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Biological activities of *Amoora rohituka* Roxb. Leaf extracts through dose-mortality, insect repellency, cytotoxicity, larvicidal and antimicrobial assays

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Abstract
Biological activities of Petroleum ether (Pet. ether), $CHCl_3$, CH_3OH and EtOAc extracts of *Amoora rohituka* leaf were tested against the red flour beetle *Tribolium castaneum* (Hbst.) adults for dose-mortality and insect repellent activity; against eggplant aphid *Aphis gossypii* Glover for repellent activity; against brine shrimp *Artemia salina* L. nauplii for cytotoxicity; against larvae of *Culex quinquefasciatus* Say for larvicidal activity; and against five bacteria and fungi for anti-microbial activity. The dose-mortality observed for Pet. ether, EtOAc and $CHCl_3$ extracts with LD_{50} values 1.769, 1.049, 0.871 and 0.249mg cm^{-2}; 2.099, 1.047, 0.927 and 0.819mg cm^{-2}; and 2.847, 2.126, 2.053, 1.806mg cm^{-2} for 12, 24, 36 and 48h of exposure respectively, whereas CH_3OH extract did not show any mortality against the adult beetles of *T. castaneum*. The $CHCl_3$, CH_3OH and EtOAc extracts showed promising repellent activity against *T. castaneum* adults at $P<0.001$, $P<0.01$ and $P<0.05$ levels of significance respectively, while the Pet. ether extract did not show repellent activity at all. Against eggplant aphid CH_3OH extract showed strong repellent activity ($P<0.001$) while EtOAc and $CHCl_3$ extracts were found moderately active ($P<0.01$) whereas Pet. ether extract did not show any repellency. The similar extracts responded through Brine shrimp lethality assay where CH_3OH extract found most effective (LD_{50} values 1302.933, 343.255, 295.546ppm and 100% mortality observed after 24h exposure) followed by Pet. ether extract (LD_{50} 245.577, 279.807, 89.122 and 39.884ppm), EtOAc extract (LD_{50} values 3796.668, 224.936, 155.737 and 57.643ppm) and $CHCl_3$ extract (LD_{50} -, 749.721, 366.104 and 62.413ppm) for 6, 12, 18, and 24h of exposure respectively. In case of larvicidal activity test against 1st instar larvae *Culex quinquefasciatus* the highest lethality perceived for Pet. ether extract (LC_{50} 340.501, 268.464, 118.579, 53.279 and 33.261ppm) followed by $CHCl_3$ extract (LC_{50} 2605.863, 1045.340, 566.034, 211.233 and 137.335ppm) and EtOAc extract (LD_{50} 551.542, 506.756, 384.259, 239.025 and 162.077ppm) for 6, 12, 18, and 24h exposure respectively; while CH_3OH extract did not show any activity. These four extracts were also propitious against five pathogenic bacteria (*Agrobacterium* sp., *Bacillus cereus*, *Escherichia coli*, *Shigella dysenteriae* and *Staphylococcus aureus*) at concentrations of 200 and 400µg/disc while a standard Penicillin 30µg/disc and three pathogenic fungi (*Aspergillus niger*, *Candida* sp. and *Saccharomyces* sp.) at concentrations of 50 and 200µg/disc with a standard Nystatin 10µg/disc were also used for comparison. CH_3OH extract showed the highest antibacterial activity with the inhibition zone of 19mm and 30mm respectively against *E. coli* bacteria in comparison to others while EtOAc extracts showed the highest antifungal activity with the inhibition zone of 12mm and 21mm respectively against *Candida* sp. The results revealed the potentiality of *A. rohituka* leaf extracts for the control of flour beetles, aphids, mosquito larvae, bacteria and fungi.

Keywords: *Amoora rohituka*, dose-mortality, repellency, cytotoxicity, larvicidal activity, antimicrobial activity

1. Introduction
Pesticides represent the only group of chemicals that are purposely applied to the environment with an aim to suppress pests of plants and animals and to protect agricultural and industrial products. The use of pesticides by Bangladeshi farmers increased by 328 percent during the past 10 years, posing a serious health hazards on human being due to its long-term residual effect, according to a study released by Bangladesh Rice Research Institute [1]. Excessive usage of pesticides in agriculture to overcome the pre-harvest and post-harvest problem was resulted in many toxic epidemics. Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs. Plant products still remain the principal source of pharmaceutical agents used in traditional medicine [2, 3]. Medicinal plants represent a rich source of antimicrobial agents [4]. Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicine [5].

