EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 3
Issue: 1
202-207
Year: 2016

CYTOTOXICITY EVALUATION OF *PROSOPIS CINERARIA* (L) DRUCE AGAINST BRINE SHRIMP

P.V.V. Satish[1], K. Somaiah[2], P. Brahmam[3], N. Sree Rekha[4] and K. Sunita[5*]

[1, 3,4,5]Department of Zoology and Aquaculture, Acharya Nagarjuna University, Nagarjunanagar 522510, Guntur, Andhra Pradesh, India.
[2]Department of Environmental Sciences, Acharya Nagarjuna University, Nagarjunanagar 522510, Guntur, Andhra Pradesh, India.

***Author for Correspondence: K. Sunita**
Department of Zoology and Aquaculture, Acharya Nagarjuna University, Nagarjunanagar 522510, Guntur, Andhra Pradesh, India.

Article Received on 23/10/2015 **Article Revised on 13/11/2015** **Article Accepted on 3/12/2015**

ABSTRACT
The present study aimed to evaluate the cytotoxicity of medicinal plant *Prosopis cineraria* against Brine shrimp. The plant *Prosopis cineraria* were collected from Acharya Nagarjuna University Campus, Nagarjunanagar, Guntur district, Andhra Pradesh, India. Crude extracts from dried leaves, stem, flowers and roots of *P. cineraria* were prepared through soxhlet extraction using methanol, chloroform, hexane, ethyl acetate and aqueous sequentially. These extracts were tested *in vivo* against laboratory cultured brine shrimp larva. The phytochemical screenings were also conducted with standard methods. The methanol, chloroform, hexane, ethyl acetate and aqueous extracts of leaves, stem, flowers and roots shown LC_{50} values ranging from, 315.00µg/mL to 1425.00µg/mL. Out of the 20 tested extracts, four exhibited moderate toxicity (LC_{50} 100-500µg/mL), 7 extracts displayed weak (LC_{50} 500-1000µg/mL) toxicity, 9 extracts were nontoxic (LC_{50} >1000µg/mL). The phytochemical screening has revealed the presence of alkaloids, triterpenes, flavonoids, tannins, coumarins, carbohydrates, phenols, saponins, phlobatannins and steroids.

KEYWORDS: *Prosopis cineraria*, Phyotochemicals, LC_{50}, cytotoxic activity, Brine Shrimp.

INTRODUCTION
Plants have been an exemplary resource of medicine. Ayurveda and other traditional medicinal systems reveal the use of plants in treatment of various human ailments. India has about 45,000 plant species and among them, a number of plants have been claimed to possess medicinal properties. Extensive research has been carried out in last few decades on plants mentioned in ancient literature or used traditionally for centuries.[1] Some of the traditional medicine involves the use of crude plant extracts which may contain an extensive diversity of molecules, often with indefinite biological effects. However, most of the available information regarding the medicinal potency of these plants is not provided with credible scientific data. For this reason, several researches have been conducted to determine the toxicity of medicinal plants.[2]

Plants have over the years, proved to be a good source of chemotherapeutic agents. Today, many of the drugs have been derived from plant resources such as Quinine, Chloroquine and Artemisinin. Historically, medicinal plants have provided a source of inspiration for novel therapeutic drugs, as plant-derived medicines have made large contributions. According to the World Health Organization (WHO), now a days, 80% of the world's population rely on plants for their primary health care.[3] Plants are producing secondary metabolites for their defense, which play an important role of physiological activities in human body.[4] The medicinal value of plants is due to the substances that it contains, which produce a physiological action on the human body. Some examples of these plants are alkaloids, essential oils, tannins, resins and many others.[2] India had remarkable biodiversity and rich cultural traditions of plant use. Interestingly, today many of the pharmaceutical companies are utilizing such plant-based formulations in treatment of various diseases and disorders worldwide.[5]

P. cineraria (L.) Druce is a species of flowering tree in the pea family belongs to the family Mimosoideae and is a large shrub that grows up to 10m height with prickly branches, prickles curved and compressed. It is indigenous to dry portions of Western Asia and the Indian sub-continent, including Afghanistan, Iran, India, Oman, Pakistan, Saudi Arabia, the United Arab Emirates and Yemen. It is an exotically introduced species in parts of Southeast Asia, including Indonesia. It is distributed all over the India particularly in the parts of Rajasthan, Gujarat, Haryana, Uttar Pradesh, Tamilnadu and Andhra pradesh.[1]

