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Full Length Research Paper

Phytochemical and antimicrobial investigations of different fractions of the methanolic extract of *Corchorus capsularis* leaves

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The purpose of this study was to understand and investigate the phytochemical and antimicrobial effects of different fractions of the methanolic extract of *Corchorus capsularis* leaf. For the extraction of lipophilic compounds with the help of methanol solvent system, cold extraction process was used, where the grinded coarse powder of the leaf was soaked in methanol and kept for four days for proper extraction. After that, the extract was concentrated by using rotatory evaporator and dried in room temperature. Compounds in the extract were separated according to their polarity by using vacuum liquid chromatography (VLC) and solvents of different polarity N-hexane, benzene, diethyl ether, chloroform, dichloromethane. Later, thin layer chromatography (TLC), flavonoid assay, disk diffusion, inhibitory action in nutrient broth was done for different fractions of the methanolic extract. Diethyl ether, chloroform, dichloromethane fractions showed good anti-oxidant property but poor antibacterial activity against different types of microorganism. Chloroform fraction showed good inhibitory activity against *Aspergilus niger* (fungus) in nutrient broth.

Key words: *Corchorus capsularis,* vacuum liquid chromatography (VLC), thin layer chromatography (TLC), disc diffusion method, nutrient broth, Bangladesh.

INTRODUCTION

Bangladesh is known as a growing and populated country where the majority of the community lives below the poverty level, particularly the rural areas. The general public in Bangladesh is habituated together with the local and indigenous foods. Vegetables are extremely popular in their day-to-day meals (Ullah et al., 2013). Drug

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development from purely natural origins demonstrated that natural products or natural product-derived medication made up of approximately 28% of all fresh chemical entities unveiled to the market. These are typically originated from terrestrial plant life, microorganisms, marine microorganisms, etc. However, till recently, an unimportant part of the plants has been clinically examined for their therapeutic attributes (Quader et al., 1987). Jute (*Corchorus capsularis* L.) is a yearly bast fiber plant. It can be located primarily within Southeast Parts of Asia Just after cotton. Plant seeds of *C. capsularis* L. comprised of corchorin, corchortoxin helveticoside, cardiac glycosides, corchoroside A and B, olitoriside, erysimoside, strophantidol glycosides, biosides, oliogosaccaride and olitoriside; whilst leaves comprised of saponins, flavonoids, glucoside, capsularin steroids triterpenes and several various secondary metabolites. The pharmacological experiments unveiled the fact that the plant possessed anti-inflammatory, analgesic, antipyretic, cardiac, antioxidant, antimicrobial, insecticidal and several additional pharmacological properties (Al-Snafi, 2013). Jute (white jute, *C. capsularis* L.; nalta jute or tossa jute; Tiliaceae family), known as a fiber plant, is a time-honored medicinal vegetable in North Africa, the Middle and Near East and Southeast Asia (Furumoto et al., 2002). The leaves from *C. capsularis* have been reported to hold demulcent, laxative, appetizer, stimulant and stomachic and its infusion is customarily used to cure constipation, dysentery, fevers, liver problems as well as dyspepsia. Additionally, a decoction of the roots, as well as unripe fruits, has long been used to combat dysentery. The leaves of *C. capsularis* are actually consumed as vegetables in numerous area of the world such as Bangladesh, Africa, Middle East and Southeast Parts of Asia, which include Malaysia, for a long period (Zakaria et al., 2007). In the present study, phytochemical and antimicrobial investigations of different fractions of the methanolic extract of *C. capsularis* leaf were screened. Most jute plantations are in India and Pakistan. Other producing countries are China, Taiwan and Brazil. It contains 60 to 65% cellulose, an amount lower than flax, ramie and cotton. It's woody substance, lignin makes it less durable than other fibers. About 75% of the world's jute is used for coarse woven fabrics, sacking or burlap. It is also used in twines and carpet yarns. White jute is more commonly grown for fiber than the closely related, tussa jute (*Corchorus olitorius*) (Roecklein, 1958). The *C. capsularis* is reported to have cardiotonic, carminative, diuretic, antidysenteric, purgative, etc. (Chopra et al., 1958; Satyavathi, 1971; Rao, 1972). Natural products, particularly the ones made from higher plant life, have fascinated researchers from ancient time for their likely beneficial values. Bangladesh is actually an affluent repository of medical plants, a lot of which can be widely used in the Unani, Ayurvedic, herbal and various traditional techniques of treatments (Ramadevi, 2013; Kaisar et al., 2011). This study reported the phyto-chemical and antimicrobial investigations of different fractions of the methanolic extract of *Corchorus capsularis* leaves.