A. rohituka Roxb. (Meliaceae) is a traditional medicinal plant used extensively in Asian countries. Its synonym is *Aphanamixis polystachya*. It has been shown the high potential and the elevate number of secondary compounds and their medicinal properties. It is an aboriginal of most of the hotter parts of India, as well as the lowlands and hill forests of Bangladesh, Malay and Ceylon [6, 7]. It is also useful in chronic malarial fevers and leucorrhoea. Constipation is a prominent feature [8]. The production of rohitukine from *Amoora rohituka* possesses anti-inflammatory, anti-cancer and immuno-modulatory properties [9]. Rohitukine is an important precursor for the synthesis of potential anticancer drugs flavopiridol (Sanofi-Aventis) and P-276-00 (Piramal Healthcare Limited, Mumbai, India) [10]. Rohitukine was indeed found to be an antiadipogenic molecule [9, 11] reported that Methyl-amoorain (methyl-25-hydroxy-3-oxoolean-12-en-28-oate, AMR-Me) exerts a striking chemopreventive effect against 7, 12-dimethylbenz (*a*) anthracene (DMBA)-induced rat mammary tumorigenesis to achieve breast cancer chemoprevention. Bark of *Amoora* appears to be an effective immuno-suppressive drug similar to prednisolone. A 50% ethanolic extract of the stems showed anti-cancerous activity [12]. In another experiment it was mentioned that the methanol extract of *Amoora rohituka* bark has showed a pronounced DPPH radical scavenging activity comparable to ascorbic acid, a standard antioxidant drug [13]. The ethanol extract of *Amoora rohituka* stem bark showed Cytotoxicity against MCF-7 cell lines derived from human mammary adenocarcinoma [14]. The seeds are acrid with sharp taste; refrigerant, laxative, anthelmintic [12, 15] cures ulcers, diseases of the blood, eye and ear; lessen muscular pain and have insecticidal and antifeedant activity [16]. The plant pacifies liver disorders, tumor, ulcer, dyspepsia, intestinal worms, skin diseases, diabetes, eye diseases, jaundice, hemorrhoids, burning sensation, rheumatoid arthritis and leucorrhoea [17]. However, information on its biological activity, as well as of phytochemical information is still scanty. This is why the plant was taken into consideration for investigating of its bioactive potentials.

2. Materials and Methods

2.1 Collection and preparation of test materials

The fresh leaves of *A. rohituka* were collected from Rajshahi University campus, identified and kept in the herbarium of the Department of Botany, University of Rajshahi. The leaves were chopped into small pieces, dried under shade and powdered using an electric grinder, weighted and placed in separate conical flasks to add Pet. ether, EtOAc $CHCl_3$ and CH_3OH (Merck, Germany) (100gm × 300ml × 2times) for 48h. Filtration was done by Whatman filter paper (made in USA) at 24h interval in the same flask followed by evaporation until the extract was left. The extracts were then removed to glass vials and preserved in a refrigerator at 4 ^{0}C with proper labeling.

2.2 Collection and culture of the test insects

Tribolium castaneum used in the present experiment was reared in glass beakers (500 ml) in a standard mixture of whole-wheat flour with powdered dry yeast (19:1) [18] in an incubator at 30°C ±0.5°C without light and humidity control for continuous supply of adults.

Aphids are very soft and tiny creature and have highly reproducing capability. At first some mature aphids were collected from affected plants and released on the new fresh eggplants for further production. They multiply in a good number within short time. Aphids were collected repeatedly from the culture field with a fine camel hair-brush in a Petri dish and used in the experiments.

2.3 Dose-mortality test against *T. castaneum*

The dose-mortality responses of *A. rohituka* were observed by surface film method. The concentrations used were 2.037, 1.528, 1.019, 0.509, 0.255mgcm^{-2}; 3.055, 2.546, 2.037, 1.528mgcm^{-2} and 2.037, 1.528, 1.018, 0.509, 0.255mgcm^{-2} for Pet. ether, $CHCl_3$ and EtOAc extracts respectively. No mortality has been observed for CH_3OH extract against the beetles from the *'Ad Hoc" experiments*. Each of the doses were diluted in 1ml of solvent, poured into Petri dishes and allowed to dry. Ten adult beetles were released in each Petri dish, and the experiment of all the doses for each of the extracts were replicated three times. The mortality of the beetles was assessed after 12, 24, 36, 48, 60 and 72h of exposures.

2.4 Statistical analysis

The mortality (%) was corrected using Abbott's formula [19]:

$P_r = \frac{P_o - P_c}{100 - P_c} \times 100$; Where, P_r = Corrected mortality (%), P_0= Observed mortality (%), P_c= Control mortality (%). The data were then subjected to Probit analysis according to Finney and Busvine to calculate the LD_{50} values [20, 21].

