Anticancer investigations on *Carissa opaca* and *Toona ciliata* extracts against human breast carcinoma cell line

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Abstract: This study was aimed to determine the effectiveness of two ethnobotanically important plant species *Carissa opaca* and *Toona ciliata* against cancer cells. Antiproliferative activity of the plant extracts and their fractions was tested against MCF-7 breast cancer cell line using MTT assay. A concentration dependent inhibition was observed for both crude extracts. *C. opaca* crude extract showed 78.5% inhibition while *T. ciliata* showed 57% activity against cancer cells at 500 µg/ml. Fractions were tested at 200 µg/ml concentration and were more active than crude extracts. Chloroform fraction of *C. opaca* showed maximum inhibition 99% followed by ethyl acetate and methanol fraction of *C. opaca* exhibiting 96% and 94% inhibition, respectively. Ethyl acetate fraction of *T. ciliata* showed 78% inhibition of cancer cells at the same concentration. Preliminary phytochemical screening revealed the chemical composition of *C. opaca* extract containing alkaloids, flavonoids, tannins and saponins while *T. ciliata* had tannins and coumarins. Present investigation suggests that tested plant species possess potent anticancer compounds specially chloroform, ethyl acetate and methanol fractions of *C. opaca* and ethyl acetate fraction of *T. ciliata* can be an important source of anticancer drugs.

Keywords: *Carissa opaca*, MCF-7 breast cancer cell line, MTT assay, *Toona ciliate*

INTRODUCTION

Cancer is the major cause of mortality in the world and it claims more than 6 million lives each year (Chermahini *et al*. 2010). Methods commonly used for the treatment of cancer although possess some benefits but still there is a significant need to improve current cancer therapies and search for novel compounds. Plant-derived products can be valuable source for the discovery and development of unique anticancer drugs (Shoeb, 2006). During the last few decades medicinal plants has gained significant importance for the discovery and development of novel drugs, based on their traditional use in the different parts of the world (Sharma *et al*., 2009). Bioactivity is an important tool to determine pharmacological activity and a way to obtain new drugs bio-friendly in nature (Shrimali *et al*., 2001). *Carissa opaca* (Apocynaceae) is distributed in many mountainous parts of Indian subcontinent from Punjab to Himalayas in Pakistan and India, and Burma and Sri Lanka. The plant is used to cure fever and eye disorders (Ahmad *et al*., 2009) and its decoction is effective against jaundice and hepatitis (Abbasi *et al*., 2009). *Toona ciliata* (Meliaceae) is a timber tree mainly grown in the tropical areas of Asia. Bark of plant is used to treat dysentery, fever, and menstrual disorders in Chinese folk medicine. *Toona ciliata* crude extract has been reported to possess antifungal and analgesic activities (Malairajan *et al*., 2006). The deficiencies of the presently available anticancer drugs together with the scientific interest and economical consideration have drawn our attention to our local flora for treatment of cancerous diseases. Present study was undertaken to investigate anticancer potential of *C. opaca* and *T. ciliata* extracts and their fractions against MCF-7 breast cancer cell line.

MATERIALS AND METHODS

Collection of Plant Material

The fresh plant material (Leaves) of *C. opaca* and *T. ciliata* was collected from Peer Sohava Islamabad and Quaid-i-Azam University campus Pakistan, respectively. The taxonomic identification of the plants was done by Department of Plant Sciences, Quaid-i-Azam University Islamabad Pakistan.

Preparation of Extracts

The fresh plant material of each plant was rinsed with tap water and air dried under shade. Dried material was grinded to powder form. The powdered material 6/Kg of *C. opaca* and 4/Kg of *T. ciliata* was soaked in methanol for 14 days at room temperature. The mixture was then filtered using a clean muslin cloth followed by Whatman No.1 filter paper. The filtrates were then evaporated under vacuum by using a rotary evaporator. Extracts 210/g of *C. opaca* and 236/g of *T. ciliata* were stored at 4ºC till further use.

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Fractionation

Four organic and one aqueous fraction was prepared from crude extract by suspending 200/g crude extract in 100/ml of water and then partitioning with organic solvents in increasing order of polarity (Hexane<chloroform<ethyl acetate<methanol<aqueous) in separating funnel.

