- Short communication

Evaluating Phytochemical, Antioxidant, Ascorbic Acid Equivalent Capacity and Antimicrobial and Synergistic Effects of Stolon of *Colocasia esculenta* Against Clinical Pathogens

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People have been using medicinal plants to treat various human diseases since the dawn. Almost 80% of Africans use medicinal herbs to treat prevalent illnesses.[1] As newer and more potent therapeutic agents have developed from medicinal plants and occupy a prominent position in most research facilities worldwide.

The microorganisms that are resistant to antibiotics are being discovered at this time and are increasing exponentially due to the abuse of antibiotics. To find and create new and more affordable antimicrobial agents to cure infections by resistant microorganisms, more medicinal plants must now be thoroughly screened. Despite being extensively cultivated in tropical areas, *Colocasia esculenta*, a perennial herbaceous plant, inhabits Bangladesh and India. When combined with other plant components, a decoction of the leaves is traditionally consumed to encourage menstruation and is also used to treat cysts and soothe stomach discomfort. Because of cyanoglucoside is present, *C. esculenta* has been noted to have hypoglycemia effects.[2] Additionally, discovered and linked to arabinogalactan, mono, and digalactocyl diacylglycerols and arabinogalactan are hypolipidemic activities.[3,4] The abundance of cystatin has been linked to reports that it has antifungal properties.[5]

The current state of bacterial antibiotic resistance has reached a critical point, yet discovering new effective drugs to tackle the issue is inadequate. It is worth noticing that components of plants are biologically active and some modern drugs are analogs of these phytochemicals.[6] A new route for overcoming bacterial drug resistance is being explored at the moment: the synergy of commercially accessible antibiotics and medicinal plant extracts. In reality, some plants (*Salvadora persica*, *Nymphaea tetragona* and *Syzygium aromaticum*) have succeeded in this direction.[7, 8]

Plant extracts and medications have a documented synergistic action against both Gram-positive and Gram-negative bacteria. Plant-based ingredients have several benefits, two of which are that they are safer than synthetic equivalents and can be used to cure illnesses at reasonable costs.[2]

One such powerfully therapeutic plant *Colocasia esculenta* is a member of the Araceae family and is

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thought to be among the oldest plants. There is history of use in traditional medicine, particularly in tropical and subtropical areas, in many different nations worldwide. This plant has been used to cure a variety of illnesses, including otalgia, otorrhoea, adenitis, asthma, arthritis, internal hemorrhage, hepatomegaly, neurological diseases, skin problems, and others since it was first discovered to have healing powers in ancient cultures. Recent studies have demonstrated the antibacterial, antifungal, anti-inflammatory, analgesic, anti-hepatotoxic and antimicrobial activities of its extract.[9,10]

It is unclear whether these plant extracts and antibiotics have synergistic effects. In this study, we wanted to investigate any antibacterial activity of several solvent extracts of *C. esculenta* against MRSA & numerous other pathogens to determine its synergistic impact with antibiotics.

The stolon of *Colocasia esculenta* (in Bangali Kochur loti) was collected from the local market of Mirpur, Dhaka, Bangladesh, in December 2022. The voucher specimen was deposited to the National Herbarium Bangladesh for documentation (accession number 87287). The stolon was cleaned and washed with tap water and then cut into small pieces. The samples were sun-dried and finally dried in a drying oven at 40°C. The dried sample was then ground into a coarse powder by using a laboratory mill equipment and kept in an appropriate condition for further use.

A quantity of 400 g powdered sample was soaked overnight in chloroform. The suspension obtained was then filtered using Whatman filter paper with the aid of a vacuum pump and the procedure was repeated three times. The combined filtrate was then concentrated using a rotary vacuum evaporator at 40°C and finally lyophilized. The dry extract obtained was then kept in a refrigerator at 4°C before use. The above extraction procedure was followed for aqueous extraction by soaking 200 g of powdered samples in 4.6 l of distilled water. The filtrate was stored in a labeled container and preserved at 4°C until it was used. The filtrate of the aqueous extract was then dried using a rotary vacuum evaporator and finally dried with a freeze-dryer. The organic extract was suspended in dimethyl sulfoxide (DMSO) for antimicrobial testing.

