Evaluation of cytotoxic activity of two species of *Cadaba* Forsk.

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Summary

Absolute alcohol and aqueous extracts of *Cadaba fruticosa* and *Cadaba trifoliata* were evaluated for cytotoxic activities using MTT-dye assay. Alcohol extracts of both species exhibited cytotoxicity against Vero (primary monkey kidney cell line), RD (Rhabdo myosarcoma) and Hep-2 (human epithelioma cell lines of the larynx) cell lines. Aqueous extracts showed weak activity when compared with quercetin.

Keywords: *Cadaba,* cell lines, cytotoxicity, Invitro anticancer activity, MTT-dye assay,

Introduction

Cadaba fruticosa (L.) Druce (Capparaceae) a wasteland shrub reported to contain various constituents such as cadabine, stachydrine and a dilactone cadabalone (1-3). It possess various medicinal uses such as purgative, anthelmintic, antiphlogistic, antispasmodic and antipyretic (4-8). *Cadaba trifoliata* (Roxb.) Wt. & Arn. (Capparaceae) possess medicinal properties like antirheumatic, antibacterial, anthelmintic, antiphlogistic, purgative, and useful for indigestion in children, amenorrhea, dysmenorrhea, antisyphilic and emmenagogue (4-11). No reports are available on the constituents of *C.trifoliata*. Many alkaloidal drugs obtained from plants possess anticancer and cytotoxic property against different cell lines. These two plants also contain alkaloid as chief constituents hence the present study is taken up.

Materials and Methods

Leaves of *C. fruticosa* and *C. trifoliata*. were collected from Tirunelvely, Tamil Nadu, South India, in September 2005. Specimens were identified with the help of available literature and confirmed by Dr.S.N.Yoganarasimhan, Research Coordinator, Department of Pharmacognosy, M.S.Ramaiah College of Pharmacy, Bangalore, India.

The voucher specimens (015 and 016) are deposited in the Department of Pharmacognosy, M.S.Ramaiah College of Pharmacy, Bangalore.

The leaves of both plants were shade dried separately at 25- 30° C for 5 days and powdered. Both powders (100 g) each were extracted with 200 ml of absolute alcohol and water in a Soxhlet apparatus. It was then evaporated to dryness under reduced pressure.

Cytotoxicity by MTT-dye assay using Vero (primary monkey kidney cell lines), RD (Rhabdo myosarcoma cell lines) and Hep 2 (Human epithelioma cell lines of the larynx) cell lines and IC_{50} (effective concentration of sample required to inhibit cell growth by 50 %) was determined by probit analysis. Quercetin was used as positive control drug (12). All the cell lines were cultured in EMEM medium (with glutamine) supplemented with 10% heat-inactivated newborn calf serum, 50 IU/ml penicillin G sodium, 50 µg/ml streptomycin sulphate and 0.125 µg/ml amphotericin B. Cells were maintained at 37ºC in 5% CO_2 atmosphere with 95% humidity. According to growth profiles, the optimal plating density was determined to be 2000 cells/ well to ensure the exponential growth throughout the experimental period and to ensure a linear relationship between absorbance and cell number when analyzed by SRB assay (13). The cytotoxic activity was assessed according to previously described protocol (14). In brief, the tumour cells were seeded in 96-well plates and incubated to allow for cell attachment (18-24 hr). Cell viability (% survival) after exposure to test samples (serial dilutions) was determined colorimetrically (SRB assay) at 492 nm (Power Wave X plate reader: Bio-TEK Instruments, Inc.). Cell survival of the treated wells was measured as the percentage of absorbance compared to the control wells (non-treated cells, taken as 100 % survival). The final mixture used for treating the cells contained not more than 0.5% of the vehicle, the same as in vehicle-control wells, which showed no effect on cell growth. The IC_{50} value was calculated from dose-response curves plotting between %inhibition and concentrations.

Results and Discussion

Yield of the extracts, IC_{50} values and phytochemical screening results are presented in Table 1. Quercetin produced cytotoxicity against Vero, RD and Hep 2 cell lines with the IC_{50} values of 48.23, 50.34 and 54.21 µg/ml respectively. Alcohol extract of *C. fruticosa* produced comparable IC_{50} values against Vero, RD and Hep 2 cell lines 48.31, 80.35 and 28.92 µg/ml respectively whereas aqueous extract produced an increase in IC_{50} values.

Alcohol extract of *C. trifoliata* showed IC_{50} values of 45.62, 96.38 and 75.86 µg/ml for Vero, RD, and Hep 2 cell lines and aqueous extract produced increased IC_{50} values when compared to standard quercetin. Fig. 1-3 represents the cytotoxicity of the extracts in three different cell lines.

From the graph it is evident that for **Vero** cell lines 100% inhibition was exhibited by 100 µg of alcoholic extracts of *C. fruticosa* and *C. trifoliata*. Aqueous extract of *C. trifoliata* produced dose dependant inhibitory activity and aqueous extract of *C. fruticosa* produced 100% inhibition with 100 µg concentration.