Cytotoxicity/anticancer assay

Breast cancer cell line (MCF-7) was used for the determination of cytotoxic activity. Cells were maintained in DMEM (Dulbeccos Modified Eagles Medium) supplemented with FBS (Foetal bovine serum) and Penicillin/Streptomycin-L-Glutamine and cultured in a humified atmosphere of 5% $C0_2$ and 95% air at 37ºC in Thermo Hera Cell 150 incubator. Cytotoxic profiles of the extracts were assessed using the MTT viability assay described by Bibi *et al*. (2012a).

Cell suspensions were seeded into 96-well plates at the density of 5,000 cells/well in 100 µl RPMI 1640 medium. After 24/h various concentrations of the crude extracts and the fractions were added to the cells and the plates were incubated for additional 24/h. MTT reagent (10µl) was added to each well and plates were further incubated for 4 h after which the media was removed. DMSO (100 µl) was added to each well to solubilize the formazan crystals. The plates were read for optical density at 570/nm, using a plate reader. Experiments for each extract were carried out in triplicate including untreated cell control and a blank cell-free control. Each concentration was tested in triplicate. The inhibitory rate of cell proliferation was calculated by the following formula.

$$\text{Percentage, inhibition} = \text{OD}\frac{\text{Contro} - \text{OD, treated}}{\text{OD, control}} \times 100$$

Phytochemical screening of plant extracts

Phytochemical screening was carried out of the crude extracts to determine phytochemical class in each extract e.g tannins, alkaloids, cardiac glycosides, flavonoids and saponins by using different biochemical tests as described by (Bibi *et al*., 2012b).

STATISTICAL ANALYSIS

Analysis of variance (ANOVA) and Least Significant Difference (LSD) test at $p<0.05$ was carried out using Mstatc to determine the significance of percentage inhibition values between the extracts against MCF-7 cell line.

RESULTS

For extraction from both plants cold maceration technique was used that resulted in 210 g extract of *C. opaca* and 236 g extract of *T. ciliate*. The fractionation disseminated the chemicals on polarity basis and fashioned varying heft of crude fraction (table 1).

Crude extract of *C. opaca* showed significant anticancerous activity against MCF-7 cell line while the *T. ciliata* crude extract showed moderate level of activity. However, a dose dependent response was observed. Crude extracts of *C. opaca* and *T. ciliata* were found effective against MCF-7 cell line. Anticancer activity against breast cancer cell line MCF-7 through MTT assay reveals that maximum inhibition of 78.5% and 57.25% was found at 500 µg/ml in the case of *C. opaca* and *T. ciliata* crude extracts, respectively (fig. 1). The concentration 200 µg/ml was found optimum; above to IC_{50} value that's why the fractions were established at this concentration. The results indicate that maximum activity (99%) was exposed by chloroform fraction of *C. opaca* (fig. 2). Ethyl acetate and methanol fractions of same plant also exhibited >90% activities. *T. ciliata* crude extract fractions also inhibited cancer cell growth at remarkable level. The variations of cell inhibition by different factions might be due to presence of more than one active principle of different polarities. Ethyl acetate and methanol fractions of *C. opaca* also proved highly potent and exhibited 96.2 and 94.04% activities, respectively. Aqueous fractions of *C. opaca* and *T. ciliata* exhibited 64 and 39% cell inhibition, respectively. Hexane fraction of *T. ciliata* also appeared to be significantly active with 71% activity.

Phytochemical analysis of *C. opaca* crude extract revealed the presence of alkaloids, flavonoids, tannins and saponins while *T. ciliata* leaves extract indicated the presence of tannins and coumarins (table 1). Methanol fraction of *C. opaca* indicated the presence of high concentration of alkaloids and this fraction also presented good anticancer activity