The extract solution was then sterilized by passing it through a syringe filter. After that, the filtered and unfiltered sample solutions were inoculated on Mueller Hinton (MH) agar and incubated at 37°C for 24 hours to check whether or not the sample had been sterilized. The agar plate was divided into two parts to compare with the filtered sample subjected to the first half and the unfiltered sample to the second half. After incubation, the first half showed no evidence of microbial development.

Preliminary phytochemical screening was performed on both extracts for the presence of tannins, saponins, flavonoids, terpenoids, phenols, glycosides, steroids and alkaloids by chemical test methods.[11,12]

The antioxidant activity of aqueous and chloroform extract of *C. esculenta* was assessed using 2,2-diphenyl-1-picrylhydrazyl free radical following the DPPH method developed earlier.[13,14] The 2.0 ml of DPPH solution (0.1mm) was added to 2.0 ml of extract at different concentrations 4.88, 9.75, 19.5, 39,78, 156, 312,625 µg/ml, mixed well and was kept in the dark at room temperature for 30 minutes. Then the absorbance was measured at 517 nm against methanol as a blank solution by UV-Vis 1800 spectrophotometer (Shimadzu, Japan). Ascorbic was used as a reference standard compound and the DPPH solution was treated as a control solution. The lowest absorbance of the reaction mixture indicated the highest free radical.[15] IC_{50} was calculated using a straight-line equation obtained from the inhibition curve.[16] The percentage of inhibition was calculated against the control solution: $\%I = [(A_{control} - A_{sample})] / A_{control}] \times 100$ where, $A_{control}$ is the absorbance of the control solution and A_{sample} is the absorbance of the sample solution. The ascorbic acid equivalent antioxidant capacity (AEAC) of the extracts was calculated comparing the IC_{50} of ascorbic acid with the IC_{50} of extracts.

ATCC Staphylococcus aureus, V. cholera, pseudomonas aeruginosa, escherichia coli, klebsiella

pneumonia, enterococcus faecalis and methicillin-resistant staphylococcus aureus (MRSA) isolates were obtained from BIHS General Hospital, Dhaka. However biochemical tests were performed in order to further identification of the organisms.

Suspension was made with the test microorganisms, compared with 0.5 McFarland standard and then evenly spread on the Muller Hinton agar medium before discs were added. Filter paper discs (6 mm in diameter), which were dried and sterile, were soaked in 10 µl of various extract concentrations (25 mg/ml, 50 mg/ml and 100 mg/ml) were placed on inoculated MH agar. The positive control was a standard antibiotic disc. The media were incubated at 37°C for 18–20 hours.[17] In the case of antimicrobial activity, it is possible to identify the sensitivity categories: (I) Sensitive: the zone size of the test strain measured is larger than, equal to or not more than 3 mm smaller than that of the control strain; (ii) Resistant: The zone size of the test strain is smaller than 3 mm. (iii) Intermediate: The zone size of the test strain is at least 3 mm but also 3 mm smaller than that of the control.[18]

The summarized results are presented in tables 1 and 2. The inhibition percentage vs Concentration curves shows in Figure 1, 2 and 3 for Chloroform extract, aqueous extract and ascorbic acid, respectively.

The plant extract was tested against some clinical pathogens at different concentrations (25 mg/ml, 50 mg/ml and 100 mg/ml) of the chloroform and aqueous extract, table 2 evaluates the antimicrobial activity of the aqueous and chloroform extracts**.**

Table 1. The presence (+) and absence (-) of phytochemicals in aqueous and chloroform extracts.

Tests	Phytochemicals	Result	
		Aq. extract	Chloroform extract
Hager's	Alkaloids	+	+
Shinoda	Flavonoids	-	-
Salkowski	Terpenoids	-	-
Salkowski	Steroids	-	-
Froth	Saponins	+	-
Potassium-ferro-cyanide	Phenol	+	+
Ferric-chloride solution	Tannins	-	+
Killer-killani	Glycosides	-	+

Table 2. Evaluation of the antimicrobial activity of the aqueous and chloroform extract.

