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Biological Screening of Medicinal Plants Collected from Eastern Ghats of India Using *Artemia salina* (Brine Shrimp Test)

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Abstract: Medicinal plants constitute important components of flora and are widely distributed in different regions of India. Based on ethnomedical significance, we have collected several medicinal plants used in traditional medicine from Eastern Ghats of India and evaluated for their biological activity. In the present study, a method utilizing brine shrimp (*Artemia salina* Leach) lethality was used to screen medicinal plants for their biological activity. Aqueous extracts from 118 Indian medicinal plants were screened by the brine shrimp lethality assay and found eleven out of the 118 extracts showed significant toxicity to the brine shrimp (<60 µg/ml). *Polygonum cuspidatum* and *Syzygium cumini* extracts have exhibited potent activity with LC_{50} 13.5 and 20, respectively. The results were analyzed within the context of the available traditional knowledge and uses for these plants. Present study could be useful in the search for new antitumor compounds from the Indian flora.

Keywords: *Artemia salina*; cytotoxicity, brine shrimp; Indian medicinal plants

1. Introduction

During the past decade, traditional systems of medicine have become increasingly important in view of their safety. Current estimates suggest that, in many developing countries, a large proportion of the population relies heavily on traditional practitioners and medicinal plants to meet primary health care needs. Although modern medicine may be available in these countries, herbal medicines (phytomedicines) have often maintained popularity for historical and cultural reasons. Concurrently, many people in developed countries have begun to turn to alternative or complementary therapies, including medicinal herbs [1].

India possesses rich floristic wealth and diversified genetic resources of medicinal plants. It has a widely ranging tropical and the agro climatic conditions, which are conducive for introducing and domesticating new and exotic plant varieties. The use of the plants, plant extracts and pure compounds isolated from natural sources provided the foundation to modern pharmaceutical compounds. The

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well known Indian systems of Medicine, namely, the Ayurveda, Siddha and Unani use predominantly plant based raw materials. Most of these traditional preparations and formulations have been found to be a reservoir of pharmaceuticals [2].

The brine shrimp lethality assay consists of exposing larvae to test sample in saline solution and lethality is evaluated after 24 h. The commercial availability of inexpensive brine shrimp eggs, the low cost and ease of performing the assay make brine shrimp lethality assay, a very useful bench-top method [3]. A number of studies have demonstrated the use of the brine shrimp assay to screen plant extracts [4-6]. Lethality assay has been used successfully to biomonitor the isolation of cytotoxic [7], antimalarial [8], insecticidal [9], and antifeedent [10] compounds from plant extracts. In this report, results of a screening of water, hydroalcoholic and alcoholic extracts of some important medicinal plants used in the traditional medicine (collected from the Eastern Ghats of India) for lethality towards *Artemia salina* larvae are presented.

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 on deposit at the herbarium of Laila Impex Research Centre, India.

2.2 Preparation of extracts

The plant materials were dried under shade and ground to a coarse powder. The powdered plant materials (each 25 g) were individually extracted with water / hydro-alcohol / alcohol (200 ml) and then filtered. Filtrates were concentrated individually, dried under vacuum and used for screening the brine shrimp lethality.

2.3 Brine shrimp lethalityy bioassay

Brine shrimp lethality bioassay [11], 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^{-1} 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^{-1}) 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 Statistic 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

Brine shrimp lethality is the simple bioassay

useful for screening large number of extracts in the drug discovery process from the Indian Medicinal plants. The procedure of Mayer et al [11], was adopted to determine the lethality of plant extracts to brine shrimp. The method allows the use of smaller quantity of the extracts and permits larger number of samples and dilutions within shorter time than using the original test vials [12].

The LC_{50} values of the brine shrimp obtained for extracts of these medicinal plants and that of the positive control, podophyllotoxin, have been presented in Table 1. The degree of lethality was found to be directly proportional to the concentration of the extract. In the evaluation for general toxicity using brine shrimp, maximum mortalities took place at a concentration of 1000 µg/ml whereas; least mortalities were at 10 µg/ml concentration. The most promising extracts were the aqueous extracts of *Polygonum cuspidatum* and *Syzygium cumini* which have been used in the traditional medicine for the treatment of antitumor and anti-inflammatory diseases and the lethality (LC_{50}) values are 13.5 and 20 ug/mL, respectively (Table 1). In addition, the extracts of *Ocimum sanctum*, *Lagerstroemia reginae*, *Cissampelos pareira*, *Acacia conccina*, *Punica granatum*, *Aconitum species*, *Rosa damascene*, *Cinchona species*, *Bacopa monnieri*, *Symplocos racemosa* and several species of *Piper* showed significant lethality to brine shrimp. The LC_{50} values were found to be lower than 100. The activity results of species belonging to *Piperaceae* family were found to be consistent with existing phytochemical knowledge of these plants as a source of cytotoxic and antitumor compounds [13]. In few cases, complete analysis of the cytotoxicity of several plant parts allow to understand the location of cytotoxic substances.