It is adopted as a state tree of Rajasthan (India). A large and well known example of the species is the Tree of Life in Bahrain – approximately 400 years old and growing in a desert devoid of any obvious sources of water. This is also the national tree of the United Arab Emirates. *Prosopis* was introduced in 1880 and has become a serious problem as an invasive species.[6,7]

P. cineraria is a small tree generally grows 3 – 5 m height. Evergreen or nearly so, it forms an open crown and has thick, rough gray bark with deep cracks. Its branches are slight glabrous and armed with fairly compressed, straight and scattered spines of 3-4 cm length. This plant has alternately arranged bipinnate compound leaves. The leaflets of leaves are in 15–18 pairs and shaped oblong with an entire margin, apiculate apex, obtuse base, glabrous surface, reticulate venation, petiolate and the petiole is 0.5–4 cm long. The average size of leaf is 2.5 cm (length) and 1 cm (breadth). Leaves are green in colour and are odorless with a bitter taste. Flower is in the form of axillary spikes with the length of 7-11 cm, either solitary or in terminal panicles. Flowers are small and creamy yellow corolla, attracting large number of insects including large number of *Apis florea* and numerous other wild bees in the month of December and April and followed by seeds in pods.[8,9]

The species is established throughout India, extending to Persia. The entire plant is used in native systems of medicine (ISM) and is called 'Kalpa Plant' in Ayurveda and Siddha literature. A literature survey exposed that the plant has been used in the treatment of leprosy, dysentery, bronchitis, asthma, leucoderma, muscular tremors and rheumatism. It is also known to have anthelmintic, antibacterial, antifungal, antiviral and anticancer activities.[10]

2. MATERIALS AND METHODS

Collection of plant materials

Fresh samples of leaves, stem, roots and flowers from P. cineraria (L) Druce were collected from ANU campus, Nagarjunanagar of Guntur district, Andhra Pradesh, India. The plant *P. cineraria* were deposited in the Department of Botany, Acharya Nagarjuna University and voucher specimen was deposited in the department. All the collected plant parts were washed thrice with tap water and twice with distilled water to remove the adhering salts and other associated animals. The authentication of the plant species were done by Prof. K. Khasim, Department of Botany, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India.

Extract preparation

Shade-dried plant samples were subjected for in 90% different organic solvents methanol, chloroform, hexane, ethyl acetate and water at 50-60^{0}C in a Soxhlet apparatus. After complete extraction, the filtrates were concentrated one by one by rotary vacuum evaporation and then freeze dried (−80°C) to obtain solid residue. The extraction percentage was deliberated by using the following formula: percentage of extraction = weight of the extract (g)/weight of the plant sample (g) ×100. The extracts of plant were screened for the presence of phytochemical constituents by following the methods of Kepam[11] and Sofowora.[12] The plant extracts were dissolved in dimethyl sulphoxide (50 mg/mL- for all experiments) and double distilled water (50mg/ml- for all experiments) filtered through Millipore sterilized filters (mesh 0.20µm).The filtrate was used for testing at different concentrations of 100 to 1600µg/mL.

Brine shrimp lethality bioassay

In this study, the eggs of *Artemia salina* were hatched for 24 h at room temperature (25-30^{o}C) in artificial sea water (20 g NaCl and 18 g Table salt in 1 L of distilled water) to obtain nauplii (shrimp larvae). Test samples (methanol, chloroform, hexane, ethyl acetate and aqueous extract of leaves, stem, flowers and roots of *P. cineraria*) dissolved in DMSO were added in test tubes, each contains 4 ml of sea water with sample concentrations of 100, 200, 400, 600, 800, 1000, 1200, 1400, 1600 and 1800µg/mL where concentration of solvent should be not more than 5%, which was shown not to have any destructive effects on the larvae. The same procedure was followed for the standard drug chloramphenicol. The final volume for each test tube was made up to 10 mL with artificial sea water with 10 living nauplii in each test tube. The same process was followed in control test tubes containing 10 living nauplii in 10 ml of artificial sea water. After 24 h of period, the test tubes were observed and the number of survived nauplii in each test tube was counted and the resulted data was noted. The percentage of dead nauplii in the test and standard group was established by comparing with that of control group. The percentage of mortality plotted against log- concentrations and the lethal concentrations (LC_{50}) was deliberate by analysis of dose response logistic curves. The general toxicity activity was considered weak when the LC_{50} ranged from 500 and 1000 µg/mL, moderate when the LC_{50} ranged from 100 and 500µg/mL and strong when the LC_{50} ranged from ≤ 100µg/mL.[13]

