also in supermarkets and online platforms. It is estimated that nearly four billion people in developing countries rely primarily on herbal remedies for their healthcare needs, with such practices deeply embedded in cultural traditions [1].

Herbal medicines are generally considered safe due to their natural origin. The increasing toxicity and adverse effects of many allopathic drugs have driven global interest in plant-based alternatives, encouraging the growth of the herbal medicine industry. These preparations exhibit a broad spectrum of pharmacological activities, including anti-inflammatory, hepatoprotective, analgesic, antispasmodic, antidiabetic, anxiolytic, and antioxidant effects [2]. However, factors such as soil quality, pH, climate, and standardised preparation methods are crucial in ensuring the safety, efficacy, and consistency of herbal formulations.

Medicinal plants are valued as significant alternatives or complements to modern synthetic drugs, largely due to their chemical diversity and potential to yield novel therapeutic agents. Among them, Epiphyllum oxypetalum (family: Cactaceae) is an ornamental cactus widely cultivated for its large, fragrant, night-blooming flowers. Known by various names such as Brahma Kamal, Dutchman's Pipe, Queen of the Night,

and Nishagandhi, this species is used in traditional medicine to manage conditions including cholecystitis, cancer, gallstones, liver infections, urinary tract infections, renal calculi, gynaecological inflammation, hypercholesterolaemia, and certain skin disorders [3,4]. Despite its long-standing folk use, the biological activities of E. oxypetalum remain insufficiently explored through modern scientific methods.

Cancer is a group of over 100 diseases characterised by uncontrolled cell proliferation, tissue invasion, and, in many cases, metastasis. The term "cancer" derives from Hippocrates' observation of tumour morphology, later adopted into Latin as "cancer" from the Greek karkinoma. Advances in cellular theory by Hooke (1600s) and Virchow (1800s) established that cancers originate from abnormal cells arising from pre-existing ones [5]. Historical epidemiological observations such as high lung cancer rates among German miners exposed to radioactive pitchblende and scrotal cancer among chimney sweeps in 18th-century England revealed environmental links to carcinogenesis [6,7].

Cancer develops through a multistep process involving genetic mutations that affect proto-oncogenes and tumour suppressor genes. These alterations disrupt normal cell cycle regulation, leading to progressive changes:

1. Initiation – genetic mutation in a single cell.
2. Promotion/Hyperplasia – abnormal but morphologically normal cell proliferation.
3. Dysplasia – altered cell morphology and disorganisation.
4. Carcinoma in situ – cancerous transformation confined to the original site.
5. Malignant stage – invasion of surrounding tissues and metastasis [8–12].

Although conventional therapies such as chemotherapy, radiotherapy, and targeted drugs remain the mainstay of treatment, they are often associated with significant limitations, including systemic toxicity, severe side effects, and the emergence of multidrug resistance. Consequently, there is growing interest in plant-derived agents that may act as safer alternatives or adjuvants in cancer therapy.

Ayurveda, India's traditional medical system, has a rich heritage of using plant-based preparations for therapeutic purposes. Among its formulations, Asava and Arishta are well-known fermented liquid medicines prepared using natural fermentation. Asava is produced without prior boiling of herbs, while Arishta involves decoction before fermentation. Both contain 5–12% self-generated alcohol, which enhances extraction of phytoconstituents and improves bioavailability [13,14]. References to these preparations appear in classical Ayurvedic texts such as Charaka Samhita, Sushruta Samhita, and Bhaishajya Ratnavali, where they were prescribed for a variety of ailments, including digestive, respiratory, metabolic, and cardiovascular disorders [15].

The preparation of Asava involves infusion of medicinal herbs (raw or powdered) with natural sweeteners such as jaggery, sugar, or honey, and fermentation initiators like Dhataki pushpa (Woodfordia fruticosa). The mixture is fermented anaerobically for 15–40 days, leading to biotransformation of herbal constituents and generation of mild alcohol that aids in preservation and solubilisation of active phytochemicals [16,17]. The resulting formulation contains a complex phytochemical profile alkaloid, flavonoids, tannins, glycosides, saponins, and polyphenols each contributing to pharmacological activities such as antioxidant, anti-inflammatory, hepatoprotective, immunomodulatory, and gastroprotective effects [18–20].

Recent interest has emerged in the potential anticancer applications of Asava formulations. The self-generated alcohol facilitates enhanced delivery of lipophilic bioactive, while fermentation may yield novel secondary metabolites with potent bioactivity. Such properties make Asava a promising candidate for integrative oncology, either as a direct cytotoxic agent or as an adjuvant to conventional therapy, potentially reducing required drug doses, mitigating side effects, and overcoming drug resistance. Given E. oxypetalum's ethnomedicinal reputation and rich phytochemical composition, developing an Asava from its leaves and evaluating its anticancer activity represents a valuable step toward validating traditional knowledge through modern scientific investigation.

