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SCREENING OF *GLYCOSMIS PENTAPHYLLA* FOR CYTOTOXIC AND REPELLENT POTENTIALS AGAINST *ARTEMIA SALINA* NAUPLII AND *TRIBOLIUM CASTANEUM* (HERBST) ADULTS

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Abstract: Chloroform extracts of the leaf, stem bark, stem wood and roots of *Glycosmis pentaphylla* (Retz.) were tested against the brine shrimp, *Artemia salina* nauplii for cytotoxic activity in which the dose-mortality assay revealed LC_{50} values of 28.579, 28.659, 57.213 and 84.111 ppm respectively, for the plant parts efficacy of which could be arranged in the order of leaf > stem bark > stem wood > root. The same extracts were tested for repellency against *Tribolium castaneum* (Herbst) adults where the root bark and root wood were separately extracted and used. The F values of the arcsin transformed data were 60.983, 14.177, 19.437, 15.429 and 1.082 respectively for the leaf, stem bark, stem wood, root bark and root wood extracts. Except for the root wood extract, strong repellent activity was observed for the rest of the extracts (P<0.001).

Key words: *Artemia salina, Tribolium castaneum, Glycosmis pentaphylla,* cytotoxic activity, repellent activity.

mvivsk: evBb wk‡¤úi bIwc-i (*Artemia salina* nauplii) Ici *Glycosmis pentaphylla* (AwU‡kviv ev fvU) Mv‡Qi cvZv, Kv‡Êi evKj, Kv‡Êi KvV I †kK‡oi †Kv‡ivdg wbhvm c‡qv‡M †WvR-gUwjwU cix¶Y c×wZ‡Z mvB‡UvUw·K cwZwµqv ch‡e¶Y Kiv nq hv‡Z LC_{50} cvIqv hvq h_vµ‡g 28.579, 28.659, 57.213 I 84.111 wcwcGg; Ges KvhKwiZvi w`K w`‡q gv‡bi wbgµgvbmv‡i mvRv‡j djvdj cvIqv hvh cvZv> Kv‡Êi evKj> Kv‡Êi KvV> †kKo | H GKB wbhvm¸wj AvUvi ‡cvKv *Tribolium castaneum* (Herbst)-Gi Dci weZvob KvhKwiZvI ch‡e¶Y Kiv nq (G‡¶‡Î †kK‡oi evKj I †kK‡oi KvV Avjv`v Avjv`vfv‡e cix¶v Kiv nq)| AvKwmb i/cvšwiZ Dcv‡Ëi Analysis of variance K‡i F-gvb cvIqv hvq cvZv, Kv‡Êi evKj, Kv‡Êi KvV, †kK‡oi evKj I †kK‡oi Kv†Vi Rb¨ h_vµ‡g 60.983, 14.177, 19.437, 15.429 I 1.082| †kK‡oi KvV e¨ZxZ meKÕwU wbhv‡mi wbZvob gvÎv wQj D"P ZvrchcY (P <0.001)|