Other plant species, however, showed no significant differences in percentage mortalities between different concentrations within the same species indicating that no brine shrimp lethality, compared to that of control. The LC_{50} values of the plant extracts (24 h) were obtained by a plot of percentage of the shrimps killed against the concentrations of the extracts and the best-fit line was obtained from the data by means of regression analysis. LC_{50} was obtained from the best-fit line method.

4. Conclusions

From the preliminary screening, we have identified numerous extracts of Indian medicinal plants with pharmacological activity against brine shrimp. The fact that eleven out of the 118 plants screened for toxicity against brine shrimp had LC_{50} values less than 60 µg ml^{-1} is interesting and lend support to the traditional use of these plants. Based on the possible relationship between brine shrimp lethality and plant bioactivity, this work could serve for further ethnobotanical and phytochemical research.

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Tabe 1. Brine shrimp lethality data of Indian medicinal plants

Plant	Part used	Traditional use	Brine shrimp lethality (LC_{50}, 24h)
Lamiaceae			
1. *Mentha arvensis*	Herb	Anti-fertility	140
2. *Ocimum sanctum*	Whole Plant	Adaptogenic	30
3. *Rosmarinus officinalis*	Herb	Dyspepsia, flatulence	1,551
Lauraceae			
4. *Cinnamomum zeylanicum*	Bark	Anti-spasmodic, antiflatulent	2,050
5. *Cinnamomum tamala*	Leaf	Hypoglycemic	870
Liliaceae			
6. *Aloe barbadensis*	Gum	Hypocholestremic, cosmetic application	1,900
	Leaf	Treating liver disorders, appetite stimulant	4,050

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

Plant	Part used	Traditional use	Brine shrimp lethality (LC_{50}, 24h)
7. *Gloriosa superba*	Root	Anti-fungal and abortifacient	177.5
8. *Allium sativum*	Cloves	Antihypertensive, hypocholesterimic	>5,000
9. *Smilax* sp.	Tuber	Rheumatoid arthritis, psoriasis	2,430
10. *Polygonatum cirrhifolium*	Rhizome	Anti-bacterial	1,300
11. *Allium cepa*	Bulb	Anthelmintic	>5000
12. *Chlorophytum tuberosum*	Tuber	Treating impotency	580
13. *Asparagus racemosus*	Root	Activate Lipase and Amylase	1,150
Lobeliaceae			
14. *Lobelia nicotianaefolia*	Leaf	Anti-asthmatic	142.5
Lythraceae			
15. *Lagerstroemia reginae**	Leaf	Anti-diabetic	55
16. *Woodfordia fruticosa*	Flowers	Treating Leukorrhea	230
17. *Lawsonia inermis*	Leaf	Anti-Inflammatory	1,300
Malvaceae			
18. *Abelmoschus esculentus*	Fruit	Diuretic	147
19. *Abelmoschus moschatus*	Seed	Diuretic and stomachic	160
20. *Abutilon indicum*	Seed	Anti-inflammatory & Anthelmintic	165
21. *Bobax malabarica*	Bark	Treating skin eruptions	>5,000
22. *Gossypium herbaceum*	Root	Treating Nerve inflammation	>5,000
	Seed	Anti-fertility	183
23. *Hibiscus rosasinensis*	Flowers	Antiasthama	460
	Bark	Anti-fertility	950
24. *Hibiscus cannabinus*	Leaf	Purgative and aperient	590
25. *Sida cordifolia*	Whole plant	Antiobese, antiasthamatic and antiinflammatory	2,650
Meliaceae			
26. *Melia azedarach*	Fruit	Anti-viral	170
	Leaf	Anti-Malarial	875
27. *Azadirachta indica*	Seed	Anti-viral	440
	Bark	Anti-bacterial	370

