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Bioactive Potentials of *Cassia renigera* Benth. Extracts against the Stored Product Pests *Callosobruchus chinensis* L., *Sitophilus oryzae* L. and *Tribolium castaneum* (Hbst.)

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Abstract

Petroleum (Pet.) ether, $CHCl_3$ and CH_3OH extracts of *Cassia renigera* Benth. were tested against *Callosobruchus chinensis* L. for dose-mortality and repellency; and against *Sitophilus oryzae* L. and *Tribolium castaneum* (Hbst.) for repellent activity test. The Pet. ether extract of leaf was found active against *C. chinensis* adults through dose mortality assay and the LD_{50} values established were 9.270, 4.932, 2.032, 1.721, 1.671, 1.543, 1.513, 1.503 and 1.478 mg cm^{-2} for 0.5, 6, 12, 18, 24, 30, 36, 42 and 48 h of exposure respectively. However, the leaf extracts of $CHCl_3$ and CH_3OH; as well as the Pet. ether, $CHCl_3$ and CH_3OH extracts of the stem-bark did not give mortality to the test organism. The $CHCl_3$ extract of leaf showed repellent activity against *Tribolium castaneum* at 0.1% level of significance ($P < 0.001$), and the Pet. ether extracts of the leaf and the CH_3OH extracts of the stem-bark offered repellency at 1% level of significance ($P < 0.01$) between dose levels. Only the $CHCl_3$ extracts of the stem-bark gave repellency at 5% level of significance ($P < 0.05$), while the CH_3OH extracts of leaf and Pet. ether extract of stem-bark did not show any significant repellency at all.

Keywords: Dose mortality; Repellency; *Cassia renigera*; *T. castaneum*; *S. oryzae*; *C. chinensis.*

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1. Introduction

Cassia renigera L. (Family: Caesalpinaceae) an annual under shrub grows all over the tropical countries and in wasteland as a rainy season weed is known as Burmese Pink Cassia. Over 1000 species have belonged to *Cassia* over the years and *C. renigera* is one of them [1,2]. *C. renigera* is a small tree not more than 20 feet but in spring it presents a strikingly beautiful picture, enhanced by the varied tones of pink in each dense cluster [3]. Various medicinal properties have been attributed to this plant in the traditional medicine.

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Sennosides, which are well known for their medicinal importance, have been detected in the leaves of the plant [4]. The extracts of *Cassia* species have been used as a remedy for various skin ailments, rheumatic disease and as laxatives [2,5,6]. The extract of *Cassia* species leaves has been found to possess significant hepatoprotective activity and anti-inflammatory activity [7,8]. Pharmacologically, the plant has been investigated for its antitumour [9] antioxidant [10] anti-inflammatory [11] antimicrobial [12] antifeedant and larvicidal [13] activities. Many plants have been recognized to show repellent activities to the termites [14,15]. These have shown very high mortality in stored grain [16] pests and efficiently control grain damage and seed weight loss [17]. Different parts of the plant, *C. renigera* has been taken to find out the bioactive potential especially of pesticidal activity and repellent effects against some store grain pests (e.g. *Tribolium castaneum, Sitophilus oryzae* and *Callosobruchus chinensis*). *T. castaneum* (Family: Tenebrionidae) is a worldwide pest of stored products and of Indo-Australian in origin [18]. These beetles have chewing mouthparts, but do not bite or sting. The red flour beetle may elicit an allergic response [19]. The eggs are microscopic and the slender larvae are creamy yellow to light brown in color. The adult is a small reddish-brown beetle. Total life cycle completes independently, while for egg incubation 8.8 days, larval development 22-100 days depending on temperature, pupal development 4.5 days, and for reproductive maturation 5-4 days [20]. *S. oryzae* (rice weevil) (Family: Curculionidae) is a serious stored product pest which attacks several crops and worldwide in distribution. The adult rice weevil is a dull reddish-brown to black in color. The larval rice weevil must complete its development inside the seed kernel. The larva develops within the seed, hollowing it out while feeding. Total life cycle from egg to adult took 34 to 49 days with an average of 42 days at 15 to 34°C and 58 to 89 per cent relative humidity [21]. *C. chinensis* (Family: Chrysomelidae) is a common species of beetle found in many stored legumes [22]. The eggs are cemented to the surface of pulses and are smooth, domed structures with oval, flat bases. The larvae and pupae are normally only found in cells bored within the seeds of pulses [23].The developmental period from egg to adult takes 20-25 days [24,25].

