Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(3): 601-605
Received: 05-03-2017
Accepted: 06-04-2017

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Bioactive potentials of *Ficus racemosa* L. and *Momordica charantia* L. through brine shrimp lethality, insect dose-mortality and repellent activity tests

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Abstract
Screening of *Ficus racemosa* (leaf and stem) and *Momordica charantia* (Stem) extracted in petroleum ether, $CHCl_3$ and CH_3OH were conducted through lethality against Brine shrimp *Artemia salina* L. nauplii, dose-mortality and repellent activity against *Tribolium castaneum* (Hbst.) adults. For Pet. ether extract of *F. racemosa* leaf and stem the lethality against *A. salina* nauplii were LC_{50} 72.62, 38.78, 20.47 and 16.78ppm and 1802.91, 137.67, 57.14 and 27.33ppm for 12, 18, 24 and 30h of exposure respectively. For $CHCl_3$ extract the were 218.67, 187.76, 38.96 and 15.20ppm and 191.94, 62.15, 39.85 and 23.84ppm respectively; while for the CH_3OH extracts the LC_{50} values were -, 525.15, 107.71 and 41.67ppm and 47.83,41.42, 26.91 and 20.20ppm for 12, 18, 24 and 30h nof exposure respectively. For Pet. ether extract of *M. charantia* stem no mortality was recorded, however the $CHCl_3$ and CH_3OH of the same offered LC_{50} values 69.58, 25.00, 23.02 and 16.20ppm and 91.83, 28.15, 20.18 and 15.22ppm respectively for 12, 18, 24 and 30h of exposure respectively. For *F. racemosa* leaf and stem and *M. charantia* stem extracts of Pet.ehter and CH_3OH extracts no insecticidal activity was recorded, while the $CHCl_3$ extract gave mortality to the beetles of *T. castaneum*. The LD_{50} values for *F. racemosa* leaf and stem were 32.27, 4.14, 3.64, 3.34 and 3.05 and 7.57, 5.21, 3.84, 2.65 and 1.72mg cm^{-2} respectively after 24, 30, 36, 42 and 48h of exposure respectively; and for *M. charantia* stem these were 5.97, 4.99, 3.82, 3.09 and 2.89mg cm^{-2} respectively for the same exposure time. In repellent activity test, the $CHCl_3$ leaf extracts of *F. racemosa* and the $CHCl_3$ stem extracts of *M. charantia* found abstemiously repellent ($P<0.01$) and the CH_3OH leaf extracts of of *F. racemosa* showed mild repellency ($P<0.05$) against *T. castaneum* between dose levels while the Pet.E. leaf extracts, $CHCl_3$ stem extract of *F. racemosa* and CH_3OH stem extracts of *M. charantia* didn't show any significant repellent activity.

Keywords: Bioactive potentials, *Ficus racemosa* L., *Momordica charantia* L.

1. Introduction

F. racemosa (Family: Moraceae) is a huge tropical, deciduous, ever- green tree with greater than 800 species. It is locally known as 'Jagadumur' in Bangladseh and 'Fig' in English occurring cosmopolitan in distribution. It is goolar, to 30m high; bole buttressed; bark 8-10 mm thick, surface reddish-brown or yellowish-brown smooth, coarsely flaky, fibrous; blaze creamy pink; latex milky; young shoots and twigs finely white hairy, soon glabrous; branchlets 1.5-3mm thick, puberulous[1]. Leaves are ovate-oblong or elliptic lanceolate, entire tapering to a bluntish point at the apex. Flowers unisexual; inflorescence a syconia, on short leafless branches or warty tubercles of trunk or on larger branches, subglobose to pyriform, smooth, often lenticellate-verrucose; peduncle 3-12mm long, stout, orifice plane or slightly sunken, closed by 5-6 apical bracts [2]. Bark is reddish grey or grayish green, soft surface, uneven and often cracked, 0.5-1.8cm thick, inner surface light brown, fracture fibrous, taste mucilaginous without any characteristic odour. Unlike the banyan, it has no aerial roots [3-4]. The decoction of the bark of *F. racemosa* is claimed as an antidiuretic and its potential is evaluated in rats using three doses (250, 500 or 1000mg/kg). It had a rapid onset (within 1h), peaked at 3 h and lasted throughout the study period (5h) [5]. In traditional systems of medicine, different parts (leaves, stem, root, fruit, seeds, latex and even whole plant) of *F. racemosa* Linn have been recommended for the treatment of diarrhea, diabetes, hypertension, gastric ulcer, wound healing etc. *F. racemosa* Linn. showed a wide range of pharmacological actions like hypoglycemic, hypolipidemic, renal anti-carcinogenic, anti-diuretic, anti-tussive, hepatoprotective, radioprotective, anti-ulcer, anti-inflammatory, anti-diarrhoel and anti-fungal.
β-sitosterol, glauanol acetate, the active constituent present in *F. racemosa* L., has been found to be largely responsible for the therapeutic potentials of gular [6].

