

## Preliminary evaluation of some medicinal plants of Sundarbans mangrove forest on central nervous system

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### SUMMARY

The Sundarbans mangrove forest has a rich biodiversity of flowering plants and many of these have been used in traditional medicine although the flora remains comparatively uninvestigated scientifically. *Xylocarpus granatum*, *Xylocarpus moluccensis* and *Excoecaria agallocha* methanolic extract showed a central nervous system depressant activity on the hole cross and open field test at 800 mg/kg dose level. The most significant depressant activity was observed in *Xylocarpus granatum* followed by *Xylocarpus moluccensis* and *Excoecaria agallocha*. There was no depressant activity observed in the models for *Sarcolobus globosus*. Further studies are required to confirm the activity and to explain the mechanism.

**Key words:** Sundarbans; Neuropharmacological; *Xylocarpus granatum*; *Xylocarpus moluccensis*; *Excoecaria agallocha*; *Sarcolobus globosus*

### INTRODUCTION

The natural world, especially the plant kingdom, is recognized as a valuable source of diverse molecules. There is a revival of interest in the use of plants as a source of new lead drug molecules and from the general public who are using plant extracts in many ways in conventional and complementary therapies. The Sundarbans mangrove forest has a rich biodiversity of flowering plants and many of these have been used in traditional medicine although the chemistry and bioactivity of the flora remains comparatively uninvestigated scientifically. It provides a safe habitat for many rare medicinal plant species, which can provide an important

range of novel drug compounds for human kind. These plants may not only provide useful dietary adjuncts to existing therapies, but may also represent a source of new lead drug compounds for characterization and subsequent development of pharmaceutical modalities. Thus we can utilize the natural environment and its resources in the Sundarbans through the sustainable utilization and management of medicinal plant resources in the Sundarbans (Miles *et al.*, 1997).

*Xylocarpus granatum* Koen. (Meliaceae); *Xylocarpus moluccensis* Koen. (Meliaceae) and *Excoecaria agallocha* Linn. (Euphorbiaceae) are moderate size trees, and *Sarcolobus globosus* is a large climber; these grow in the Sundarbans, the coasts of Indo-China and Malaya (Prain, 1903; Kiritkar and Basu, 1999). *Xylocarpus granatum* and *Xylocarpus moluccensis* barks are astringent and used for dysentery, diarrhoea and other abdominal troubles and as febrifuges (Yusuf *et al.*, 1994; Ghani, 1998). Previously

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both of the plants displayed significant free radical scavenging activity in DPPH assay (Uddin *et al.*, 2004) and *Xylocarpus moluccensis* showed antibacterial and antidiarrhoeal activity (Uddin *et al.*, 2005). In traditional Thai medicine the bark and wood of *Excoecaria agallocha* is used against flatulence (Karalai *et al.*, 1994). In Sri Lanka the smoke of the burning wood has been used in the treatment of leprosy, while the root has been used as an embrocation for swelling hands and feet (Jayaweera, 1980). In Bangladesh the plant is used as caustic in obstinate ulcers and leprosy sores. It is used as purgative and alternative. Latex is abortifacient, boiled in oil is applied in rheumatism, leprosy and paralysis. Decoction of leaves is used in epilepsy and ulcers. Bark is used as purgative and emetic (Ghani, 1998). *E. agallocha* showed significant antitumor activity (Konishi *et al.*, 1996; Konoshima *et al.*, 2001), anti-HIV activity (Erickson *et al.*, 1995), antioxidant activity (Masuda *et al.*, 1999), anti-termite activity (Miki *et al.*, 1994), antifungal activity (Sakaki *et al.*, 1994), and growth inhibitory activity (Rajia *et al.*, 2006). *Sarcolobus globosus* has been used in traditional medicine against rheumatism, dengue and fever (Johnson, 1999). *Sarcolobus globosus* seeds showed cardiotoxic action (Jabbar and Khan, 1982), neuromuscular blocking activity (Mustafa *et al.*, 1990) and smooth muscle inhibitory activity (Mustafa, 1993); and stems were reported for free radical scavenging and antioxidant activity (Wangensteen *et al.*, 2003). Limonoids (Alvi *et al.*, 1991; Kokpol *et al.*, 1996, Wu *et al.*, 2004), lignins, tannins (Shinoda *et al.*, 1985) have been isolated from *X. granatum*. A number of limonoids have previously been reported from *X. moluccensis* (Connolly *et al.*, 1976; Taylor, 1983; Mulholland and Taylor, 1992). From stem, root and wood of *E. agallocha* several new diterpenes, excoecarins and agallochins have been isolated (Konishi *et al.*, 1998, 2003; Anjaneyulu *et al.*, 2002). New rotenoids and isoflavonoids were recently isolated from *Sarcolobus globosus* (Wangensteen *et al.*, 2005).