2.5 Repellent activity test against *T. castaneum* adults and eggplant aphids

The methodology for repellency test used in the experiment was adopted from the method (No. 3) of McDonald *et al*. [22] with some modifications by Talukder and Howse [23, 24]. For *T. castaneum* half filter paper discs (Whatman No. 40, diameter 9 cm) were treated with the selected doses 0.314, 0.157, 0.079, 0.039 and 0.019mgcm^{-2} concentrations for Pet. ether extract and then were attached lengthwise, edge-to-edge, to a control half-disc with adhesive tape and placed in Petri dishes. Ten adult *T. castaneum* were released in the middle of each of the filter paper discs. For aphids instead of petri dish fresh eggplant leaves were used. Stalks of each of the leaves were wetted with water soaked cotton to keep them fresh. A CD marker was used to draw round circle (3.6 cm diam.) on leaves. A general concentration for each of the plant extracts was selected as stock dose for repellency and other successive doses were prepared by serial dilution to give 0.079, 0.039, 0.019, 0.009 and 0.004mgcm^{-2} concentrations for Pet. ether extract. Ten aphids were released in the middle of each of the leaves 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 process was done for $CHCl_3$, CH_3OH and EtOAc extracts.

2.6 Observation and analysis of repellency data

Repellency was observed for one-hour interval and up to five successive hours of exposure, just by counting the number of insects from the non-treated part of the filter paper spread on the floor of the 90mm Petri dish (for *T. castaneum*) and non-treated part of the restricted circle (3.6 cm) on eggplant leaf. The average of the counts was converted to percent repellency (*PR*) using the formula of Talukder and Howse: *PR* = (Nc-5) × 20, where, Nc is the percentage of insects on the untreated half of the disc or circle [23, 24].

2.7 Brine shrimp nauplii lethality test

Brine shrimp eggs were purchased from Kalabagan, Dhaka and kept in aerated sea water at room (25-30°C) temperature and they took 30-48h to give nauplii. The series of concentration were 100, 50, 25, 12.5 and 6.25ppm for Pet. ether and EtOAc extracts, 200, 100, 50, 25 and 12.5ppm for CH_3OH extract and 400, 200, 100, 50 and 25ppm for $CHCl_3$ extract. Ten freshly hatched nauplii were added to each of the test tubes with different concentrations mentioned earlier and observed mortality after ½, 6, 12, 18 and 24h of exposures. The data was then subjected to Probit analysis.

2.8 Larvicidal activity test

To perform larvicidal activity mosquito rafts (eggs) were collected from different drains of Rajshahi University then placed in a new beaker containing normal pond water and kept in a dark place inside the laboratory for hatching. Hatched larvae were collected after 24h and used in the experiment. The plant extracts were dissolved in 10µl of DMSO for its solubility in water. Ten freshly hatched larvae were treated with the plant extracts in doses 200, 100, 50, 25 and 12.5ppm for Pet. ether extract and 400, 200, 100, 50 and 25ppm for $CHCl_3$ and EtOAc extracts and mortality was observed after 6, 12, 18, 24 and 30h of exposure. The data was then subjected to Probit analysis.

2.9 Growth and maintenance of test microorganism for antimicrobial studies

Strains of bacteria and fungi, were obtained from Department of Pharmacy, University of Rajshahi, Bangladesh, were used for antimicrobial test. The bacteria were maintained on nutrient broth (NB) at 37°C and fungi were maintained on Potato dextrose agar (PDA) at 28°C.

2.10 Anti-bacterial Activity

Pet. ether, $CHCl_3$, CH_3OH and EtOAc extracts of *A. rohituka* leaves were tested by the disc diffusion method. Two concentrations (200 and 400µg) of the extracts were prepared by reconstituting with required solvents. The test microorganisms were seeded into respective medium by spread plate method with 24h cultures of bacteria growth in nutrient broth. After solidification the filter paper discs (5 mm in diameter) impregnated with the extracts separately were placed on test organism-seeded plates. *Agrobacterium* sp., *Bacillus cereus, Escherichia coli, Shigella dysenteriae and Staphylococcus aureus* were used for antibacterial test. Penicillin (30µg/disc) was used as Standard. The antibacterial assay plates were incubated at 37°C for 24h. The diameters of the inhibition zones were measured in mm.

2.11 Antifungal Activity

Antifungal activity was tested by disc diffusion method. Potato dextrose agar plates were inoculated with each fungal culture (10 days old) by point inoculation. Filter paper discs (5mm in diameter) impregnated with two (50 and 200µg/disc) concentrations of the extracts were placed on test organism-seeded plates. Solvents were used to dissolve the extracts and were completely evaporated before application on test organism-seeded plates. Nystatin (10µg/disc) were used as standard. The activity was determined after 24-48h of incubation at 28°C. Diameters of the inhibition zones were measured in mm.

