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### RESEARCH ARTICLE

#### IN VITRO-CYTOTOXICITY STUDIES OF METHANOLIC LEAF EXTRACT OF *MEMOCYLON UMBELLATUM* BURM.F. AGAINST BREAST CANCER.

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#### Abstract

The *Memocylon umbellatum* is a shrub widely found in the western Ghats of Karnataka, India. The leaf extracts of the plant is being used for various medicinal purposes such as used to cure diseases Ghonerria and also used for the treatment for eye irritations. Our study was aimed to analyze the phytochemical constituents present in the methanolic leaf extract of *Memocylon umbellatum* and further to study its cyto-toxic activity on the MCF-7 (Breast cancer line). Extraction of phytochemicals was done by using soxhlet apparatus. The methanolic extract was tested for the preliminary phytochemical analysis by using standard methods. Test showed the presence of phytochemicals such as Tannins, Lignin, Flavanoids, Steroids, Phenols, and Glycosides. Further, the cytotoxic nature of methanolic extract was explored by performing MTT assay on MCF-7 cell line. The methanolic leaves extract of *Memocylon umbellatum* evidenced the inhibition of MCF-7 cancer cell lines. Thus, active compounds of the extract could be of potential medicinal application.

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#### Introduction:-

Since ancient times, traditional medicines are in practice. Studies also have evidenced the use of traditional medicine is more than 40% of world's population (Subban M, *et al* 2011). Since traditional medicine has been in practice from 200 BC. Traditional knowledge is far better than modern synthetic medicines, so the present world of research is intensified on the Native and Natural medicine. Systematic screening of the medicinal plants may yield some of the novel compounds. To find out the cure for diseases there have been research activities for different sources such as plant, animals, birds, sea -animals, and even from aquatic plants. The plant preferred by us is noted for its medicinal value (Puttaswamy, *et al* 2013) i.e *Memocylon umbellatum* is a shrub belonging to family Melastomataceae used in the traditional medicine such as Ayurveda, unani, sidda. The plant is called as "Anjani" in Sanskrit, "HuliSoppu" in Kannada and as "Iron-wood" tree in English. It is mainly found in the Deccan plateau, Western Ghats of India and some parts of Andaman and Nicobar islands. It is a large shrub or tree which can reach height upto 8 meters, inflorescence of the plant is deep blue in color (Arunachalam KD, *et al* 2013), it has numerous medicinal values such as the decoction of the roots of the plant was given to stop heavy pains of menstrual cycle (S. Satya *et al*, 2003) used in the treatment of some of the deadly diseases such as gonorrhoea (Padmavathy J *et al*, 2010). Furthermore, the plant extracts are said to be used for eye irritation treatment. Hypoglycemic activity was shown by the roots and leaves of the plant. Some of the major activity such as anti-inflammatory, anti-helminthic, anti-insect activity anti-

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spasmodic, anti-tumour, neuro-leptic activity were shown by this plant (Kantha D Arunachalam *et al*, 2013). The shade dried leaf syrup of the plant was used to cure diabetes (Ayanar M, *et al*, 2008). The leaf was also used for snake bites as an anti-venom agent (Kshiragsagar RD and Singh NP, 2001). The plant was also used in creation of gold and silver nano particles (Arunachalam KD, *et al* 2013). This plant play a significant role in lowering of blood glucose level (Amalraj T and Ignacimuthu, 1998). This plants contains many phyto-chemical compounds such as oleanolic acid, Ursolic acid, Sitosterol, organic acid, calcium oxalate (Krishnamurthy S.R. and Asha B, 2011). Studies have shown that many of these antioxidant compounds possess anti-inflammatory, anti-atherosclerotic, anti-tumor, anti-ulcer, anti-mutagenic, anti-carcinogenic, anti-bacterial and anti-viral activities (Agarwal SK and Rastogi RP, 1978). A new constituent of *M.umbellatum* i.e. (4-hydroxymethyl-3-methyl-but-2-ene4,1-olide) was isolated by Agarwal and Rastogi (Han, X, *et al*, 2007). The present study aims to explore the plant's leaf extract is possessing the cure for the Breast cancer. As cancer has high rate of drugresistance and due to its hazardous side effect of its treatment it has led us to concentrate the on anti-cancerous activity of the plant sample. Breast cancer which is been widely spread and increase in the search for the cure of cancer has made researches to explore most possible ways to find the cure. The present work was performed to find the anti-cancer activity of the plant extract. Different plants have been the inspiration for this work, this plant may have many novel compounds which will be examined by performing phyto-chemical screening. The cyto-toxicity assay was also performed to know the cell inhibition property of the plant. The cyto-toxicity assay was asseccesed on the MCF-7 Breast cancer cell line.