The present study was performed as an ongoing process to investigate the preliminary effect of *Xylocarpus granatum*; *Xylocarpus moluccensis*; *Excoecaria agallocha* and *Sarcolobus globosus* on central nervous system by hole cross and open field test.

## MATERIALS AND METHODS

### Plant materials

*Xylocarpus granatum* stem bark; *Xylocarpus moluccensis* stem bark; *Excoecaria agallocha* stem bark and *Sarcolobus globosus* stems were collected from the Burigoalini Range of the Sundarbans forest, Khulna, Bangladesh, and identified from the Bangladesh National Herbarium, Mirpur, Dhaka. The plants were air and oven (40 to 45°C) dried and grinded by hammer mill to a fine powder.

### The extract

The plant was extracted by cold extraction method. The dried powder was soaked in 80% of methanol in a glass container for six days. The extract was separated from the plant debris by filtration and was concentrated by evaporation using rotary vacuum evaporator. They were dried under vacuum to give the methanol extract.

### Animals

Nonfasted, female mice (*Swiss-Webster* strain, 20-25 g body weight) bred in the animal house of the Department of Pharmacy, Jahangirnagar University were used for the experiments. The animals were provided with standard food and tap water *ad libitum*. The animals were maintained at natural day night cycle. The extract was administered intraperitoneally (i.p.) at a dose level of 200, 400, and 800 mg/kg body weight. The control animals were given equal volume of physiological saline.

### Hole cross test

The experiment was performed by Takagi *et al.* (1971) method. Spontaneous movement of the animals through the hole from one chamber to

another was counted for two min in this test. The observations were made on 0, 60, 120, and 180 min after i.p. administration of the test drug.

### Open field test

In this experiment, the method of Gupta, 1971, was employed. The animals were transferred carefully to the corner of the field and the number of squares travelled by the mouse were recorded for a period of two minutes. The observations were made on 0, 60, 120, and 180 min after i.p. administration of the test drug.

### Statistical Analysis

Data obtained from the experiments were expressed as mean and standard error of the mean (Mean  $\pm$  S.E.M.). Unpaired *t*-test was performed by computer software SPSS (Statistical Package for Social Science) release 10.0 for Windows, to test the level of significance. Probability (*P*) value ( $P < 0.05$ ) was considered as significant.

## RESULTS AND DISCUSSION

Our present study was carried out to investigate the preliminary neuropharmacological properties

of some medicinal plants of Sundarbans *Xylocarpus granatum*; *Xylocarpus moluccensis*; *Excoecaria agallocha* and *Sarcolobus globosus* in laboratory mice models. Hole cross and open field tests are classical animal models for the preliminary evaluation of central activity.