3. Results and Discussion

3.1 Bioassay on *T. castaneum* adults

In the present study the highest and the lowest mortality (LD_{50} 0.174mgcm^{-2}) and (LD_{50} 0.843mg cm^{-2}) have been observed for EtOAc and $CHCl_3$ extracts after 72h of exposure against the adult beetles while CH_3OH extract of *A. rohituka* leaf did not show any mortality. The efficacy of the extracts could be arranged in a descending order of EtOAc> Pet. ether> $CHCl_3$ extract of the test plant. The results of dose-mortality assay of Pet. ether, $CHCl_3$ and EtOAc extracts of *A. rohituka* leaves are represented in Table 1. These findings receive supports from the previous researchers' works. Guruprasad *et al.* demonstrated that among methanol, petroleum ether and ethyl acetate solvent extracts of *Clerodendron inerme* leaf, methanol extract exhibited strong repellent effect to the red flour beetle [25]. Methanolic extract of *Clerodendron inerme* leaf offered dose-mortality action against *T. castaneum*, *R. dominica* and *Callosobruchus chinensis* adults. Roly *et al.* showed the presence of insecticidal properties of Pet. ether, $CHCl_3$ and CH_3OH extracts of *Rauvolfia canescens* (whole plant), *Desmodium heterocarpon* (whole plant) and leaf extract of *Vitex negundo* as well as traces of repellent potential against adult beetles of *T. castaneum* [26]. For repellency *D. heterocarpon* (Pet. ether) extract and *V. negundo* (leaf /Pet. ether) extract were weakly active (*P <0.05*) and *R. canescens* (whole plant /Pet. ether) extract and *V. negundo* (root /Pet. ether) extract were mildly active (*P<0.01*). Similar results were also concluded by Sagheer *et al.* (2011, 2013) while working on the effects of different repellent plant extracts towards the repellent action [27, 28]. $CHCl_3$ extracts (leaf, root and stem extracts) of *Urena sinuate* showed high toxicity and repellent activity for root and stem extracts against *T. castaneum* adults were at *P<0.01* and *P<0.05* levels of significance respectively Abdulla *et al*. [29]. Our experiments proved a significant impact of plant extracts on the test insect that cause damage to processed and stored commodities.

Table 1: LD_{50} values of Pet. ether, $CHCl_3$, and EtOAc extracts of *A. rohituka* leaf against *T. castaneum* adults.

Type of extract	LD_{50} (mgcm^{-2}) at different exposures (in hours)			
	12	24	36	48
Pet. ether	1.769	1.049	0.871	0.249
EtOAc	2.099	1.047	0.927	0.819
$CHCl_3$	2.847	2.126	2.053	1.806
CH_3OH	NA	NA	NA	NA

3.2 Repellent effects against *T. castaneum*

EtOAc, $CHCl_3$ and CH_3OH extracts of *A. rohituka* leaves offered a promising repellent effects against *T. castaneum* adults at level of significance *P<0.05*, *P<0.001* and *P<0.01* respectively; whereas Pet. ether extract did not show any repellency. The data are given in Table 2.

Table 2: Repellency effect of Pet. ether, EtOAc, $CHCl_3$ and CH_3OH and extracts of *A. rohituka* leaves against *T. castaneum* adults.

Types of extract	Between doses (df = 4)		Between time interval (df = 4)	
	F-value	Level of significance	F-value	Level of significance
Pet. ether	5.394	-	0.368	-
EtOAc	14.942	*P<0.05*	3.337	-
$CHCl_3$	27.749	*P<0.01*	1.398	-
CH_3OH	22.573	*P<0.01*	0.162	-

3.3 Repellent effect against eggplant aphid

Present study revealed that CH_3OH extract of *A. rohituka* leaves showed strong repellent activity (*P<0.001*) while EtOAc and $CHCl_3$ extracts of *A. rohituka* leaves were found moderately active (*P<0.01*) against eggplant aphids (*A. gossypii*) whereas Pet. ether extracts of *A. rohituka* leaves did not show any repellency. This study is a preliminary investigation in aphid control and more studies are needed to bioassay the activity of specific compounds against aphid species and other pests. Results of the repellency effect of Pet. ether, $CHCl_3$, CH_3OH and EtOAc extracts of *A. rohituka* leaves are represented in Table 3. Crude leaf extract of *T. minuta* and *T. vogelii* was significantly repellent against red spider mites with the level of significance (Fisher's LSD test *P< 0.05*) [30]. Among three solvent extracts $CHCl_3$, CH_3OH extracts of *Phyllanthus niruri* offered a promising repellent effect against eggplant aphids at the level of significance *P<0.01* and *P<0.05* respectively while Pet. ether extract did not offer repellent activity [31].