Microorganism	Zone of inhibition (mm)			
	Extract concentration (100mg/ml)			
	Aqueous		Chloroform	
Klebsiella pneumonia	CL	CL + Extract	CL	CL + Extract
	15 mm	18 mm	10 mm	No zone of inhibition
ATCC Staphylococcus aureus	AM	AM + Extract	AM	AM + Extract
	33 mm	35 mm	33 mm	33 mm
Vibrio cholerae	DO	DO + Extract	Not tested	
	27 mm	32 mm		
Pseudomonas aeruginosa	CN	CN + Extract	CN	CN + Extract
	25 mm	23 mm	24 mm	12 mm
Enterococcus faecalis	AM	AM + Extract	AM	AM + Extract
	23 mm	24 mm	23 mm	27 mm
MRSA	Not tested		CN	CN + Extract
			25 mm	32 mm

When the zone of inhibition (ZOI) of the antibiotic combined with the extract is greater than the ZOI of the antibiotic alone known as the synergistic activity of the extract. If the ZOI is equal to the ZOI of the antibiotic alone, it has no impact on activity. Finally, it shows antagonistic activity if the ZOI of the combination of antibiotic and extract is less than the ZOI of the antibiotic zone alone.

Plants have an abundant range of phytochemicals that have antioxidant and disease-preventive capabilities against cancer, plant pathogens, and certain antibacterial activity.[19,20] Chemical tests are widely used for qualitative phytochemical analysis to screen the chemical constituents in the plant extract. Table 1 shows the presence of alkaloids and phenols in both aqueous and chloroform extracts. However, it shows the presence of tannins and glycosides in the chloroform extract.

The antioxidant activity, IC_{50} of the chloroform (Figure 1) and aqueous extracts (Table 2) of stolon was estimated to 0.349 and 1.553 mg/mL, respectively which shows weak activity, whereas the IC_{50} of ascorbic acid is 2.1 µg/ml. In figure 3 the inhibition power of ascorbic acid nearly 100% at concentration 8 µg/ml. The ascorbic acid equivalent capacity (AEAC) of chloroform and aqueous extracts are 0.601 and 0.155 g/100g, respectively. Here the free radical scavenging power of water extract is more than the scavenging power of chloroform extract.

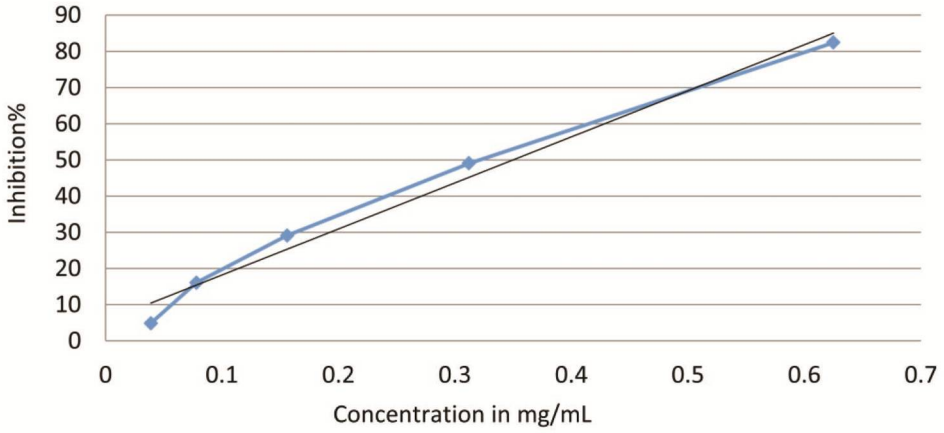

Figure 1. Percentage inhibition curve of chloroform extract of Stolon.