The plant also possesses potent inhibitory activity against six species of fungi, viz. *Trichophyton mentagrophytas*, *Trichophyton rubrum*, *Trichophyton soundanense*, *Candida albicans*, *Candida krusei* and *Torulopsis glabrata* [7-8]. The another plant *M. charantia* is called bitter melon, bitter gourd or bitter squash in English, and 'Korolla' in Bengali. It is a tropical and subtropical vine of the family cucurbitaceae, widely grown and known in Africa, Asia (including Bangladesh), and the Caribbean for its edible fruit, which is extremely bitter. In Bangladesh bitter melon is used as vegetable mainly and it has lots of medicinal values. Bitter melon has been used in various Asian and African herbal medicine systems for a long time [9-11]. In Turkey, it has been used as a folk remedy for a variety of ailments, particularly stomach complaints [12]. According to the Memorial Sloan-Kettering Cancer Center, *M. charantia* has a number of purported uses. While it has shown some potential clinical activity in laboratory experiments, 'further studies are required to recommend its use'. Several animal studies and small scale human studies have demonstrated a hypoglycemic effect of concentrated bitter melon extracts [13-15]. The present work on bioactive potentials of *F. racemosa* and *M. charantia* have been carried out against *A. salina* and *T. castaneum*. The rust-red flour beetle, *T. castaneum* (Hbst.) (Coleoptera: Tenebrionidae) is one of the most serious pests of stored products. It is commonly known as 'red flour beetle' (Coleoptera: Tenebrionidae). Mouthparts of this pest insect are not adapted to feed on hard whole grains and they are thus found in almost any kind of flour, cracked grains etc. [16]. The red flour beetle is reddish-brown in color and its antennae end in a three-segmented club[17]. Not only pulses and millets, cereals are also been attacked by this beetle [18]. In severe infestation, the flour turn grayish and moldy and has a pungent and disagreeable odor making it unfit for human consumption (Good, 1936). Although small beetles, about ¼ of an inch long, the adults are long-lived and may live for more than three years [19]. *T. castaneum* contaminates more than they consume. The *A. salina* (Family: Artemiidae) belong to a genus of very primordial crustacean (crawfish - crayfish) the Anostraca (Fairy Shrimps). It has three eyes and 11 pairs of legs and can grow to about 15 millimetres (0.6 in) in size. Males differ from females by having the second antennae markedly enlarged, and modified into clasping organs used in mating [20]. This genus just have a divided exoskeleton made of chitin enhanced protein, no usual crust of chitin (escutcheon) as other crawfish have. There are many species within the genus of *Anostraca*, but the *A. salina* are used as laboratory agent since they are very nice to grow, and the rate of successful hatches is very high. However, previous workers investigated these plants giving emphasis mostly on the chemical constituents and their medicinal uses, but information on their various biological activities is still scanty. The present investigation was carried out to find out the potential of its lethality against the brine shrimp, *Artemia salina* L. nauplii; insecticidal and repellent activity against the red flour beetle, *T. castaneum* (Herbst).

2. Materials and Methods

2.1 Collection and preparation of test materials

Fresh plants of *F. racemosa* and *M. charantia* were collected from the Rajshahi University Campus and identified by the Department of Botany while a voucher specimen is kept in the herbarium. After collecting leaves and stem were chopped into small pieces separately to dry under shade in a well-ventilated room. Dried materials were then powdered in a grinder, weighted and placed in separate conical flasks to add solvents (Pet. ether followed by $CHCl_3$ and CH_3OH; 100g × 300ml × 3 times) for 48h. Filtration was done by Whatman filter paper at 24h interval in the same flask followed by evaporation until the extract was left. It was then removed to glass vials and preserved in a refrigerator at 4°C with proper labeling.

2.2 Collection and culture of the test agents

Brine shrimp cysts were purchased from Katabon, Dhaka and kept in simulated seawater in aerated condition (3.8% sodium chloride or 38g salt/1000ml of water) at room (25-30 ^{0}C) temperature. It normally takes 24-36h to give nauplii under the laboratory conditions. For the adult beetles of *T. castaneum* used in the present experiment were reared in glass beakers (500 ml) in a standard mixture of whole-wheat flour with powdered dry yeast (19:1) (Park, 1962; Zyromska-Rudzka, 1966) in an incubator at 30 °C ± 0.5 °C without light and humidity control for continuous supply of adults. The test insects used in the present investigation were collected from the stock cultures of the Crop Protection Laboratory, Department of Zoology, University of Rajshahi.