MATERIALS AND METHODS

The study of taxon of *C. capsularis*

Jute thrives nicely where the annual rainfall is 1500 mm, with a minimum of 250 mm in each of the months of March, April and May. Optimum necessary temperature is 18-33°C. Jute (*C. capsularis*) is harvested during the wet season. Farming primarily depends on pre-monsoon showers as well as moisture conditions. *C. capsularis* is much more water tolerant and for that reason, it can be typically grown in minimal lands, and in many cases under water logging circumstances (Islam et al., 2013).

Collection of plant and identification

C. capsularis L. was collected from Lalmohan (Ward No. 3) is an Upazila of Bhola District in the Division of Barisal, Bangladesh in the month of January, 2014 and was identified by the taxonomist of Bangladesh National Herberium, Dhaka, Bangladesh.

Methanolic extraction process

Fresh leaves were collected from the plant and dried under the sun light. Dry leaves were weighed again in electronic balance (SHIMADZU ATX2204) to measure the total weight of the dry leaves. Then, the dry leaves were converted to fine powder by using a blender (Miyako, Japan) then weight of the blended leaves measured and preserved in an air tight container. Methanol (Merck, India) was selected as the solvent for extraction from leaves of *C. capsularis* L. The powdered leaf (325 g) of *C. capsularis* L. was soaked in methanol (~ 970 ml) for 4 days and filtered by using of filter paper Filter paper (Double Rings 100 – 11 cm, HANGZHOU XINHUA PAPER Industry Co. Ltd., China). It was stirred with a clean glass rod to ensure the maximum amounts of constituents present in the blended leaves become soluble into methanol (Merck, Germany). To prevent the evaporation of the solvent and volatile compounds from the medium, the beaker was covered by aluminium foil. The extract was then concentrated with a Buchii rotary evaporator (IKA RV-10, Biometra). An aliquot of the crude methanolic extract (32.5 g) was fractionated by vacuum liquid chromatographic (VLC) technique using silica gel 10 mesh (Merck, Germany) N-hexane (Merck, Germany), benzene (Merck, Germany), diethyl ether (Merck, Germany), chloroform (Merck, Germany), dichloromethane (Merck, Germany) in increasing order of polarity. Two parts were obtained after filtration: residue and filtered part, filtered part was poured into 1000 ml round bottomed flask (Borosil, Japan) and was concentrated with the help of rotary evaporator at low temperature of 45°C and RPM of 120. When evaporation was completed, di-ethyl-ether (Merck, Germany) was used for collecting the extract (Faisal et al., 2016).

Thin layer chromatography (TLC)

Different solvent fractions were analyzed by performing thin layer chromatography (TLC) to determine the composition of each

extract. TLC was done under two solvent systems. Successively, the polarity of the solvent systems was increased to get a clear graph of all the possible compounds present in the extract. The compositions of various solvent systems used for TLC: Intermediate polar solvent: Chloroform 5 ml, ethyl acetate 4 ml, formic acid 1 ml; Polar solvent: Chloroform 6.3 ml, methanol 3.5 ml, distilled water 1 ml; Spotting: It was done with capillary tube in the following sequence. Same solvent was used to prepare samples solutions for same fraction. It was then put into the TLC with the solvent. After the solvent reached the upper limit, it was seen under UV light and was exposed to 10% sulphuric acid solution, dried and then heated to 80-90°C for charring purpose. This helped in the fixation of spot and allowed it to be prominently visible. A second batch was dipped into 0.4% DPPH solution and dried while keeping in a dark place (Saha et al., 2016a).