From the hole cross test (Table 1) of *Xylocarpus granatum* we can observe that the extract has a tendency to reduce the movements on hole cross study at 200 and 400 mg/kg body weight dose level, but the reduction was only significant at 60 min ( $P < 0.05$ ) for 200 mg/kg dose and at 180 min ( $P < 0.05$ ) for 400 mg/kg dose. The drug eventually stopped the hole cross movement at 800 mg/kg dose from 60 to 180 min giving highly significant result ( $P < 0.001$ ). When the open field experiment was done it also showed a similar results (Table 2), at 200 mg/kg dose level at 60 min ( $P < 0.05$ ) it significantly reduced the number of open field scores; and at 400 mg/kg dose level it significantly decreased the movements at 60 min ( $P < 0.001$ ) and 180 min ( $P < 0.05$ ). The reduction was highly significant ( $P < 0.001$ ) at 800 mg/kg dose level throughout the experimental period from 60 min to 180 min. The extract of *Xylocarpus granatum* thus has the central nervous system depressant activity

**Table 1.** Effect of the medicinal plants on hole cross experiment

Group	Dose (mg/kg)	0 min	60 min	120 min	180 min
Control (n = 12)		0.75 $\pm$ 0.37	1.25 $\pm$ 0.48	1.33 $\pm$ 0.56	3.08 $\pm$ 0.73
<i>X. granatum</i> (n = 7)	200	1.57 $\pm$ 0.48	0.14 $\pm$ 0.14*	1.57 $\pm$ 0.65	1.71 $\pm$ 0.64
	400	1.86 $\pm$ 0.83	0.86 $\pm$ 0.7	0.71 $\pm$ 0.56	0.71 $\pm$ 0.45*
	800	1.43 $\pm$ 0.53	0.00 $\pm$ 0.00*	0.00 $\pm$ 0.00*	0.00 $\pm$ 0.00***
<i>X. moluccensis</i> (n = 7)	200	1.71 $\pm$ .42	2.86 $\pm$ 0.55*	4.14 $\pm$ 0.77**	3.14 $\pm$ 0.55
	400	1.71 $\pm$ 0.42	2.57 $\pm$ 0.87	2.86 $\pm$ 0.55	1.71 $\pm$ 0.64
	800	3.42 $\pm$ 1.10*	1.14 $\pm$ 0.86	1.71 $\pm$ 0.91	1.00 $\pm$ 0.58*
<i>E. agallocha</i> (n = 7)	200	1.14 $\pm$ 0.63	1.14 $\pm$ 0.83	0.57 $\pm$ 0.43	0.43 $\pm$ 0.43**
	400	1.00 $\pm$ 0.53	0.86 $\pm$ 0.55	2.00 $\pm$ 0.87	1.29 $\pm$ 0.64*
	800	0.86 $\pm$ 0.55	0.57 $\pm$ 0.43	0.57 $\pm$ 0.43	1.14 $\pm$ 0.40*
<i>S. globosus</i> (n = 7)	200	4.00 $\pm$ 1.02**	4.00 $\pm$ 0.76**	2.57 $\pm$ 0.84	3.71 $\pm$ 0.36
	400	2.14 $\pm$ 0.51*	2.57 $\pm$ 0.57	2.43 $\pm$ 0.75	2.43 $\pm$ 0.72
	800	4.57 $\pm$ 0.92**	2.29 $\pm$ 0.99	2.14 $\pm$ 0.67	1.86 $\pm$ 0.63

Values are expressed as Mean  $\pm$  S.E.M n = number of mice. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