DISCUSSION

Cold maceration technique was used for extract preparation. This technique is widely used for extraction from plant materials that leads to maximum isolation of phytochemicals present in the plants. While partitioning of crude extract is valuable to detach chemicals on polarity basis. Despite the fact, bioactivities of fractions may also be accommodating to isolate the bioactive compound in lesser time period than conventional method (Bibi *et al*., 2010; Waheed *et al*., 2013). Anticancer activity of *C. opaca* was higher than *T. ciliata.* This can be due to difference in nature of plants. Both plants show liner inhibition patterns that progressed as concentration of crude extracts increased. Such pattern has been reported in many studies including anticancer activity against breast carcinoma cell line (Bibi *et al*., 2011; Abdolmohammadi *et al*., 2008; Phonnok *et al*., 2010). Aqueous extracts were also found to be active against cancer cell line presuming that these plants also contain some water soluble anticancer compounds as isolated from other medicinal plants (Izevbigie, 2003). Hexane

Table 1: Pyhtochemical analysis of crude extracts and fractions

Plants	Extracts/Fractions (g)	Phytochemical Tests				
		Alkaloids	Tannins	Saponins	Coumarins	Flavonoids
Carissa opaca	Crude (210)	+++	++	+++	-	++
	Hexane (18)	-	-	-	-	+
	Chloroform (24)	-	-	-	-	+++
	Ethyl acetate (31)	-	+	+	-	++
	Methanol (37)	+++	+	++	-	+
	Aqueous (47)	-	+++	-	-	-
Toona ciliate	Crude (236)	-	++	+++	-	-
	Hexane (19)	-	-	++	-	-
	Chloroform (28)	-	-	++	-	-
	Ethyl acetate (31)	-	-	+++	-	-
	Methanol (36)	-	-	-	-	-
	Aqueous (40)	-	++	-	-	-

Key: -= Absent, +=Low, ++= Moderate and +++= High

fraction of *T. ciliata* also exhibited good activity. A cytotoxic hydroxy steroidal ketone from the petroleum ether extract in a bioassay guided phytochemical investigation of *T. ciliata* has also been isolated. This steroidal compound was found cytotoxic in a brine shrimp lethality bioassay (Chowdhury, 2004). This activity can be linked with presence of such type of compounds. *C. opaca* crude extract revealed the presence of alkaloids, flavonoids, tannins and saponins during phytochemical screaning while *T. ciliata* leaves extract indicated tannins and coumarins Methanol fraction of *C. opaca* indicated the presence of high concentration of alkaloids and this fraction also presented good anticancer activity.

CONCLUSION

In conclusion *C. opaca* and *T. ciliata* can be a better candidate for isolation of cytotoxic and anticancer compounds specially chloroform, ethyl acetate and methanol fraction of *C. opaca* and ethyl acetate fraction of *T. ciliata*. On the basis of present investigation these plant species can be further investigated for pharmaceutical applications and achievement of novel anticancer compounds.

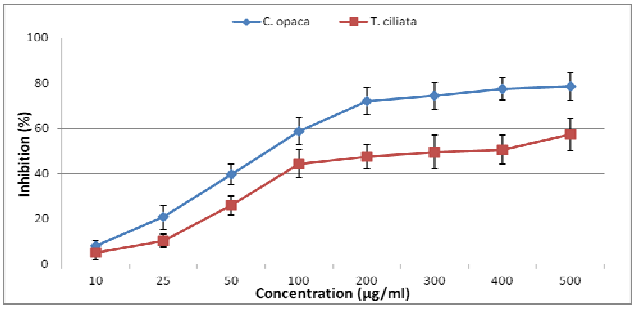

Fig. 1: Effects of crude extracts on cell inhibition against MCF-7 cell line

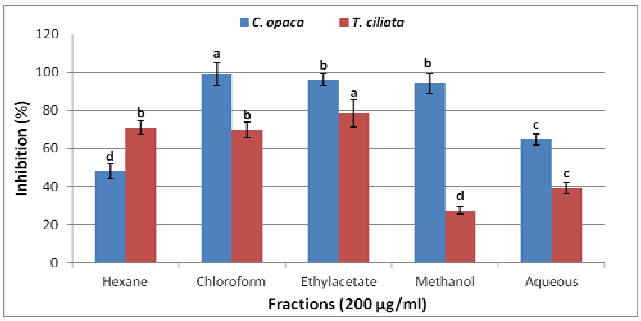

Fig. 2: Anticancer potential of *C. opaca* and *T. ciliata* fractions against MCF-7 cell line. Alphabets on bars represent LSD values at $p<0.05$.

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