2. Materials and Methods

2.1. *Collection and preparation of test materials*

C. renigera was collected from the Rajshahi University Campus and was identified by the Department of Botany, Rajshahi University where a voucher specimen is kept in the herbarium. The plant parts were sliced and chopped into small pieces, dried under shade and powered using an electric grinder, weighed and placed in separate conical flasks to add Pet. ether, $CHCl_3$ and CH_3OH (Merk, Germany) (200 g × 600 mL× 2 times) for 48 h.

Filtration was done by Whatman filter paper (made in USA) at 24 h interval in the same flask followed by evaporation until the extract was left. The extracts was then removed to glass vials and preserved in a refrigerator at 4°C with proper labeling.

2.2. *Collection and culture of test insects*

To carry on tests for repellent and insecticidal efficacy of extractives of the *C. renigera*, samples *T. castaneum, S. oryzae, C. chinensis* were selected, because these are easy cultivable and noble laboratory animals. Moreover, they are important stored grain pests in a wide variety of cereal products and highly producing insects. The test insects *T. castaneum, S. oryzae* and *C. chinensis* used were collected from the stock cultures of the Crop Protection Laboratory, Department of Zoology, Rajshahi University.

2.3. *Dose-mortality tests*

2.3.1. *Dose-mortality test on C. chinensis*

For insecticidal activity test each of the extracts were dissolved in its solvent of extraction at different concentrations to go through *Ad Hoc* experiments to set considerable mortality and that were considered as doses. Plant extracts were dissolved in 1 mL of solvent and mixed with the prepared food in Petri dishes. However, being volatile the solvent was evaporated out shortly. A set of concentrations of dose for Pet. ether leaf extract of *C. renigera* were obtained as 2.037, 1.783, 1.528, 1.273, and 1.019 mg cm^{-2} for *C. chinensis*. For each of the tests ten insects were released in the treated food medium and 3 replications for each of the doses were maintained. The mortality was assessed for 0.5, 6, 12, 18, 24, 30, 36, 42 and 48 h of exposure.

2.3.2. *Statistical analysis*

The mortality (%) was corrected using Abbott's formula [26]. $P_r = \frac{P_o - P_c}{100 - P_c} \times 100$; where, P_r = Corrected mortality (%), P_o = Observed mortality (%), P_c = Control mortality (%). The data were then subjected to probit analysis [27,28].

2.4. *Repellent activity*

The repellency test was adopted from the method of McDonald *et al*., [29] with some modifications in the process. A general concentration for each of the plant extracts was selected as a stock dose for repellency while other successive doses were prepared by serial dilution. The quantity of the applied doses were 0.628, 0.314, 0.157, 0.078, 0.039 mg cm^{-2} and 1.414, 0.707, 0.353, 0.176, 0.088 mg cm^{-2} of Pet. ether, $CHCl_3$ and CH_3OH extracts of leaf and stem-bark for *T. castaneum* and *S. oryzae* respectively. For the application of *T. castaneum* half filter paper discs were prepared and selected doses of all the extracts separately applied onto each of the half-discs and allowed to dry out as exposed in the air for 10 min. Each treated half-disc was then attached length wise, edge-to-edge, to a control half-disc with adhesive tape and placed in a petri dish (9 cm). Ten adult insects were released in the middle of each filter-paper circle. Each concentration

was tested five times. Whereas, in case of *S. oryzae* a Petri dish (9 cm) was divided into three parts and marked with two narrow sticks through the adhesive tape. Then both the sides filled with food where in one side with treated food and other side with non-treated food followed by the concentration except the middle one. Then ten adult insects were released into the middle of the petri-dish. Insects were counted after 1 h and then at hourly intervals for 5 h. The average of the counts was converted to percentage repellency using the formula: PR = (Nc-5) × 20

Where, *Nc* was the average hourly observation of insect on the untreated half of the disc [30,31].