Alcohol extract of *C. fruticosa* and aqueous extract of *C. trifoliata* produced 100% inhibition with 75 µg of the extracts whereas aqueous *C. fruticosa* and alcoholic *C. trifoliata* produced 100% inhibition with 100 µg of the extracts against **RD** cell lines.

For **Hep 2** cell lines all the four extracts exhibited maximum inhibition with 100µg.

Table 1 Cytotoxicity of alcohol and aqueous extracts of two species of Cadaba

Plant	Extract	Yield (%w/w)	Phytochemical screening	Vero IC_{50}[a](µg/ml)	RD IC_{50}[b](µg/ml)	Hep 2 IC_{50}[c](µg/ml)
Quercetin	-	-	-	48.23	50.34	54.21
C.fruticosa	Aqueous	7.37	Glycosides, phenolic compounds, tannins	198.03	196.21	248.56
	Alcohol	15.51	Alkaloids, Glycosides, phenolic compounds, tannins,flavonoids, steroids, triterpenoids	48.31	80.35	28.92
C.trifoliata	Aqueous	12.04	Glycosides, phenolic compounds, tannins, steroids	202.32	203.41	193.22
	alcohol	9.21	Alkaloids, glycosides, phenolic compounds, tannins, flavonoids and steroids	45.62	96.38	75.86

Results are the mean values of three replications

[a] VERO- IC_{50}, 50% inhibitory concentration of primary monkey kidney cell lines.

[b] RD- IC_{50}, 50% inhibitory concentration of Rhabdo myosarcoma cell lines.

[c] Hep 2- IC_{50}, 50% inhibitory concentration of human epithelioma cell line of the larynx.

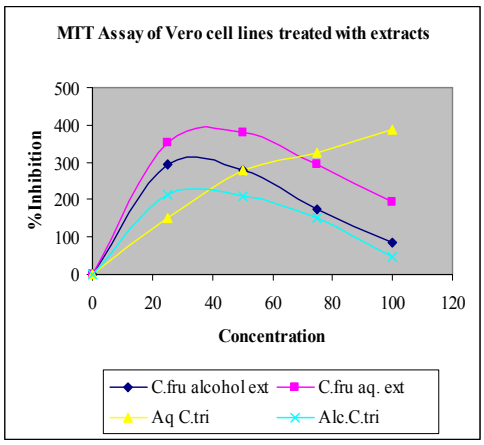

Fig.1. Cytotoxic activity of extracts against Vero cell lines

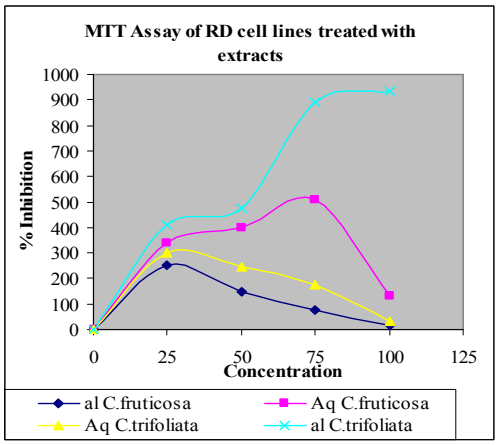

Fig.2. Cytotoxic activity of extracts against RD cell lines

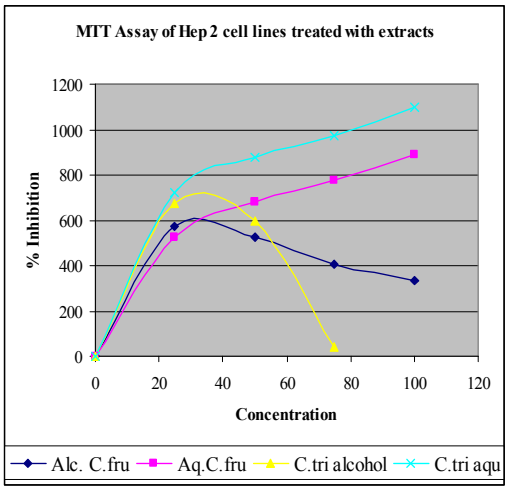

Fig.3. Cytotoxic activity of extracts against Hep 2 cell lines

Conclusion

Phytochemical screening of aqueous extracts of plants revealed presence of glycosides, phenolic compounds, tannins and steroids. Alcohol extract showed alkaloids, glycosides, steroids, tannins and triterpenoids as main principles. The results of the present study suggest that tested plant materials have moderate to potent cytotoxic activity and/or invitro anticancer scavenging activity against the tested cell lines. However, we do not know what components in the plant extracts show these activities. More detailed studies on chemical composition of the plant extracts, as well as other in vivo assays are essential to characterize them as biological cytotoxic agents which are beyond the scope of this study. It should also be kept in mind that anticancer activity measured by in vitro methods may not reflect in vivo effects of anticancer (15). Many other factors such as absorption/metabolism, nature of cell lines are also important. The findings of this study support this view that some medicinal plants are promising sources of potential anticancer agents.

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