Anti-oxidant test

Total flavonoid assay

Initially, 1.5 ml methanolic leaf solution (1 mg/ml) of three fractions (dichloromethane, chloroform and diethyl ether) was taken in test tubes and different dilution of standard solution of quercetin (2.5, 5.0, 10, 20, 30 and 40 µg/ml) were added to 10 ml volumetric flask containing 6 ml of deionized water. To the above mixture, 0.45 ml of 5% $NaNO_2$ was added. After 6 min, 0.45 ml of 10% $AlCl_3$ was added into the mixture. After 6 min, 6 ml 4% (w/v) NaOH was added and the total volume was made up to 10 ml with distilled water. Finally, 0.6 ml deionized water was added to the sample for 15 min incubation. Then, the solution was mixed well and the absorbance was measured against a freshly prepared reagent blank at 510 nm. Total flavonoid content of the extracts was expressed as mg of quercetin equivalent per g dry weight of sample (Sanadhya et al., 2013).

Anti-microbial assay

The antibacterial activity was carried out by the disc diffusion method using. Three different bacterial strains of Gram positive, different strains of Gram negative bacteria and fungus were used to carry out this assay. In this study, fractions obtained after VLC were tested for antimicrobial activity by disc diffusion method. Dried and sterilized filter paper discs (6 mm diameter) containing the test samples of known amounts are placed on nutrient agar medium (TECHNO PHARMCHEM, India) uniformly seeded with the test microorganisms. The antibacterial activity was carried out by using 50 µL of suspension containing ~103 CFU/mL of microorganism spread on nutrient agar medium. A disc was soaked with solutions of 50 µl of test samples and dried placed. Standard antibiotic discs (erythromycin 15 µg/disc) together with blank disks were used as a positive and negative control. Those plates are retained at the lower temperature (4°C) for 24 h to allow for highest diffusion of the test components to the neighboring media. These plates are next inverted as well as incubated at 37°C for 24 h to get the best possible growth of the microorganisms. The test components possessing antimicrobial property restrict microbial growth within the media neighboring the discs and in so doing provide a transparent, distinct area understood to be the zone of inhibition. This antimicrobial actions belonging to the test agent is then simply determined by calculating the dimension of zone of inhibition depicted in millimeter (mm). Petridishes and other glassware were sterilized by autoclaving (Hirayama, Japan) at a temperature of 121°C and a pressure of 15-lbs/sq. inch for 20 min. Micropipette tips, cotton, forceps, blank discs etc. were also sterilized. After incubation at 37°C for 24 h, the antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in mm. After incubation, the antimicrobial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter with a transparent scale (Saha RK et al., 2016b).

RESULTS AND DISCUSSION

Thin layer chromatography (TLC)

The results obtained after TLC of the fractions obtained from methanolic extract of the *C. capsularis* leaf is shown in Figure 1 and Table 1.

Anti-oxidant tests (total flavonoid assay)

Determination of total flavonoids content of the fractions of methanolic extract of *C. capsularis* (leaves) requires a standard curve which was obtained from a series of different quercetin concentrations.

Anti-microbial assay

Disc diffusion method

After incubation of 24 h at 37°C, Petri-dishes were observed for zone of inhibition for different fractions of methanolic extract of *C. capsularis* leaf. The results are shown in Table 3.

Inhibitory effect in nutrient broth

Absorbance's of the samples, positive control and blank before and after incubation are shown in Tables 4 and 5.