**Table 2.** Effect of the medicinal plants on open field experiment

Group	Dose (mg/kg)	0 min	60 min	120 min	180 min
Control (n =12)		97.58 ± 10.23	65.67 ± 10.87	67.67 ± 9.71	59.00 ± 12.23
<i>X. granatum</i> (n = 7)	200	84.00 ± 14.36	30.71 ± 10.40 <sup>†</sup>	50.14 ± 15.02	54.29 ± 16.76
	400	96.00 ± 10.47	14.71 ± 7.81 <sup>***</sup>	36.58 ± 11.73	25.86 ± 10.76 <sup>*</sup>
	800	19.43 ± 10.70	15.85 ± 7.08 <sup>***</sup>	0.00 ± 0.00 <sup>***</sup>	2.71 ± 1.60 <sup>***</sup>
<i>X. moluccensis</i> (n = 7)	200	114.29 ± 20.40	87.71 ± 15.05	71.0 ± 7.78	59.43 ± 8.08
	400	134.43 ± 13.24 <sup>†</sup>	94.28 ± 15.43	78.71 ± 16.35	75.57 ± 18.97
	800	121.57 ± 14.42	29.86 ± 8.41 <sup>**</sup>	14.00 ± 5.21 <sup>***</sup>	27.43 ± 14.42
<i>E. agallocha</i> (n = 7)	200	98.43 ± 7.03	39.86 ± 13.95	35.00 ± 9.78 <sup>†</sup>	29.00 ± 11.41
	400	93.29 ± 9.60	64.86 ± 10.78	53.71 ± 15.53	45.14 ± 14.98
	800	108.71 ± 13.16	30.29 ± 10.15 <sup>†</sup>	38.29 ± 9.60 <sup>†</sup>	39.00 ± 6.93
<i>S. globosus</i> (n = 7)	200	108.57 ± 12.19	94.29 ± 10.60	93.00 ± 16.87	79.86 ± 8.12
	400	97.43 ± 11.89	78.43 ± 8.38	54.14 ± 10.33	68.29 ± 12.00
	800	123.29 ± 9.80	42.86 ± 14.29	67.14 ± 17.77	45.00 ± 10.14

Values are expressed as Mean ± S.E.M n = number of mice. <sup>\*</sup>*P* < 0.05, <sup>\*\*</sup>*P* < 0.01, <sup>\*\*\*</sup>*P* < 0.001.

at 800 mg/kg dose level.

*Xylocarpus moluccensis* showed a variable hole cross result (Table 1) in the experiment, both at 200 mg/kg and 400 mg/kg dose level at 60 min and 120 min it showed an increase in the hole cross movements. At 800 mg/kg dose level the initial high movements was reduced by the extract in the following time period of the experiment. The open field experiment showed (Table 2) the number of scores was not significantly changed at the 200 mg/kg and 400 mg/kg dose level, but at 800 mg/kg dose level it significantly reduced the open field scores at 60 min (*P* < 0.05) and 120 min (*P* < 0.001). Thus the *Xylocarpus moluccensis* extract has slight central nervous system depressant activity only at 800 mg/kg dose level.

The *Excoecaria agallocha* showed a significant (*P* < 0.05) decrease in the hole cross test (Table 1) only at 180 min interval for all the dose level (200, 400 and 800 mg/kg) tested. Whereas the extract reduced the open field scores (Table 2) at 200 mg/kg dose level but only significant at 120 min (*P* < 0.05). There was a non significant slight reduction tendency on the open field movements at 400 mg/kg dose and the reduction was significant (*P* < 0.05) at 800 mg/kg at 60 min and 120 min. The results suggest

a slight central nervous system depressant activity of the extract of *Excoecaria agallocha* at 800 mg/kg dose level.

*Sarcolobus globosus* showed an increase in the hole cross movements (Table 1) from beginning of the experiment. In the open field scores, there was no major change observed in the extract group compared to control (Table 2). So, the high movements in the hole cross experiment was considered not to be related to the central nervous system. There could be some other factors (e.g. irritation) contributed to this high movements.

## CONCLUSION

*Xylocarpus granatum*, *Xylocarpus moluccensis* and *Excoecaria agallocha* methanolic extract showed a mild central nervous system depressant activity at higher dose level 800 mg/kg. The most significant depressant activity was observed at *Xylocarpus granatum* followed by *Xylocarpus moluccensis* and *Excoecaria agallocha*. There was no depressant activity observed in the models for *Sarcolobus globosus*. Further studies are required to confirm the activity and to explain the mechanism.

