



A STUDY ON BIOASSAY GUIDED IDENTIFICATION OF ANTIOXIDANT PROPERTY, INVITRO CYTOTOXICITY AND ANTICANCER POTENTIAL OF AEGLE MARMELLOS CRUDE EXTRACT

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ABSTRACT

Aegle marmelos is known for its various medicinal properties, whose invitro cytotoxicity and anticancer properties of various crude extracts of leaves was carried out in the present study. Moreover an attempt was made to identify the antioxidant potent fraction from crude solvent extract. The acetone extract of *A. marmelos* leave sample showed the presence of antioxidant fraction (Rf 0.79). Among the various extract ethanol and ethyl acetate extract of *A. marmelos* leaves showed higher anticancer activity with an IC₅₀ value of 116 and 130µg ml⁻¹ respectively.

KEY WORDS: Anti oxidant, Anticancer, Cytotoxicity, TLC Bioautography, *Aegle marmelos*

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INTRODUCTION

Medicinal plants are characteristic attributed with bioactive components which can be used to treat various ailments (1). From the time unknown plants have been used as traditional medicine. Antioxidant, antimicrobial, cytotoxicity and other bioactivity of these medicinal plants are due to the effects of different kind of phytochemicals present (2,3). Recently use of such medicinal plants as raw material for production of new drugs has become common, so as implying the necessity for implanting strategies for standardization the scientific base of herbal medicine (4). In this aspect of bioprospecting of medicinal plant is a perfect alternative to tap the bioactive potency (5). On such bioprospecting is identifying antioxidant property. Imbalance between free radical produced during oxidative stress and antioxidant defense mechanism results in the various pathogenesis of disease conditions due to the breakdown of biological macromolecules like proteins and DNA that lead to cell death. Antioxidants are proven to act against these free radicals and believed to prevent cancer. Antioxidant attracts investigators to search for more cost effective and active phytochemical which pose antioxidant activity. TLC bioautography for plant extract is useful for finding new antioxidants (2, 6). In this study, *A.marmelos* was exploited to study their antioxidant ,cytotoxicity and anticancer potentials.

MATERIALS AND METHODS

Extraction

Aegle marmelos leaves were collected and shade dried. After drying, leaves were subjected for various solvent extractions viz. ethanol, ethyl acetate, acetone, chloroform and petroleum ether by cold extraction method.

Thin layer chromatography

30 µl of the extracts were applied at 2.5 cm from the base of the prepared Silica gel 60 F254 thin layer chromatography (TLC) . After drying, the TLC plates were developed with

Chloroform:methanol(5:1) as the developing solvent and were run in duplicate. One set was used as the reference chromatogram and other set was used for bioautography. The reference TLC plates were then developed using Iodine vapour .

TLC-bioautography analysis

The antioxidant activities of fractions of *A.marmelos* leave extract was studied by qualitative DPPH method - TLC bioautography (6,7,8). Around 30µl of extracts were added on to TLC plate and phytochemical fractionation was carried out on aluminium-backed TLC plates (F²⁴⁵, Merck) using chloroform: methanol (5:1) eluent system in a saturated compartment. Thus separated components were analyzed for the presence of antioxidant fraction by spraying DPPH . Decolourization of the DPPH to yellow colour around the active fractions was noted and the Rf values were calculated.

Cytotoxicity and Anticancer activity of crude extract of A. marmelos

Anticancer activity of crude extracts of *A. marmelos* were determined through MTT assay (9). Hep-2 (Liver cancer cell line) - cancer cell line and Vero (African green kidney cells) - normal cell line (control) was used in this study. Cell lines were grown as monolayer culture in Tissue culture flask containing RPMI1640 medium supplemented with 10% FCS (Fetal calf serum) and grown at 37°C under humidified atmosphere of 95% air and 5% CO₂. Cells were regularly passaged and maintained before experimentation. Cell lines (100µl) were seeded in 96-well plates at a concentration of 6×10^3 cells ml⁻¹ and incubated for 24h at CO₂ incubator. 100 µl of various concentrations of crude extracts of *A. marmelos* added to all the wells. DMSO was used as vehicle control. The plates were further incubated for 48h in CO₂ incubator. Thereafter, 50µl of MTT solution [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] was added to each well and plates were further incubated for 3h in

incubator and developed colour was measured using Synergy H4 Micro plate reader at 570nm.