Table 3: Repellency effect of Pet. ether, EtOAc, $CHCl_3$ and CH_3OH extracts of *A. rohituka* leaves against eggplant aphid.

Types of extract	Between doses (df = 4)		Between time interval (df = 4)	
	F-value	Level of significance	F-value	Level of significance
Pet. ether	8.672	-	2.024	-
EtOAc	39.756	P<0.01	1.621	-
$CHCl_3$	29.672	P<0.01	0.819	-
CH_3OH	104.722	P<0.001	0.734	-

3.4 Brine shrimp lethality effect

This experiment as a lethality test of *A. rohituka* leaves extracts on Brine shrimp nauplii is in agreement with the above studies where CH_3OH extract offered 100% mortality after 24h of exposure, followed by Pet. ether, EtOAc and $CHCl_3$ extracts. These suggest that brine shrimp bioassay is simple, reliable and convenient method for assessment of bioactivity of medicinal plants and that the four extracts of *A. rohituka* leaf contains useful potent bioactive compounds that can be harnessed and purified into useful therapeutic drugs**.** Brine shrimp lethality results and LC_{50} values obtained are shown in and Table 4. Juzavil *et al*. showed that absolute ethanol extracts of *Phyllanthus niruri* and *Passiflora foetida* were toxic after 24h of exposure against *A. salina* with LC_{50} values 251.19µg/ml and 749.89µg/ml respectively [32]. Jeda *et al*. reported that ethanol extract showed toxicity effect after 6h and 24h exposures with LC_{50} values 944.07 and 266.07ppm respectively [33]. In comparison to ampicillin trihydrate (LC50: 7.21 ± 0.47 µg/mL) used as positive control, the cytotoxicity exhibited by compound-1 was promising with the LC50 values of 15.26 ± 0.57 µg/mL whereas the LC50 of compound-2 was 27.67 ± 0.40 µg/mL [34]. Acetone extract of *A. arctotoides* and hexane extracts of *A. arctotoides* and *G. bicolor* exhibited significant brine shrimp lethality with LC_{50} values 0.87, 0.89 and 0.82mg/ml, respectively [35]. Pet. ether, $CHCl_3$ and CH_3OH extracts of *S. nodiflora* showed lethality effect against brine shrimp with LC_{50} values 140.866, 22.161 and 248.325ppm for 30h of exposures respectively [36].

Table 4: LC_{50}values of Pet. ether, EtOAc $CHCl_3$ and CH_3OH extracts of *A. rohituka* against *A. salina* nauplii.

Type of extract	LC_{50} values (ppm) at different exposures (in hours)			
	6	12	18	24
Pet. ether	279.807	245.577	89.122	39.884
EtOAc	3796.668	224.936	155.737	57.643
$CHCl_3$	NA	749.721	366.104	62.413
CH_3OH	1302.933	343.2552	295.546	All dead

3.5 Larvicidal activity effect

Results of this experiment indicate that the extracts of *A. rohotuka* leaves except CH_3OH can be used as potential larvicide in vector control programs as field application of these extracts can be done. The larvicidal activity for Pet. ether, $CHCl_3$, CH_3OH and EtOAc extracts of *A. rohituka* against *C. quinquefasciatus* represented in Table 5. Similar findings were found in the findings of the previous researchrs. LC_{50} values of methanol extract of *Murraya exotica* and *Lawsonia inermis* against 3rd and 4th instar larvae and pupae were 135.539, 154.361, 178.571ppm and 139.057, 163.630, 188.151ppm respectively after 24h of exposure [37]. Naser *et al*. (2014) revealed that Pet. ether, $CHCl_3$ and CH_3OH extracts of *Phyllanthus niruri* offered cytotoxic activity against *Culex quinquefasciatus* with LC_{50} values 3.39, 3.42 and 259.86ppm after 30h of exposure respectively [38]. Resent study was assessed for larvicidal activity test of four solvent extracts of *A. rohotuka* leaves against *Culex quinquefasciatus* where Pet. ether extract showed highest activity with LC_{50} values 33.261ppm, followed by $CHCl_3$ and EtOAc extracts with LC_{50} values 137.335 and 162.077ppm respectively after 30h of exposure; while CH_3OH extract did not show larvicidal activity.

