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Invitro cytotoxicity study on combined plants extracts (*Cissus quadrangularis* and *Aegle marmelos*)

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ABSTRACT

The paper details a biological investigation on Cissus quadrangularis and Aegle marmelos. The Cytotoxicity of samples on colon: HT-29 cells were determined by the MTT assay .The ethanolic and ethyl acetate crude extract of Cissus quadrangularis and Aegle marmelos have been screened for antitumor potentials using in vitro assay for Cytotoxicity activity (MTT assay) with eight different concentrations. Measurements were performed and the concentration required for a 50% inhibition of viability (IC50) was determined graphically. The effect of the samples on the proliferation of HT-29 was expressed as the % cell viability. From the graphs the concentration of ethanolic and ethyl acetate crude extract yields the value of LC_{50} (50% mortality) as 62.5µg/ml and 62.5µg/ml respectively for Cissus quadrangularis and Aegle marmelos. In the same way the concentration at which 88% mortality occurs for the ethanolic extract and 83% mortality occurs for the ethyl acetate extract are obtained from the graphs and the values have been found to be 7.8 µg/ml for Cissus quadrangularis and Aegle marmelos respectively.

Keywords: *Cissus quadrangularis, Aegle marmelos, Medicinal plant,* Cytotoxicity, MTT Assay.

INTRODUCTION

Natural products and related drugs are used to treat 87% of all categorized human diseases including bacterial infection, cancer and immunological disorders [1]. About 25% of prescribed drugs in the world originate from plants [2] and over 3000 species of plants have been reported to have anticancer properties [3]. About 80% of the populations in developing countries rely on traditional plant based medicines for their primary health care needs [4]. Bangladesh has a rich and prestigious heritage of herbal medicines among the South Asian countries. More than 500 species of medicinal plants are estimated as growing in Bangladesh and about 250 species of them are used for the preparation of traditional medicines. However, the majority of these plants have not yet undergone chemical, pharmacological and toxicological studies to investigate their bioactive compound(s) [5]. Traditional records and ecological diversity indicate that Bangladeshi plants represent an exciting resource for possible lead structures in drug design. Traditional medicines have been used for many centuries by a substantial proportion of the population

of India[6].The World Health Organization (WHO) estimated that 80% of the populations of developing countries rely on traditional medicines, mostly plant drugs, for their primary health care needs[7], [8]. Pharmacological research on the medicinal properties of phytochemicals has become mandatory, to establish the claimed medicinal properties of herbs [9]. Medicinal plants play an important role in the discovery of novel drugs used in modern medicine[10]. Many works have been done which aim at knowing the different antimicrobial and phytochemical constituents of medicinal plants and using them for the treatment of microbial infections (both topical and systemic applications) as possible alternatives to chemically synthetic drugs to which many infectious microorganisms have become resistant. During the last ten years the pace of development of new antimicrobial drugs has slowed down while the prevalence of resistance (especially multiple) has increased astronomically [11]. The cost of drugs in use today is too expensive for the majority of the population in the third world countries and therefore the search for some cheap sources of antimicrobial substances in nature become inevitable. [12] Antibacterial resistance among bacterial pathogens in recent time is a critical area of public health concern.[13] The usual causative agents of infectious diseases (especially bacteria) are becoming increasingly resistant to some or most antibiotics.[14] In developing countries, thousands of rural communities still depend mainly on folklore medicine to cure diseases. [15] Medicinal plants are cheap and handy to most of the populations on the globe [16]. As a result of proximity, reliability and age long practice, people still depend largely on traditional medicine for their health care delivery [17]. Though the therapeutic uses of plants by the primitive people lack scientific explanations [18]. There is a great awareness in the use and significance of these medicinal floras by the World Health Organization in several resource- poor nations WHO [19]. India has a rich heritage of traditional knowledge and is home to several important time-honored systems of health care like Ayurveda, Siddha and Unani. It has been estimated that the proportion of medicinal plants in India (7,500 of the 17,000 higher plant species are medicinal plants) is higher than any country of the world with respect to the existing flora of that respective country.