DISCUSSION

TLC plates were seen under UV light and different compound were found to be separated in the plates. Numbers 4 and 5 show some spots in dichloromethane, chloroform and diethyl ether. So, compunds are present in this fraction. Number 3 show spots in N-hexane and benzene after charring done in 10% sulphuric acid solution. Charring with H_2SO_4 in high temperature separated compound transformed into black color. Finally, numbers 1 and 2 show pale yellow color in dichloromethane, chloroform & diethyl ether fractions, which indicate flavonoids present in the fractions. To evaluate the antioxidant activities of VLC fraction of *C. capsularis* (leaves) total flavonoid assay was used. Total flavonoid content of different fractions of *C. capsularis* is

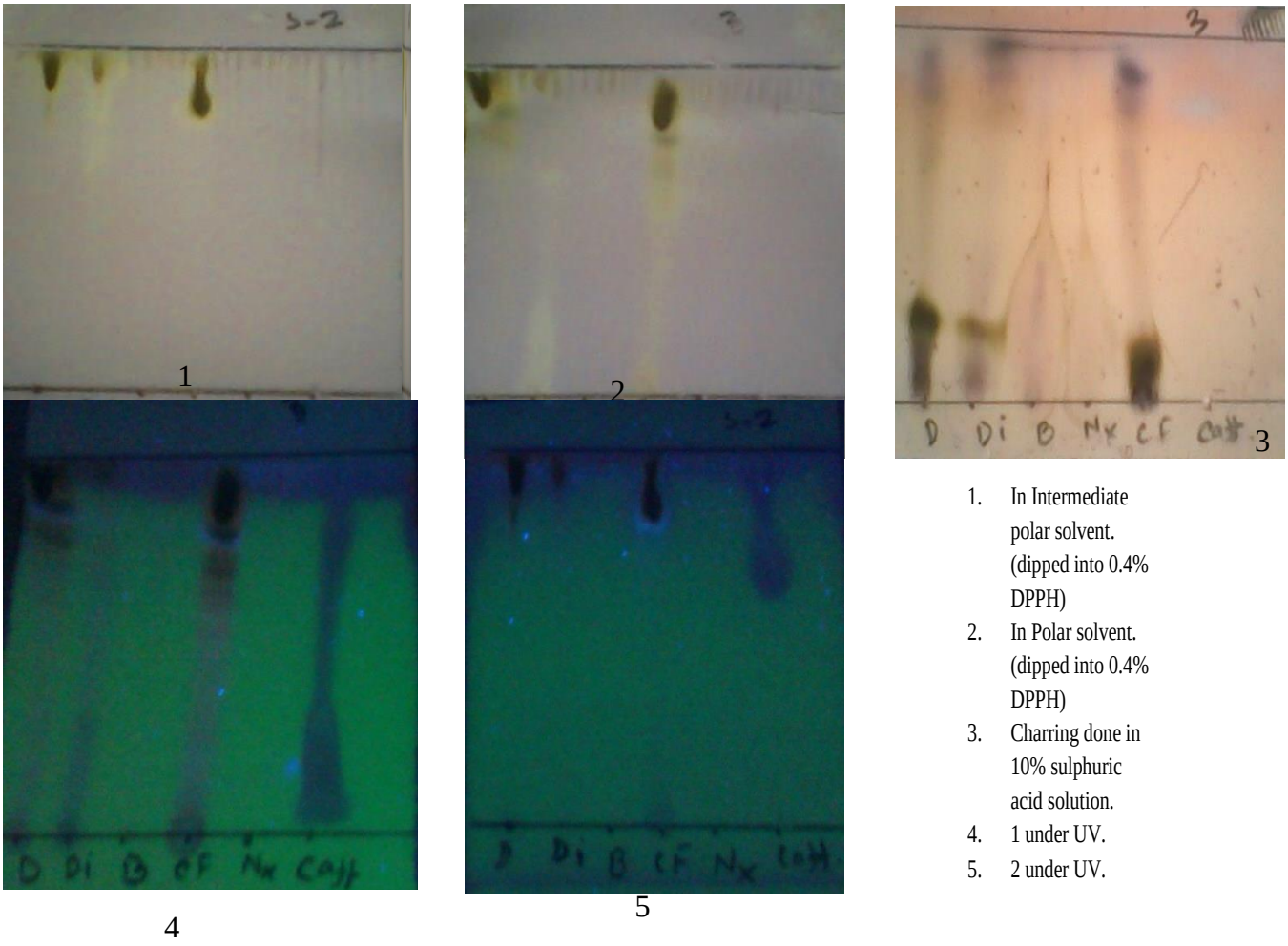

Figure 1. TLC results of the fractions. Numbers 1 and 2: Showing pale yellow color in dichloromethane, chloroform and diethyl ether fractions, which indicate flavonoids are present