Table 5: LC_{50} values of Pet. ether, $CHCl_3$ and EtOAc extracts of *A. rohotuka* leaf against *Culex quinquefasciatus* larvae.

Type of extract	LC_{50} values (ppm) at different exposures (in hours)			
	12	18	24	30
Pet. ether	268.464	118.579	53.279	33.261
$CHCl_3$	1045.340	566.034	211.233	137.335
CH_3OH	-	-	-	-
EtOAc	506.756	384.259	239.025	162.077

3.6 Effect of antimicrobial activity

The present study offered a promising result of four solvent extracts of *A. rohituka* leaves against five pathogenic bacteria and three pathogenic fungi. CH_3OH leaf extract of *A. rohituka*

leaves showed maximum antibacterial activity with the inhibition zone of 19 and 30mm for 200µg/disc and 400µg/disc application against *E. coli* bacteria and EtOAc extract of *A. rohituka* leaves exposed highest antifungal activity with the inhibition zone of 12 and 21mm for 50µg disc^{-1} and 200µg disc^{-1} application against *Candida* sp. in comparison with standard penicillin (30µg/disc) and standard Nystatin (10µg/disc) with inhibition zone 11mm and 7mm respectively. Some previous researchers revealed these potentials. The results of antimicrobial activity of *A. rohituka* leaves extracts against the selected bacterial and fungal strains are shown in Table 6 and 7.

Pathogenic bacteria like *Pseudomonas aeruginosa*, *Staphylococcus aureus* and fungus *Aspergillus niger* were inhibited in presence of ethanolic extract of *Hildegardia Populiolia* [39]. Methanol extracts of *Andrographis paniculata* and *Melia azadirach* were found with potential antimicrobial activity with the zone of inhibitions 22, 21, 14mm and 10, 14, 14mm respectively for 50µl disc^{-1} against *Pseudomonas aueruginosa, Vibrio cholera* and *Salmonella typhi* [40]. Methanolic extracts of *Amoora cucullata* stems and leaves showed potent antimicrobial activity against *Escherichia coli, Shigella dysenteriae, Salmonella typhi* and *Salmonella paratyphi* with the zone of inhibition 9, 8, 7, 10mm and 7, 9, 7, 9mm respectively in comparison with the standard Kanamycin(30µg/disc) with inhibition zone 13, 19, 20, 32mm [41]. Ethanolic extract of dried stem bark of *Aphanmixis polystachya* showed significant antimicrobial activity against *Pseudomonas auruginosa* and *Staphylococcus aureus* bacterial strains with the zone of inhibition 18 and 10mm respectively in comparison to kanamycin (20.5mm) which is a broad spectrum antimicrobial agent [42].

Table 6: Antibacterial activity of *A. rohituka* leaf extracts and standard Penicillin

Bacterial strains	Zone of inhibition (in mm)								
	Pet. ether		$CHCl_3$		CH_3OH		EtOAc		Standard Penicillin (30µg/disc)
	200µg	400µg	200µg	400µg	200µg	400µg	200µg	400µg	
Agrobacterium sp.	14	15	12	13	11	12	15	16	6
Bacillus cereus	12	14	11	12	6	8	13	15	6
Escherichia coli	14	17	12	13	19	30	12	14	11
Shigella dysenteriae	15	16	17	20	16	20	17	22	30
Staphylococcus aureus	12	17	11	12	14	18	21	23	16

Table 7: Antifungal activity of *A. rohituka* leaf extracts and standard Nystatin

Fungal strains	Zone of inhibition (in mm)								
	Pet. ether		$CHCl_3$		CH_3OH		EtOAc		Standard Nystatin (10µg/disc)
	50µg	200µg	50µg	200µg	50µg	200µg	50µg	200µg	
A. niger	6	6	10	13	6	7	10	11	7
Candida sp.	6	20	10	13	6	7	12	21	7
Saccharomyces sp.	7	10	11	15	6	7	8	12	7