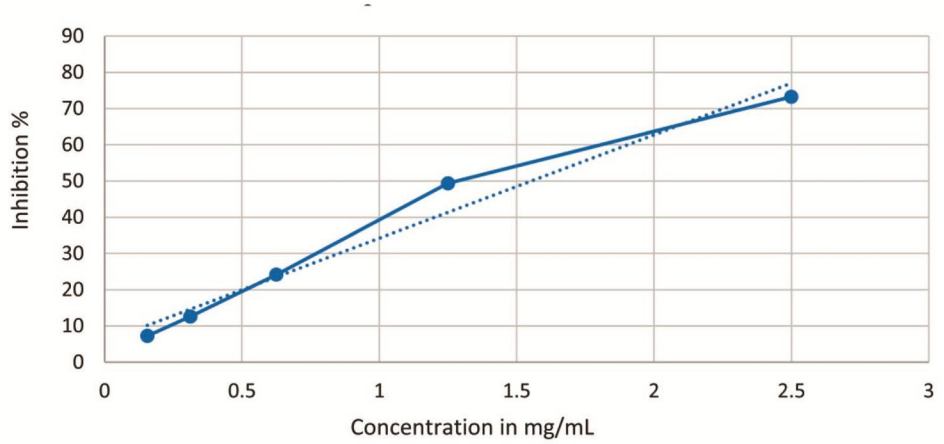

Figure 2. Percentage inhibition curve of aqueous extract of Stolon.

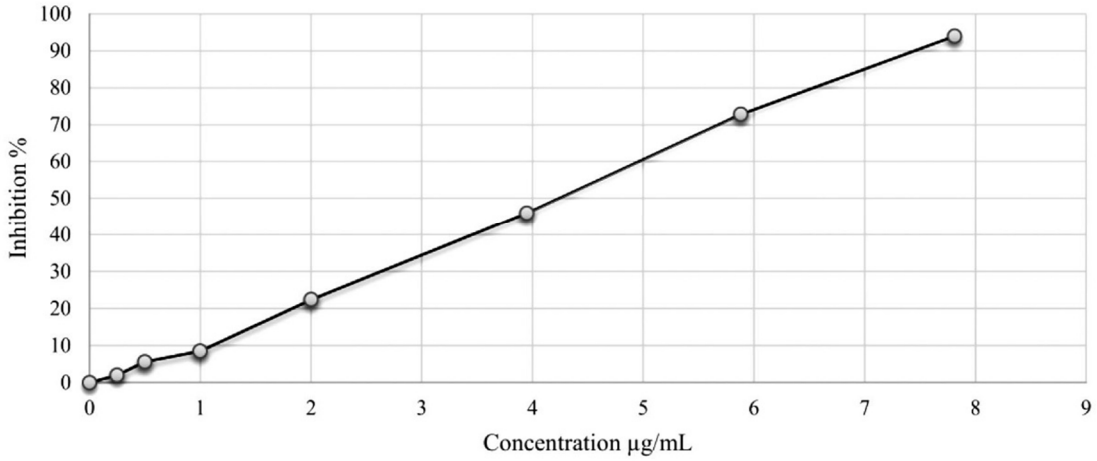

Figure 3. Percentage inhibition vs. concentration of ascorbic acid.

Infectious diseases have been successfully treated with antibiotics, but sometimes drug resistance has failed the treatment. One method of eradicating these multidrug-resistant microorganisms is using antibiotics with plant extracts. Evaluation of the antibacterial and antioxidant qualities of stolon was the main goal of this work. The disc diffusion method is used to assess the plants' antibacterial efficacy against seven pathogens. In our study, the stolon did not show any antimicrobial activity against bacteria. Chloroform and aqueous extract of stolon were selected for further study for synergistic activity with standard antibiotics. Aqueous extract demonstrated synergistic activity in the majority species, antagonistic activity in Pseudomonas and indifferent effects in *E. coli*. The chloroform extract had a heterogeneous pattern of activity that included antagonistic, synergistic and neutral effects. One organism had neutral activity, two synergistic and three antagonistic activities.

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