RESULTS AND DISCUSSION

Extracts from the dry, finely grounded powder of *A. marmelose* was done different solvent such as petroleum ether, acetone, chloroform, ethyl acetate and ethanol. This extract was stored in dry container and used for future analysis. TLC fingerprint of the medicinal plant extract is a method of choice for identification of compounds. This identification is done on the basis of retention factor or R_f value. Among chromatographic technique TLC is the most easiest and cheapest method for analysis of natural products which does not require more sophisticated equipment. (10) The extracts were separated on the basis of polarity in TLC plate using different solvent system. The band separated were measured and represented as R_f (Figure 1)(Table 1) Antioxidant potential of the TLC separated bands were analysed using TLC bioautography with 0.25% of DPPH(11,12) as the developing agent. DPPH based method is a common method used to identify antioxidant compound. DPPH when reduced change its colour from violet to fluorescent yellow due to formation of diphenylpicryl hydrazine when react with antioxidant. (13). The bioautographs showed one distinct region with DPPH reduction in TLC plates, which had R_f value of 0.79 (Figure 2). It confirmed the presence of antioxidant component in the

acetone extract of the *A. marmelos* leaves. This kind of confirmation has been well documented (6,7,8). Antioxidant activity in Bamboo was shown using the TLC bioautography method. (14) Antibacterial potent fraction of *A. marmelos* were reported earlier using TLC bioautography (15) An attempt was also given to evaluate the in-vitro cytotoxicity of crude extract against Hep-2 (Liver cancer cell line) and Vero (African green monkey kidney cell line) using MTT cytotoxicity assay. Results showed dose dependent growth inhibition of cell line (Fig 2 and 3). Among various organic solvent extracts of *A. marmelos*, ethanol and ethyl acetate extract showed higher reduction in proliferation of cancer cells and recorded least IC₅₀ value of 116 and 130 $\mu\text{g ml}^{-1}$, respectively. Nevertheless, other crude extracts recorded considerably higher IC₅₀ value ranged between 245 and 349 $\mu\text{g ml}^{-1}$ (Table 1). Chockalingam et al (16) found hydroalcoholic extract of *A. marmelos* to have antitumor as well as antioxidant activities in animal models i.e DLA bearing mice. On the other hand, the crude extracts tested for cytotoxicity activity against the normal cell line (Vero) recorded markedly higher concentrations towards exhibiting 50% inhibition (IC₅₀) and it ranged from 240 to 500 $\mu\text{g ml}^{-1}$ (Table 2). This indirectly indicates that crude extracts of *A. marmelos* did not show much toxicity on normal cell line (Vero). Samrot et al (17) found LD₅₀ (lethal dose) of crude extract of *A. marmelos* against peripheral blood mononuclear cells (PBMC) as $\leq 140 \mu\text{g/ml}$.

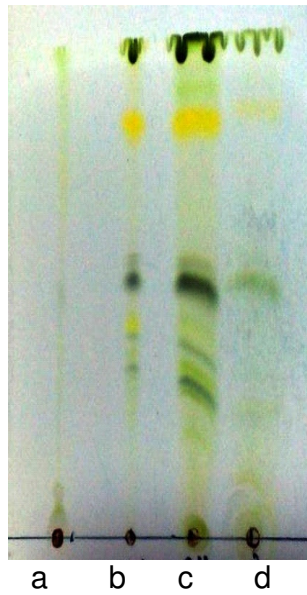


Figure 1
TLC plate a) Petroleum ether b) chloroform c) acetone d) ethylacetate

EXTRACT	Rf Values
Petroleum Ether	0.64,0.78
Chloroform	0.39,0.42,0.46,0.51,0.52,0.84
Acetone	0.39,0.4,0.42,0.46,0.51,0.52,0.84
Ethyl acetate	0.51,0.84

Table 1
Rf Value for the component separated using TLC.

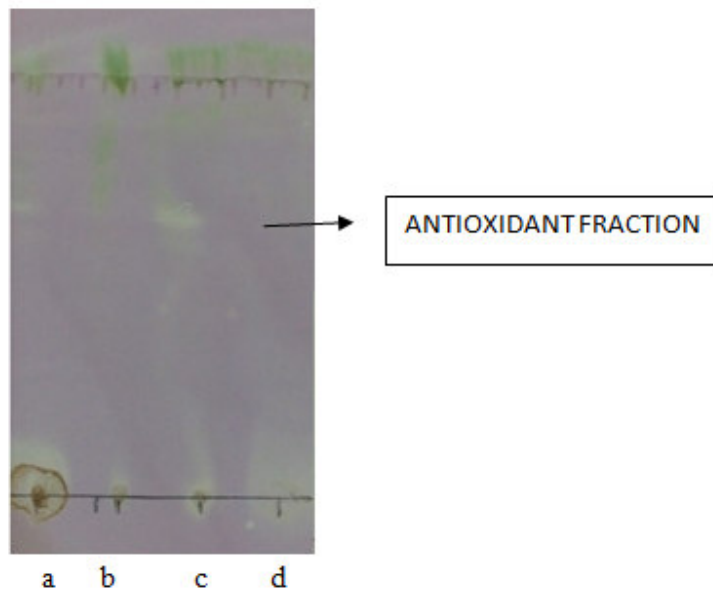


Figure 2
Bioautograph for antioxidant fraction a) Petroleum ether b) chloroform c) acetone d) ethylacetate

