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# PHYTOCHEMICAL ANALYSIS, TOTAL PHENOL CONTENT AND *IN VITRO* ANTICANCER ACTIVITY OF IMMATURE BARK OF *AEGLE MARMELOS*

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## ABSTRACT

The aim study is to evaluate phytoconstituents and *in vitro* anticancer activity of immature bark of *Aegle marmelos.* Total phenol content of extract was found to be 898mg/g. Chloroform extract showed potent anticancer activity in VERO, 3T3-L1 cell lines.

**Key Words:** *Aegle marmelos,* Immature bark, Anticancer activity, Total phenol content

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## INTRODUCTION

Medicinal plants have been in use from time immemorial and their utility has been increasing day by day in the present world. Naturally obtained compounds are considered safer and easily biodegradable than synthetic compounds and the problem of drug resistance observed in synthetic drugs is also reduced (1). Bael (*Aegle Marmelos* (Linn), family Rutacae, is also known as Bale fruit tree, is a moderate sized, slender, aromatic tree, 6.0 -7.5 m in height, and 90 to 120 cm in girth, with a somewhat fluted bole of 3.0-4.5 meter growing wild throughout the deciduous forests of India, ascending to an altitude of 1200 meter in the western Himalayas and also occurring in Andaman island (2). Leaves, fruit, stem and roots of this tree at all stages of maturity are used as ethno medicine against various human ailments. The different parts of Bael are used for various therapeutic purposes, such as for treatment of Asthma, Anaemia, Fractures, Healing of Wounds, Swollen Joints, High Blood Pressure, Jaundice, Diarrhoea Healthy Mind and Brain Typhoid Troubles during Pregnancy.

The ripe fruit is a good and simple cure for dyspepsia. The pulp of unripe fruit is soaked in gingelly oil for a week and this oil is smeared over the body before bathing. This oil is said to be useful in removing the peculiar burning sensation in the soles. The roots and the bark of the tree are used in the treatment of fever

by making a decoction of them. The leaves are made into a poultice and used in the treatment of opthalmia. The leaf part of the plants have been claimed to be used for the treatment of inflammation, asthma, hypoglycemia, febrifuge, hepatitis and analgesic (3-14). Although tremendous progress has been made in basic cancer biology and in the development of novel cancer treatments, cancer remains a leading cause of death in the world.

## MATERIALS AND METHODS

### Collection and authentication of plant

The immature bark of *Aegle marmelos* were collected from the West Godavari district, Andhrapradesh. They were identified and authenticated in Department of Botany, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India and a specimen (No-CRR/COG/1/2014) was preserved.

### Preparation of the extract

Immature bark of *Aegle marmelos* were collected and air dried in shade under the room temperature. Around 500 g of finely powdered immature bark material was evenly packed in a soxhlet apparatus and the extraction was done with chloroform for 48 hours. The solvent was then evaporated under reduced pressure. The percentage (%) yield of the extract was calculated.

### Phytochemical analysis of chloform extract of immature bark of *Aegle marmelos*

The chloroform extract of immature bark of *Aegle marmelos* of the plant was subjected to chemical test for the identification of various phyto constituents as described Kokate (15).

### Total phenol content

Total phenol content in extract is determined by Folin-Ciocalteu method. This test is based on the oxidation of phenolic groups – with phosphomolybdic and phosphotungstic acids. After oxidation a green blue complex obtained measurable at 765 nm. The total phenol content of a material is being related to the antioxidant activity shown by it (15).

### *In vitro* anticancer activity

### Cell lines

Three cell lines VERO, 3T3-L1 and HeLa were purchased from NCCS, Pune, India and were cultured in RPMI 1640 cell culture medium containing 10% fetal bovine serum

### Determination of Total Cell Protein Content by Sulphorhodamine B (SRB) assay (16)

After 72 hours, 50ml of 50% trichloro acetic acid was added to the walls gently such that it forms a thin layer over the drug dilutions to form an overall concentration of 10%. The plates were incubated at 4° C and were flicked and washed five minutes with tap water to remove traces of medium, drug serum and then air dried. The air dried plates were stained with Sulphorhodamine B for 30 minutes. The unbound dye was then removed by rapidly washing four times with 1% acetic acid. The plates were then air dried. 100ml of 10mM Tris base was then added to the walls to solubilise the dye and plates were shaken vigorously for 5 minutes. The absorbance was measured using Microplate reader at a wavelength of 540nm and calculated by the formula

**% Growth inhibition = Mean O.D. of test group/ Mean O.D. of control group X 100**

## RESULTS AND DISCUSSION

### Extraction

Extraction of *Aegle marmelos* was carried by Soxhlet extraction method. The yield of chloroform extract was found to be 7.31gm.

