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Assessment of Bioactivity of Indian Medicinal Plants Using Brine Shrimp (*Artemia salina*) Lethality Assay†

Alluri V. Krishnaraju[a], Tayi V. N. Rao[a], Dodda Sundararaju[a], Mulabagal Vanisree[b], Hsin-Sheng Tsay[b], and Gottumukkala V. Subbaraju[a*]

[a] *Laila Impex Research Centre, Unit I, Phase III, Jawahar Autonagar, Vijayawada –520 007, Andhra Pradesh, India*
[b] *Institute of Biotechnology, Chaoyang University of Technology, Wufong, Taichung 413, Taiwan*

Abstract: Medicinal plants constitute an important component of flora and are widely distributed in India. The pharmacological evaluation of substances from plants is an established method for the identification of lead compounds which can leads to the development of novel and safe medicinal agents. Based on the ethnopharmacological literature, several species of medicinal plants used in traditional medicine in India were collected. In the present study, aqueous extracts of these medicinal plants were screened for their cytotoxicity using brine shrimp lethality test. Out of the 120 plants tested, *Pistacia lentiscus* exhibited potent brine shrimp lethality with LC_{50} 2.5 μg. *Aristolochia indica* (Aristolochiaceae), *Boswellia serrata* (Burseraceae), *Ginkgo biloba* (Ginkgoaceae), *Garcinia cambogia* (Clusiaceae), and *Semecarpus anacardium* (Anacardiaceae) have also showed significant cytotoxicity with LC_{50} 13, 18, 21, 22, and 29.5 μg respectively. The present study supports that brine shrimp bioassay is simple reliable and convenient method for assessment of bioactivity of medicinal plants and lends support for their use in traditional medicine.

Keywords: *Artemia salina*; brine shrimp lethality test; medicinal plants; cytotoxicity.

1. Introduction

The importance of medicinal plants and traditional health systems in solving the health care problems of the world is gaining increasing attention. Because of this resurgence of interest, the research on plants of medicinal importance is growing phenomenally at the international level, often to the detriment of natural habitats and mother populations in the countries of origin. Most of the developing countries have adopted traditional medical practice as an integral part of their culture. Historically, all medicinal preparations were derived from plants, whether in the simple form of raw plant materials or in the refined form of crude extracts, mixtures, etc. Recent estimates suggest that several thousands of plants have been known with medicinal applications in various cultures [1].

Some of these plants have been subjected to the isolation of the active ingredients (chemical compounds) and their subsequent

* Corresponding author: e-mail: lailarescen@sify.com
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modification. A large proportion of such medicinal compounds have been discovered with the aid of ethno-botanical knowledge of their traditional uses. The rich knowledge base of countries like India and China in medicinal plants and health care has led to the keen interest by pharmaceutical companies to use this knowledge as a resource for research and development programs in the pursuit of discovering novel drugs. India is a varietal emporium of medicinal plants and it is one of the richest countries in the world as regards genetic resources of medicinal plants. It exhibits a wide range in topography and climate, which has a bearing on its vegetation and floristic composition. More over the agro climatical conditions are conducive for introducing and domesticating new exotic plant varieties. At present majority of the people are relying for their primary health care on traditional medicine [2].

In continuation of our efforts to verify the efficacy of traditional medicine we have collected several medicinal plants from various geographical locations based on the ethnopharmacological information. In order to study the toxicity of these medicinal plants we performed brine shrimp lethality bioassay which based on the ability to kill laboratory cultured brine shrimp *(Artemia nauplii)*. The brine shrimp assay was proposed by Michael et al. [3], and latter developed by Vanhaecke et al. [4], Sleet and Brendel [5]. The assay is considered a useful tool for preliminary assessment of toxicity and it has been used for the detection of fungal toxins, plant extract toxicity, heavy metals, pesticides and cytotoxicity testing of dental materials [6-10].

The brine shrimp assay is very useful tool for the isolation of bioactive compounds from plant extracts [11]. The method is attractive because it is very simple, inexpensive and low toxin amounts are sufficient to perform the test in the microwell scale. In the present work, we report the cytotoxicity studies on various medicinal plant species collected from various geographical locations of India based on their ethnopharmacological information and the results obtained were described.