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5. References

1. Islam ASM. Pesticide use in Bangladesh tripled 10 years. BRRI. Agriculture Economic Diversity. 2010. (http://news.agropages.com)
2. Carson R. Pesticide action network. Environmental effects of pesticides, Europe, 2010.
3. Ibrahim MB. Anti-microbial effects of extract leaf stem and root bark of *Anogeissus Leiocarpus* on *Staphylococcus aureus, Staphylococcus pyogenes, Escherichia coli* and *Proteus vulgaris*. Journal of Pharmaceutical Development. 1997; 2:20-30.
4. Ogundipe O, Akinbiyi O, Moody JO. Antibacterial activities of essential ornamental plants. Nigeria Journal of Natural Products & Medicine. 1998; 2:48-47.
5. Maheswaran R, Sathis S, Ignacimuthu S. Larvicidal activity of *Leucas aspera* (Wild) against the larvae of *Culex quinquefasciatus* Say and *Aedes aegypti*. International Journal of Integrated Biology. 2008; 2(3):214-217.
6. Mann A, Banso A, Clifford LC. An antifungal property of crude plant extracts from *Anogeissus leiocarpus* and *Terminalia avicennioides*. Tanzania Journal of Health Research. 2008; 10(1):34-38.
7. Kirtikar KR, Basu BD. Indian Medicinal Plants. Basu Publication, Allahabad, 1981: I:552.
8. Gupta M, Sarkar K, Baral R, Laskar S. Some chemical investigation of *Amoora rohituka*. Journal of Food Chemistry. 2010; 119(5):1057-1067.
9. Kumar V, Hart AJ, Wimalasena TT, Tucker GA, Greetham D. Expression of RCK2 MAPKAP (MAPK-activated protein kinase) rescues yeast cells sensitivity to osmotic stress. Journal of Microbial Cell Factories. 2015; 10(7):1-10.
10. Boericke W. New Manual of Homoeopathic Materia Medica with Repertory. Edn 9, M/s B. Jain Publishers (P) Ltd., New Delhi, 2007, 973.
11. Mandal A, Mandal S, Park MH. Genome-Wide Analyses and Functional Classification of Proline Repeat-Rich Proteins: Potential Role of eIF5A in Eukaryotic Evolution. Oral and Pharyngeal Cancer Branch, NIDCR, National Institutes of Health, Bethesda, Maryland, United States of America, 2014, 1-12.
12. Varshney S, Shankar K, Beg M, Balaramnavar VM. Rohitukine inhibits *in vitro* adipogenesis arresting mitotic clonal expansion and improves dyslipidemia *in vivo*. Journal of Lipid Research. 2015; 55(6):1019-1032.
13. Ramesh LL, Aruna LH, Amarvani PK. Potential Investigation of *in Vitro* Antioxidant, Anti-Inflammatory and Anti-Haemolytic Activities from Polar Solvent Extracts of *Pterocarpus marsupium*. International Journal of Pharmacognosy and Phytochemical Research. 2017; 9(1):100-107.
14. Mahajan A, Go MJ, Zhang W, Below JE, Gaulton KJ, Ferreira T *et al.* Production of rohitukine in leaves and

