Research Article

Cytotoxicity Screening of the Commonly Used Indigenous Medicinal Plants Using Brine Shrimp Lethality Bio-Assay

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ABSTRACT

Novel cytotoxic, antitumor, and pesticidal compounds can be isolated from potential plant sources through the assessment of cytotoxic activity against brine shrimps. In the present work commonly used Indian medicinal plants were tested for brine shrimp lethality in order to detect potential sources of novel cytotoxic, antitumor and pesticidal compounds and also to correlate cytotoxicity results with known pharmacological activities of the plants. The alcoholic and aqueous extracts of the selected plants were tested for their cytotoxicity. Ten nauplii were added into three replicates of each concentration of the plant extract. After 24 hours the surviving brine shrimp larvae were counted and percentage viability was reported. Amongst the tested extracts, alcoholic extracts of *Piper longum, Curcuma longa, Ocimum sanctum*, *Zingiber officinalis, Eclipta alba* and *Azadiracta indica* showed significant cytotoxic activity, amongst which the most potent was found to be *Piper longum with* LD_{50} value of 33.33µg/ml. Thus, the results support the uses of these plant species in traditional medicine and can be further evaluated for their cytotoxic effects on the cell lines.

Keywords: Cytotoxicity, Brine shrimp larvae, Medicinal plants, LD_{50} value.

INTRODUCTION

The medicines derived from plants have made a large contribution to the field of pharmaceutical drug discovery. Almost one-half of all the licensed drugs that were registered worldwide in the past 25 years, prior to 2007 were natural products or their synthetic derivatives. The major contribution of the isolated pure compounds is in the field of anti-infective and anti-cancer therapies.[1] From the ancient times till the present day, nearly all cultures have been partially or fully dependent on natural products.[2] Likewise the ethno-medicine is also widespread in India but majority of the plants have not been investigated for their toxicity, inspite of their prolonged history in the treatment of various ailments.

The present work was aimed to screen the commonly used medicinal plants with different known therapeutic activities for their cytotoxic effect on *Artemia salina* and to correlate the toxicity results with their known ethnopharmacological activities.

Brine shrimp lethality assay have been used as a bench-top bioassay for the discovery and purification of bioactive natural products. It was primarily proposed by Michael and his team in the year 1956 and was later developed by Vanhaecke and his team in 1981.[3-4] Brine shrimp, *Artemia* species are also known as sea monkeys. They are marine invertebrates about 1 mm in size. The cysts last for several years and can be hatched without special equipment. Brine shrimp lethality assay is based on the ability to kill the laboratory-cultured *Artemia nauplii* brine shrimp.

This assay serves as a useful tool for preliminary assessment of toxicity, for the detection of fungal toxins, plant extract toxicity, and cyanobacteria toxins.[5-8] Brine shrimp tests are usually performed to draw inferences on the safety of the plant extracts and also depict trends of their biological activities upto an extent.

Twenty Indian medicinal plants were selected randomly as shown in Table 1, mainly based on their availability and apart from their traditional uses.

MATERIALS AND METHODS

Collection and authentication of plant material

The plant materials were collected from the fields of Gandhinagar District, Gujarat and from the local supplier of herbal drugs, Lallu Vrajlal Gandhi, Ahmedabad. The plant material was authenticated by various morphological characters and voucher specimens were deposited at Department of Pharmacognosy, KBIPER, Gandhinagar.

Preparation of extract of plants

Each plant material was dried under shade and powdered. 10 g of the powdered crude drug was extracted with methanol using Soxhlet apparatus and with water by heating for 1 hour and occasional shaking respectively. The filtrate was concentrated to dryness and stored in an air tight container for further use.