Statistical analysis

The mean and standard deviations of the treated and control group were calculated at 95% confidence interval levels. The results were analyzed statistically using two – tailed Student's t-test (Minitab 11. 12. 32. Bit software) to identify the differences between treated group and controls. The data was considered significant at $P < 0.05$ and $P < 0.001$.

RESULTS

The phytochemical studies revealed that the methanol, chloroform, hexane, ethyl acetate and aqueous extracts of leaf, stem, flower and root of *P. cineraria* have variety of phytochemical constituents namely alkaloids, triterpenes, flavonoids, tannins, coumarins, carbohydrates, phenols, saponins, phlobatamins and steroids represented in Table 1.

Table 1: Preliminary screening for phytochemical constituents of *Prosopis cinearia* in different extracts from leaves, stem, flowers and roots.

Tested compounds2	**Leaves**					**Stem**					**Roots**					**Flowers**				
	Me	Ch	He	EA	Aq	Me	Ch	He	EA	Aq	Me	Ch	He	EA	Aq	Me	Ch	He	EA	Aq
Alkaloids	+	+	+	+	-	+	+	-	+	-	+	+	+	-	-	+	-	-	+	-
Coumarins	-	-	-	-	+	-	-	-	+	-	-	-	-	+	+	+	-	-	-	-
Carbohydrates	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
Phenols	+	-	+	-	-	+	-	-	-	-	-	-	+	+	-	+	-	+	-	-
Saponins	-	-	-	+	+	+	-	-	+	-	+	-	-	+	-	-	-	-	-	+
Tannins	+	-	+	+	-	+	-	+	+	-	+	-	+	-	-	-	-	-	-	-
Flavanoids	-	-	-	-	+	-	-	-	-	+	-	-	-	-	+	-	-	-	-	+
Terpenoids	-	-	-	+	-	+	+	-	-	-	+	-	-	+	-	-	+	-	-	-
phlobatannins	+	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-
Steroids	+	-	+	-	-	+	+	+	-	-	-	+	+	+	-	-	-	-	-	-

Me= Methanol; Ch= Chloroform; He= Hexane; EA= Ethyl Acetate; Aq = Aqueous.
+ Present, - Absent.

In this cytotoxic evaluation the methanol, chloroform, hexane, ethyl acetate and aqueous extracts of leaves, stem, flowers and roots shown LC_{50} values ranging from, 315.00µg/mL to 1425.00µg/mL. The general toxicity activity was considered to be nontoxic when the LC_{50} >1000µg/mL, weak when the LC_{50} is between 500µg/mL to 1000µg/mL, moderate when LC_{50} is between 100µg/mL to 500µg/mL and strong when the LC_{50} is < 100µg/mL.[13] Basing on the above classification, out of the 20 extracts tested, four exhibited moderate toxicity (LC_{50} 100-500µg/mL), 7 extracts displayed weak (LC_{50} 500-1000µg/mL) toxicity, 9 extracts were nontoxic (LC_{50} >1000µg/mL) as shown in Table 2. Among these extracts none of the extract showed LC_{50} below 100. The methanol (1160.00µg/mL), chloroform (1425.00µg/mL), ethyl acetate (1223.30µg/mL) extracts of leaves; chloroform (1293.33µg/mL) and ethyl acetate (1023.30µg/mL) extracts of bark; methanol (1010.00µg/mL) and chloroform (1148.33µg/mL) extracts of flowers; chloroform (1150.00) and ethyl acetate (1015.00) extracts of roots showed LC_{50} >1000µg/mL. The hexane (956.70µg/mL) and aqueous (577.30µg/mL) extracts of leaves; methanol (863.63µg/mL) and hexane (533.67µg/mL) extracts of bark; hexane (502.67µg/mL) and ethyl acetate (941.70µg/mL) extracts of flowers; methanol (730.33µg/mL) extract of root showed LC_{50} ranged between 500- 1000µg/mL. The aqueous extracts of bark (342.70µg/mL); aqueous extract of flower (382.70µg/mL); hexane (358.30µg/mL) and aqueous (315.00µg/mL) extracts of root showed LC_{50} <500µg/mL (Table 2).