MATERIALS AND METHODS

Method A:

Collection and Preparation of the Extract

Cissus quadrangularis and *Aegle marmelos* were collected from in and around area of Nandyal, Andhra Pradesh. The above mentioned plants were examined, identified and authenticated by Dr.Prasad Rao,Professor, Department of Botany, P.S.C & K.V.S.C. Govt. Degree College, Nandyal. The stem part of *Cissus quadrangularis* and the fruit pulp of *Aegle marmelos* were air dried and pulverized into powder. About 25gm of the powdered sample of each medicinal plant were weighed into 100 ml of ethanol and ethyl acetate extract in a Soxhlet apparatus separately and the process is carried out for 7 days at 40-50˚c. The filtrate was evaporated to dryness at 40˚ c in a rotary evaporator. And the above process was repeated for several times, until the sufficient amount of extract is produced. The concentrated extract of each plant was stored at 4˚ c until when required for use.

Cell line and culture

Colon cancer- HT-29 cell lines were obtained from National centre for cell sciences Pune (NCCS). The cells were maintained in Minimal Essential Media supplemented with 10% FBS,penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO_2 at 37 °C.

Reagents

MEM was purchased from Hi Media Laboratories Fetal bovine serum (FBS) was purchased from Cistron laboratories Trypsin, methylthiazolyl diphenyl- tetrazolium bromide (MTT), and Dimethyl sulfoxide (DMSO) were purchased from (Sisco research laboratory chemicals Mumbai). All of other chemicals and reagents were obtained from Sigma Aldrich Mumbai.

In vitro assay for Cytotoxicity activity (MTT assay)

The Cytotoxicity of samples on colon: HT-29 cells were determined by the MTT assay [20-23]. Cells (1×10^5/well) were plated in 1ml of medium/well in 24-well plates (Costar Corning, Rochester,NY). After 48 hours incubation the cell reaches the confluence. Then, cells were incubated in the presence of various concentrations of the samples in 0.1% DMSO for 48h at 37°C. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 200µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromide cells(MTT) phosphate- buffered saline solution was added. After 4h incubation, 0.04M HCl/ isopropanol were added. Viable cells were determined by the absorbance at 570nm. Measurements were performed and the concentration required for a 50% inhibition of viability (IC50) was determined graphically. The absorbance at 570 nm was measured with a

UV- Spectrophotometer using wells without sample containing cells as blanks. The effect of the samples on the proliferation of HT-29 was expressed as the % cell viability, using the following formula:

% cell viability = A570 of treated cells / A570 of control cells × 100%.

Table 1. Anticancer effect of *Sample 1- Ethyl Acetate Extract* on HT-29 cell line

S.No	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)
1	1000	Neat	0.04	9.5
2	500	1:1	0.09	21.4
3	250	1:2	0.14	33.3
4	125	1:4	0.19	45.2
5	62.5	1:8	0.22	52.3
6	31.2	1:16	0.29	69.0
7	15.6	1:32	0.34	80.9
8	7.8	1:64	0.37	88.0
8	Cell control	-	0.42	100

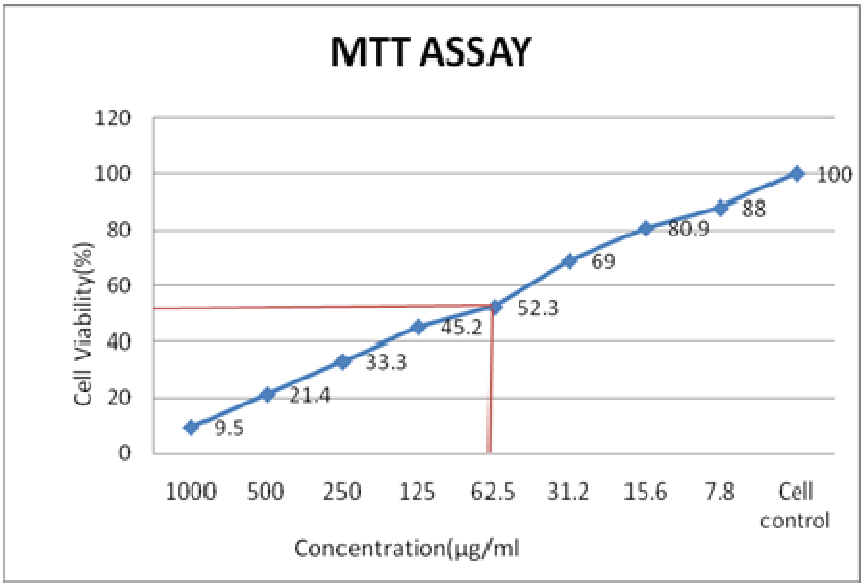


Fig 1. Anticancer effect of *Sample 1- Ethyl Acetate Extract* on HT-29 cell line

Table 2. Anticancer effect of *Sample 2- Ethanol Extract* on HT-29 cell line

S.No	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)
1	1000	Neat	0.02	4.7
2	500	1:1	0.08	19.0
3	250	1:2	0.12	28.5
4	125	1:4	0.18	42.8
5	62.5	1:8	0.21	50.0
6	31.2	1:16	0.27	64.2
7	15.6	1:32	0.33	78.5
8	7.8	1:64	0.35	83.3
8	Cell control	-	0.42	100