Table 1: Information about the selected Indian Medicinal Plants

Name of the plant	Family	Common name	Parts Used	Uses
Adhatoda vasica	*Acanthaceae*	Ardusa	Leaves	Respiratory conditions[9]
Andrographis paniculata	*Acanthaceae*	Kalmegh	Aerial parts	Chronic fever, boils, scabies, skin erruptions[10]
Azadiracta indica	*Meliaceae*	Neem	Leaves	Bitter tonic, insecticide[11]
Boerhavia diffusa	*Nyctaginaceae*	Punarnava	Whole plant	Arthritis, renal oedema, dysuria[12]
Cassia angustifolia	*Leguminosae*	Senna	Pods	Laxative, purgative[13]
Centella asiatica	*Apiaceae*	Brahmi	Herb	Memory booster, Adaptogenic[14]
Curculigo orchioides	*Hypoxidaceae*	Kali Musli	Rhizomes	Diuretic, cancer, aphrodisiac[15]
Curcuma longa	*Zingiberaceae*	Turmeric	Rhizomes	Anti-inflammatory, antioxidant, anti-cancer[16]
Eclipta alba	*Asteraceae*	Bhangro	Leaves	Anti-hepatotoxic, hair growth promoter[17]
Emblica officinalis	*Euphorbiaceae*	Amla	Fruits	Antioxidant, anticarcinogenic, anti-inflammatory[18]
Glycyrrhiza glabra	*Leguminosae*	Jethimadh	Roots, stolons	Expectorant, treatment of peptic ulcer[19]
Nardostachys jatamansi	*Valerianaceae*	Jatamansi	Roots	Neuroprotective, anticonvulsant,tranqilizing[20]
Ocimum sanctum	*Labiateae*	Tulsi	Aerial parts	Bronchitis, antimicrobial, anticancer[21]
Phyllanthus niruri	*Phyllanthaceae*	Bhoyamli	Aerial parts	Hepatoprotective, hypolipidemic, anti obesity[22]
Piper longum	*Piperaceae*	Lindi piper	Fruits	Bio-availability enhancer, immunomodualtory[23]
Saraca indica	*Leguminosae*	Ashoka	Stem barks	Uterine tonic, oxytocic[24]
Teriminalia chebula	*Combretaceae*	Harde	Fruits	Fever, cough, astringent[25]
Terminalia arjuna	*Combretaceae*	Arjun	Stem barks	Cardiotonic, hypotensive[26]
Withania somnifera	*Solanaceae*	Ashvagandha	Leaves	Anti-inflammatory, anti-tumour, anti-stress,anti-oxidant[27]
Zingiber officinalis	*Zingiberaceae*	Sunth	Rhizomes	Anti-emetic, anti-inflammatory, anti-viral[28]

Table 2: Brine shrimp toxicity (% Lethality) of the extracts of selected medicinal plants

Name of the plant	Alcoholic Extract Conc. (µg/ml)			Aqueous Extract Conc. (µg/ml)		
	100	500	1000	100	500	1000
Adhatoda vasica	3.3	13.3	16.7	0.0	6.7	6.7
Andrographis paniculata	6.7	26.7	60.0	6.7	26.7	43.3
Azadiracta indica	56.7	76.7	100	10.0	33.3	40.0
Boerhavia diffusa	0.0	3.3	13.3	0.0	0.0	10.0
Cassia angustifolia	6.7	10.0	20.0	0.0	6.7	10.0
Centella asiatica	6.7	33.3	63.3	13.3	30.0	43.3
Curculigo orchioides	3.3	13.3	26.7	0.0	0.0	6.7
Curcuma longa	26.7	66.7	100.0	0.0	0.0	10.0
Eclipta alba	13.3	43.3	73.3	0.0	3.3	13.3
Emblica officinalis	13.3	26.7	33.3	0.0	10.0	20.0
Glycyrrhiza glabra	6.7	16.7	23.3	0.0	0.0	3.3
Nardostachys jatamansi	6.0	13.3	13.3	0.0	0.0	0.0
Ocimum sanctum	63.3	100.0	100.0	16.7	53.3	66.7
Phyllanthus niruri	10.0	23.3	40.0	0.0	6.7	3.3
Piper longum	90.0	100.0	100.0	10.0	30.0	40.0
Saraca indica	0.0	6.7	10.0	0.0	0.0	3.3
Teriminalia chebula	0.0	3.3	10.0	3.3	13.3	26.7
Terminalia arjuna	6.7	13.3	20.0	0.0	6.7	10.0
Withania somnifera	10.0	20.0	36.7	3.3	16.7	13.3
Zingiber officinalis	40.0	100.0	100.0	13.3	60.0	90.0