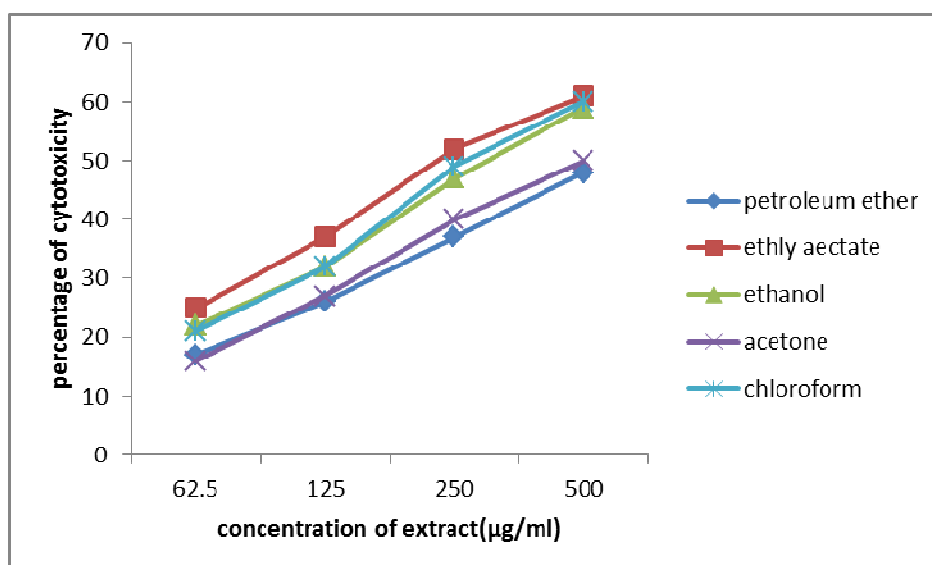


Figure 3
Cytotoxicity of various extracts of A. marmelos against vero cell line

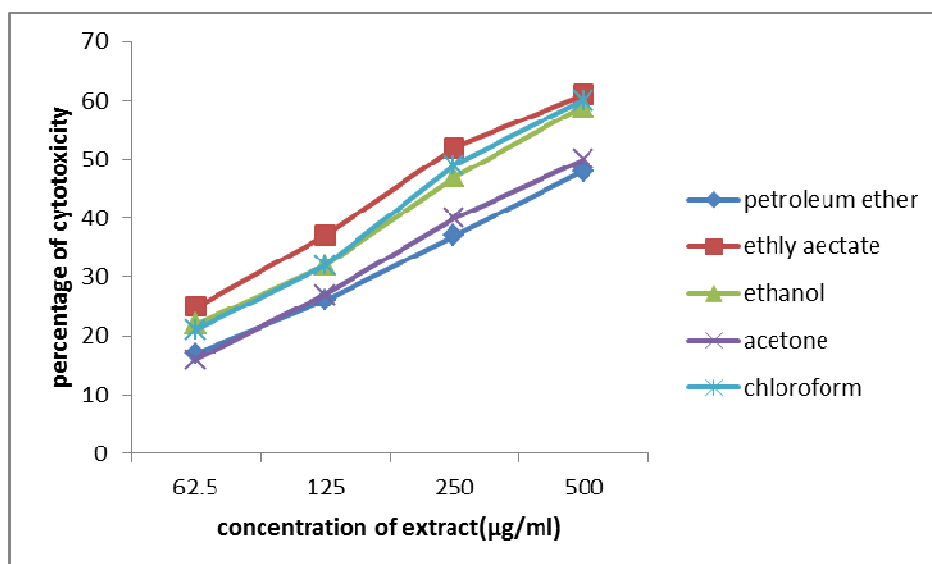


Figure 4
Anticancer activity of various extracts of A. marmelos against Hep2 cell

EXTRACT	ic50(µg/ml)
Petroleum ether	480
Ethyl acetate	260
Ethanol	240
Acetone	500
Chloroform	245

Table 1
IC50 value of various extracts of A. marmelos against Vero cell line

EXTRACT	ic50($\mu\text{g/ml}$)
Petroleum ether	260
Ethyl acetate	130
Ethanol	116
Acetone	245
Chloroform	349

Table 2
IC50 value of various extracts of *A.marmelos* against Hep2 cell line

CONCLUSION

The results of the present study clearly, emphasize that the active principles reside within the crude extracts of *A. marmelos* has significantly inhibited the growth of cancer cell lines than non-cancer normal cell line.

ACKNOWLEDGMENT

The authors are grateful for the support by Sathyabama University ,Chennai, Tamil Nadu, India, for carrying out the above research work. We would like to express our heart full thanks to Dr. Bhavanilatha, Assistant Professor, Department of Biotechnology Sathyabama University ,Chennai for her assistance in anticancer activity.

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