Introduction

Glycosmis pentaphylla (Retz.) is a wild, oriental and an unarmed shrub or small tree distributed throughout the roadsides, under large trees or on uncultivated lands in Bangladesh, Sri Lanka, Eastern part of India, Southern Indochina, Malaysia, Indonesia and in Eastern Australia. In Bangladesh and India it is popularly known as Ash-sheura, Bon-nimbu, Bon-nebu, Atishoti, Vat and Dantan-gaas (Rastogi *et al.*, 1980). In Ayurvedic medicine the plant has been used traditionally in bilious complaints, cough, worms, jaundice and fever (Anon, 1952). Its medicinal virtues came into light when it was found that people who were cleansing their teeth with little sticks of *G. pentaphylla* do not suffer from Kala-azar (Kirtikar and Basu, 1935). Its growth-inhibiting activity against larvae of *Diaprepes abbreviatus* is also known (Shapiro *et al.*, 1997). Different species of *Glycosmis* contain a wide variety of compounds with potential biological activity (Stone, 1985). These include terpenoids (Chakravarty *et al.*, 1996), amides (Greger *et al.*, 1996; Hofer *et al.*, 1995a, 1998), imides (Hofer *et al.*, 1995b), alkaloids (Wu *et al.*, 1983; Wurz *et al.*, 1993; Ono *et al.*, 1995), coumarins (Rahmani *et al.*, 1998), and flavonoids (Wu *et al.*, 1983). Compounds exhibiting antifungal and insecticidal activities have already been isolated from several *Glycosmis* species (Greger *et al.*, 1996). However, previous workers investigated this plant giving emphasis mostly on the chemical constituents and their medicinal profile but information on its various biological activities is still scanty. Since this plant is being used as a toothbrush, bioactive potentials other than killing of the test insects were taken into consideration and accordingly, cytotoxic and repellent activity tests were carried out on the brine shrimp, *Artemia salina* nauplii and the red flour beetle, *Tribolium castaneum* (Herbst).

Materials and Methods

Brine shrimp lethality assay: It is a bench top bioassay method recently developed in the arena of bioassay for the bioactive compounds, which indicates cytotoxicity, as well as, a wide range of pharmacological activities, *e.g.* anticancer, antiviral, pesticidal, AIDS, etc. of the compounds. Brine shrimp eggs were purchased and kept in aerated seawater (3.8% sodium chloride or 38gm salt/1000ml) of water at room (25-30 ^{o}C) temperature. It normally takes 30 – 48 h to give nauplii under these conditions. For the leaf and stem bark, samples (2 mg) were initially dissolved in 100 μl of dimethyl sulfoxide (DMSO) to make it hydrophilic before adding 19.98 ml

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of water and diluted up to 20 ml with brine water to obtain a series of concentration, viz. 100, 50, 25 and 12.5 ppm. For the stem wood and root samples 5 mg of each were initially dissolved in 200 µl of DMSO before adding 19.98 ml of water and diluted up to 20 ml with brine water to obtain a series of concentration, e.g. 250, 125, 62.5, 31.25 and 15.625 ppm. After 48 h, 10 freshly hatched nauplii were added to each test tube with different concentration of the extracts of *G. pentaphylla* samples. After 24 h the number of nauplii killed in each tube was counted and compared to controls. LC_{50} values were calculated by probit analysis.

Repellent activity test: The repellency test used in this investigation was adopted from the method (No. 3) of McDonald *et al*. (1970) with some modifications by Talukder and Howse (1993, 1994) and the test insect was *T. castaneum* adults. For the continuous supply of the adult beetles the test insect was mass-cultured. A standard mixture of whole wheat flour with powdered dry yeast (19:1) was used as food medium throughout the experimental period (Park and Frank, 1948). Both the flour and the powdered dry yeast were sterilized at 60^0C for six hours in an oven. Food was not used until at least 15 days after sterilization to allow its moisture content to equilibrate with the environment (Khan and Selman, 1981). Half filter paper discs (Whatman No. 40, diameter 9 cm) were prepared and selected doses of all the $CHCl_3$ extracts were separately applied onto each of the half-discs and allowed to dry out as exposed to the air for 10 minutes. Each treated half-disc was then attached lengthwise, edge-to-edge, to a control half-disc with adhesive tape and placed in petri dishes. The orientation of the same was changed in the replica to avoid the effects of any external directional stimulus affecting the distribution of the test insects. Ten adult insects were released in the middle of each of the filter-paper circles. Each concentration was tested for five times. Insects that settled on each half of the filter paper discs were counted after 1 h and then at hourly intervals for 5 hours. The average of the counts was converted to percent repellency (*PR*) using the formula of Talukder and Howse (1993, 1995): *PR* = 2(C - 50), where, C is the percentage of insects on the untreated half of the disc. Positive values expressed repellency and negative values for attractant activity. The data obtained as percent repellency, was again developed by arcsin transformation for the calculation of analysis of variance (ANOVA).