II. MATERIAL AND METHOD

1.Collection and Authentication of Plant Material
Collection: the plant epiphyllum oxypetalum was collected from local region of Kolhapur.
Authentication: This crucial task carried out by Prof. M.N. Patil, The head of department of botany, Yashwantrao Chavan Warana Mahavidyalaya, Warananagar, Kolhapur.

2. Preparation of Plant Material
Fresh Epiphyllum oxypetalum was cleaned with tap and distilled water, shade-dried (25–30 °C, 1 day), crushed to obtain fresh juice, and stored in amber containers.

3. Preparation of Asava
Ingredients (per Ayurvedic Pharmacopoeia):

- Fresh juice: 500 mL
- Jaggery: 400 g
- Distilled water: 1.6 L
- Woodfordia fruticosa flowers (Dhataki): 100 g

Method:

1. Juicing & Hydration – Juice mixed with water, covered, and kept 24 h.
2. Sugar Addition – Jaggery dissolved completely.
3. Fermenting Agent – Dhataki flowers added.
4. Fermentation – Anaerobic fermentation (30 days, 27–30 °C, dark).
5. Filtration – Filtered to obtain clear amber liquid.
6. Storage – Amber bottles, airtight, labelled.

4. Evaluation & Characterization
4.1 Ethanol Content – 25 mL sample distilled, SG measured (0.980), ethanol content 7.5 % v/v.
4.2 Total Solids – 10 mL dried at 105 °C, residue weighed; total solids = 32.8 mg/mL.
4.3 HPTLC – Methanolic extract prepared, applied (5 µL) to silica gel 60 F_{254} plates; mobile phase: toluene: ethyl acetate: formic acid: methanol (5:4:1:0.5); detection at 254 nm & 366 nm; derivatization with anisaldehyde–sulfuric acid; scanned for Rf comparison with 4-hydroxy-2-methylacetophenone standard.

5. In Vitro Antioxidant Activity (DPPH Assay)
DPPH (0.1 mM in methanol) mixed with sample dilutions (10–50 µg/mL); incubated 30 min (dark, RT); absorbance measured at 517 nm; % inhibition calculated; IC_{50} determined from inhibition vs. concentration plot.

6. In Vitro Anticancer Activity (MTT Assay)
Cell lines: MCF-7 (breast adenocarcinoma), Colo-205 (colon adenocarcinoma). Culture: DMEM/RPMI + 10 % FBS + antibiotics; 37 °C, 5 % CO_2.
Procedure:

- Cells seeded (5×10^3/well, 96-well plates), incubated 24 h.
- Treated with Asava extract (0–200 µg/mL) for 24–48 h.
- MTT (20 µL, 5 mg/mL) added; incubated 4 h.
- Supernatant removed; DMSO (100 µL) added to dissolve formazan.
- Absorbance read at 570 nm; % cell viability calculated.

III. RESULT

Phytochemical Screening
The ethanolic and aqueous extracts of Epiphyllum oxypetalum Asava showed the presence of flavonoids, phenolics/tannins, steroids/triterpenes, and saponins/glycosides, while alkaloids and carbohydrates were absent.

Alcohol Content
The ethanol content of Epiphyllum oxypetalum Asava was determined by distillation followed by alcoholometry. From 25 mL of sample, ~20 mL distillate was collected with a specific gravity of 0.980 at 27 °C, corresponding to 7.5% v/v ethanol. This level is within the Ayurvedic standard range (5–10%) and indicates successful anaerobic fermentation by Woodfordia fruticosa, with alcohol acting as a natural preservative.

Total Solid Content
Using gravimetric analysis, 10 mL of Asava yielded 0.328 g of residue, equivalent to 32.8 mg/mL total solids. This fraction comprises phytochemicals, sugars, minerals, and other dissolved or suspended components of the formulation.

IV. HPTLC ANALYSIS

High-Performance Thin-Layer Chromatography (HPTLC) of Epiphyllum oxypetalum Asava was performed using ethanol: chloroform (20:80) as the mobile phase. The reference standard (Track 1) showed a distinct band at an Rf value corresponding to 4-hydroxy-2-methylacetophenone. The sample extract (Track 2) exhibited a band at the same Rf, confirming the presence of this compound. Band intensity indicated a detectable concentration of the marker phytoconstituent.

UV–Vis Spectroscopy

UV–Vi's analysis of Epiphyllum oxypetalum Asava (200–400 nm) showed a sharp absorbance rise near 220 nm, followed by a high, stable plateau (~5.0 AU) between 250–350 nm. This profile is characteristic of aromatic and conjugated phenolic compounds. The spectrum supports the presence of 4-hydroxy-2-methylacetophenone, complementing the HPTLC findings.