in the fractions. Number 3– Showing spots in N-hexane and benzene after charring done in 10% sulphuric acid solution. Numbers 4 and 5- Showing spots in dichloromethane, chloroform and diethyl ether. So, compounds are present in this fraction.

Table 1. Absorbance of different concentrations of quercetin.

Concentration of quercetin (µg/ml)	Absorbance (415 nm)
2.5	0.0365
5	0.0417
10	0.0521
20	0.0735
30	0.0906
40	0.1127

shown in Table 2. Antimicrobial screening leaves of *C. capsularis* showed activity against Gram positive bacteria (*Staphylococcus aureus*) and fungi (*A. niger*) as shown in Table 3 and Figures 2 to 5. The positive control used in this study was erythromycin. The results showed possible benefits of VLC fraction of *C. capsularis* (leaves) as an antiviral therapeutics. Total flavonoid assay, disk diffusion and inhibitory effect in nutrient broth were done for

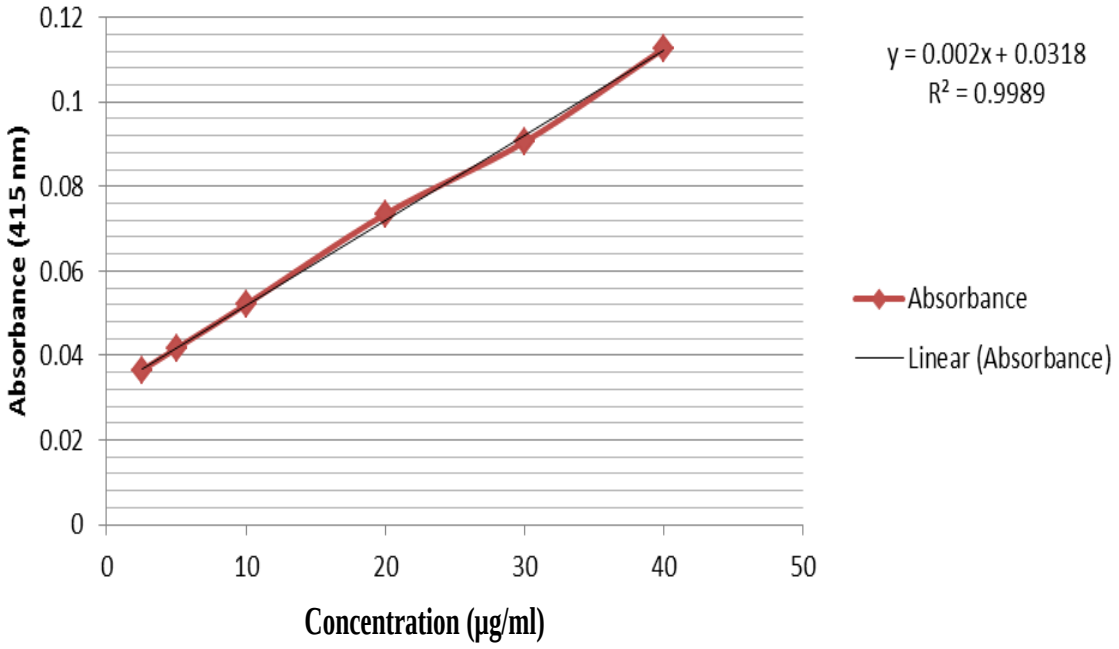

Figure 2. Concentration versus absorbance graph of quercetin.