Result and Discussion

For the leaf, stem bark, stem wood and root extracts the LC_{50} values established were 28.579, 28.659, 57.213 and 84.111 ppm respectively (Table 1). The findings of Muthukrishnan *et al.* (1999) resembles corroborate with the results. The quinazolone of arborine isolated from the ethyl acetate fraction of *G. pentaphylla* leaf extract to water resulted in 83 to 100% mortality of *Culex quinquefasciatus* larvae. Except the root wood extract strong repellent activity was found. The repellency due to differences between doses were highly significant ($P<0.001$). The F values have been established through ANOVA with the arcsine transformed data being 60.983, 14.177, 19.437, 15.429 and 1.082 for leaf, stem bark, stem wood, root bark and root wood extracts respectively (Table 2 and 3). The P values were established as 2.03E-11, 5.41E-6, 4.62E-7, 2.48E-6 and 0.4002 for the analysis between doses for the leaf, stem bark, stem wood, root bark and root wood extracts respectively, and the degree of repellent activity could be arranged in a descending order: leaves> stem wood> root bark> stem bark. This finding has been supported by Chopra *et al.* (1956) who mentioned that the leaves of this plant are used to keep insects away from sweets and other edible items that are taken by natives in India and Australia.

Table 1. Dose mortality effect of *G. pentaphylla* extracts against *A. salina* nauplii after 24 hours of exposure

Plant parts	LC_{50} value (ppm)	95% confidence limits		Regression equations	χ^2 value (Df)
		Upper	Lower		
Leaves	28.579	33.840	24.136	Y = -0.901 + 4.053 X	4.056 (2)
Stem bark	28.659	41.419	19.831	Y = 1.639 + 2.306 X	1.402 (1)
Stem wood	57.213	68.863	47.535	Y = -1.436 + 3.662 X	5.870 (2)
Root	84.111	109.118	64.836	Y = 1.007 + 2.074 X	3.013 (3)

Table 2. Repellency of *T. castaneum* adults by chloroform extract of leaf, stem bark, stem wood, root bark and root wood of *G. pentaphylla*