Table 2: Cytotoxicity against brine shrimp larva of different crude extracts from *Prosopis cineraria*.

Parts	Extracts	Concentration of crude extract (µg/mL)									
		100	200	400	600	800	1000	1200	1400	1600	LC_{50}
		% of mortality (M±SD, P-value)									
Leaves	ME	0.00 ±0.00 NS	0.00 ±0.00 NS	2.71 ±0.69 0.021	5.35 ±2.82 0.051	12.15 ±2.69 0.0097	28.74 ±3.10 0.0022	54.79 ±0.38 0.0000	86.14 ±2.76 0.0002	100.00 ±0.00 0.0000	1160.00 ±4.58 (1148.40-1171.38
	CH	0.00 ±0.00 NS	0.00 ±0.00 NS	0.00 ±0.00 NS	1.60 ±0.46 0.030	4.71 ±1.95 0.044	11.78 ±1.69 0.0050	20.80 ±2.02 0.0030	46.24 ±5.33 0.0034	73.60 ±3.42 0.0005	1425.00 ±31.20 (1347.40-1506.60
	HE	3.29 ±0.24 0.0008	3.59 ±1.35 0.033	8.08 ±1.81 0.0098	13.71 ±2.75 0.0073	24.35 ±3.05 0.0029	56.76 ±3.91 0.0008	77.83 ±1.02	99.27 ±0.33 0.0000	100.00 ±0.00 0.0000	956.70 ±20.80 (905.00-1008.40
	EA	0.00 ±0.00 NS	0.00 ±0.00 NS	0.00 ±0.00 NS	4.50 ±0.63 0.0013	9.08 ±0.19 0.0000	22.77 ± 0.0055	46.28 ±5.21 0.0027	67.63 ±2.01 0.0002	91.50 ±3.78 0.0004	1223.30 ±32.10 (1143.50-1303.20
	AQ	7.52 ±1.75 0.011	16.68 ±2.22 0.0032	29.50 ±2.52 0.0013	52.51 ±7.15 0.0031	79.45 ±0.65 0.0000	98.24 ±1.47 0.0000	100.00 ±0.00 0.0000	100.00 ±0.00 0.0000	100.00 ±0.00 0.0000	577.30 ±36.50 (486.80-667.90
Bark	ME	2.02 ±0.42 0.011	6.83 ±2.69 0.029	10.71 ±1.13 0.0023	19.04 ±2.07 0.0021	37.86 ±3.29 0.0013	74.83 ±4.04 0.0005	99.01 ±1.60 0.0001	100.00 ±0.00 0.0000	100.00 ±0.00 0.0000	863.63 ±16.07 (823.41-903.26
	CH	0.00 ±0.00 NS	0.00 ±0.00 NS	0.00 ±0.00 NS	5.67 ±0.86 0.0055	8.30 ±0.39 0.0001	17.15 ±1.78 0.0021	31.25 ±3.20 0.0026	67.86 ±2.93 0.0004	94.43 ±4.09 0.0004	1293.33 ±15.28 (1255.39-1331.28
	HE	4.50 ±1.53 0.025	14.44 ±4.22 0.015	26.23 ±0.37 0.0000	60.78 ±3.44 0.0005	82.70 ±2.31 0.0001	99.14 ±0.16 0.0000	100.00 ±0.00 0.0000	100.00 ±0.00 0.0000	100.00 ±0.00 0.0000	533.67 ±10.26 (508.17-559.16
	EA	0.00 ±0.00 NS	0.00 ±0.00 NS	5.05 ±0.11 0.0011	11.23 ±1.07 0.0019	23.65 ±2.16 0.0015	45.66 ±4.46 0.0017	83.49 ±3.52 0.0003	100.00 ±0.00 0.0000	100.00 ±0.00 0.0000	1023.30 ±13.30 (966.60-1080.70
	AQ	15.01 ±2.09 0.0036	29.06 ±0.62 0.0008	58.06 ±4.93 0.0012	79.26 ±1.83 0.0001	98.30 ±1.19 0.0000	100.00 ±0.00 0.0000	100.00 ±0.00 0.0000	100.00 ±0.00 0.0000	100.00 ±0.00 0.0000	342.70 ±25.30 (279.80-405.60)
Flowers	ME	0.00 ±0.00 NS	4.78 ±.146 0.017	8.54 ±2.94 0.020	14.81 ±2.04 0.0024	25.76 ±3.32 0.0028	48.00 ±4.68 0.0017	87.11 ±2.88 0.0002	99.11 ±0.60 0.0000	100.00 ±0.00 0.0000	1010.00 ±17.30 (967.00-1053.00)
	CH	0.00 ±0.00 NS	0.00 ±0.00 NS	2.10 ±0.68 0.024	8.12 ±0.84 0.0021	12.30 ±0.22 0.0000	28.59 ±2.46 0.0014	57.13 ±2.44 0.0004	90.63 ±2.20 0.0002	100.00 ±0.00 0.0000	1148.33 ±10.41 (1122.48-1174.19
	HE	4.29 ±1.07	13.10 ±1.28	31.08 ±1.29	68.54 ±0.53	91.41 ±1.95	99.25 ±0.27	100.00 ±0.00	100.00 ±0.00	100.00 ±0.00	502.67 ±15.53 (464.08-