Table 2. Total flavonoid content of different fractions of *C. capsularis* leaf.

Fractions	Amount (ml)	Absorbance (415 nm)	Conc.Form Std. Curve	Flavonoid Content (mcg Quercetin Equivalents/g of sample)
Dichloromethane (1 mg/ml)	1.5	0.037	2.6	3900
Chloroform (1 mg/ml)	1.5	0.038	3.1	4650
Diethyl ether (1 mg/ml)	1.5	0.045	6.6	9900

Table 3. Results of disc diffusion method.

Microorganisms		Sample names with zone of inhibition (mm)				Positive Control (Erythromycin 15 µg disc)
Positive strain	Negative strain	DCM	CF	DIE	NHX	
Staphylococcus aureus(+)		8	-	-	NT	9
Shigella boydii(-)		7	-	-	NT	20
Saccharomyces cerevisiae(yeast)		7	-	-	NT	22
Aspergillus niger(Fungus)		-	8	-	NT	24
Salmonella typhi(-)		7	-	7	-	18
Vibrio mimicus (-)		7	-	-	-	20
Candida albicans(yeast)		7	7	-	-	26
Staphylococcus saprophyticus (+)		-	7	-	NT	NT
Beta-hemolytic streptococci(+)		-	7	-	NT	NT

DCM- Dichloromethane, CF- Chloroform, DIE - Diethyl ether, NHX- N-hexane, NT – Not tested. Sample disc size = 6 mm. The diameters of zones of inhibition (mm) are expressed as mean ± SD (n=3); a diameter less than 7 mm was considered inactive.

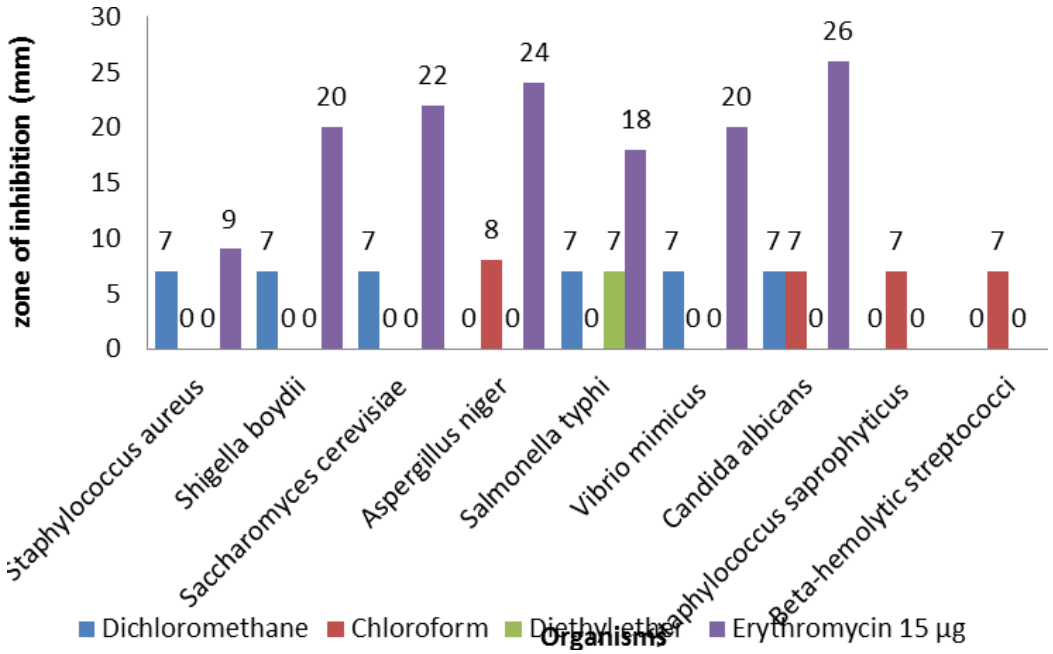

Figure 3. Zones of inhibition of different fractions of methanolic extract of *C. capsularis* (leaves) against various microorganisms.

