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CYTOTOXIC STUDIES ON SELECTED MEDICINAL PLANTS

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ABSTRACT
Methanolic extract of flower bud of Syzygium aromaticum (L.) Merr. & L.M.Perry**,** leaves of Volkameria inermis L. (syn. Clerodendrum inerme L.) whole plant of Sphaeranthus amaranthoides Burm.f. and combination of these three drugs were studied cytotoxic activity against a human hepato cellular carcinoma cell line, Hep G2 in different concentrations (10, 20, 40 & 80µg/ml) along with standard drug Adriamycin (Doxorubicin)(ADA)(Positive control compound). The results showed that all the extracts were non-cytotoxic at the dose levels studied and all of them showed GI50 only at concentration > 80 µg/ml.

KEYWORDS: Cytotoxic study, Syzygium aromaticum L. Merr. & L.M.Perry, Volkameria inermis L, Sphaeranthus amaranthoides and Adriamycin (ADA).

INTRODUCTION

Plants have been used as a source of medicine since ancient time (Plotkin, 1991). The description of the use of a variety of plant-derived medications was written in different ancient literature of India (Veda, Purana and Upanishad) and China (Ahmad et al, 2006).Cloves are the aromatic flower buds of Syzygium aromaticum **(**L.) Merr. & L.M.Perry**,** family Myrtaceae. It is an evergreen tree, grows up to 8–12 m tall, with large leaves and sanguine flowers grouped in terminal clusters. The flower buds initially have a pale hue, gradually turn green and then transition to a bright red when ready for harvest. Cloves are harvested at 1.5–2.0 cm long, and consist of a long calyx that terminates in four spreading sepals, and four unopened petals that form a small central ball. A major component of clove taste is imparted by the chemical eugenol (Kamatou et al, 2012) and the quantity of the spice required is typically small.

Volkameria inermis L**.** a straggling shrub occurring abundantly near the coastal region of India and Ceylon. Fresh and dry leaves possess alternative and febrifugal properties. The leaves contain an amorphous bitter principle resembling that found in chiretta, a resin, a gum and a brown coloring matter. Steam distillation yields a stearopten – like body having the fruity odour of the fresh plant. The ether extract is fragrant. The ash of the leaves is rich in sodium chloride (Anonymous, 1950).

Sphaeranthus amaranthoides is a small procumbent herb found in semi-aquatic environment. The flowers of the plant have depurative, cooling and tonic effect. Seeds and root are used as stomachic and anti-helmintic (Murugesa mudaliar, 1951). Dried and powdered leaves are useful in the treatment of chronic skin diseases, urethral discharges, and jaundice (A.K. Nadkarni, 1976). The leaves have been reported for their antioxidant, antimutagenic and antimicrobial activities (Prabakaran et al, 2011). The healing potential of ethanolic extract of aerial part has been reported (Swarna Latha et al, 2009) for treatment of dermal wounds in Wistar rats studied on excision wound models.

The traditional practitioners use the combination of above mentioned drugs for treatment of Cancer. Hence, we have taken this study to evaluate the Cytotoxic activity of combined and individual drugs.

MATERIAL AND METHODS

SAMPLE COLLECTION

The flower bud of Syzygium aromaticum (L.) Merr. & L.M..Perry was purchased from raw drug dealers, Chennai. Leaves of Volkameria inermis L., and whole plant of Sphaeranthus amaranthoides Burm f. were collected from Mettur, Salem Dt., Tamilnadu and authenticated by Dr. Sorna Subramaniyan, Research Officer (Botany), SMPG, Mettur Dam.

EXTRACTION

Methanolic Extract

All the three drugs were shade dried and coarsely powdered separately (1 kg) and extracted with methanol in an aspirator bottle by cold percolation method at room

temperature (48 hr). Each extraction was carried out twice. All extracts were filtered through Whatman No. 1 filter paper. Nearly 80% of the solvent was removed by distillation on a water bath at atmospheric pressure and the last traces were removed under reduced pressure.

The studied cells are

(a) **Hep G2** is a human liver carcinoma Cell Line
b) PLC/PRF/5 (Alexander) Hepatoma Cell Line
Both Cells lines were obtained from Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Mumbai.

EXPERIMENTAL PROCEDURE FOR SRB ASSAY

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 microtiter 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 microtiter plates were incubated at 37° C, 5 % CO2, 95 % air and 100 % relative humidity for 24 h prior to addition of experimental drugs.

Experimental drugs were initially solubilized in dimethyl sulfoxide at 100mg/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 (1mg/ml) was thawed and diluted to 100 µg/ml, 200 µg/ml, 400 µg/ml and 800 µg/ml with complete medium containing test article. Aliquots of 10 µl of these different drug dilutions were added to the appropriate microtiter wells already containing 90 µl of medium, resulting in the required final drug concentrations i.e.10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml.

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 incubated for 60 minutes at 4°C. 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 an plate reader at a wavelength of 540 nm with 690 nm reference wavelength.

Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells. Percent Growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells * 100.

Using the six absorbance measurements [time zero (Tz), control growth (C), 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: [Ti/C] x 100 % (Skehn, 1990 & Vanicha et al., 2006).