3. Results and Discussion

3.1. *Dose mortality effects on C. chinensis*

Dose mortality results of Pet. ether extracts of *C. renigera* against *C. chinensis* are represented in Table 1. The Pet. ether extract offered highest mortality giving LD_{50} values ranged between 1.478 mg cm^{-2} to 9.270 mg cm^{-2} against *C. chinensis* for 48 h and 0.5 h respectively.

Table 1. LD_{50} values of Pet. ether extracts of *C. renigera* L. leaf against *C. chinensis.*

Solvent	LD_{50} (mg cm^{-2}) at different exposures (h)								
	0.5	6	12	18	24	30	36	42	48
Pet. ether	9.270	4.932	2.032	1.721	1.671	1.543	1.513	1.503	1.478

3.2. *Repellent effects on T. castaneum and S. oryzae*

The Pet. ether, $CHCl_3$ and CH_3OH extracts of leaf and stem-bark of *C. renigera* offered promising repellent activity against *T. castaneum* at 0.1, 1.0 and 5.0% levels of significance while the extracts didn't show any significant repellent activity against *S. oryzae* between doses (Tables 2 and 3).

The results of the present study revealed that only the Pet. ether extract of *C. renigera* leaf contains insecticdial potential against *C. chinensis.* However, the $CHCl_3$ extracts of leaf gave repellency at 0.1% ($P < 0.001$); Pet. ether extract of leaf and CH_3OH extract of stem-bark at 1% ($P < 0.01$) and $CHCl_3$ extract of stem-bark at 5% ($P < 0.05$) level of significance against *T. castaneum* between dose levels. None of the extracts of leaf and stem bark showed any significant repellent activity against *S. oryzae*. These findings receive supports from works done by previous researchers. Works on *C. renigera* extracts for insect mortality is scanty; however a lot of work has been done on larvicidal potentiality. Pavananundt *et al.* [32] conducted experimentation of *Cassia* sp. against mosquito larvae. The efficacy of mosquito larvicidal of *Cassia siamea* leaf aqueous extract was investigated by determining the median lethal concentration, LC_{50}, within 24, 48, 72, and 96 h which is similar with this investigation as an insecticidal agent. The 24,

48, 72 and 96 h LC_{50} values were 394.29, 350.24, 319.17 and 272.42 ppm, respectively. The present study reveals that aqueous *C. siamea* leaf extracts have natural biopesticide properties. Kamaraj *et al.* [33] also supports our work as it was established the toxic effect of leaf methanol extract of *C. siamea*, seed methanol extract of *C. cyminum*, leaf ethyl acetate extract of *N. nucifera*, leaf ethyl acetate and methanol extract of *P. amarus* and seed methanol extract of *T. ammi* were showed 100% mortality against *A. stephensi* and *C. quinquefasciatus* after 48 h exposure. This investigation gets complete support from Hossain *et al.* [34] who found *C. renigera* is an evergreen tree containing antioxidant, brine shrimp lethality and antimicrobial properties. Jothy *et al.* [35] conducted a promising research using *Cassia* since *Cassia spectabilis* had been traditionally employed by healers for many generations which matches with our investigation supportively. The cytotoxicity result indicated that the extract is nontoxic as was clearly substantiated by a half maximal inhibitory concentration (IC_{50}) value of 59.10 µg/mL. The effects of the extract on the cell wall proteins illustrated that there were three major types of variations in the expression of treated cell wall proteins: the presence of new proteins, the absence of proteins, and the amount of expressed protein.

So, no doubt this plant is a potential source of promising biologically active compounds, and thus further investigation should be attempted on this natural resource.