Type of extracts	Dose (µg/cm^2)	Average of hourly observations (Nc)					Percent repulsion (PR) PR = (Nc – 5) ×20%				
		1h	2h	3h	4h	5h	1h	2h	3h	4h	5h
Leaf	78.634	9.33	9.33	9.00	9.66	9.66	86.6	86.6	80.0	93.2	93.2
	39.317	9.00	9.66	7.33	7.33	7.00	80.0	93.2	46.6	46.6	40.0
	19.659	6.00	6.66	7.00	7.33	6.66	20.0	33.2	40.0	46.6	33.2
	9.829	8.00	7.00	7.00	6.33	6.00	60.0	40.0	40.0	26.6	20.0
	4.915	5.33	3.66	4.33	3.66	3.66	6.6	-26.8	-13.4	-26.8	-26.8
	2.457	4.66	3.66	4.00	3.00	1.33	-6.8	-26.8	-20.0	-40.0	-73.4
Stem bark	78.634	7.00	7.00	4.33	4.00	7.66	40.0	40.0	-13.4	-20.0	53.2
	39.317	8.33	8.00	4.33	7.66	6.33	66.6	60.0	-13.4	53.2	26.6
	19.659	7.00	7.00	6.66	5.66	6.66	40.0	40.0	33.2	13.2	33.2
	9.829	3.66	3.00	3.33	4.00	4.66	-26.8	-40.0	-33.4	-20.0	-6.8
	4.915	4.00	4.66	4.00	3.00	2.66	-20.0	-6.8	-20.0	-26.8	-46.8
	2.457	2.66	3.00	3.00	3.66	4.00	-46.8	-40.0	-40.0	-26.8	-20.0
Stem Wood	78.634	7.66	7.33	6.66	7.66	7.33	53.2	46.6	33.2	53.2	46.6
	39.317	5.33	3.66	4.33	3.66	3.66	6.6	-26.8	-13.4	-26.8	-26.8
	19.659	4.66	5.33	5.66	4.00	1.66	-6.8	6.6	13.2	-20.0	-66.8
	9.829	7.00	7.33	7.00	6.00	6.33	40.0	46.6	40.0	20.0	26.6
	4.915	3.66	3.33	4.00	3.33	4.33	-26.8	-33.4	-20.0	-33.4	-13.4
	2.457	5.00	4.00	3.66	4.33	4.33	00.0	-20.0	-26.8	-13.4	-13.4
Root bark	78.634	10.0	10.0	10.0	10.0	10.0	100	100	100	100	100
	39.317	9.00	9.00	8.66	5.66	5.66	80.0	80.0	73.2	13.2	13.2
	19.659	9.00	8.33	7.00	7.33	7.00	80.0	66.6	40.0	46.6	40.0
	9.829	6.00	5.33	5.33	4.66	4.33	20.0	6.6	6.6	-6.8	-13.4
	4.915	7.00	7.33	6.00	4.66	4.33	40.0	46.6	20.0	-6.8	-13.4
	2.457	3.66	3.33	4.00	3.33	5.00	-26.8	-33.4	-20.0	-33.4	00
Root wood	78.634	8.33	9.66	9.33	10.00	9.66	66.6	93.2	86.6	100.0	93.2
	39.317	9.00	9.66	9.66	9.66	9.33	80.0	93.2	93.2	93.2	86.6
	19.659	8.00	9.66	9.66	9.66	10.00	60.0	93.2	93.2	100.0	100.0
	9.829	7.00	7.66	8.66	8.66	8.66	40.0	53.2	73.2	80.0	73.2
	4.915	8.33	7.33	9.00	9.00	9.00	66.6	46.6	80.0	66.6	80.0
	2.457	6.33	6.66	8.66	8.66	8.33	26.6	33.2	73.2	53.2	66.6

Table 3. ANOVA results of repellency by chloroform extract of leaf, stem bark, stem wood, root bark and root wood of *G. pentaphylla* against *T. castaneum* adults.

Sources of variation	SS	Df	MS	F-values
Leaf				
Between doses	42904.49	5	8580.897	60.98325***
Between hourly observations	1419.20	4	354.800	2.521516ns
Error	2814.18	20	140.709	
Total	47137.87	29		
Stem bark				
Between doses	26948.51	5	5389.703	14.17715***
Between hourly observations	2745.83	4	686.457	1.805666ns
Error	7603.37	20	380.168	
Total	37297.71	29		
Stem wood				
Between doses	24779.38	5	4955.876	19.43674***
Between hourly observations	1339.77	4	334.943	1.313633ns
Error	5099.49	20	254.975	
Total	31218.64	29		
Root bark				
Between doses	20117.29	5	4023.457	15.42936***
Between hourly observations	3617.81	4	904.452	3.46844*
Error	5215.33	20	260.766	
Total	28950.42	29		
Root wood				
Between doses	2576.38	5	515.276	1.081782ns
Between hourly observations	2423.46	4	605.864	1.271965ns
Error	9526.43	20	476.322	
Total	14526.27	29		

*=P<0.05; *** =P<0.001; ns= not significant

Since types or category of compounds of this plant is already known it is also known that this plant might have some bioactive potential. The result of this investigation has been added a clue in this connection showing cytotoxic and repellent activity of the chloroform extract of *G. pentaphylla*. Chinese researchers reviewed the plants used for pest insect control in China and found that there was a strong connection between medicinal and pesticidal plants (Jembere *et al.*, 1995). So, being a medicinal plant and originally being confined to a certain area of Asia and Australia this plant deserves attention to be investigated especially for its bioactive potentials.

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