2. Materials and methods

2.1. Plant material

Authenticated medicinal plants were collected from the Eastern Ghats of Southern India during November 2002. The botanical identification was made by Dr. K. Hemadri and voucher specimens were deposited at the herbarium of Laila Impex Research Centre, India.

2.2. Preparation of extracts

The plant materials were dried under shade and grinded to a coarse powder. Powdered plant materials (each 25 g) were individually extracted with water / hydro-alcohol / alcohol (200 ml) and then filtered. Filtrates were concentrated dried under vacuum and subjected for activity studies.

2.3. Cytotoxicity bioassay

Brine shrimp lethality bioassay was carried out to investigate the cytotoxicity of extracts of medicinal plants of India. Brine shrimps (*Artemia salina*) were hatched using brine shrimp eggs in a conical shaped vessel (1L), filled with sterile artificial seawater (prepared using sea salt 38 g/L and adjusted to pH 8.5 using 1N 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 vial containing 4.5 ml of brine solution. In each experiment, 0.5 ml. of the plant extract was added to 4.5 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 (1-5000

μg/ml) of the test substances in a set of three tubes per dose.

2.4. Lethality concentration determination

The percentage lethality was determined by comparing the mean surviving larvae of the test and control tubes. LC_{50} values were obtained from the best-fit line plotted concentration verses percentage lethality. Podophyllotoxin was used as a positive control in the bioassay.

2.5. Statistical analysis

The percentage lethality was calculated from the mean survival larvae of extracts treated tubes and control. LC_{50} values were obtained by best-fit line method.

3. Results and discussion

The brine shrimp lethality assay represents a rapid, inexpensive and simple bioassay for testing plant extracts bioactivity which in most cases correlates reasonably well with cytotoxic and anti-tumor properties [12]. In the present study the brine shrimp lethality of extracts of 120 medicinal plants used in Indian traditional medicine to brine shrimp was determined using the procedure of Meyer et al [13]. The LC_{50} values of the brine shrimp obtained for extracts of these medicinal plants and that of the positive control, podophyllotoxin, are given in Table 1. Alcoholic extract of *Pistacia lentiscus* showed most prominent activity with LC_{50} 2.5 μg. The plant extracts *Aristolochia indica* (Aristolochiaceae), *Boswellia serrata* (Burseraceae), *Ginkgo biloba* (Ginkgoaceae), *Garcinia cambogia* (Clusiaceae), and *Semecarpus anacardium* (Anacardiaceae) exhibited significant brine shrimp lethality with LC_{50} values 13, 18, 21, 22, and 29.5 μg respectively. In addition, the aqueous extracts of *Quercus infectoria* (Fagaceae), *Phyllanthus emblica* (Euphorbiaceae), *Phyllanthus reticulatus* (Euphorbiaceae), *Trigonella foenum graecum* (Fabaceae), *Operculina turpethum* (Convolvulaceae), and *Terminalia chebula* (Combretaceae), have also shown moderate brine shrimp lethality and the LC_{50} values were found to be lower than 100 and noted in Table 1. The degree of lethality was found to be directly proportional to the concentration of the extract. Maximum mortalities took place at a concentration of 1000 μg/ml whereas least mortalities were at 10 μg/ml concentration. Other plant species, however, showed no significant differences in percentage mortalities between different concentrations within the same plant species indicating no brine shrimp lethality compared to that of control. The LC_{50} values of the plant extracts were obtained by a plot of percentage of the shrimp nauplii killed against the concentrations of the extracts and the best-fit line was obtained from the data by means of regression analysis.

This significant lethality of several plant extracts to brine shrimp is an indicative of the presence of potent cytotoxic components which warrants further investigation.

4. Conclusions

Although the brine shrimp lethality assay is rather inadequate regarding the elucidation of the mechanism of action, it is very useful to assess the bioactivity of the plant extracts. In the course of our studies, the brine shrimp lethality assay actually has proven to be a convenient system for monitoring biological activities of several plant species that are used in the Indian traditional medicine. Out of the several plants screened for toxicity against the brine shrimp, some species showed LC_{50} values less than 100 μg/ml and these interesting results lend further support to their traditional use.