## **Materials and Methods:-**

### **Plant collection:-**

The *Memcydon umbellatum* was collected in Anashi Forest the Western Ghats of Karnataka, India and the plant was identified by the taxonomist. The leaves were collected in the month of March-April. Leaves were washed under running tap water to remove debris present on the leaves, then were allowed to sundry for about 5-6 days to remove the moisture content present on leaves. Dried leaves were ground to coarse powder and packed into a sterile sealed container for the further use.

### **Extraction:-**

Powdered leaves were used for obtaining methanolic extract by warm extraction. Briefly, dried leaf powder was soaked in methanol and kept for stirring for 48-72 hours. Later, methanolic mixture was filtered using filter paper and collected into a sterile glass beaker. Filtered mixture was kept for drying, during which it was loosely covered with aluminum foil. After the methanol traces are evaporated, leaf extract was used for further experiments.

### **Soxhlet Extraction:-**

A known amount of shade dried leaf powder is taken and filled in a thimble and the extraction was done successively with various solvent of increasing in there polarity value that is from petroleum ether to distilled water through soxhlet extractor for 48 hrs and all the extracts were concentrated by flash evaporator. After the extraction, the extracts were preserved in an air tight bottles in 5°C. Further, the samples were subjected for preliminary screening of primary and secondary metabolites.

### **Phyto-chemical Analysis:-**

The plant was identified and brought to the lab and was washed under running tap water and washed with alcohol to avoid dust contamination and was shade dried. The leaves were converted into coarse powder with the use of electric blender. The dried leaves were packed into an air tight packet and stored for further analysis.

### **Preliminary screening of secondary metabolites:-**

Samples obtained from hot extraction was subjected for preliminary phytochemical screening of primary and secondary metabolites. The test of metabolites was done by their respective tests. Flavonoids were tested by (Pews Test, Slenods Test, NaOH Test), Glycosides were tested by (Keller kellani Test, Glycosides Test, Molish Test), Phenols were tested by (Ellagic acid Test, Phenols Test), Lignins were tested by (Lobats Test), Steroids were tested by (Libemam-Burchard Test, Salkovaski Test), Tannins were tested by (Gelatin Test, Lead Acetate Test), Anthraquinone (Borntrager's Test) Phlobataninns, Volatile oil. The preliminary phytochemical analysis results are given in the table below [Table 1].

After completion of the preliminary phytochemical analysis. The Methanolic leaf extract of *Memocylon umbellatum* was further subjected to known its cyto-toxicity. The cyto-toxicity assay was done by conducting MTT assay

**Cytotoxic Activity:-****Cell Culture:-**

The cells of the MCF-7 (Epithelial cells of breast cancer) were purchased from NCCS (National Center For Cell Sciences) Pune. Cells were nourished with DMEM-HG media and kept for incubation at 37°C, 5% CO<sub>2</sub> until they reach confluence. Upon the cells reaching 80-90% confluence, cells were passaged. After passaging cells were used for experiment. MCF-7 cells were seeded in 96 well plate at  $2.2 \times 10^4$  cells/well. Cells were allowed to attached and grow for 24 hours.

Number of wells in 96 well plate was considered depending on experimental setup and concentrations of plant extract considered. Five different concentrations of plant extracts were used to test the cyto-compatiblity. Standard drug (Cisplatin) and untreated cultures were considered as control groups. Experiments were performed in duplicates.

The seeded cells were observed under microscope and confirmed the attachment of cells and formed a monolayer.

**MTT Assay:-**

After 48 hours of drug treatment cells were taken for performing MMT assay and cytotoxic effects of drug on MCF-7 cells was done. Briefly, cells were treated with pre-defined concentration of plant extract and controls for 24 hours. Later, 0.5mg/ml MTT was added to each well and incubated for 3 hours. Post MTT incubation, culture plate was observed under microscope to check the needle like formazan, crystals formation. Formazan crystals were dissolved by adding DMSO to cells and supernant media was read for optical density at 625nm using spectrophotometer.

**Results:-**

The methanolic leaf extract of *M.umbellatum* plant leaves is said to have medicinally important phytochemicals which were estimated by preliminary phytochemical analysis. It is evident from preliminary phytochemical analysis that the plant contains Flavanoids, Steroids, Glycosides, Tannins and traces of phenols. The phytochemicals were analyzed by following standard procedures.

Tests	Methanolic extraction
<b>Test for Flavanoids</b>	
Pews Test	+
Slenods Test	+
NaoH Test	-
<b>Glycosides Test</b>	
Keller kellani Test	++
Glycosides Test	+
Molish Test	++
<b>Phenols</b>	
Ellagic acid Test	-
Phenols Test	++
<b>Lignin Test</b>	
Lobats Test	+
<b>Steroids Test</b>	
Libemam-Burchard Test	++
Salkovaski Test	++
<b>Tannins Test</b>	
Gelatin Test	++
Lead Acetate Test	+
<b>Aturoquinne Test</b>	
Bamtragers Test	+
<b>Test for Pholobatanins</b>	
	+

**Table 1:-** Results of Phytochemical analysis

Further the test was carried out by performing MTT assay. The assay revealed the plant has an efficiency to terminate the breast cancer cell at 100µg/ml.

