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In-vitro Anticancer Activity of *Aegle Marmelos Linn*

Miss. Pallavi V Ganvir[1], Mr. Rupesh V Ingole[2], Dr. S R Butle[3], Mr. Mohd Ameen M[4], Mr. Mohammad Zishan Ibrahim[5], Mr.Shaikh Abid[6]

Department of Pharmaceutical Chemistry, Kandhar College of Pharmacy, Balantwadi Ghodag Road Kandhar.

Department of Pharmaceutical Chemistry, School of pharmacy Nanded, SRTM University Nanded

ABSTRACT

In present study. Phytochemical investigation and in-vitro anticancer activity of Aegle marmelos Linn. was performed. Standardization of powder is done by determination of ash value, extractive value and moisture content of bark and leaf of Aegle marmelos Linn Thin layer chromatography was performed for the separation of active constituents from ethyl acetate, chloroform, methanol and aqueous extract. Methanol fractions of leaf & bark and aqueous extract of leaf of Aegle marmelos L leaf and bark were tested for in-vitro anticancer activity. In-vitro anticancer activity was carried out by using SSC 40 oral cancer cell line. Characterization of methanolic extract of bark and leaf was carried out by using NMR (nuclear magnetic resonance) spectroscopy and methanolic extract of leaf by using HR-LCMS (High resolution- liquid chromatography mass spectroscopy) analysis.

The obtained ash value is in the range of 12.8% w/w and the yield of leaf extract was found to be in the range of 5.78% and the yield of bark extract was found to be in the range of 6.04. RF value was found to be 0.91. Phytochemical screening confirmed the presence of alkaloids, glycosides, carbohydrates, flavonoids, phenol amino acids and proteins. At any concentration SSC 40 cell line didn't shows inhibition of cancer cells, observed by calculating LC50, TGI and G150. At 20 ug/ml concentration, aqueous extract shows more cell inhibition in oral cancer cell line than methanolic leaf extract and methanolic bark extract. Phytochemical investigations of methanol extract were performed by NMR and HR-LCMS analysis. Result indicates that methanolic extract of leaf and bark and aqueous extract of leaf of Aegle marmelos L having significant anticancer activity and various compounds are identified by using HR-LCMS analysis.

Keywords; *Cancers, Aegle Marmelos Linn, Anti-cancer activity, Oral cancer.*

INTRODUCTION

Cancer can be defined as a disease in which a group of abnormal cells grow uncontrollably by disregarding the normal rules of cell division. Normal cells are constantly subject to signals that indicate whether the cell should divide, resulting in uncontrolled growth and proliferation. If this proliferation is allowed to continue and spread,

it can be fatal. In fact, almost 90% of cancer-related deaths are due to tumours spreading a process called metastasis. The cancer known medically as neoplasm is a broad group of various disease all involving unregulated cell growth. In cancer, cell divides and grows uncontrollably forming the malignant tumour and invades nearby parts of body. The cancer may also spread to more distinct part of body through the lymphatic system or bloodstream. Not all tumours of cancerous. Benign tumours do not grow uncontrollably do not invade neighbouring tissues, and do not spread through the body. [1]

Oral cancer is a type of cancer that begins in the mouth or throat. It's quite common, but if caught and treated early, it can be cured. This cancer can affect different parts of the mouth like the lips, tongue, and throat. There are two main types: oral cavity cancer and oropharyngeal cancer. Signs of oral cancer include easy bleeding and slow healing sores in the mouth. Men are more likely to get it than women, and age increases the risk, although younger people can also develop it. Pain that doesn't go away in the mouth is another warning sign. Oral cancer often starts as a small white or red spot that's hard to notice, and it can spread to other parts of the body. Dentists can help find it early, which improves the chances of survival. Other signs include problems in the mouth like pain or sores that don't heal. [2]

Risk factor of oral cancer

1. Tobacco use and alcohol: Most people with oral cancers use tobacco. The reason of developing this increase with the amount chewed or smoke and the duration of the habit. About 70% of all patient oral cancer is heavy drinkers. The combination of smoking and drinking increases a person's risk of oral cancer.
2. Sun exposure: Many patients with cancers of the lip have outdoor jobs associated with prolonged exposure to sunlight.
3. Diet: A diet low in fruits and vegetables is associated with an increased risk of developing cancer of the oral cavity.
   HPV infection: The number of oropharyngeal cancer linked to Human Papilloma Virus (HPV). Has risendramatically over the past few decades. HPV DNA is now found in about 2 out oropharyngeal cancers and in a smaller fraction of oral cavity cancers.