Tabel 1. Brine shrimp lethality data of extracts of Indian medicinal plants

Plant	Part used	Traditional use	Brine shrimp lethality (LC_{50}, µg/mL, 24h)
Acanthaceae			
1. *Andrographis paniculata*	Whole plant	Antiviral, Antipyretic	2,500
2. *Hygrophila spinosa*	Herb	Anti-diabetic	200
3. *Barleria prionitis*	Whole plant	Antipyretic, used in respiratory diseases	2,610
4. *Adhatoda vasica*	Leaf	Antiasthamatic	>5,000
Amaranthaceae			
5. *Amaranthus spinosus*	Herb	Antidiarrheal	920
Anacardiaceae			
6. *Mangifera indica*	Kernel	Antiviral	365
7. *Semicarpus anacardium*	Nut	Anti-inflammatory, anti-arthritic	29.5
8. *Pistacia interrima*	Leaf gall	To treat Respiratory disorders	240
9. *Pistacia lentiscus***	Gum resin	Cytotoxic, antimicrobial	2.5
Annonaceae			
10. *Annona squamosa*	Seed	Anti-diabetic	510
Apiaceae			
11.*Trachyspermum roxburghianum*	Fruit	Anti-tumor	145
12. *Trachyspermum amni*	Fruit	Antibacterial, antifungal	2,200
13. *Centella asiatica**	Herb	Memory booster, adaptogenic	500
14. *Daucus carota var. sativa*	Seed	Antihypertensive, cardiotonic	1,110
15. *Apium graveolens*	Fruit	Anti-arthritic	900
16. *Coriandrum sativum*	Fruit	Used in sore throat, flatulence	700
17. *Anethum sowa*	Fruit	Antifungal	>5,000
18. *Ferula assafoetida*	Resin	Anticoagulant	1,131
19. *Cuminum cyminum*	Fruit	Antifungal, stimulant	106
20. *Foeniculum vulgare*	Fruit	Appetite stimulant, galactagogues	1,940

Table 1. Brine shrimp lethality data of extracts of Indian medicinal plants (continued)

Plant	Part used	Traditional use	Brine shrimp lethality (LC_{50}, µg/mL, 24h)
Apocynaceae			
21. *Holarrhena antidysenterica*	Bark	Anti-dysentric,	250
22.*Rauvolfia serpentina**	Root	Anti-hypertension, anti-migraine	230
23. *Wrightia tinctoria*	Whole plant	Antioxidant, antinociceptive	>5,000
24. *Vinca rosea*	Leaf	Anti-diabetic, anti-cancer	170
Araceae			
25. *Acorus calamus*	Rhizome	Anti-arthritic, hepatoprotective	>5,000
Aristolochiaceae			
26. *Aristolochia indica*	Root	Gastric stimulant	13
27. *Aristolochia bracteolata*	Whole plant	Anti-inflammatory	1,600
Asclepiadaceae			
28. *Hemidesmus indicus*	Root	Antioxidant, anti-inflammatory	1,800
29. *Leptadenia reticulata*	Whole plant	Respiratory stimulant	4,400
30. *Gymnema sylvestre*	Leaf	Anti-diabetic	630
31. *Pentatropis capensis*	Herb	Cooling agent	3,000
32. *Tylophora indica*	Leaf	Anti-asthamatic	145
33. *Calotropis gigantea*	leaf	Anti-fertility	1,200
Asteraceae			
34. *Achillea miliefolium*	Flower	Anti-inflammatory	370
35. *Anacyclus pyrethrum*	Root	Cardiotonic, to treat typhus fever,	460
36. *Eclipta alba*	Whole plant	Anti-hypertensive, cardiotonic	235
37. *Calendula officinalis*	Flower	Anti-inflammatory, wound healing	245
38. *Elephantopus scaber*	Herb	Antidiarrheal	>5,000
39. *Sphaeranthus indicus*	Flower	Insecticidal	170
40. *Cichorium intybus*	Root	Used in liver diseases	>5,000