Table 2. ANOVA results of repellency by the *C. renigera* L. (leaf and stem-bark) extracts of Pet. ether, $CHCl_3$ and CH_3OH against *T. castaneum* and *S. oryzae.*

Part of plant	Extract of *C. renigera*	Stored product insect pest used	Source of variation			F-ratio with level of significance		P - value	
			Between doses	Between time interval	Error	Between doses	Between time interval	Between doses	Between time interval
Leaf	Pet. ether	*T. castaneum*	4	4	16	53.750**	1.411	4.47E-09	0.275
	$CHCl_3$	*T. castaneum*	4	4	16	366.316***	1.198	1.65E-15	0.349
	CH_3OH	*T. castaneum*	4	4	16	3.093	2.791	0.045	0.062
	Pet. ether	*S. oryzae*	4	4	16	2.082	0.553	0.130	0.699
	CH_3OH	*S. oryzae*	4	4	16	2.396	0.598	0.093	0.668
	CH_3OH	*S. oryzae*	4	4	16	2.204	1.078	0.114	0.399
Stem -bark	Pet. ether	*T. castaneum*	4	4	16	2.082	0.553	0.130	0.699
	$CHCl_3$	*T. castaneum*	4	4	16	11.932*	3.442	0.001	0.032
	CH_3OH	*T. castaneum*	4	4	16	24.626**	1.322	1.15E-06	0.304
	Pet. ether	*S. oryzae*	4	4	16	4.315	6.660	0.0147	0.002
	$CHCl_3$	*S. oryzae*	4	4	16	1.035	0.908	o.419	0.482
	CH_3OH	*S. oryzae*	4	4	16	1.070	0.365	0.403	0.829

*** = Significant at 0.1% level (P < 0.001), ** = Significant at 1% level (P < 0.01) * = Significant at 5% level (P < 0.05)

Table 3. Repellent effect of the Pet. ether, $CHCl_3$ and CH_3OH extracts of leaf and stem-bark of *C. renigera* against *T. castaneum* and *S. oryzae.*

Solvents	Between doses (df = 4)		Between time interval	
	F - values	level of significance	F - values	level of significance
Pet. ether(leaf)	53.750**	P<0.01	1.411	-
$CHCl_3$(leaf)	366.316***	P<0.001	1.198	-
$CHCl_3$(stem-bark)	11.932*	P<0.05	3.442	-
CH_3OH(stem-bark)	24.626**	P<0.01	1.322	-

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References

1. A. H. M. Rahman, American Life Sci. **1(3)**, 105 (2013).
2. S. K. Jain, Medicinal Plants (National Book Trust, New Delhi, 1968) pp. 378.
3. H. A. Pawar, P. M. D. Mello, Int. J. Pharm. Sci. Res. **2(9)**, 2286 (2011).
4. K. Raghunathan, V. Hariharan, and S. Rangaswami, Ind. J. Chem. **12**, 1251 (1974).
5. J. D. Hooker, The Flora of British India, Vol. II, L. (Reeve and Co., England, 1879) pp. 26.
6. T. Bhakta, P. K. Mukherjee, K. Mukherjee, S. Banerjee, S. C. Mandal, T. K. Maity, M. Pal, and B. P. Saha, J. Ethnopharmacol. **66(3),** 277 (1999). https://doi.org/10.1016/S0378-8741(98)00220-7
7. T. K. Maitya, S. C. Mandal, B. P. Saha, and M. Pal, *Nat. Pro. Sci.* **4(4)**, 226 (1998).
8. L. V. Asolkar, K. K. Kakkar, and O. J. Chakre, Second Supplement to Glossary of Indian Medicinal Plants. 11th Edition (PID, CSIR, New Delhi, 1992) pp. 80.
9. M. Gupta, U. K. Mazumder, N. Rath, and D. K. Mukhopadhyay, J. Ethnopharmacol. **72**, 151 (2000). https://doi.org/10.1016/S0378-8741(00)00227-0
10. A. Luximon-Ramma, T. Behorun, M. A. Soobrattee, and O. I. Aruoma, Agri. Food Chem. **50**, 5042 (2002). https://doi.org/10.1021/jf0201172
11. I. Raju, M. Moni, and V. Subramanian, African Trad. Chem. **2(1)**, 70 (2005).
12. K. R. Aneja, C. Sharma, and R. Joshi, Jundishapur J. Microbiol. **4(3)**, 175 (2011).
13. V. Duraipandiyan, S. Ignacimuthu, and P. M. Gabriel, Saudi Biol. Sci. **18**, 129 (2011). https://doi.org/10.1016/j.sjbs.2010.12.009
14. I. K. Park and S. C. Shin, Agric. Food Chem. **53**, 4388 (2005). https://doi.org/10.1021/jf050393r
15. W. Ding and X. P. Hu, Insect Sci. **17(5)**, 427 (2010).
16. D. K. Weaver and B. Subramanyam, Botanicals, ed. B. Subramanyam et al. Alternative to Pesticides in Stored product IPM. (Kluwer Academic Publishers, Massachusetts, USA, 2000) pp. 303–320.
17. M. M. Sabbour, E. Shadia, Abd-Al-Aziz, Agri. Biol. Res. Sci. **3(2)**, 67 (2007).
18. E. H. Smith and R. C. Whitman, Field Guide to Structural Pests. National Pest Management Association, (Dunn Loring, VA, 1992).
19. K. Alanko, T. Tuomi, M. Vanhanen, M. Pajari-Backas, L. Kanerva, and K. Havu, Allergy **55,** 879 (2000). https://doi.org/10.1034/j.1398-9995.2000.00572.x
20. N. E. Good, USDA Technical Bull. **5**, 27 (1936).

