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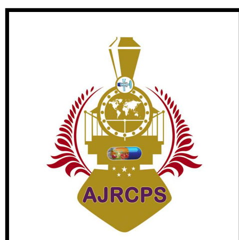

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ANTIMICROBIAL AND INSECTICIDAL ACTIVITIES OF *CORCHORUS CAPSULARIS* SEEDS EXTRACT

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ABSTRACT

Antimicrobial and insecticidal activities of different extractives (petroleum ether, chloroform, ethyl acetate, and methanol) as well as three unidentified compounds isolated from the seeds extract of *Corchorus capsularis* have been investigated. It was observed that all the fractions and the isolated compounds showed antimicrobial and insecticidal activities against four pathogenic bacteria *V.cholerae, Shigella* spp., *Salmonella* spp., *E.coli* and the insect *T. castaneum*. The antimicrobial activity test revealed that the crude methanol extracts have the highest antibacterial activity with inhibition zone of 26-27 mm and the isolated compounds showed the activity with zone inhibition of 20-15 mm. In the insecticidal activity test, the petroleum ether fraction showed the highest toxicity than that of the other fractions. The isolated compound also showed similar toxicity against *T.castaneum*. This study shows the promising potential of *Corchorus capsularis* in the perspective of the development of new therapeutic agents.

KEYWORDS

Corchorus capsularis, Insecticidal activity, *T. castaneum*, *V.cholerae*, *Shigella* spp. and *Salmonella* spp.

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INTRODUCTION

Corchorus capsularis (*CC*) known as "Jute" is an herbaceous and annually grown plant in Bangladesh. It has a long historical role in the socioeconomic development of Bangladesh. Once, Jute was considered as a golden fiber of Bangladesh and it was a good source of different grades of pulp[1]. Jute is a native plant of tropical Africa and Asia but also has been spread to Australia, South America and some parts of Europe. It has been grown extensively in India, Bangladesh, China, Myanmar and Nepal. The leaves are consumed as vegetables and used in

preparing different types of delicacies in different parts of the world[2]. The plant has an antioxidant activity with a significant α-tocopherol equivalent vitamin E. The leaves of ***CC*** have been claimed to possess stimulant, demulcent, laxative, appetizer and stomachic effects. The infusion of the leaves is traditionally used to treat fever, constipation, dysentery, liver disorders and dyspepsia[3]. The chloroform extract of *CC* has been found to contain significant anti-nociceptive activity and an inflammatory type of pain response[4]. Anti-tumor-bioactive components were isolated from the plant *CC*[5]. Cardiac glycosides, polysaccharides, water-soluble polysaccharides, tri-terpenes, phenolics and sterols have been reported from different parts of the plant[6-9]. Lipophilic extractive was isolated from *CC* fiber that was used for the high-quality paper pulp manufacturing[10]. Dammarane tri-terpene, capsugenin, was isolated from the plant[11]. Full-length cDNA coated as CCOAOMT and cDNA as designated *CcUGP* gene has been isolated[12, 13]. From the point of economical and medicinal importance, it is essential to perform in detail phytochemical, antimicrobial and insecticidal investigations of the plant *CC*. Although, enormous researches have been done on the bark, leaves, and roots of these plants which contain a number of bioactive components, but detail phytochemical, antimicrobial and insecticidal investigations on the seeds of *CC* have not been done well enough. From the point of view of finding the presence of bioactive constituents in different seed extract of *CC*, a chemical investigation on antimicrobial and insecticidal activity has been performed in this study.

METHODS AND MATERIALS

All chemicals used in this study were of analytical grade. Methanol, ethyl acetate, chloroform, petroleum ether were purchased from Merck Germany and Fluka. All the solvents were used further purification by distillation.

Seed collection and extraction process

The seeds of ***CC*** were collected from the local market during the month of May-June. The collected seeds were washed with clean water manually to remove extraneous materials and were dried in direct sunlight. The dried seeds were pulverized in an electric grinder and were stored in an airtight container. An airtight plastic container was used for the extraction process in which methanol was used as solvent. The mixture of *CC* seeds and methanol was kept for 24h and after then the resulting methanolic juice was decanted off from the container. The resulting juice was filtered through a Whatman filter paper and concentrated under reduced pressure at 45°C using the Buchi Rota-vapor (R-200). The crude extract was preserved in a refrigerator. The process was repeatedly done for another 10 times to increase the amount of crude extract to 791g. After completing the extraction process, methanol was removed by flowing air with constant stirring with a glass rod. This methanol free extract was then transferred in a 1 litre round bottom flask and was referred to as the mother liquor and was preserved in a refrigerator. Mother liquor was then triturated with petroleum ether, chloroform, ethyl acetate and methanol and all the triturates were subjected to the biological activity test. Compound-1, compound-2 and compound-3 were separated from the petroleum ether triturate using column chromatography with the solvent system ethyl acetate and petroleum ether in the ratio of 1:16, 1:8, and 1:4, respectively.