Table 1. Brine shrimp lethality data of extracts of Indian medicinal plants (continued)

Plant	Part used	Traditional use	Brine shrimp lethality (LC_{50}, µg/mL, 24h)
41. *Silybum marianum*	Seed	Hepatoprotective	>5,000
42. *Inula racemosa*	Root	Antidiabetic, hypocholestremic	1,475
43. *Wedelia chinensis*	Whole plant	Anti-inflammatory	1,150
Baringtoniaceae			
44. *Careya arborea*	Flower	Demulcent	>5,000
Berberidaceae			
45. *Berberis aristata*	Root	To treat Liver and spleen diseases	550
Betulaceae			
46. *Betula utilis*	Bark	Bronchitis	>5,000
Bignonaceae			
47. *Tecomella undulate*	Bark	Cardiotonic	220
Bixaceae			
48. *Bixia orellana*	Seed	Used in cosmetics	470
Bobaceae			
49. *Bombax ceiba*	Galls	Chronic inflammation	>5,000
Bombacaceae			
50. *Ceiba pentandra*	Young bud	Laxative	165
Brassicaceae			
51. *Lepidium sativum*	Seed	Bone fracture healing	124
52. *Raphanus sativum*	Seed	To treat Gonorrhea	182.5
Burseraceae			
53. *Boswellia serrata**	Gum resin	Anti-inflammatory	18
54. *Commiphora wightii*	Gum resin	Anti-reheumatic	1,600
55. *Commiphora myrrha*	Oleoresin	Anti-asthamatic	>5,000
Caesalpiniaceae			
56. *Cassia fistula*	Fruit	Laxative	250
57. *Saraca asoca*	Bark	Treating gynecologic disorders	1,225
58. *Cassia auriculata*	Seed	Anti-dysentry, anti-diabetic	100
59. *Cassia tora**	Seed	Treating skin diseases	725

Table 1. Brine shrimp lethality data of extracts of Indian medicinal plants (continued)

Plant	Part used	Traditional use	Brine shrimp lethality (LC_{50}, µg/mL, 24h)
60. *Griffonia simplicifolia**	Seed	Anti-depressant	1,725
61. *Cassia occidentalis*	Seed	Treating the whooping cough	200
62. *Bauhinia purpurea*	bark	Anti-diarrheal	>5,000
63. *Cassia angustifolia*	Leaf	Laxative	1,550
64. *Caesalpinia digyna*	Root	Anti-diabetic	335
Capparidaceae			
65. *Capparis spinosa*	Root	Analgesic, antitussive	330
66. *Crataeva nurvala*	Bark	Diuretic	1,125
Caprifoliaceae			
67. *Viburnum opulus*	Bark	Antispasmodic	>5,000
Caricaceae			
68. *Carica papaya*	Fruit	Treating skin diseases	310
Celastraceae			
69. *Celastrus paniculatus**	Seed	Antispermatogenic	375
70. *Salacia chinensis**	Root	Antidiabetic	950
Chenopodiaceae			
71. *Beta vulgaris*	Tuber	Treatment of Cardiovascular diseases	292
Clusiaceae			
72. *Garcinia cambogia*	Fruit	Anti-obese	22
73. *Mesua ferrea*	Flower buds	Treatment of bleeding piles, menorrhagia	177
Combretaceae			
74. *Terminalia arjuna*	Bark	Cardiotonic, hypocholestremic	110
75. *Terminalia belerica*	Fruit	Antipyretic	170
76. *Terminalia chebula*	Fruit	Stomatic sore throat	82
Convolvulaceae			
77. *Ipomoea nil*	Seed	Laxative	1,545
78. *Operculina turpethum*	Root	Used in jaundice	81
79. *Evolvulus alsinoides*	Whole plant	Antipyretic	3,175

Table 1. Brine shrimp lethality data of extracts of Indian medicinal plants (continued)