21. M. I. Bhuiyah, N. Islam, A. Begum, and M. A. Karim, Bang. J. Zool. **18**, 67 (1990).
22. Chandra and Girish "*Callosobruchus chinensis* The Pulse Beetle Cowpea Bruchid". Ias zoology.com. (IAS Zoology. Ret. 28 November, 2014).
23. L. K. Vats, Ind. J. Entomol. **36(1)**, 17 (1974).
24. A. K. Raina, Ind. J. Entomol. **32(4)**, 303 (2009).
25. L. S. Blumer and C. W. A. Beak, Handbook on Bean Beetles, *Callosobruchus maculatus*. (Retrieved from http://www. beanbeetles. Org/handbook, 2012).
26. W. S. Abbott, J. Econ. Entomol. **18,** 265 (1925). https://doi.org/10.1093/jee/18.2.265a
27. D. J. Finney, Probit Analysis: A Statistical Treatment of the Sigmoid Response Curve, (Campridge University Press, London, 1947) pp. 333.
28. J. R. Busvine, A Critical Review of the Techniques for Testing Insecticides (Commonwealth Agricultural Buereux, London, 1971) pp. 345.
29. L. L. McDonald, R. H. Guy, and R. D. Speirs, Preliminary Evaluation of New Candidate Materials as Toxicants Repellents and Attractants against Stored-product Insects (Marketing Res. Report, Agricultural Res. Service, US Department of Agriculture, Washington DC, 882, 1970).
30. F. A. Talukder and P. E. Howse, J. Chem. Ecol. **19(11)**, 2463 (1993). https://doi.org/10.1007/BF00980683
31. F. A. Talukder and P. E. Howse, Efficacy of Pithraj (*Aphanamixis polystachya*) Seed Extracts against Stored-product Pests - *Proc. Int. Working Conf. on Stored-prod. Protect*. **2**, 848 (1994).
32. P. Pavananundt, K. Jiraungkoorskul, P. Kosai, and W. Jiraungkoorskul, Int. Modern Agri. **2(1),** 1 (2013).
33. C. Kamaraj, A. A. Rahuman, A. Bagavan, G. Elango, A. A. Zahir, and T. Santhoshkumar, Asian Pacific Trop. Med. **4(9)**, 698 (2011). https://doi.org/10.1016/S1995-7645(11)60177-6
34. M. M. Hossain, M. Mondal, M. P. Hossain, M. S. Uddin, N. Uddin, M. M. Hasan, M. S. Hossain, A. F. M. M. Islam, and M. S. Rana, Food Meas. Charact. **9(1)**, 68 (2015). https://doi.org/10.1007/s11694-014-9211-6
35. S. L. Jothy, A. Aziz, Y. Chen, and S. Sasidharan, Alt. Med. **2012**, Article ID 561284, (2012).