DEFINITIONS AND NOTES	
GI50	Growth inhibition of 50 % (GI50) calculated from [(Ti-Tz)/(C-Tz)] x 100 = 50, drug concentration resulting in a 50% reduction in the net protein increase
TGI	Drug concentration resulting in total growth inhibition (TGI) will calculated from Ti = Tz
LC50	Concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of 50% cells following treatment is calculated from [(Ti-Tz)/Tz] x 100 = -50.
ADR	Adriamycin (Doxorubicin). Known drug.
GI50 value of ≤ 10^-6 (i.e. 1 µmole) or ≤ 10µg/ml is considered to demonstrate activity in case of pure compounds. For extracts, GI50 value ≤ 20µg/ml is considered to demonstrate activity.	
Yellow highlighted test values under GI50 column indicate activity.	

RESULTS AND DISCUSSION

The Cytotoxic activitity on flower bud of Syzygium aromaticum(L.)Merr.&L.M. Perry, leaves of Volkameria inermis L. whole plant of Sphaeranthus amaranthoides Burm.f. and combination of these three drugs were studied against human liver carcinoma Cell Line Hep G2 and Hepatoma Cell Line PLC/PRF/5 (Alexander).The in-vitro studies were carried out at the dose level 10,20,40 and 80 µg/ml (Table 1and 3).The results are given in Table 2 and 4. The plant extracts are non-cytotoxic when compared with positive control Adriamycin (ADR). But the activity was observed at the dose level > 80 µg/ml.

CONCLUSION

The Cytotoxic activitity on Syzygium aromaticum(L.) Merr. & L.M. Perry, Volkameria inermis L**.** Sphaeranthus amaranthoides Burm.f. and combination of these three drugs were studied against human liver carcinoma Cell Line Hep G2 and Hepatoma Cell Line PLC/PRF/5 (Alexander) showed noncytotoxic at the dose levels studied . All of them showed activity of GI50 only at concentrations > 80 µg/ml.

Table: 1

	Human Hepatoma Cell Line HEPG2															
	% Control Growth															
	Drug Concentrations (µg/ml)															
	Experiment 1				Experiment 2				Experiment 3				Average Values			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
SA	100.0	100.0	100.0	89.2	100.0	100.0	90.1	77.0	100.0	94.5	86.8	82.3	100.0	98.2	92.3	82.8
SK	100.0	98.5	95.5	92.4	100.0	100.0	84.4	80.9	100.0	97.0	95.5	91.8	100.0	98.5	91.8	88.4
VI	100.0	100.0	100.0	99.9	100.0	100.0	98.8	95.3	100.0	100.0	100.0	100.0	100.0	100.0	99.6	98.4
CSS	100.0	100.0	100.0	100.0	100.0	100.0	98.3	94.8	100.0	100.0	100.0	100.0	100.0	100.0	99.4	98.3
ADR	19.0	-4.1	-10.6	-15.6	-22.8	-42.6	-46.1	-50.7	-19.8	-24.4	-34.5	-41.3	-7.9	-23.7	-30.4	-35.9

SA - Methanolic extract of flower bud of Syzygium aromaticum(L.) Merr. & L.M.. Perry
SK - Methanolic extract of whole plant of Sphaeranthus amaranthoides Linn.
VI - Methanolic extract of leaves of Volkameria inermis L.
CSS - Combination of selected three drugs
ADR - Adriamycin (Doxorubicin), Positive control compound.

Table: 2

Drug concentrations (µg/ml) calculated from graph			
HEPG2	**LC50**	**TGI**	**GI50**
SA	>80	>80	>80
SK	>80	>80	>80
CI	>80	>80	>80
CSS	>80	>80	>80
ADR	73.9	31.4	<10

Table: 3

	Human Hepatoma Cell Line PLC-PRF-5															
	% Control Growth															
	Drug Concentrations (µg/ml)															
	Experiment 1				Experiment 2				Experiment 3				Average Values			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
SA	87.6	87.5	78.1	62.7	89.2	81.1	78.1	63.6	80.0	79.4	76.8	65.2	85.6	82.6	77.7	63.8
SK	88.3	77.3	62.9	48.1	85.1	70.7	66.4	53.7	75.9	65.6	59.2	54.1	83.1	71.2	62.8	52.0
CI	97.0	96.0	94.3	86.0	96.6	91.0	87.6	85.8	86.2	83.2	81.7	81.5	93.3	90.1	87.9	84.4
CSS	98.3	93.1	89.5	73.9	95.4	89.8	85.8	76.7	86.7	80.7	78.2	68.6	93.5	87.8	84.5	73.1
ADR	- 63.4	- 72.1	- 77.9	- 80.9	- 62.9	- 70.2	- 76.8	- 78.8	- 64.8	- 70.8	- 75.2	- 78.0	- 63.7	- 71.0	- 76.7	- 79.2

Table: 4

Drug concentrations (µg/ml) calculated from graph			
PLC-PRF-5	**LC50**	**TGI**	**GI50**
SA	>80	>80	>80
SK	>80	>80	75.4
CI	>80	>80	>80
CSS	>80	>80	>80
ADR	39.3	<10	<10

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