2.3 Bioassay techniques

2.3.1 Brine shrimp lethality

For the lethality response by brine shrimp nauplii a certain concentration of the extracts of leaves and stem of *F. racemosa* and stem of *M. charantia* were selected through *Ad Hoc* experiments by adding 2mg (diluted by using DMSO) of each of the extracts diluted separately in 10ml of seawater to apply in 20cc test-tubes and by increasing or decreasing the amount of extracts in a repeated manner until the suitable mortality range was obtained. Selected doses were ranged between 100 to 6.25ppm for the final experimentation. Observation of mortality was made after 6h of application with 6 hours interval up to 30 hours. The series of concentration were 500, 400, 300, 200, 100, 50 ppm; 400, 200, 100, 50, 25, 12.5 ppm and 500, 400, 300, 200, 150, 100 ppm for Pet. ether, $CHCl_3$ and CH_3OH extracts respectively. Ten freshly hatched nauplii were released to each of the test tubes with different concentrations mentioned earlier and the mortality was observed after 6, 12, 18, 24 and 30h of exposures. The data was then subjected to probit analysis.

2.4 Dose-mortality tests

It was revealed in the *Ad Hoc* experiments that the test extractives showed efficacy against the rust-red flour beetle, *T. castaneum* and were subject to dose-mortality tests.

2.4.1 Dose-mortality on *T. castaneum*

For the dose-mortality response through surface film method several doses of the leaves and stems of *F. racemosa* and stems of *M. charantia* were selected by putting 50mg of each of the extracts diluted separately in 1ml of solvent to apply in 50mm Petri dishes and by increasing or decreasing the amount of extracts in a repeated manner until the suitable mortality range was obtained. The doses selected for the final experiment were ranged between 4.074 to 2.547mg cm^{-2}. Each of the doses were diluted in 1ml of solvent, poured into a Petri dish and allowed to dry out. Ten adult beetles were released in each of the Petri dishes and the experiment of all the doses for each of the extracts were set in triplicates. The mortality of the beetles was assessed at 12h intervals.

2.5 Statistical analysis

The mortality (%) was corrected using Abbott's formula [21].

$P_r = \frac{P_o - P_c}{100 - P_c} \times 100$; where, P_r = Corrected mortality (%), P_o = Observed mortality (%), P_c = Control mortality (%). The data were then subjected to Probit analysis according to Finney [22] and Busvine [23].

2.6 Repellent activity test against *T. castaneum* adults

The methodology for repellency test used in the experiment was adopted from the method (No. 3) of McDonald *et al*. [24] with some modifications. Half filter paper discs (Whatman No. 40, diameter 9 cm) were treated with the selected doses of 0.079, 0.039, 0.020, 0.010 and 0.005mg cm^{-2} of crude extracts. After selection of a general effective concentration other successive doses were prepared through serial dilution. For the application of insects half filter paper discs were prepared and selected doses of all the extracts separately applied onto each of the half-disc and allowed to dry out as exposed in the air for 10 minutes. 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 (9cm). Ten adult insects were released in the middle of each filter-paper circle. The orientation was changed in the 2 remaining replicates to avoid the effects of any external directional stimulus affecting the distribution of the test insects. The same was done for each of the doses at least in tree replicates. Observations were made five times with 1h interval. The average of the counts were converted to percentage repellency (*PR*) using the formula of Talukder and Howse: [25-26] *PR* = (Nc - 5) × 20; where Nc is the average hourly observation of insect on the non-treated half of the disc. Positive values expressed repellency and negative values for attractant activity.