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

Plant	Part used	Traditional use	Brine shrimp lethality (LC_{50}, 24h)
Menispermaceae			
28. *Tinospora cordifolia*	Stem	Adaptogenic	>5,000
29. *Anamirta cocculus*	Seed	Treating scabies, epilepsy	840
30. *Cissampelos pareira*	Root	Antidiabetic, Antiasthamatic	39
Mimosaceae			
31. *Acacia nilotica*	Bark	Antidiabetic, antioxidant	300
32. *Acacia catechu*	Bark	Treating Liver diseases	450
33. *Mimosa pudica*	Seed	Diuretic	1,125
34. *Acacia conccina*	Fruit	Cosmetic application	59.5
35. *Albizzia lebbeck*	Bark	Anti-inflammatory	120
Moraceae			
36. *Ficus benghalensis*	Bark	Antidiabetic, antidiarrhoeal	1,000
37. *Ficus glomerata*	Bark	Anthelmentic	850
38. Ficus religiosa	Leaf	Treating skin diseases	>5,000
Moringaceae			
39. *Moringa oleifera*	Bark	Anti-inflammatory	>1,000
	Seed	Anti-inflammaroty	400
	Leaf	Anti-inflammaroty	1,525
Musaceae			
40. *Musa paradisica*	Tuber	Treating peptic ulcer	>5,000
Myricaceae			
41. *Myrica esulenta*	Fruit	Sedative, stomachic	430
Myristicaceae			
42. *Myristica fragrans*	Nut	Anti-arthritics & anti-inflammatory	555
Myrsinaceae			
43. *Embelia ribes*	Fruit	Anti-inflammatory	463
Myrtaceae			
44. *Psidium guajava*	Leaf	Anti-diarrhoeal	880
45. *Syzygium cumini*	Seed	Anti-diabetic	475
46. *Syzygium aromaticum*	Flower buds	Anti-inflammatory	20
Nymphaeaceae			
47. *Nelumbo nucifera*	Flower	Astringent, stops bleeding	185
	Seed	Treating GI disorders and diarrhoea	2,200
Orchidaceae			
48. *Orchis sp.*	Tuber	Treating diarrhoea, indigestion	2,325
Papaveraceae			

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

Plant	Part used	Traditional use	Brine shrimp lethality (LC_{50}, 24h)
49. *Papaver somniferum*	Seed	Analgesic, CNS stimulants	3,562
Papilionaceae			
50. *Phaseolus roseburghii*	Fruit	Urolithotriptics	580
Parmeliaceae			
51. *Parmelia perlata*	Whole plant	Diuretic and sedative	730
Passifloraceae			
52. *Passiflora* sp.	Leaf	Anti-hypertensive, used in gastric disorders	2,075
Pinaceae			
53. *Cedrus deodara*	Wood	Anti-asthmatic	300
Piperaceae			
54. *Piper betle*	Leaf	Improve digestion	240
55. *Piper cubeba*	Fruit	Antitussive	750
56. *Piper nigrum*	Fruit	Appetite stimulants	30
57. *Piper longum*	Fruit spike	Anti-diarrhoeal	45
	Root	Anti-diarrhoeal	95
Plumbaginaceae			
58. *Plumbago zeylanica*	Root	Treating skin diseases, cytotoxic	2,410
Poaceae			
59. *Cynodon dactylon*	Whole plant	Astringent	>5,000
60. *Andropogon muricatus*	Root	Anti-spasmodic, diuretic	4,050
61. *Vetiveria zizanioides*	Root	Perfumes	690
Polygonaceae			
62. *Polygonum cuspidatum*	Root	Anti-tumor	13.5
63. *Rheum emodi*	Root	Hepatoprotective, blood purifier	1,152
64. *Rumex crispus*	Root	Anti-inflammatory, used in dermatitis	1,125
Punicaceae			
65. *Punica granatum*	Fruit rind	Anti-diarrhoeal And Anti-dysenteric	45

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

Plant	Part used	Traditional use	Brine shrimp lethality (LC_{50}, 24h)
Ranunculaceae			
66. *Aconitum* sp.	Tuber	Antiarthritic, anti-inflammation	44
67. *Nigella sativa*	Fruit	Anti-bacterial	414
68. *Hydrastis canadensis**	Root	Treating Gastritis, peptic ulcer	320
Rosaceae			
69. *Rosa damascena*	Flower	Cosmetic application	80
Rubiaceae			
70. *Cinchona* spp.	Bark	Appetite stimulant	47
71. *Gardenia gummifera*	Gum resin	Antispasmodic, expectorent	126.5
72. *Paedaria foetida*	Whole plant	Treating piles	2,300
73. *Rubia cordifolia*	Root	Anti-bacterial	370
Rutaceae			
74. *Aegle marmelos*	Fruit pulp	Anti-Dysentric & Anti-Diarrhoeal	4,120
	Root	Antihypertensive and cardiotonic	3025
	Leaf	Anti-diabetic	430
75. *Murraya koenigii*	Leaf	Antidysentric and antidiarrhoeal	>5,000
76. *Citrus medica var. limon*	fruit	Cardiac tonic and palpitation	147
	Fruit peel	Anti-scurvy	92.5
77. *Citrus reticulata*	Fruit peel	Anti-oxidant	500
78. *Citurs aurantium*	Fruit	Treating atherosclerosis	580
Santalaceae			
79. *Santalum album*	Heart wood	Treating skin diseases	>5000
Sapindaceae			
80. *Sapindus emarginatus*	Fruit	Anti-bacterial	118