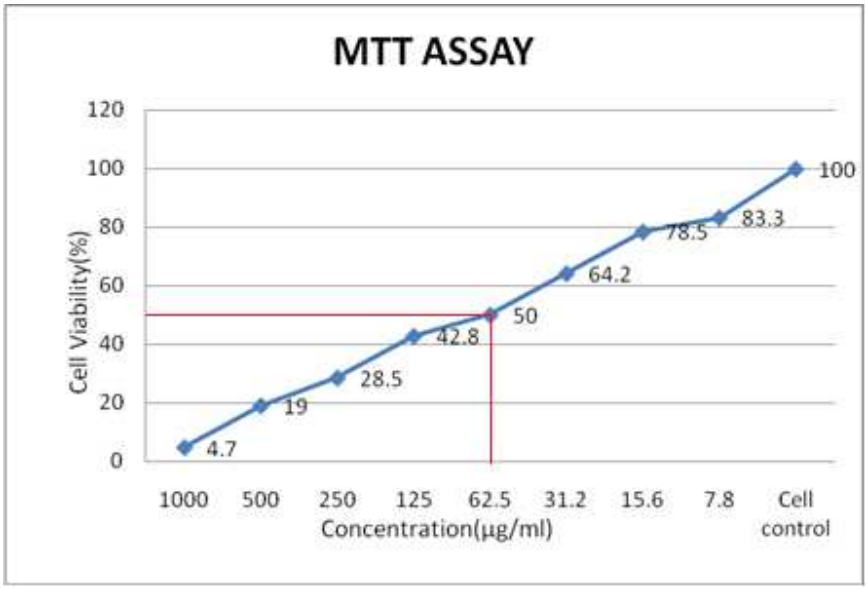
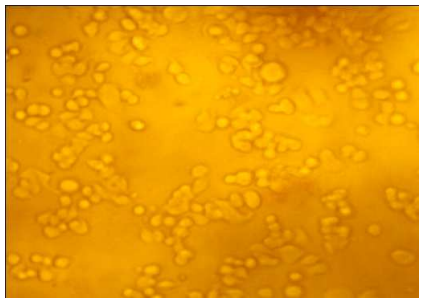
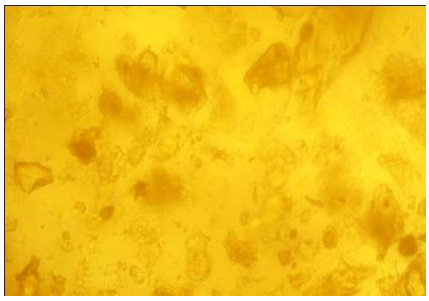


Fig 2. Anticancer effect of *Sample 2- Ethanol Extract* on HT-29 cell line

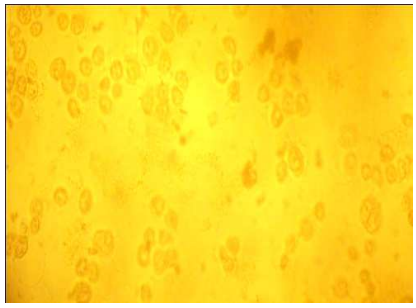
Fig 3. Anticancer effect of *Sample 1- Ethyl Acetate Extract* on HT-29 cell line