### Qualitative phytochemical analysis of chloroform extracts of *Aegle marmelos*

Qualitative phytochemical analysis of *Aegle marmelos* showed the presences of alkaloids, steroids, terpenoids, tannins, flavonoids, glycosides and saponins.

### Estimation of Total Phenolic Content

The chlorofrom extract of *Aegle marmelos* was subjected to determination of total phenolic content by Folin-Ciocalteu method. The results are shown in the table-2

### *In vitro* anticancer activity of chloroform extracts of *Aegle marmelos*

*In vitro* anticancer activity of chloroform extracts of *Aegle marmelos* was carried out by Sulphorhodamine B assay. The results are given in table-2, 3 and 4. Beneficial effects of crude drugs are believed to be attributed to plant phytochemicals (various factors in plant foods), such as carotenoids, antioxidative vitamins, phenolic compounds, terpenoids, steroids, indoles, and fibers, etc (17). These are the effective elements considered to be responsible for reducing cancer risk.

**Table-1 Estimation of Total Phenolic Content by Folin-Ciocalteu method**

| S.No | Extract | Total Phenolic Content (mg/g) |
|---|---|---|
| 1 | Chloroform | 898 |

**Table-2 Percentage cell growth inhibition of chloroform extract of *Aegle marmelos* on VERO cell lines by Sulphorhodamine B (SRB) assay**

| S.no | Concentration of chloroform extract (µg/ml) | Absorbance | % inhibition |
|---|---|---|---|
| 1 | 500 | 0.599±0.07 | 53.14 |
| 2 | 250 | 0.487±0.04 | 43.21 |
| 3 | 125 | 0.356±0.08 | 31.58 |
| 4 | 62.5 | 0.256±0.08 | 22.71 |
| 5 | 31.25 | 0.156±0.19 | 13.84 |
| 6 | Control | 1.127±0.15 | 0 |

**Table-3 Percentage cell growth inhibition of chloroform extract of *Aegle marmelos* on 3T3-L1 cell lines by Sulphorhodamine B (SRB) assay**

| S.no | Concentration of chloroform extract (µg/ml) | Absorbance | % inhibition |
|---|---|---|---|
| 1 | 500 | 0.499±0.17 | 45.77 |
| 2 | 250 | 0.377±0.24 | 34.58 |
| 3 | 125 | 0.254±0.18 | 23.30 |
| 4 | 62.5 | 0.154±0.08 | 14.12 |
| 5 | 31.25 | 0.099±0.22 | 9.08 |
| 6 | Control | 1.09±0.24 | 0 |

**Table-4 Percentage cell growth inhibition of chloroform extract of *Aegle marmelos* on HeLa cell lines by Sulphorhodamine B (SRB) assay**

| S.no | Concentration of chloroform extract (µg/ml) | Absorbance | % inhibition |
|---|---|---|---|
| 1 | 500 | 0.402±0.27 | 40.22 |
| 2 | 250 | 0.312±0.04 | 31.26 |
| 3 | 125 | 0.214±0.08 | 21.44 |
| 4 | 62.5 | 0.134±0.28 | 13.42 |
| 5 | 31.25 | 0.079±0.21 | 7.91 |
| 6 | Control | 0.998±0.20 | 0 |

## CONCLUSION

Immature bark of *Aegle marmelos* was extracted with chloroform using Soxhlet apparatus for 48 hours and yield was found to be 7.31gm. Qualitative phytochemical analysis of *Aegle marmelos* showed the presences of alkaloids, steroids, terpenoids, tannins, flavonoids, glycosides and saponins. Total phenolic content was determined by Folin-Ciocalteu method and the content was found to be 898mg/g. *In vitro* anticancer activity was carried out by Sulphorhodamine B assay using VERO, 3T3-L1 and HeLa cell lines. Chloroform extract showed potent

anticancer activity in VERO, 3T3-L1 cell lines and moderate activity in HeLa cell lines.

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