		0.012	0.0017	0.0003	0.0001	0.0001	0.0000	0.0000	0.0000	0.0000	541.26)
	EA	0.00 ±0.00 NS	1.20 ±0.18 0.0051	5.07 ±1.01 0.0077	11.31 ±1.90 0.0013	27.26 ±1.36 0.0005	59.59 ±5.15 0.0013	87.67 ±1.46 0.0001	99.09 ±0.13 0.0000	100.00 ±0.00 0.0000	941.70 ±23.60 (883.00-1000.40
	AQ	12.65 ±2.49 0.0067	24.20 ±1.29 0.0005	52.40 ±4.44 0.0012	79.00 ±0.32 0.0000	99.14 ±0.51 0.0000	100.00 ±0.00 0.0000	100.00 ±0.00 0.0000	100.00 ±0.00 0.0000	100.00 ±0.00 0.0000	382.70 ±25.20 (324.20-449.20)
Roots	ME	2.12 ±0.93 0.041	4.50 ±1.06 0.011	11.00 ±1.88 0.0051	26.23 ±1.79 0.0008	61.50 ±1.57 0.0001	93.30 ±3.17 0.0002	99.20 ±0.68 0.0000	100.00 ±0.00 0.0000	100.00 ±0.00 0.0000	730.33 ±0.58 (728.90-731.77)
	CH	0.00 ±0.00 NS	0.00 ±0.00 NS	2.31 ±0.20 0.0005	6.62 ±1.08 0.0050	13.17 ±2.89 0.0083	29.31 ±0.28 0.0000	56.26 ±3.87 0.0010	72.18 ±1.77 0.0000	100.00 ±0.00 0.0000	1150.00 ±22.90 (1093.10-1206.90)
	HE	11.21 ±0.90 0.0012	24.71 ±0.80 0.0002	56.01 ±3.68 0.0007	82.24 ±1.72 0.0001	99.06 ±0.80 0.0000	100.00 ±0.00 0.0000	100.00 ±0.00 0.0000	100.00 ±0.00 0.0000	100.00 ±0.00 0.0000	358.30 ±17.60 (314.70-402.00)
	EA	6.04 ±1.98 0.019	13.33 ±1.11 0.0012	27.63 ±2.69 0.0016	52.64 ±3.65 0.0008	75.36 ±1.37 0.0001	96.25 ±3.39 0.0002	100.00 ±0.00 0.0000	100.00 ±0.00 0.0000	100.00 ±0.00 0.0000	1015.00 ±5.00 (1002.58-1027.42)
	AQ	13.30 ±1.14 0.0013	29.13 ±1.91 0.0007	65.03 ±3.84 0.0006	91.12 ±3.08 0.0002	99.00 ±0.67 0.0000	100.00 ±0.00 0.0000	100.00 ±0.00 0.0000	100.00 ±0.00 0.0000	100.00 ±0.00 0.0000	315.00 ±5.00 (302.58-327.42)