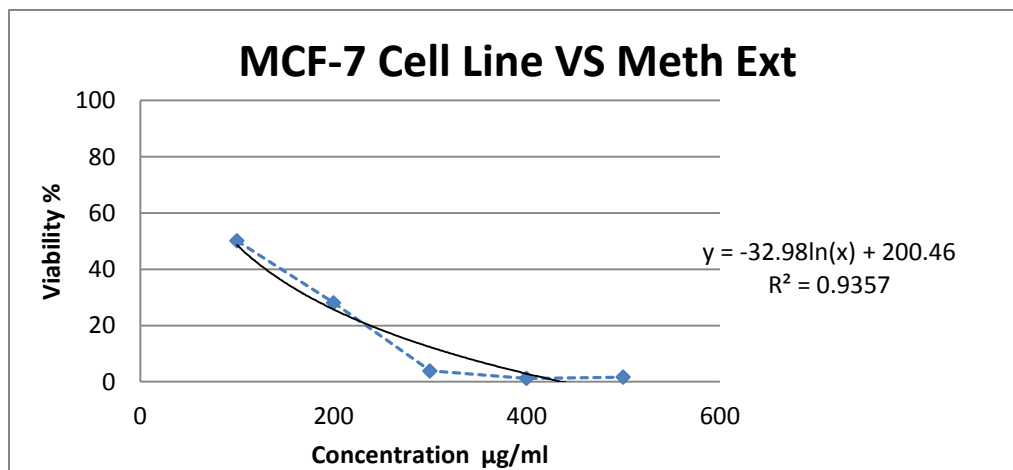


Figure 1:- Cytotoxic activity of *M.umbellatum* on MCF-7

### Discussion:-

The plant is known for its medical importance, which has the property to heal many severe diseases and which is achievable due to the bioactive compounds present in the plant's leaf extract. The methanolic leaf extract of the plant showed loaded amount of secondary metabolites during phytochemical analysis (Table No. 1). Some of the major secondary metabolites and their medicinal properties are phenols which is having biological activity such as anti-aging, anti-carcinogen, anti-inflammation (Orhan I, *et al.* 2007). According to some reports, Glycosides are said to have the property of lowering the blood pressure (Nyarko, *et al.* 1990). Steroids are known for their nutrition value and their use in cosmetics (Orhan I, *et al.* 2007). Flavonoids exhibit their events through property on membrane permeability, reticence of membranebound enzymes such as the ATPase and phospholipase A2 (Hausteen B, 1983). Tannins are used in treatment of cardiovascular protection and enhancement of endothelial function and reticence of angiogenesis and cell proliferation actions and anti-diarrhea (Killedar S and More H, 2011). Lignins are also present in the leaf extract. Cytotoxic activity was performed on the MCF-7 cell line, the results were obtained from the Spectrophotometer analysis at 625 nm and are as above. The standard drug (Cisplatin) shows the cell viability count of 4.189% were almost all the cancer cell were dead and the remaining were in the stage of apoptosis. The plant extract which was treated on MCF-7 cell line also showed cytotoxic activity. The cytotoxic activity was very effective in the first concentration of 100µg/ml and the major activity was seen in 300µg/ml. The cell viability was 50.14%, 28.10% (Figure 1). The present study reports that some of the major compounds present in the plant extract have shown cytotoxic effect on cancer cells, which could be developed further for a potent anti-cancer drug.

### Conclusion:-

The investigation of the phytochemical test done on the methanolic leaf extract of *Memocylon umbellatum* leaves reveals the presence of bio active novel compounds of pharmacological importance. Although, the systematic pharmacological activity of this plant is still unknown but is used extensively in native medication. The results obtained from the preliminary phytochemical analysis showed positive results for the test of Phenols, Lignins, Glycosides, Steroids, Tannins however, alkaloids were not recorded. Further, cytotoxic assay was conducted by performing MTT assay, methanolic leaf extract of *Memocylon umbellatum* was tested on the MCF-7 (Breast cancer cell line) cell line. The test exposed that the plant leaves extract showed cytotoxic activity on the cancer cell line MCF-7 inhibiting the cell at very low concentration at 100µg/ml the IC<sub>50</sub> was seen. It has inhibited cells greater than that of standard anti-cancer drug (Cisplatin). The cytotoxic effect shown by *Memocylon umbellatum* can be used in production of noble compounds by pharmaceutical industry to harness its useful biological activity. This concludes that the data obtained from the present study shows its usefulness in treating various diseases. The isolation and purification of the individual component may reveal its medicinal usage and their structure.

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