## REFERENCES

- Alvi KA, Crews P, Aalbersberg B, Prasad R. (1991) Limonoids from the Fijian medicinal plant dabi (*Xylocarpus*). *Tetrahedron* **47**, 8943-8948.
- Anjaneyulu ASR, Rao VL, Sreedhar K. (2002) Ent-kaurane and beyerane diterpenoids from *Excoecaria agallocha*. *J. Nat. Prod.* **65**, 382-385.
- Connolly JD, MacLellan MODA, Taylor DAH. (1976) Limonoids from *Xylocarpus moluccensis* (Lam.) M Roem. *J. Chem. Soc.* **1**, 1993-1996.
- Erickson KL, Beutler JA, Cardellina JH, McMahon JB, Newman DJ, Boyd MR. (1995) A novel phorbol ester from *Excoecaria agallocha*. *J. Nat. Prod.* **58**, 769-772.
- Ghani A. (1998) *Medicinal Plants of Bangladesh*, 1st ed, p. 233, Asiatic Society of Bangladesh, Dhaka.
- Gupta BD, Dandiya PC, Gupta ML. (1971) A Psychopharmacological Analysis of Behavior in Rat. *Jpn. J. Pharmacol.* **21**, 293-298.
- Jabbar A, Khan MR. (1982) Pharmacological studies on the glycosides from *Sarcolobus globosus*. *J. Bangladesh Academy Sci.* **6**, 171-173.
- Jayaweera DMA. (1980) *Medicinal Plants Used in Ceylon*, pp. 214-215, National Science Council, Sri Lanka.
- Johnson T. (1999) *CRC Ethnobotany Desk Reference*. p. 747, CRC Press, Boca Raton, Florida.
- Karalai C, Wiriyachitra P, Operkuch HJ, Hecker E. (1994) Cryptic and free skin irritants of the daphnane and tigliane in latex of *Excoecaria agallocha*. *Planta Med.* **60**, 351-355.
- Kirtikar KR, Basu BD. (1984) *Indian Medicinal Plants*. 2nd ed., Vol III; Bishen Singh Mahendra Pal Singh, Dehra Dun, India.
- Kokpol U, Chavasiri W, Tip-pyang S, Veerachato G, Zhao F, Simpson J, Weavers RT. (1996) A limonoid from *Xylocarpus granatum*. *Phytochemistry* **41**, 903-905.
- Konishi T, Fujiwara Y, Kiyosawa S, Konoshima T. (1996) Structures of diterpenes from the wood of *Excoecaria agallocha*. *Tennen Yuki Kagobutsu Toronkai Koen Yoshishu* **38**, 319-324.
- Konishi T, Konoshima T, Fujiwara Y, Kiyosawa S. (1998) Stereostructure of excoecarin H, a novel seco-labdane-type diterpene from *Excoecaria agallocha*. *Chem. Pharm. Bull.* **46**, 721-722.
- Konishi T, Yamazoe K, Konoshima T, Maoka T, Fujiwara Y, Miyahara K. (2003) New bis-secolabdane diterpenoids from *Excoecaria agallocha*. *J. Nat. Prod.* **66**, 108-111.
- Konoshima T, Konishi T, Takasaki M, Yamazoe K, Tokuda H. (2001) Anti-tumor-promoting activity of the diterpene from *Excoecaria agallocha* II. *Biol. Pharm. Bull.* **24**, 1440-1442.
- Masuda T, Yonemori S, Oyama Y, Takeda Y, Tanaka T, Andoh T, Shinohara A, Nakata M. (1999) Evaluation of the antioxidant activity of environmental plants: activity of the leaf extracts from seashore plants. *J. Agric. Food Chem.* **47**, 1749-1754.
- Miki T, Sakaki T, Shibata M, Inukai Y, Hirose H, Ikema Y, Yaga S. (1994) Soxhlet extraction of mangrove and biological activities of extracts. *Kyushu Kogyo Gijutsu Kenkyusho Hokoku* **53**, 3347-3352.
- Miles DH, Kokpol U, Chittawong V, Pyang ST, Tunsuwan K, Nguyen C. (1997) Mangrove forests—the importance of conservation as a bioresource for ecosystem diversity and utilization as a source of chemical constituents with potential medicinal and agricultural value. *International Conference on Biodiversity and Bioresources: Conservation and Utilization*, 23-27 November, Phuket, Thailand.
- Mulholland DA, Taylor DAH. (1992) Limonoids from Australian members of the Meliaceae. *Phytochemistry* **31**, 4163-4166.
- Mustafa MR, Hadi A, Hamid A. (1990) Neuromuscular blocking activity of a glycosidic extract of the plant *Sarcolobus globosus*. *Toxicol.* **28**, 1237-1239.
- Mustafa MR. (1993) Inhibition of calcium-dependent contractions of the isolated guinea-pig ileal longitudinal muscle and taenia coli by the total glycosidic extract of the plant *Sarcolobus globosus*. *Toxicol.* **31**, 67-74.
- Prain D. (1903) *Flora of the Sundribuns*. pp. 231-390, Record of the Botanical Survey of India, Superintendent of Government Printing, Calcutta.
- Rajia R, Alamgir M, Shahriar M, Choudhuri MSK. (2006) Bioactivity of the methanol extract of *Excoecaria agallocha* Linn. (Euphorbiaceae). *Orient. Pharm. Exp. Med.* **6**, 102-107.
- Sakaki Y, Shibata M, Inukai Y, Miki T, Hirose H, Ikema Y, Yaga S. (1994) Extraction of mangrove with supercritical carbon dioxide. II. Extraction characteristics of mangrove trees and biological activities of extracts. *Kyushu Kogyo Gijutsu Kenkyusho Hokoku* **52**, 3247-3251.
- Shinoda Y, Ogisu M, Inaba M, Tajima T. (1984)