Values are represented as mean of 3 replicates ± standard deviations at 95% confidence intervals and P- value is significant at <0.05 and 0.001, NS – not significant; ME- methanol, CH-chloroform, HE-hexane, EA-ethyl acetate, AQ-aqueous.

DISCUSSION

In the present study, crude extracts of methanol, hexane, chloroform, ethyl acetate and aqueous from leaves, stem, flowers and roots of *P. cineraria* were evaluated for their cytotoxicity against Brine shrimp due its traditional usage.

Plants have over the years, proved to be a good source of chemotherapeutic agents. Today, many of the drugs have been derived from plants resources such as quinine, chloroquine and artemisinin. Historically, medicinal plants have provided a source of inspiration for novel therapeutic drugs, as plant-derived medicines have made large contributions. According to WHO, now a days, 80% of the world's population rely on plants for their primary health care.[3] Plants are producing secondary metabolites for their defense, which play an important role of physiological activities in human body.[4] The medicinal value of plants is due to the substances that it contains, which produce a physiological action on the human body. Some examples of these plants are alkaloids, essential oils, tannins, resins and many others.[2] India had remarkable biodiversity and rich cultural traditions of plant use. Interestingly, today many of the pharmaceutical companies are utilizing such plant-based formulations in treatment of various diseases and disorders worldwide.[5]

P. cineraria have been used in traditional medicine for treatment of leprosy, dysentery, bronchitis, asthma, leucoderma, muscular tremors and rheumatism. It is also used to possess anthelmintic, antibacterial, antifungal, antiviral and anticancer activities. *Prosopis* spices have also been used in indigenous system of medicine as a folk medicine for various ailments. The bark is dry, acrid, bitter, with sharp taste; cooling antihelmintic, tonic; cures leprosy dysentery, asthma, leucoderma, piles tremors of the muscle, wandering of the mind. The flowers are pounded mixed with sugar and used during pregnancy as safeguard against miscarriage. The ashes of bark are rubbed over the skin to remove hair. The smoke of the leaves is good for eye troubles. Fresh Leaves juice mixed with lemon juice is used for dyspepsia; extract of crushed pods is used for earache, toothache, pain relief from fractured bones. Aqueous extract of bark and leaves applied externally to treat skin disease disinfects wounds and promotes healing.[1,14]

Some of the traditional medicine involves the utilization of crude plant extracts which may contain an extensive diversity of molecules, often with unclear biological effects. However, most of the available information regarding the medicinal potency of these plants is not provided with credible scientific data. For this reason, several researches have been conducted toxicity assays to find out the toxicity of medicinal plants. A general bioassay that appears capable of detecting a board spectrum of bioactivity present in plant crude extracts is the Brine Shrimp (Artemia sp) Lethality assay (BSLA). BSLA is used as an indicator for general toxicity and also as a guide for the detection of pesticidal and antitumor compounds. The low cost and ease of performing the assay and the commercial availability of inexpensive brine shrimp eggs makes BSLA is very useful bench top method. *In vivo* cytotoxic test has been successfully used as a preliminary study of antitumor and

cytotoxic agents. Thus, the findings of this present work would give baseline information on the most promising plant species that might be used as a basis for the development of new tools of great therapeutic importance. The general toxicity activity was considered weak when the LC_{50} was between 500 and 1000µg/mL, moderate when the LC_{50} was between 100 and 500µg/mL, and strong when the LC_{50} ranged from ≤ 100µg/mL.[15] According to the above hypothesis, these have weaker toxic properties hence these are safer for therapies.