ANTIMICROBIAL ACTIVITY TEST

Four pathogenic bacteria *(i) V.cholerae (ii) Shigella* spp. *(iii) Salmonella* spp. *(iv) E.coli*. Were used in the antimicrobial activity test.

Sterilization

Antimicrobial screening was carried out in a laminar air flow unit. Precautions were highly maintained in order to avoid all types of contamination during the test. Petri dishes and other glass wares were sterilized by autoclaving at a temperature of 121°C and a pressure of 15 lbs/sq inch for 20 min. Nutrient agar medium was used in the study. The constituents of nutrient agar was accurately weighed and dispersed in water. It was then placed in a water bath to dissolve the ingredients until a transparent solution was obtained.

Preparation of fresh culture

The nutrient agar media were dispensed to a number of clean test tubes, each containing 5 ml of the prepared slants. The test tubes were plugged with cotton and sterilized in an autoclave at 121°C and 15 lbs/sq-inch pressure for 15 min. After sterilization the test tubes were kept in an inclined position for solidification. These were then incubated at 37.5°C to ensure the sterilization. Finally the slants were streaked with pure culture of the test organisms in the laminar air flow and incubated at 37.5° C for 24hrs.

Preparation of test plates

The test plates were prepared according to the following procedure. Nutrient agar media was poured in 15.0 mL in clean test tubes and plugged with cotton. The test tubes were sterilized by autoclaving and allowed to cool at about 50°C. The media in the test tubes were incubated with fresh culture. Bacteria were agitated to ensure uniform dispersion of organisms into the media. Finally, the media were poured into sterile Petri dishes in aseptic condition. The petri dishes were rotated several times, first clockwise and then anticlockwise to assure homogeneous distribution of the test organisms. Thus the plates were ready for sensitivity test and stored it in a refrigerator at 4°C.

Preparation of sample and standard discs

The sample solution was prepared in methanol in such a manner that 10 μl contained 200 μg of the antibiotic. Filter paper discs were taken in a petri dish and sterilized by autoclaving. 20 μl of the test solution was applied on a disc and thus the disc containing 400 μg of the antibiotic prepared. These discs were left for few min in aseptic condition for complete removal of the solvent. The standard discs were used as positive control to ensure the activity of the standard antibiotic against the test organisms as well as for comparison of the response produced by the known antibacterial agent. In this study, Kanamycin (K-30) containing 30 μg/disc of antibiotic was used as standard disc for comparison. The sample impregnated discs and standard antibiotic discs were placed gently on solidified agar plates seeded with the organisms to ensure contact with the media. The plates were kept in a refrigerator 4°C for 24hrs and then incubated at 37.5°C for 24hrs. The antibacterial activity was determined by standard disc diffusion method by measuring the zone of inhibition and was compared to that of the standard disc[14].

INSECTICIDAL ACTIVITY TEST

Culture of red flour beetles (*Tribolium castaneum*)

Red flour beetles were sorted out to start a fresh culture. Mass cultures were maintained in earthen pot and subcultures in beakers with food medium. The beakers were kept in an incubator at 30°±0.5°C. Each earthen pot and beaker contained 500g and 250g of food respectively. A crumpled filter paper was placed inside each earthen pot and beaker for easy movement of the beetles. The earthen pots and beakers were covered with a pieces of cotton cloth tightly fixed to avoid possible escape of the beetles.

Preparation and application of doses

The residual film method has been used for the application of doses in which the adult red flour beetles were used. Ten beetles were used in each replication. The extractives/compounds was weighed and diluted in methanol and pilot experiments was carried out to obtain doses in which mortality rates was in between 10 to 90% for the 6 to 10 days old beetles. The actual doses were calculated from the amount of insecticide present in 1.0 ml of solution. The calculated amount of the insecticide was expressed in $\mu g/cm^2$. Different extracts of *CC* (80.0 mg, 40.0 mg, 20.0 mg, and 10.0 mg) were dissolved in 4mL methanol, respectively. Each fraction was applied to petri dishes (9.2cm diameter). Calculated doses were $1.20 mg/cm^2 = 1200 \mu g/cm^2$, $0.60 mg/cm^2 = 600 \mu g/cm^2$, $0.30\ mg/cm^2 = 300\ \mu g/cm^2$ and $0.15\ mg/cm^2 = 150\ \mu g/cm^2$. After evaporation and drying of solvent, the dishes were kept at room temperature. 20 adult beetles were transferred to each dish and left for 24, 48 and 72h. Control dishes were treated with solvent only. Those insects that did not move when prodded gently with a brush were considered to be dead. The mortality of adult beetles was recorded after 24, 48 and 72h treatment. The mortality percentage was corrected by using the Abbott's formula[15].