seeds of Dysoxylum binectariferum: an alternate renewable resource. Journal of Pharmacological Biology. 2015; 53(7):968-74.
15. Anonymous. The Useful Plants of India. Publication & Information Directorate, Council of Scientific & Industrial Research, New Delhi, 1986, 46.
16. Rabi T, Gupta RC, Gulati AK. Influence of *Amoora Rohituka* on MCF-7 Human Mammary Adenocarcinoma Cells *in Vitro*. Indian Journal of Pharmaceutical Science. 1994; 56(4):136-137.
17. Kritikar KR, Basu BD. Indian Medicinal Plants. International book distributers, Rajpur Road, Dehradun, Uttarakhand, 1999, 551.
18. Malviya S, Jain S, Patel R, Malviya V. Pharmacological review on *Amoora rohituka*. World Journal of Pharmaceutical Science. 2012; 1(1):7-16.
19. Abbott WS. A method of computing the effectiveness of an insecticide. Journal of Economical Entomology. 1925; 18:265-267.
20. Finney DJ. Probit analysis: a statistical treatment of the sigmoid response curve. Cambridge University Press, London, 1947, 333.
21. Busvine JR. A critical review of the techniques for testing insecticides. Commonwealth Agricultural Bureaux, London, 1971; 345.
22. McDonald LL, Guy RH, Speirs RD. Preliminary evaluation of new candidate materials as toxicants, repellents and attractants against stored-product insects. Marketing Research Report No. 882. Agricultural Research Service, US Department of Agriculture, Washington, DC, 1970.
23. Talukder FA, Howse PE. Deterrent and insecticidal effects of extracts of Pithraj, *Aphanamixis polystachya* (Meliaceae), against *Tribolium castaneum* in storage. Journal of Chemical Ecology. 1993; 19:2463-2471.
24. Talukder FA, Howse PE. Repellent, toxic and food protectant effect of Pithraj, *Aphanamixis polystachya* extracts against pulse beetle, *Callosobruchus chinensis* in storage. Journal of Chemical Ecology. 1994; 20:899-908.
25. Guruprasad BR, Pasha A. Biological Screening of *Clerodendron Inerme* Leaf Extracts for Repellency and Toxicity Potentials Against Stored Product Insrcts. Munis Entomology and Zoology Journal. 2015; 10(2):407-416.
26. Roly ZM, Islam MM, Shaekh MPE, Shahik SD, Moududee SA, Nur SM *et al*. Repellent Activities of Some Indigenous Medicinal Plants against the Stored Grain Pests Tribolium Castaneum (Hbst.) (Coleoptera: Tenebrionidae). International Journal of Pharmacognosy. 2014; 1(9):579-586.
27. Sagheer M, Hasan M, Latif MA, Iqbal J. Evaluation of some Indigenous medicinal plants as a source of toxicant, repellent and growth inhibitors against Tribolium castaneum (Coleoptera: Tenebrionidae). Pakistan Journal of Entomologist. 2011; 33:87-91.
28. Sagheer M, Hasan M, Rehman H, Khan FZA, Gul HT, Haidri SR *et al*. Growth Regulatory Potential of Five Indigenous Plant Extracts against Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae). International Journal of Biosciences. 2013; 3(12):50-54.
29. Abdullah M, Pk AK, Saleh DKMA, Islam R, Khan AR, Islam N. Insecticidal and repellent activities of the chloroform extracts of Urena sinuata L. against Tribolium castaneum (Herbst) adults. University Journal of Zoology, Rajshahi University. 2011; 30:25-28.
30. Park Beetles T. Competition and Populations. Journal of American Association for the Advancement of Science. 1962; 138:136-1375.
31. Zyromska-Rudzka H. Abundance and emigrations of *Tribolium* in a laboratory model. Polish Journal of Ecology. 1966; 14:491-518.
32. Juzavil J, Eddie M, Olga N, Mylene U. Effect of *Phyllanthus niruri* and *Passiflora foetida* extracts on the Mortality and Survival rate of the Brine Shrimp *Artemia salina.* Research Journal of Recent Science. 2015; 4(2):61-67.
33. Jeda A, Olga N, Mylene U. Brine Shrimp (*Artemia salina*) Bioassay of the medicinal plant *Pseudelephantopus spicatus* from Iligan City, Philippines. International Research Journal of Biological Science. 2014; 3(9):47-50.
34. Habib MR, Islam MAU, Karim MR. Cytotoxic Compounds from *Calotropis gigantea* (Linn.) and *Amoora rohituka* (Roxb.). International Biological and Biomedical Journal. 2016; 2(3):120-126.
35. Mmbone S, Mulaa M, Wanjala FM, Nyukuri RW, Cheramgoi E. Efficacy of *Tagetes minuta* and *Tephrosia vogelii* Crude Leaf Extracts on *Tetranychus urticae* (Acari: Tetranychidae) and *Aphis fabae* (Homoptera: Aphididae). International Journal of Scholastic Research Gate. 2015; 3(1):13-20.
36. Otang WM, Grierson DS, Ndip RN. Antifungal activity of *Arctotis arctotoides* (L.f.) *O. Hoffm*. And *Gasteria bicolor* Haw. Against opportunistic fungi associated with HIV/AIDS. Journal of Pharmacological Management. 2012; 80:135-140.
37. Moniruzzaman, Abdullah M, Islam N. Potentiation of *Synedrella nodiflora* L. for Insecticidal Activity, Insect Repellency and Brine Shrimp Lethality in the Laboratory Conditions. Journal of Life and Earth Science. 2012; 7:79-82.
38. Naser AN, Parvin N, Abdulla M, Khan AR, Nasirujjaman K, Islam N. Dose-mortality and Repellent activity of *Phyllanthus niruri* L. extracts against eggplant aphid, *Aphis gossypii* Glover and larvicidal activity against *Culex quinquefasciatus* Say. University Journal of Zoology, Rajshahi University. 2014; 33:71-76.
39. Dass K, Mariappan P. Larvicidal activity of *Lawsonia inermis* and *Murraya exotica* leaves extract on filarial vector, *Culex quinquefasciatus*. International Journal of Mosquito Research. 2014; 1(2):25-27.
40. Lakshmi CS, Pullaiah T. Phytochemical Screening and Antimicrobial Activities of a Medical Plant *Hildegardia populiolia*. International Journal of Plant and Animal Science. 2015; 5(1):107-110.
41. Nanthini AUR, Athinarayanan G, Ranjitsingh AJA, Mariselvam R, Chairman K, Narayanan KR. International Journal of Current Research. 2013; 5(11):3563-3566.
42. Yadav R, Chauhan NS, Chouhan AS, Soni VK, Omray L. Antimicrobial screening of various extracts of *Aphanamixis polystachya* stem bark. International Journal of Advance Pharmaceutical Science. 2010; 1:147-150.