- Chemical composition of mangroves. I. *Gifu Daigaku Nogakubu Kenkyu Hokoku* **49**, 119-125.
- Shinoda Y, Ogisu M, Iwata S, Tajima T. (1985) Chemical composition of mangroves. II. *Gifu Daigaku Nogakubu Kenkyu Hokoku* **50**, 155-165.
- Takagi K, Watanabe M, Saito H. (1971) Studies on the spontaneous movement of animals by the hole cross test: effect of 2-dimethylaminoethan. Its acylesters on the central nervous system. *Jpn. J. Pharmacol.* **21**, 797-810.
- Taylor DAH. (1983) Limonoid extractives from *Xylocarpus moluccensis*. *Phytochem.* **22**, 1297-1299.
- Uddin SJ, Shilpi JA, Delazar A, Nahar L, Sarker SD. (2004) Free radical scavenging activity of some Bangladeshi plant extracts. *Orient. Pharm. Exp. Med.* **4**, 187-195
- Uddin SJ, Shilpi JA, Alam SMS, Alamgir M, Rahman MT, Sarker SD. (2005) Antidiarrhoeal activity of the methanol extract of the barks of *Xylocarpus moluccensis* in castor oil- and magnesium sulphate-induced diarrhoea models in mice. *J. Ethnopharmacol.* **101**, 139-143.
- Wangensteen H, Alamgir M, Rajia S, Samuelsen AB, Malterud KE. (2003) Radical scavenging and antioxidant activity of *Sarcolobus globosus*. p. 164, *51st Congress, Society for Medicinal Plant Research*, Kiel, Germany.
- Wangensteen H, Alamgir M, Rajia S, Samuelsen AB, Malterud KE. (2005) Rotenoids and isoflavones from *Sarcolobus globosus*. *Planta Med.* **71**, 754-758.
- Wu J, Xiao Q, Huang J, Xiao Z, Qi S, Li Q, Zhang S. (2004) Xylocensins O and P, Unique 8,9,30-Phragmalin Ortho Esters from *Xylocarpus granatum*. *Organic Lett.* **6**, 1841-1844.
- Yusuf M, Chowdhury JU, Wahab MA, Begum J. (1994) *Medicinal Plants of Bangladesh*. p. 263, BCSIR Laboratories, Chittagong, Bangladesh.