$$P_t = \frac{(P_o - P_c)}{(100 - P_c)} \times 100 \quad \text{.................................} (1)$$

Where, P_t = corrected mortality %, P_o = observed mortality %, and P_c = control mortality %.
The observed data were subjected for prohibit analysis according to Busvine[16]. Heterogeneity is tested by a chi squared test. If the probability is greater than 5% an automatic correction of heterogeneity is introduced.

RESULTS AND DISCUSSION

Antimicrobial property

The antimicrobial activities of the different fractions and that of the pure unidentified compounds isolated from the seed extract of *CC* were examined. The results are tabulated in the Table No.1. From the quantitative estimation of zone inhibitions of the crude extract and the different fractions as well as the compounds showed various range of antibacterial activity against the four different bacteria. In the antimicrobial studies, the petroleum ether fraction showed maximum inhibition against *V.cholerae*, *Shigella* spp, *Salmonella* spp., *E.coli*. with diameter of 26-27mm. This may be due to the presence of more amounts of antimicrobial compounds (phytochemicals) in the petroleum ether fraction. The methanolic crude extract and all the three isolated compounds also showed similar inhibition against all the pathogens with diameter of 20-15mm and hence the highest biological active towards the microorganism. The chloroform, ethyl acetate and methanol fraction showed moderate activity against the all test organisms with diameter of 9-6mm. This shows that the petroleum ether is one of the best solvents for extraction of phytochemicals of seed extract of *CC*. The increase in the diameter of the zone was due to the higher concentration of the fraction (400 μg/disc) used in this study. This reveals that as the concentration of the fraction increases, its antimicrobial activity also increases. However, further research such as detail phytochemical screening is necessary to establish the pharmacological properties of the extractives.

Insecticidal property

The mortality percentage of *T. castaneum* due to the effect of different fractions and three unidentified pure compounds of the seed extract of *CC* are shown in Table No.2. In the test samples, 80μg, 40μg, 20μg, and 10 μg were dissolved separately in 4.0 mL of methanol. Each fraction was applied to Petri dishes (9.2cm, diameter). The calculated doses were A=1200μg/cm^2, B=600μg/cm^2, C=300μg/cm^2, and D=150μg/cm^2. After evaporation and drying, the dishes were left at room temperature. After then 20 adult beetles were transferred to each of the dishes and left it for 24, 48 and 72h. Control dishes were treated with solvent only. Those insects that did not move when prodded gently with a brush were considered as dead. The highest mortality was recorded in the petroleum ether fraction in which all most all the beetles (19~16 out of 20) were died after 72h treatment. Therefore, the petroleum ether fraction was found to be the most toxic extractive to the beetle of *T. castaneum* than that of the other three fractions with 72h treatment. On the other hand, all the three isolated compounds were also found to have toxic effect against the *T. castaneum* in which the mortality was recorded as 17-8 at 72h treatment. The methanol fraction was mild and the chloroform and ethyl acetate fractions were less toxic. The respective LD_{50} value, 95% confidence limit, χ^2 values and regression equations for the different fractions and the pure compounds are shown in the Table No.3. The calculated LD_{50} values of the petroleum ether, chloroform, ethyl acetate and methanol fractions were (259.54, 179.49, 157.70 μg/cm^2); (2945.20, 700.81, 454.75 μg/cm^2); (1994.15, 576.72, 401.59 μg/cm^2) and (568.22, 325.05 and 208.90 μg/cm^2) at 24, 48 and 72h, respectively.
The petroleum ether fraction was the most effective toxicant and their efficiency followed the order. Petroleum ether>methanol>ethyl acetate>chloroform fraction. For pure compounds on the other hand the compound-1 was the most toxicant having the LD_{50} values 362.64μg/cm^2, 317.71μg/cm^2 and 243.74μg/cm^2 at 24, 48 and 72h interval, respectively. The calculated LD_{50} values for compound-2 and compound-3 were found to be (1031.67, 499.47 and 250.84μg/cm^2) and (889.47, 767.94 and 297.96μg/cm^2) at 24, 48 and 72h interval, respectively.