3. Results

3.1 Lethal action on Brine Shrimp

The Pet. ether, $CHCl_3$ and CH_3OH extracts of *F. racemosa* (leaf and stem) and $CHCl_3$ and CH_3OH extracts of *M. charantia* (stem) were found effective against the brine shrimp nauplii represented in Table-1. However, the Pet.E. extract of *M. charantia* stem didn't offer any mortality. For the Pet. ether extract of *F. racemosa* leaf and stem the lethality against *Artemia salina* nauplii were LC_{50} 72.62, 38.78, 20.47 and 16.78ppm and 1802.91, 137.67, 57.14 and 27.33ppm for 12, 18, 24 and 30h of exposure respectively. For $CHCl_3$ extract the were 218.67, 187.76, 38.96 and 15.20ppm and 191.94, 62.15, 39.85 and 23.84ppm respectively; while for the CH_3OH extracts the LC_{50} values were -, 525.15, 107.71 and 41.67ppm and 47.83,41.42, 26.91 and 20.20ppm for 12, 18, 24 and 30h nof exposure respectively. For Pet. ether extract of *M. charantia* stem no mortality was recorded, however the $CHCl_3$ and CH_3OH of the same offered LC_{50} values 69.58, 25.00, 23.02 and 16.20ppm and 91.83, 28.15, 20.18 and 15.22ppm respectively for 12, 18, 24 and 30h of exposure respectively.

Table 1: LC_{50} values of Pet.E., $CHCl_3$ and CH_3OH extracts of *F. racemosa* (leaf and stem) and *M. charantia* (stem) against *A. salina* nauplii.

Solvents	Plant parts	Duration of exposure			
		12h	18h	24h	30h
Pet.E.	*F. racemosa* (Leaf)	72.63	38.78	20.47	16.78
	F. racemosa (Stem)	1802.91	137.67	57.14	27.33
	M. charantia (Stem)	-	-	-	-
$CHCl_3$	*F. racemosa* (Leaf)	218.67	187.76	38.96	15.20
	F. racemosa (Stem)	191.94	62.15	39.85	23.84
	M. charantia (Stem)	69.58	25.00	23.02	16.20
CH_3OH	*F. racemosa* (Leaf)	-	525.15	107.71	41.67
	F. racemosa (Stem)	47.83	41.42	26.91	20.20
	M. charantia (Stem)	91.83	28.15	20.18	15.22

Note: (-) no mortality recorded.

3.2 Dose mortality effects on *T. castaneum*

The same extracts were subjected to dose-mortality assay against the flour beetle *T. castaneum* adults; while the $CHCl_3$ extracts of *F. racemosa* (leaf and stem) and *M. charantia* (stem) were found effective as mentioned in Table 2; however, the Pet. ether and CH_3OH extracts didn't show any insecticidal activity. The activity could be arranged in a descending order of *F. racemosa* (stem) > *M. charantia* (stem) > *F. racemosa* (leaf) extracts. No acute toxicity was recorded at all.

Table 2: LD_{50} values of the $CHCl_3$ extracts of *F. racemosa* (leaf and stem) and *M. charantia* (stem) against *T. cstaneum* adults

Plant parts	Duration of exposure						
	½h	12h	24h	36h	48h	60h	72h
F. racemosa (Leaf)	-	-	32.27	4.14	3.64	3.34	3.05
F. racemosa (Stem)	-	-	7.57	5.21	3.84	2.65	1.72
M. charantia (Stem)	-	-	5.97	4.99	3.82	3.09	2.89

Note: (-) no mortality recorded.

3.3 Repellent effects on *T. castaneum* adults

For the repellent activity test against the adult beetles of *T. castaneum* the same extracts were subjected. The highest but moderate repellent activity was observed for the $CHCl_3$ extract of leaves of *F. racemosa* (P<0.01) and the stem of *M. charantia* (P<0.01), followed by the CH_3OH extract of leaves of *F. racemosa* (P<0.05); while the Pet.E. extract of leaf and stem, and $CHCl_3$ extract of stem of *F. racemosa* and CH_3OH extracts of stem of *M. charantia* didn't offer any significant repellent activity(Table3 and 4). According to intensity of repellency the result could be arranged in a descending order: *F. racemosa* leaves ($CHCl_3$) > *M. charantia* stem ($CHCl_3$) > *F. racemosa* leaves (CH_3OH).

Table 3: ANOVA results of repellency of the Pet. E., $CHCl_3$ and CH_3OH leaf and stem extracts of *F. racemosa* and stems of *M. charantia* against *T. castaneum* adults

Types of extract			Sources of variation (df)			F-ratio with level of significance		P-value	
Plant parts		Solvents	Between doses	Between time intervals	Error	Between doses	Between time intervals	Between doses	Between time intervals
F. racemosa	Leaf	Pet.E.	4	4	16	2.334	0.566	0.099	0.691
		$CHCl_3$	4	4	16	31.974**	2.548	1.89E-07	0.079
		CH_3OH	4	4	16	13.723*	1.065	4.84E-05	0.406
	Stem	Pet.E.	4	4	16	7.957	0.673	0.001	0.620
		$CHCl_3$	4	4	16	4.983	0.166	0.008	0.953
		CH_3OH	4	4	16	4.915	0.319	0.009	0.861
M. chara-ntia	Stem		Not attempted (less amount of extract)						
		$CHCl_3$	4	4	16	19.161**	0.720	6.03E-06	0.591
		CH_3OH	4	4	16	0.771	0.744	0.559	0.576