Brine shrimp lethality bioassay

Brine shrimp lethality bioassay was carried out to investigate the cytotoxicity of the plant extracts.[29-30] Brine shrimps (*Artemia salina*) were hatched using brine shrimp eggs in a conical shaped vessel (1 L), filled with sterile artificial seawater (prepared using sea salt 38 gm/L and adjusted to pH 8.5 using 1 N NaOH) under constant aeration for 48 h. After hatching, active nauplii free from egg shells were collected from brighter portion of the hatching chamber and used for the assay. Ten nauplii were drawn through a glass capillary and placed in each well containing 2.0 ml of brine solution. In each experiment, 0.5ml of the plant extract was added to 2.0 ml of brine solution and maintained at room temperature for 24 h under the light and surviving larvae were counted. Experiments were conducted along with control (vehicle treated), different concentrations of plant extracts (100, 500 and 1000 µg/ml) of the test substances in a set of three well per dose (n=3). Each concentration was added in triplicates (n=3). LD_{50} values were calculated using Graph pad Prism.

RESULTS AND DISCUSSION

All the alcoholic and aqueous extracts were screened for their cytotoxic activity using brine-shrimp bench-top bioassay. The assay was based on the ability of extracts to kill the brine shrimp larvae. The results of brine shrimp are as shown in Table 2 where percentage mortality of the different plant extracts at various concentrations i.e. 100 µg/ml, 500 µg/ml and 1000 µg/ml was calculated. Amongst the selected plants, both alcoholic and aqueous extracts of *Adhatoda vasica, Boerhavia diffusa, Cassia angustifolia, Curculigo orchioides, Emblica officinalis, Glycyrrhiza glabra, Saraca indica, Terminalia chebula, Terminalia arjuna,* the aqueous extract of *Curcuma longa, Eclipta alba, Nardostachys jatamansi, Phyllanthus niruri* showed no cytotoxic effect against brine shrimp larvae. Whereas the alcoholic extracts of *Piper longum, Curcuma longa, Ocimum sanctum*, *Eclipta alba*, *Zingiber officinalis and Azadiracta indica* showed significant cytotoxic activity and were further calculated for their LD_{50} values as shown in Table 3.

Table 3: LD_{50} values of the plant extracts showing more than 50% mortality.

Name of the plant	Extract	LD_{50} (µg/ml)
Azadiracta indica	Alcoholic	78.7
Centella asiatica	Alcoholic	278.6
Curcuma longa	Alcoholic	143.6
Eclipta alba	Alcoholic	222.0
Ocimum sanctum	Alcoholic	94.3
	Aqueous	469.0
Piper longum	Alcoholic	33.3
Zingiber officinalis	Alcoholic	102.7
	Aqueous	357.0

The most potent extract amongst all was found to be *Piper longum alcoholic extract,* showing 90% lethality at the lower dose of 100µg/ml. At the dose of 500 µg/ml *Azadirachta indica, Curcuma longa, Ocimum sanctum* and *Zingiber officinalis* also showed 100% lethality, and hence supporting their use as anti-microbial and anti-cancer agents. These plants have high potential of having a lead therapeutic agent which can be further tested by using different cell based and microorganism based assays.

CONCLUSION

Artemia nauplii have been suggested for use as a model for several preliminary evaluations of pharmacological and ecotoxicological activities of compounds of greater complexity. The Brine shrimp lethality assay has been used for insecticidal, acaricidal, anaesthetic, and anti-tumour activity evaluations, using different methodologies. Amongst tested extracts, alcoholic extracts of *Piper longum, Curcuma longa, Ocimum sanctum*, *Eclipta alba*, *Zingiber officinalis and Azadiracta indica* showed significant cytotoxic activity. The above data suggests that alchoholic *Piper longum has* the most potent cytotoxic effect with LD_{50} value of 33.33µg/ml. The above data supports the uses of these plant species in traditional medicine and can be further evaluated for their cytotoxic effects on the cell lines using different methodologies, for the isolation of the novel compounds.

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