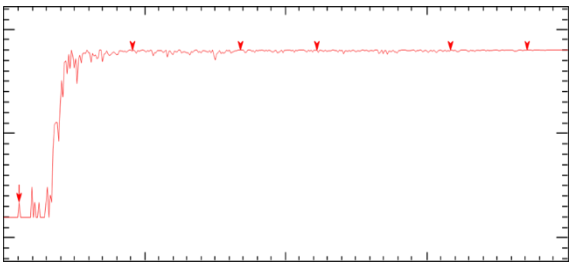


Fig -1. UV–Vis Spectroscopy

Antioxidant Activity (Reducing Power Assay)

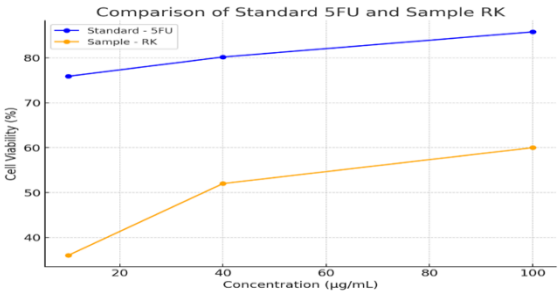
The antioxidant potential of Epiphyllum oxypetalum Asava was evaluated using the reducing power assay. Ascorbic acid (standard) showed a concentration-dependent increase in absorbance from 0.19 to 0.29 OD (250–1000 µg/mL). The Asava sample displayed lower activity, with absorbance values ranging from 0.05 to 0.11 OD over the same concentration range. Results indicate moderate electron-donating capacity, significantly lower than the standard, suggesting limited but measurable antioxidant potential.

In Vitro Cytotoxicity Against MCF-7 Cells

The cytotoxic potential of Epiphyllum oxypetalum Asava (Sample-RK) was assessed using the MTT assay on the MCF-7 human breast adenocarcinoma cell line and compared to the standard drug 5-fluorouracil (5-FU).

For the standard, 5-FU exhibited strong, dose-dependent cytotoxicity, reducing cell viability from 24.32% at 10 µg/mL to 14.66% at 100 µg/mL, with an IC_{50} value of 6 µg/mL.

In comparison, Sample-RK showed moderate but significant cytotoxicity, decreasing cell viability from 64.10% at 10 µg/mL to 40.41% at 100 µg/mL, with an IC_{50} of 52.82 µg/mL. Although the extract was less potent than 5-FU, its activity suggests the presence of bioactive constituents with potential anticancer properties against MCF-7 cells.



Graph-1: Comparative graph of effect standard drug and asava sample against MCF-7 cells.