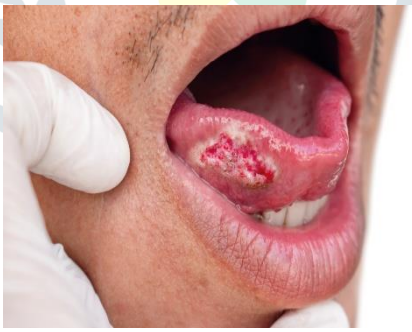

Figure No 1: Oral Cancer

Natural products are playing key role in drug discovery programs of the pharmaceutical industry and various research organizations. Early civilizations learned of planting and planned farming, leading today to well established food and tobacco, sugarcane, tea and coffee. Today there are many examples of important natural products as drugs plants were abundant and easily obtained more and more chemicals were isolated and their chemical nature elucidated. Plants have been used for medicinal purposes across history and cultures and even across species a majority of the world still relies heavily on natural products as herbal remedies for their primary health care. [3]

Aegle marmelos Linn. Is a perennial tree, wild in the sub Himalaya tract, central and south India with enormous therapeutic value in traditional medicine, use das folk medicines. The Ayurveda practioners use almost all of their parts but the greatest medicinal value ascribed to its fruits. Aegle marmelos Linn. Is an Indian plant, which has enormous therapeutic value in traditional systems of medicine. Aegle marmelos belonging to family Rutaceae and grows wild, in outer Himalayas and Shivaliks. Aegle marmelos is the only member of the monotypic genus. [4]

Figure No 2: Aegle Marmelos Linn

MATERIALS AND METHOD

Collection and authentication:

Leaf and bark of Aegle marmelos L. Collected from Sakoli Dist. Bhandara Nagpur region. After collection of leaf of Aegle marmelos L. The Herbarium sheet was prepared and deposited at department of life science, SRTM University Nanded, Maharashtra for authentication and it is identify as twig of Aegle marmelos L. belongs to family Rutaceae.

Instrument:

Hot air oven, Desiccators, Analytical weighing balance, NMR Spectrophotometer, Mass Spectrophotometer, Hip sampler, Binary pumps and Column pumps.

Chemicals and reagents:

Petroleum ether Mumbai(INDIA), chloroform CHCL (New Delhi – INDIA), ethyl acetate (Waroli road Mumbai) , n-butanol CHO (waroli road Mumbai) , hexane (Waroli Mumbai), acetone.

Preparation of extraction:

After collection of leaf and bark of Aegle marmelos L. Were shade dried at room temperature. Leaf and bark were grinded to powder by using grinder and it was passed through sieve for further use. Hot continuous soxhlet extraction was used for the extraction of powder. Successive method was employed for the extraction and different solvents were used as per the polarity. Continuous extraction was started with non-polar solvent, petroleum ether. This extract was discarded. Further, powder was treated with continuous flow of chloroform, ethyl acetate, n-butanol, acetone, n-hexane, methanol and water. After completion of extraction process, evaporation of solvents was carried out at room temperature then stored in desiccator.

Figure No: Soxhlet Apparatus

In-vitro Anti-cancer Activity

The cytotoxicity of compounds on Oral cancer: SSC 40 cells were determined by MTT assay.

SRB solution preparation:

1. Dimethyl sulfoxide 100 µg/ml and diluted to 1mg/ml using water and stored in refrigerator.
2. Sulforhodamine B solution: 50 µl at 0.4 % (w/v) in 1 % acetic acid.

Methodology

1. The cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 Mm L-glutamine. For present screening experiment, cells were inoculated into 96 well microliter plates in 100 µl at plating densities as shown in the study details above, depending on the doubling time of individual cell lines. After cell inoculation, the microliter plates were incubated at 37° C, 5% CO2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs.

2. Experimental drug were initially solubilized in dimethyl sulfoxide at 100 mg/ml and diluted to 1mg/ml using water and stored frozen prior to use. At the time of drug addition, an aliquote of frozen concentrate (1 mg/ml) was thawed and diluted to 100 ug/ml, 200 ug/ml, 400 ug/ml, and 800 ug/ml with complete medium containing test article. Aliquots of 10 ul of these different drug dilutions were added to the appropriate microliter wells already containing 90 ul of medium, resulting in the required final drug concentrations i.c., 10 ug/ml, 20 ug/ml, 40ug/ml, 80 ug/ml.

3. After compound addition, plates were incubated at standard conditions for 48 hours and assay was terminated by the addition of cold TCA. Cells were fixed in situ by the gentle addition of 50 µl of cold 30% (w/v) TCA (final concentration, 10 % TCA) and it is incubated for 60 minutes at 4°C.

4. The supernatant was discarded; the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (50 µl) at 0.4% (w/v) in 1% acetic acid was added to each of the wells,

and plates were incubated for 20 minutes at room temperature. After staining, unbound dye was recovered and the residual dye was removed by washing five times with 1% acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10 Mm trizma base, and the absorbance was read on a plate reader at a wavelength of 540 nm with 690 nm reference wavelength.

5. Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells. Percent growth was express as the ratio of average absorbance of the control wells 100. 59,60

6. Using the six absorbance measurement [time zero (TZ). Control growth ©, and test growth in the presence of drug at the four concentration levels (Ti)]. The percentage growth was calculated at each of the drug concentration levels. Percentage growth inhibition was calculated as:

RESULTS

% Control Growth, Drug concentration (ug/ml):

1.00	**Human Oral squamous Cell Line SSC 40**			
	% Control Growth			
	Drug Concentration (ug/ml)			
	Experiment I			
	10	**20**	**40**	**80**
Methanolic leaf	82.3	78.0	67.2	90.9
Methanolic bark	87.3	87.7	89.3	81.5
Aqueous leaf	80.0	71.2	54.7	9.2
ADR	-80.0	-81.9	-80.2	-73.6
	Experiment II			
	10	**20**	**40**	**80**
Methanolic leaf	114.0	112.0	90.5	106.6
Methanolic bark	142.0	154.0	99.2	110.4
Aqueous leaf	112.0	102.0	78.0	39.5
ADR	-65.7	-80.0	-81.7	-70.6

	Experiment III			
	10	**20**	**40**	**80**
Methanolic leaf	103.4	86.3	97.0	89.0
Methanolic bark	102.2	100.4	94.3	84.6
Aqueous leaf	79.8	77.5	70.2	19.9
ADR	-80.6	-82.9	-82.4	-72.2
	Average values			
	10	**20**	**40**	**80**
Methanolic leaf	99.9	92.1	84.9	95.5
Methanolic bark	110.6	114.2	94.3	92.1
Aqueous leaf	90.8	83.7	67.6	22.9
ADR	-75.4	-81.8	-81.4	-72.1

Drug Concentration (ug/ml) calculated from graph:

SSC 40	LC50	TGI	GI50*
Methanolic leaf	**NE**	**NE**	**NE**
Methanolic bark	**NE**	**NE**	**NE**
Aqueous leaf	**NE**	**NE**	**54.1**
ADR	**NE**	**<10**	**<10**

Whereas,

LC50 Concentration of drug causing 50% of cell kill.

GI50 Concentration of drug causing 50% inhibition of cell growth.

TGI = Concentration of drug causing total inhibition of cell growth.

ADR = Adriamycin, positive control compound.

NE = Non- evaluable data, experiment needs to be repeated using different set of drug Yellow highlight the test value, under GI50 indicates the activity.

DISCUSSION

In the present investigation the in-vitro anticancer activity was performed on compound using SSC-40 oral cancer cell line. Three times the experiment was performed on the plant extract methanolic leaf extract of leaves and bark and aqueous extract of leaves by using the drug concentration 10 µg/ml, 20 µg/ml, 40 µg/ml and 80 µg/m average values are calculated. GI50 of the compound were comparable with known anticancer agent Adriamycin (20 µg/ml) which demonstrate the activity for oral cancer. The result indicates that G150 indicate the activity as compared to TGI.

Oral cancer is the sixth most common cancer affecting mankind, which also presents with low rate of survival. More than 90% of oral cancers are histopathologically squamous cell carcinomas (SSC). Oral cancer mostly affects males over 40 years of age with a history of regular exposure to the risk factors, like alcohol, tobacco products. In India, the major way of treating disease s in ancient time was Ayurveda which is mainly concentrates on using natural plant products as therapeutic agents. Aegle marmelos Linn. which is commonly known as Bael is found in most of the states. Preliminary in vitro study was evaluated the effectiveness of Bael as a cytotoxic agent against oral cancer cells. This effect can be attributed to the presence of phytochemical compounds which are abundant in Bael leaves. The plant extract of leaves, fruit and bark are reported on various types of cancers cell lines such as papilloma, leukaemia, melanoma the review encompasses an overview of both in-vitro models of anticancer studies.

As per the above discussion it was proved that the aqueous extract of leaf of Aegle marmelos Linn. which has a significant anticancer activity.

CONCLUSION

The current statues of natural product are directed towards the isolation and characterization of plant extract with wide range of biological activities. We have successfully studied in-vitro anticancer activity of plant extract of

leaf and bark of Aegle marmelos Linn. And its phytochemical investigation." The various phytoconstituents present in plant extract are identified by using the method phytochemical screening, thin layer chromatography (TLC).

Researchers have studied the anticancer effects of the water extract from Aegle marmelos Linn. Leaves. The findings revealed that the water extract was more effective at inhibiting oral cancer cells at a concentration of 20 ug/ml compared to methanolic leaf and bark extracts. The value representing the effectiveness of the extract was 54.1 µg/ml. People with oral cancer need comprehensive support from a team of experts. Since cancer is a severe illness, it's crucial to explore and use various treatment options like chemotherapy and radiotherapy. Traditional herbal medicine, like Aegle marmelos, has shown promising anticancer properties. The goal of this research is to summarize existing studies on this plant's potential and identify new areas for further exploration in the fight against cancer.

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