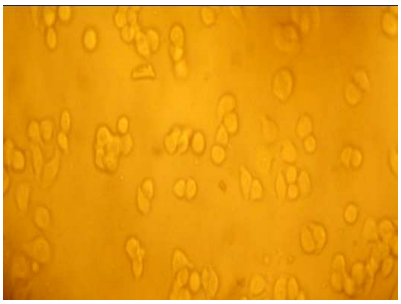
Normal HT-29 Cell line



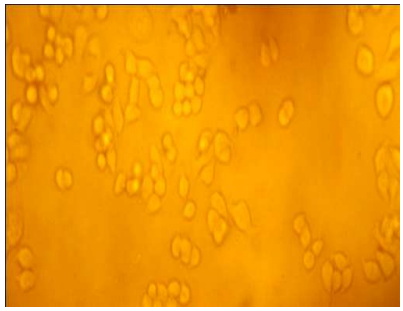
Toxicity- 1000µg/ml



Toxicity- 250µg/ml

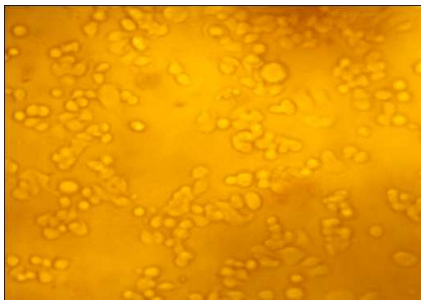


Toxicity- 62.5µg/ml



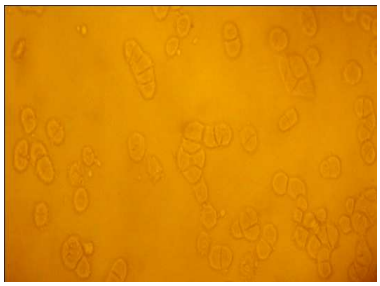
Toxicity- 31.2µg/ml

Fig 4. Anticancer effect of *Sample 2- Ethanol Extract* on HT-29 cell line



Normal HT-29 Cell line

Toxicity- 1000µg/ml



Toxicity- 250µg/ml

Toxicity- 62.5µg/ml

Toxicity- 31.2µg/ml

RESULTS AND DISCUSSION

From the above figures, we can easily determine the percentage of cell viability for eight different concentration of combined ethanolic and ethyl acetate extracts of *Cissus quadrangularis* and *Aegle marmerlos*. Eight different concentrations were 1000 µg/ml,500 µg/ml ,250 µg/ml,125 µg/ml, 62.5 µg/ml,31.2 µg/ml,15.6µg/ml,7.8 µg/ml for both the extracts respectively. The combined ethyl acetate extract of *Cissus quadrangularis* and *Aegle marmelos* at

1000 µg/ml produces 9.5% cell viability, 500 µg/ml produces 21.4% cell viability,250 µg/ml produces 33.3% cell viability, 125 µg/ml produces45.2% cell viability,62.5 µg/ml produces 52.3% cell viability , 31.2 µg/ml produces 69.0% cell viability, 15.6 µg/ml produces 80.9% cell viability,7.8 µg/ml produces88.0% cell viability. The combined ethanolic extracts of *Cissus quadrangularis* and *Aegle marmelos* at 1000 µg/ml produces 4.7% cell viability, 500 µg/ml produces 19.0% cell viability,250 µg/ml produces 28.5% cell viability, 125 µg/ml produces 42.8% cell viability,62.5 µg/ml produces 50.0% cell viability, 31.2 µg/ml produces 64.2% cell viability, 15.6 µg/ml produces 78.5% cell viability ,7.8 µg/ml produces 83.3% cell viability.

Different extracts of the plant exhibited different activity on different cell lines. This selectivity could be due to the sensitivity of the cell line to the active compounds in the extract or to tissue specific response. The effect of the samples on the proliferation of HT-29 was expressed as the % cell viability. From the graphs the concentration of ethanolic and ethyl acetate crude extract yields the value of LC_{50} (50% mortality) as 62.5µg/ml and 62.5µg/ml respectively for *Cissus quadrangularis* and *Aegle marmelos*. In the same way the concentration at which 88% mortality occurs for the ethanolic extract and 83% mortality occurs for the ethyl acetate extract are obtained from the graphs and the values have been found to be 7.8 µg/ml for *Cissus quadrangularis* and *Aegle marmelos* respectively.

CONCLUSION

In our study, we deeply believe that the cross killing occurred due to cytotoxic activity against the Colon cancer-HT-29 cell lines. The results of the present study demonstrated the potent cytotoxic activity of the combined ethanolic and ethyl acetate extracts of *Cissus quadrangular is* and *Aegle marmelos*. The phytochemical constituents such as flavanoids and terpenoids are the major components which are responsible for the potential cytotoxic activity. Further research also need for proving with other cancer models and isolating the active principle.

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