Plant	Part used	Traditional use	Brine shrimp lethality (LC_{50}, µg/mL, 24h)
Cucurbitaceae			
80. *Citrullus colocynthis*	Fruit	Used in horn cancer	1,500
81. *Momordica charantia*	Fruit	Anti-diabetic	1,300
82. *Trichosanthes cucumerina*	Whole plant	Laxative	>5,000
83. *Diplocyclos palmatus*	Seed	Anti-inflammatory	>3,400
84. *Cucumis sativus*	Fruit	Anthelmentic, purgative	570
Cupressaceae			
85. *Juniperus communis*	Fruit	Antiarthritic, anti-inflammatory	690
Cycadaceae			
86. *Cycas circinalis*	Male cone	Used in General debility	1,950
Cyperaceae			
87. *Cyperus scariosus*	Rhizome	Treating stomachache	1,412
Dioscoreaceae			
88. *Dioscorea bulbifera*	Tuber	Used in skin diseases	1,150
Dipterocarpaceae			
89. *Shorea robusta*	Gum resin	Anti-microbial	100
Euphorbiaceae			
90. *Phyllanthus emblica*	Fruit	Anti-diabetic	58
91. *Ricinus communis*	Leaf	Galactogogue	150
92. *Phyllanthus reticulatus*	Leaf	Used in bone treatment	60
93. *Mallotus philippensis*	Fruit hairs	Urolithotriptics	32.1
94. *Acalypha indica*	Herb	Antiasthamatic	>5,000
95. *Phyllanthus amarus**	Whole plant	Hepatoprotective	2,900
96. *Putranjiva roxburghii*	Bark	Treating cold and fever	1,175
Fabaceae			
97. *Dolichos biflorus*	Seed	Diuretic	1,050
98. *Pongamia pinnata*	Seed	Anti-viral	155
99. *Mucuna pruriens*	Seed	Anti-parkinsonism	>5000
100. *Glycyrrhiza glabra*	Root	Anti-inflammatory	350
101. *Teramnus labialis*	Whole plant	Used in paralysis	182.5

Table 1. Brine shrimp lethality data of extracts of Indian medicinal plants (continued)

Plant	Part used	Traditional use	Brine shrimp lethality (LC_{50}, µg/mL, 24h)
102. *Trigonella foenum graecum*	Seed	Anti-diabetic	60
103. *Vigna trilobata*	Herb	Laxative	1,350
104. *Indigofera tinctoria*	leaf	Used in liver disease	3,540
105. *Butea monosperma*	Seed	Anti-microbial	290
106. *Pterocarpus santalinus*	Heart wood	Anti-diabetic, anti-fertility	365
107. *Desmodium gangeticum*	Root	Used in fever, cough	>5,000
108. *Glycine max*	Seed cake	Memory stimulant	875
Fagaceae			
109. *Quercus infectoria*	Galls	Anti-diarrheal	52.5
Fumariaceae			
110. *Fumaria officinalis*	Whole plant	Treating leprosy	2,500
Gentianaceae			
111. *Swertia chirayita*	Whole plant	Antidiabetic, liver diseases	>5,000
112. *Enicostemma hyssopifolium*	Whole plant	Anti-inflammatory	1,900
Ginkgoaceae			
113. *Ginkgo biloba*	Leaf	Anticoagulant	21
Hypoxidaceae			
114. *Curculigo orchioides*	Rhizome	Aphrodisiac	410
Juglandaceae			
115. *Juglans regia*	Bark	Antibacterial, dentifrice	300
Labiaceae			
116. *Majorana hortensis moench*	Leaf	Treatment of rheumatism	620
Lamiaceae			
117. *Ocimum basilicum*	Seed	Antiulcer	2,150
118. *Coleus forskohlii*	Root	Antihypertensive, cardiotonic	210
119. *Leucas aspera*	Whole plant	Antipyretic	1,900
120. *Coleus vettiveroides*	Root	Antimicrobial	>5,000
Podophyllotoxin (standard)			3.1

*Alcoholic extract **Hydroalcoholic extract

Eventhough, the present study on these crude extracts is an addition to the scientific literature, detailed investigations on individual plants for the pharmacological activities and active ingredients could provide leads to interesting pharmaceuticals of plant origin.

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