Cytotoxicity is also endorsed to the occurrence of various secondary metabolites found in plant extracts. Not only their existence but also the quantity of the phytochemical components in a given plant extract will determine the scope of its bioactivity. In addition, occurrence of more than one class of secondary metabolites in a certain plant extract determines the nature and scope of its biological activity.[16] Thus, our results are in corroboration with the findings of Nguta et al.,[17]who reported cytotoxicity of stem bark of *Launae acornuta* on brine shrimp larvae (weakly toxic). The present study concludes that the plant P. cineraria has shown very minimal cytotoxic activity, so this plant will be useful for further studies and safe, for using as drug in treatments of traditional medicine.

ACKNOWLEDGEMENTS

The authors are thankful to the Co-ordinator, Dept. of Zoology and Aquaculture, Acharya Nagarjuna University for providing me necessary facilities.

REFERENCES

1. Akash G, Sanjeev KM. Review on Prosopis cineraria: A potential herb of Thar Desert. Drug Innovation Today, 2015; 5: 60-65.
2. Lilybeth FO, Olga MN. Brine shrimp lethality assay of the ethanolic extracts of three selected species of medicinal plants from Iligan city, Philippines. Int Res J Biological Sci, 2013; 2(11): 74-77.
3. Okigbo RN, Ramesh P. Effects of plants and medicinal plant combinations as anti-infectives. Af J Pharmacy and Pharmacol, 2008; 2(7): 130-135.
4. Vieira D, Padoani D, Soares J, Adriano J, Filho VC, de Souza MM, Couto AG. Development of hydroethanolic extract of Ipomoea pes-caprae using factorial design followed by antinociceptive and anti-inflammatory evaluation. Bra J of Pharmacog, 2012; 23(1): 72-78. doi.org/10.1590/S0102-695X201200 5000126.
5. Johnson M, Babu A, Irudayaraj V. Antibacterial studies on in vitro derived calli of Ocimum basilicum L. J Chem Pharm Res, 2011; 2(2): 298-305.
6. Malik A, Kalidhar SB. Phytochemical examination of Prosopis cineraria L. (Druce) leaves. Indian J Pharm Sci., 2007; 69(4): 576-578.
7. Khatri, A. Rathore, A. and Patil, U. K. "Prosopis cineraria (L.) Druce: A Boon Plant of Desert- An Overview". IJBAR, 2010; 1(5): 2010.
8. Preeti K, Avatar SR, Mala A. Pharmacology, phytochemistry and therapeutic application of Prosopis cineraria (Linn): A review. J of Plnant Sci, 2015; 3(1-1): 33-39.
9. Chaudhry P. "Prosopis cineraria (L) Druce: A Life Line Tree species of the Thar Desert in Danger. J Biodiver Ecol Sciences, 2011; 1(4): 289-293.
10. Stella R, Narayanan N, Raj KB. Antitumour activity of Prosopis cineraria (L.) Druce against Ehrlichascites carcinoma-induced mice. Nat Pro Res, 2011; 25(8): 857–862.
11. Sofowora A. Medicinal plants and traditional medicine in Africa. John Wiley and Sons, New York, 1982; 251.
12. Kepam W. Qualitative organic analysis (Spectrochemical techniques). EdII. Mc Graw Hill, London, 1986; 40-58.
13. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, Mclaughlin JL. Brine shrimp: A convenient general bioassay for active plant constituents. Planta Med, 1982; 45(5): 31-34.
14. Dharani B, Sumathi S, Sivaprabha J and Padma P.R. "In vitro antioxidant potential of Prosopis cineraria leaves" J. Nat. Prod. Plant Resour, 2011; 1(3): 26-32.
15. Padmaja R, Arun PC, Prashanth D, Deepak M, Amit A, Anjana M. 2002. Brine shrimp leathality bioassay of selected Indian medicinal plants. Fitoterapia, 2002; 73: 508-510.
16. Musila MF, Dossaji SF, Naguta JM, Lukhoba CW, Munyao JM. In vivo antimalarial activity, toxicity and phytochemical screening of selected antimalarial plants. J Ethnopharmcol, 2013; 146(2): 557-561.
17. Nguta JM, Mbaria JM. Brine shrimp toxicity and antimalarial activity of some plants traditionally used in treatment of malaria in Msambweni district of Kenya. J Ethnopharmacol, 2013; 148(3): 988-992.