Table 4. Absorbance of the samples, positive control and black before incubation.

Microplate well series	Positive control	Negative control	20 µl	10 µl	5 µl	2.5µl
Series-1	0.352	0.19	0.137	0.137	0.132	0.141
Series-2	0.333	0.244	0.13	0.14	0.135	0.131
Series-3	0.285	0.178	0.13	0.133	0.12114	0.125
Average	0.323333	0.204	0.132333	0.136667	0.12938	0.132333

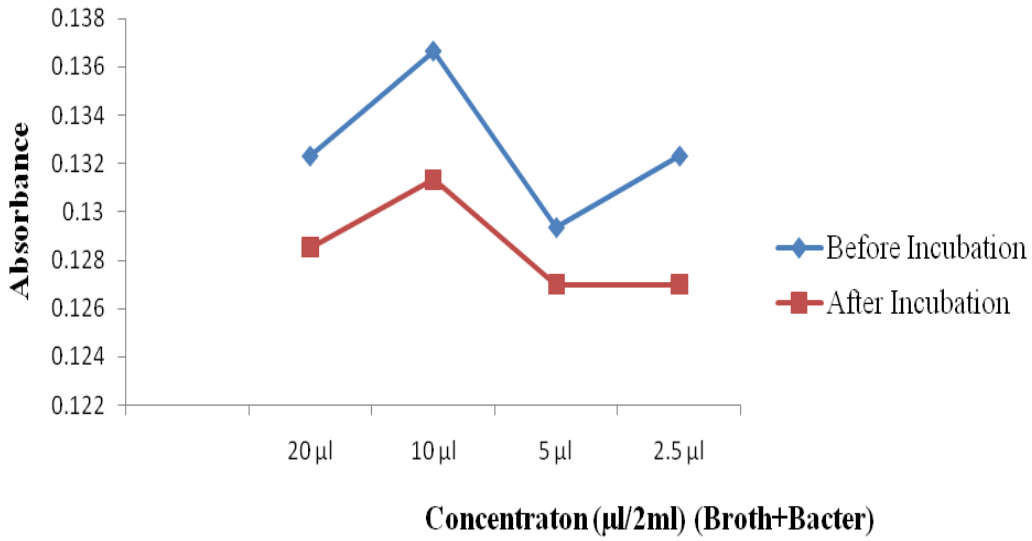

Figure 4. Concentration vs. absorbance of the samples before and after incubation.

Table 5. Absorbance of the samples, positive control and black after 24 h incubation.

Microplate well series	Positive control	Negative control	20 µl	10 µl	5 µl	2.5 µl
Series-1	0.682	0.105	0.136	0.133	0.130	0.134
Series-2	0.617	0.113	0.123	0.133	0.131	0.128
Series-3	0.676	0.103	0.126	0.128	0.120	0.119
Average	0.658333	0.107	0.128533	0.131333	0.127	0.127

Figure 5. Comparison between positive and negative control before and after incubation.

different fractions of the methanolic extract of the leaf. It is shown by this research that there is not much to do in the antimicrobial section of different fractions of methanolic extract of the *C. capsularis* leaf.

Conclusion

The results of the preliminary studies indicated phyto-chemical and antimicrobial activities of *C. capsularis* leaves. Thus, the plant warrants further investigation to isolate the active constituents responsible for these activities.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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