Table No.1: Antimicrobial activities of different fractions and that of pure compounds isolated from *Corchorus capsularis*

S.No	Extract/ Compound	Dose (µg/disc)	Inhibition zone (mm)			
			V. cholerae	*Shigella* spp.	*Salmonella* spp.	*E. coli*.
1	Methanolic crude	400	20	19	18	19
2	Petroleum ether	400	27	25	25	26
3	Chloroform	400	7	8	8	7
4	Ethyl acetate	400	6	7	7	6
5	Methanol	400	9	8	8	8
6	Compound-1	400	24	23	20	22
7	Compound-2	400	20	18	18	19
8	Compound-3	400	16	15	16	16
9	Kanamycin (K3)	400	23	21	22	22

Table No.2: Insecticidal activities of different fractions and pure compounds against *T. castaneum* of the seeds extract of *Corchorus capsularis*

S.No	Fractions/ Compound	Treatment (h)	Number of insect killed out of 20				
			Dose-A	Dose-B	Dose-C	Dose-D	Control
1	Petroleum ether	24	16	14	12	9	0
		48	18	16	15	13	0
		72	19	18	17	16	0
2	Chloroform	24	3	1	1	0	0
		48	4	2	2	1	0
		72	6	4	3	1	0
3	Ethyl acetate	24	5	3	1	0	0
		48	7	4	3	0	0
		72	8	6	4	0	0
4	Methanol	24	9	5	2	1	0
		48	10	8	6	3	0
		72	16	9	8	4	0
5	Compound-1	24	13	11	8	6	0
		48	16	13	11	9	0
		72	17	16	14	13	0
6	Compound-2	24	12	10	7	5	0
		48	15	13	10	7	0
		72	16	14	13	10	0
7	Compound-3	24	8	7	6	2	0
		48	11	12	8	5	0
		72	16	14	12	8	0

Table No.3: χ^2 values, regression equation, LD_{50} and 95% confident limits of different fractions and pure compounds of *Corchorus capsularis* against *T. castaneum* adult after 24, 48 and 72h of treatment

S.No	Extracts/ Compounds	Treatment (h)	χ^2 for heterogeneity	Regression equation	LD_{50} ($\mu g/cm^{-2}$)	95% confidence limit	
						Lower	Upper
1	Petroleum ether	24	8.53	Y = -4.15 + 3.79 X	259.54	226.54	296.78
		48	6.66	Y = -3.22 + 3.64 X	179.49	164.64	195.68
		72	6.27	Y = -2.26 + 3.30 X	157.70	143.08	173.81
2	Chloroform	24	1.81	Y = 0.68 + 1.22 X	2945.20	1171.83	74422.44
		48	4.89	Y = -1.52 + 2.99 X	700.81	580.72	845.73
		72	1.93	Y = 0.22 + 1.91 X	454.75	454.55	643.32
3	Ethyl acetate	24	4.02	Y = 0.12 + 1.48 X	1994.15	1056.66	3673.40
		48	1.49	Y = -1.65 + 2.41 X	576.77	497.50	668.08
		72	5.83	Y = -2.16 + 2.75 X	401.59	362.91	444.38
4	Methanol	24	6.69	Y = -1.58 + 2.37 X	592.19	568.22	690.05
		48	4.80	Y = -2.16 + 2.99 X	356.08	325.05	390.08
		72	1.20	Y = -2.68 + 3.26 X	228.49	208.90	249.84
5	Compound-1	24	17.14	Y = 0.41+ 2.11 X	362.64	269.86	487.33
		48	8.14	Y = 0.24 + 1.96 X	317.71	255.76	394.68
		72	10.32	Y = 0.58 + 1.85 X	243.74	198.57	313.39
6	Compound-2	24	0.874	Y = 2.19 + 0.93 X	1031.67	597.46	1781.44
		48	15.09	Y = 1.77 + 1.20 X	499.47	280.13	890.54
		72	5.63	Y = 0.347 + 1.94X	250.84	220.67	285.12
7	Compound-3	24	3.76	Y = -1.43 + 2.18 X	889.47	686.67	1152.17
		48	3.48	Y = -0.01 + 1.73 X	767.94	594.10	992.65
		72	3.35	Y = -4.15 + 3.79 X	297.96	226.97	296.78

CONCLUSION

The present study was carried out to explore anti-microbial and insecticidal potential of petroleum ether, chloroform, ethyl acetate, methanol extract, and pure compound isolated from *CC* seed. Maximum antimicrobial and insecticidal activities were observed for petroleum ether fraction and compound-1, which might be correlated to its leading phenolic and flavonoid contents as compared to its other counterparts. Significant difference in antimicrobial and insecticidal activities was noted in all of the investigated fractions and that of the isolated three compounds. On the basis of our pharmacological findings (antimicrobial and insecticidal) of petroleum ether fraction, it was selected to assess cytotoxic activity. Further research is under progress to elucidate the structure of the bioactive constituents isolated from petroleum extractive of the seed extract of *CC* to locate potential pharmacological agents.