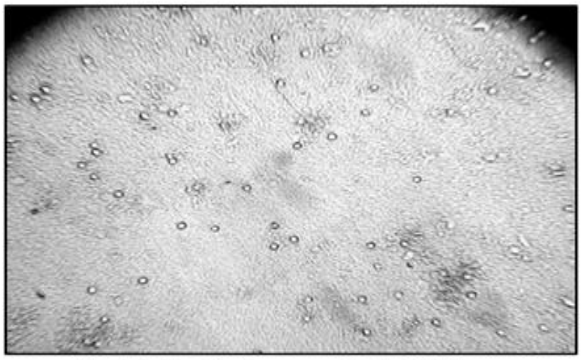


Fig. 1.A. Standard compound cell viability

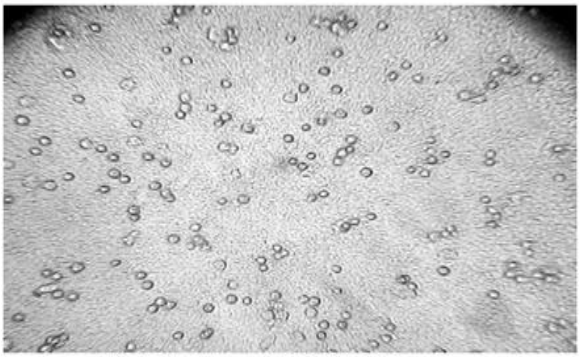


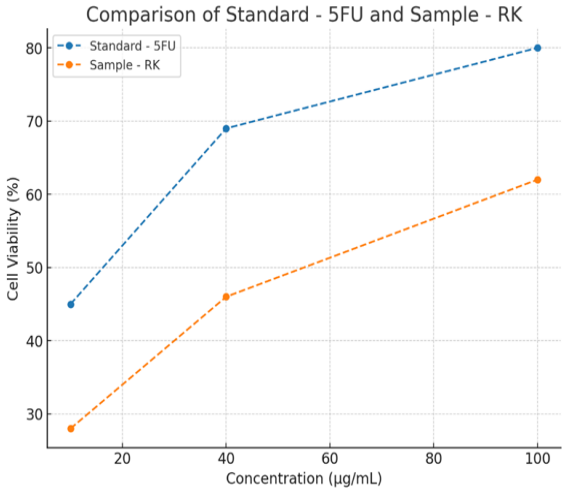
Fig B. Test Sample Cell Viability

In Vitro Cytotoxicity Against Colo-205 Cells

The anticancer potential of Epiphyllum oxypetalum Asava (Sample-RK) was evaluated on the Colo-205 human colon cancer cell line using the MTT assay and compared with the standard drug 5-fluorouracil (5-FU).For the standard, 5-FU demonstrated strong cytotoxicity, reducing cell viability from 55.36% at 10 µg/mL to 19.32% at 100 µg/mL, with an IC_{50} value of 10.92 µg/mL.

In comparison, Sample-RK showed moderate but notable cytotoxicity, decreasing cell viability from 72.58% at 10 µg/mL to 38.59% at 100 µg/mL, with an IC_{50} of 64.71 µg/mL.

Although less potent than 5-FU, the Asava formulation exhibited significant growth-inhibitory effects on Colo-205 cells, indicating the presence of bioactive compounds with potential anticancer properties.



Graph-2: Comparative graph of effect standard drug and asava sample against Colo-205 Cells.

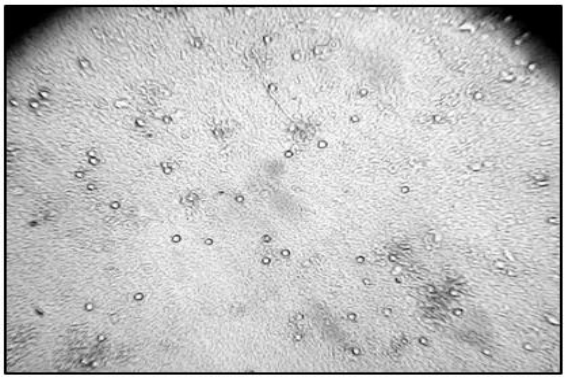


Fig.2. A. Standard compound cell viability

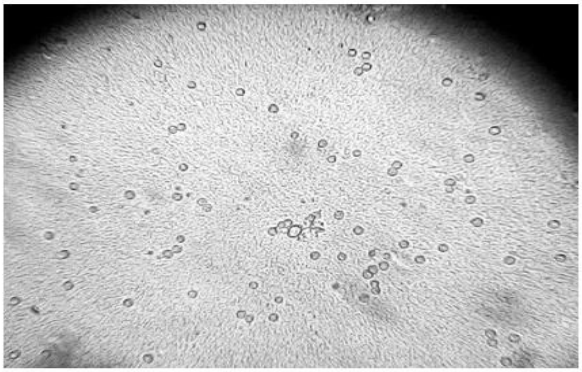


Fig B. Test Sample Cell Viability

V. CONCLUSION

This study explored the chemical and pharmacological properties of Epiphyllum oxypetalum Asava, focusing on its alcohol content, total solid content, phytochemical profile, and in vitro anticancer activity. The alcohol content was found to be 7.5% v/v, determined via distillation and alcoholometry. This falls within the Ayurvedic Pharmacopeia's acceptable range (5–10%), confirming successful self-fermentation and the preservative role of naturally produced ethanol, likely aided by Woodfordia fruticosa flowers.

The total solid content measured 32.8 mg/mL, indicating a rich concentration of dissolved phytoconstituents, sugars, and minerals components that contribute to the formulation's therapeutic potential. Phytochemical fingerprinting using HPTLC confirmed the presence of 4-hydroxy-2-methylacetophenone, a phenolic acetophenone derivative, through matching Rf values between the standard and the methanolic extract. UV-Visible spectrophotometry supported this finding, showing a broad absorbance plateau between 250–350 nm with high intensity (~5.0 AU), typical of aromatic and phenolic compounds.

The methanolic extract of the Asava was evaluated for anticancer activity using the MTT assay on MCF-7 (breast adenocarcinoma) and Colo-205 (colon cancer) cell lines. The extract showed dose-dependent cytotoxicity, with IC_{50} values of 52.82 µg/mL for MCF-7 and 64.71 µg/mL for Colo-205. Although less potent than 5-fluorouracil, the extract reduced cell viability significantly, suggesting promising anticancer potential.

In conclusion, Epiphyllum oxypetalum Asava contains notable levels of ethanol and bioactive phytoconstituents, including 4-hydroxy-2-methylacetophenone, and demonstrates moderate anticancer activity. These findings validate its traditional use and support its potential as a candidate for further pharmacological and clinical research.

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