** = Significant at 1% level ($P<0.01$) * = Significant at 5% level ($P<0.05$)

Table 4: Repellent effect of $CHCl_3$ and CH_3OH leaf and stem extracts of *F. racemosa* and stem extract of *M. charantia* against *T. castaneum* adults

Test material	Solvents	Between doses (df=4)		Between time interval	
		F- values	Level of significance	F- values	Level of significance
F. racemosa leaf	$CHCl_3$	31.974**	$P<0.01$	2.548	-
F. racemosa leaf	CH_3OH	13.723*	$P<0.05$	1.065	-
M. charantia stem	$CHCl_3$	19.161**	$P<0.05$	0.720	-

** = Significant at 1% level ($P<0.01$) * = Significant at 5% level ($P<0.05$); (-) = Not significant at any level.

4. Discussion

The present results revealed that these two selected medicinal plants *viz. F. racemosa* and *M. charantia* contain components of cytotoxicity, insecticidal and repellent activity. The findings on brine shrimp lethality through this investigation are supported by Houngbeme *et al.* (2014) [27] that revealed the dichloromethane extract from *M. charantia* were highly toxic against *A. salina* nauplii. The extracts of *Sclerocaria birrea*, *Ipomae repens*, *Momordica charantia*, *Borahaavia diffusa* and *Nauclea uculeata* showed remarkable toxicity on the brine shrimp larvae at LC_{50} values less than 60 μg/ml (Adoum, 2009) [28] A recent study carried out by Gavhane *et al*. (2016) [29] to investigate the *in vitro* cytotoxicity and anticancer activity of *F. racemosa* on MCF7 human breast cancer cell line. Effect of ethanolic extracts of tender fruits of *F. racemosa* on MCF7 human breast cancer cell lines by Sulphorodamine B (SRB) assay was carried out. Three observations *viz.* LC_{50}, TGI, GI 50 were recorded. The absorbance was recorded on an Elisa plate reader at a wavelength of 540 nm with 690 nm. *F. racemosa* showed LC_{50}, TGI and GI50 activity at ≥ 80 μg/ml concentration. Thus, it can be concluded that *F. racemosa* fruit extract has some cytotoxic and anticancer activity (*in vitro*) at ≥ 80 μg/ml concentration of plant extract on MCF7 human breast cancer cell line. In another study conducted by Khan *et al*. (2017) [30]where reported *F. racemosa* has been found to have the significant antioxidant activity in a dose-dependent manner and IC_{50} value was 150 μg/ml for DPPH and 100 μg/ml for both NO and SO scavenging assays. Further, the cytotoxicity analysis was determined against Dalton Lymphoma Ascites (DLA) cell line and the IC_{50} value was found to be 175 μg/ml for ethanolic leaf extract of *F. racemosa.* The findings through insecticidal activity tests receive supports from Rani and Devanand (2011) [31] who established that the leaf extracts of *M. charantia* exhibited considerably significant mortality against *T. castaneum* adults. Narasimhan *et al.* (2005) [32] also observed that pulp of *M. dioica* show antifeedant effect against *S. litura* larvae. Molluscidal and mosquito larvicidal efficacy of *M. charantia* has already been reported by Srivastava, *et al.* (2007) [33] and Sing, *et al*. (2006) [34]. No such reports have been reported so far on dose mortality activity of *F. racemosa* extract especially against *T. castaneum* adults. The findings on insect repellency in this investigation receive supports from Dwivedi and Shekhawat (2004) [35] who established that the acetone extract of *M. charantia* offered 74.87% repellency, and Pet.E. Extract of the same exhibited 74.47% repellency. Akhter (1997) [36] reported that the leaf extract of Bitter Ggourd showed moderate repellent effects on granary weevil. No such report havs been admitted so far on repellent activity of *F. racemosa* extract especially against *T. castaneum* adults.

5. Conclusion

A comprehensive phytochemical analyses of the test plants for their lethal, insecticidal and repellent components, as well as the physiological studies of the active ingredients are very much to be solicited for their effective use in the future pest control and pharmaceutical endeavors.

6. Acknowledgements

The authors are grateful to the University Grants Commission (UGC) of Bangladesh. They would like to extend thanks to the Chairman, Department of Zoology, University of Rajshahi, for providing laboratory facilities

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