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

Plant	Part used	Traditional use	Brine shrimp lethality (LC_{50}, 24h)
Saxifragaceae			
81. *Bergenia ligulata*	Root	Urolithotriptic	2,550
Scrophulariaceae			
82. *Bacopa monnieri**	Whole herb	Memory stimulants	90
83. *Picrorhiza kurroa*	Root	Treating viral hepatitis	3,500
Simaroubaceae			
84. *Ailanthus excelsa*	Root	Anti-tumour and cytotoxic	1,900
Solanaceae			
85. *Atropa acuminata*	Bark	Anticholinergic, nti-spasmodic	4,000
86. *Capsicum annuum**	Fruit	Anti-rheumatic	110
87. *Datura metel*	Herb	Anti-spasmodic	4,250
88. *Solanum indicum*	Root	Treating cough, nasal ulcer	195
89. *Solanum nigrum*	Fruit	Anti ulcer	130
90. *Solanum surattensis*	Whole Plant	Anti-pyretic activity anti microbials	130
91. *Solanum trilobatum*	Whole Plant	Anti-Tussive (cough)	1,250
92. *Withania somnifera**	Root	Anti-arthritic, adaptogenic	310
Sterculiaceae			
93. *Helicteres isora*	Fruit	Antidiarrhoeal	1,870
94. *Abroma augusta*	Root	Treating menstrual disorders	1,450
Strychnaceae			
95. *Strychnos nuxvomica*	Seed	Anti ulcer	430
96. *Strychnos potatorium*	Seed	Anti-hypercholestermi c & diuretic	1,100
Symplocaceae			
97. *Symplocos racemosa*	Bark	Uterine stimulant	90
Tamaricaceae			
98. *Tamarix gallica*	Gall	Treating diarrhoea	290

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

Plant	Part used	Traditional use	Brine shrimp lethality (LC_{50}, 24h)
Taxaceae			
99. *Taxus baccata*	Leaf	Antidepressant, anti-inflammatory	450
Theaceae			
100. *Camellia sinensis*	young twigs	Anti-oxidant	250
	Leaf	Anti-oxidant	150
Thymelaeaceae			
101. *Aquilaria malaccensis*	Stem wood	Diuretic	900
Urticaceae			
102. *Ficus religosa*	leaf	Treating skin diseases	>10,000
Valerianaceae			
103. *Nardostachys jatamansi*	Root	Treating peptic ulcer	375
104. *Valeriana wallichii***	Root	Sedative	3,875
Verbenaceae			
105. *Clerodendrum phlomidis*	Root	Antidiarrhoeal	3,750
106. *Clerodendrum serratum*	Root	Anti-Histamin	340
107. *Vitex negundo***	Leaf	Anti-inflammatory	282
Violaceae			
108. *Viola odorata*	Whole herb	Astringent, Diapharetic	800
Vitaceae			
109. *Vitis vinifera*	Seed	Laxative	1,600
Zingiberaceae			
110. *Amomum subulatum*	Fruit	Anti-fungal	205
111. *Curcuma longa*	Rhizome	Cytotoxic, antioxidant, treating skin diseases	1525
112. *Curcuma zedoaria*	Rhizome	Anti-dysentric and diarrhoeals	1,700

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

Plant	Part used	Traditional use	Brine shrimp lethality (LC_{50}, 24h)
113. *Hedychium spicatum*	Rhizome	Anti-spasmodics and anti-inflammatory	4,000
114. *Alpinia* sp.	Rhizome	Stomachic, carminative	5,500
114. Curcuma amada	Rhizome	Carminative, stomachic activity	6,600
115. Alpinia officinarum	Leaf	Anti-arthritic	1,875
116. Zingiber officinale	Rhizome	Anti-Inflammatory	127
Zygophyllaceae			
117. Tribulus terrestris	Fruit	Aphrodisiac	925
118. Fagonia arabica	Whole plant	Anti-microbial	900
	Whole plant	Antibacterial	525

*Alcoholic extract
**Hydroalcoholic extract