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CONFLICT OF INTEREST

All contributing authors declare no conflicts of interest.

BIBLIOGRAPHY

1. Jahan M S, Nasima Chowdhury D A, and Islam M K. Alkaline sulphite an thraqu in one methanol (ASAM) pulping of jute, *Ippta J*, 17, 2005, 37-43.
2. Oboh G, Ademiluyi A O, Akinyemi A J, Henle T, Saliu J and Schwarzenbolz U. Inhibitory effect of polyphenol-rich extracts of jute leaf (*Corchorusolitorius*) on key enzyme linked to

type-2 diabetes (a-amylaseanda-glucosidase) and hypertension (angiotensin I converting) *invitro*, *J. Funct. Foods*, 4, 2012, 450-458.

3. Whaid A and Siddiqui H H A. Survey of drugs, *IHMMR, Delhi, India,* 2nd edition, 1961.
4. Zainul A Z, Sulaiman M R, Gopalan H K, GhaniA Z D F, Nor R N S, Jais A M and Abdullah F C. Antinociceptive and anti-inflammatory properties of *Corchorus capsularis* leaves chloroform extract in experimental animal models, *Yakugaku Zasshi*, 127, 2007, 359-365.
5. Furumoto T, Wang R, Okazaki K, FerojHasan A F M, Ali MI, Kondo A and Fukui H. Antitumor promoters in leaves of jute, (*Corchorus capsularis*, *corchorusolitorius*), *Food sci. technol.Res*, 8, 2002, 239-243.
6. Karrer P and Banerjee P. Corchortoxin. A cardiac agent from jute seeds, *Helvchim Acta*, 32, 1949, 2385-2392.
7. Frerejacque M and Durgeat M. Digitalis like poisons of jute seed, *compt Rend*, 238, 1954, 507-509.
8. Schumersahl P. Semi synthetic derivatives from cardenolides of *corchorus species*, *Arch, Phar.Ber.Deutsch.Pharm.Ges*, 304, 1971, 307-314.
9. Nahar N, Mosihuzzaman M, and Mian A J. Analysis of water-soluble polysaccharides from unretted jute plant (*Corchorus capsularis*), *Carbohydrate Research*, 163, 1987, 136-142.
10. Del Rio J C, Marques G, RodriguezI M, and Gutierrez A. Chemical composition of lipophilic extractives from jute (*Corchorus capsularis*) fibers used for manufacturing of high-quality paper pulps, *Industrial Crops and Products*, 30, 2009, 241-249.
11. Choudhury M H, Islam A, Ahmed M, Waterman P G. Capsugenin. A dammaran etriterpene from *Corchorus capsularis*, *Phytochemistry*, 23, 1984, 2583-2587.
12. Zhang G, Zhang Y, Xu J, Niu X, Qi J, Tao A, Zhang L, Fang P, Lin L, and Su J. The *CCoAOMT1* gene from jute (*Corchorus capsularis* L.) is involved in lignin biosynthesis in *Arabidopsis thaliana) Gene*, 546, 2014, 398-402.
13. Zhang G, Qi J, Xu J, Niu X, Zhang Y, Tao A, Zhang L, Fang P and Lin L. Over expression of UDP-glucose pyrophosphorylase gene could increase cellulose content in Jute (*Corchorus capsularis* L.), *Biochem.Biophys, Res.Commun*, 442, 2013, 153-158.
14. Zaidan, Rain M R S, Badrul A N, AdlinA R, Norazah A and Zakiah I. *In vitro* screening of five local medicinal plants for antibacterial activity using disc diffusion method, *Trop, Biom,* 22, 2005, 165-170.
15. Abbott W S. A method of computing the effectiveness of an insecticide, *J. Econ. Entomol*, 18, 1925, 265-267.
16. Busvine J R. A critical review of the techniques for testing insecticides, *Common wealth Agricultural Buereux*, 345, 1971.

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