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Phytochemical Characterization and Evaluation of Pharmacological Activities of Leaves of a Mangrove Plant Species - *Aegiceras corniculatum* (L.)

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ABSTRACT

Aegiceras corniculatum (L.), a mangrove plant of the Sundarbans, has substantial utilization in the traditional medicinal system of the Indian subcontinent and to justify the plant utility, our study was designed to evaluate its pharmacological potentials. First, the plant's ability to scavenge different radicals namely 2, 2-diphenyl-1-picryl hydrazyl (DPPH), hydrogen peroxide and superoxide were examined. Quantitative analysis of the antioxidative secondary metabolites such as phenols, flavonoids and tannins were performed. To investigate the antihyperglycemic potential, oral glucose tolerance test (OGTT) was conducted, followed by the *α*-glucosidase enzyme inhibitory activity assay. The extract was also evaluated for analgesic, anti-inflammatory and antidiarrhoeal potential in acetic acid-induced writhing test, formaldehyde-induced paw edema test and castor oil-induced antidiarrhoeal assay, respectively. Finally, the cytotoxicity of the plant part was tested through the brine shrimp lethality bioassay. SC_{50} (concentration required to scavenge 50% radicals) values were found to be 159.7, 151.8 and 346.8 µg/mL, respectively for DPPH, hydrogen peroxide and superoxide radical scavenging assays. The total phenolic, flavonoid and tannin contents were 71 mg GAE/gm, 151 mg QE/gm and 32 mg GAE/gm. Substantial reduction of blood glucose level was observed in OGTT and the concentration required to inhibit 50% of the *α*-glucosidase enzyme (IC_{50}) was found to be 10.483 mg/mL. The extract also demonstrated significant analgesic, anti-inflammatory and antidiarrhoeal potential compared to the respective standard drugs. The LC_{50} of the extract was found to be 10.87 µg/mL in brine shrimp lethality bioassay. All of the results justified the plants utilization in folklore medicinal system.

Keywords: *Aegiceras corniculatum*, antioxidant, antihyperglycemic, analgesic, antidiarrhoeal.

Introduction

Medicinal plants may be defined as a group of plants that possess some special properties which qualify them as drugs or therapeutic agents and are used for medicinal purposes.[1] Even with the advent of technologies like combinatorial chemistry or computer-aided drug design, medicinal plants containing bioactive chemical constituents are extensively responsible for the development of new drugs.[2]

Molecules containing one or more unpaired electrons are called free radicals. In our body during the normal oxidation of foods, free radicals are constantly generated and about 1-4% of oxygen taken up in the body is converted to free radicals by oxidase enzymes.[3] For these free radicals, lipid peroxidation occurs which is responsible for the oxidation of sulfhydryl groups, protein deformation, DNA bases fragmentation which can result in the mutation of genetic sequences. Free radicals cause different diseases such as cancer, inflammation, respiratory and cardiovascular disorder, muscular dystrophy, diabetes and neurological disorder.[4] To neutralize these free radicals, antioxidant molecules are crucial. Mangrove plants are a potential source of antioxidants and other pharmacological components as they could withstand harsh environmental conditions like low oxygen, high salinity, water-logging, high temperature and wind, etc.[5]

Aegiceras corniculatum, a mangrove plant from the Primulaceae family, known as Khalsi in Bengali, is widely distributed in Indian subcontinent, China, Singapore, Australia, Papua New Guinea and Solomon Islands. This fruits, leaves and stem-barks of the plant have been used in traditional medicinal systems for their anti-asthma, antiarthritic, antidiabetic, analgesic, anti-inflammatory, cytotoxic and hepatoprotective properties.[6,7] Different bioactive secondary metabolites such as flavonoids, polyphenolic groups, tannins, alkaloids, vitamins, terpenoids, glycosides, etc. have also been reported in the leaves of the plant and therefore, we selected the leaves of *A. corniculatum* in the present study.[6,8]

The aim of this study was to investigate the antioxidant and pharmacological properties of *A. corniculatum* leaves through conducting different qualitative and quantitative antioxidant assays and some *in vivo* biological tests while determining secondary metabolic antioxidative contents (phenols, flavonoids, tannins).

Materials and Methods

Chemicals

Analytical grade reagents such as 2,2-diphenyl-1-picryl hydrazyl (DPPH) (Sigma, USA), Folin–Ciocalteu (FC) reagent (Merck, India) along with Na_2CO_3 (Loba, India), $NaNO_2$ (Loba, India), $AlCl_3$ (Loba, India), NaOH (Loba, India), H_2O_2 (Merck, Germany), Na_2HPO_4. $2H_2O$

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(Loba, India), $NaH_2PO_4.2H_2O$ (Loba, India), $FeCl_3$ (Merck, Germany), tert-butyl alcohol (TBA) (Sigma, USA), phenazine methosulfate (PMS) (Sigma, USA), ascorbic acid, nitro blue tetrazolium (NBT) (Sigma, USA), potassium ferricyanide (Merck, Germany), trichloroacetic acid (TCA) (Merck, Germany) and *α*-glucosidase enzyme from *Saccharomyces cerevisiae* were used. All of the standard drugs used for *in vivo* pharmacological tests were purchased from the Square Pharmaceuticals Ltd. and Incepta Pharmaceuticals Ltd., Bangladesh.

Collection and extraction of plant material

Leaves of *A. corniculatum* were collected from South Betagi, Sundarbans, Khulna, Bangladesh in August, 2016. The plant sample was identified by Dr. Mohammad Sayedur Rahman, senior scientific officer of Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh and the provided authentication no. was DACB-43818. The collected leaves were shade-dried, ground into coarse powder and then extraction was carried out through maceration on 250 gm powered leaves with 1000 mL of 96% ethanol for 15 days. The yield of extraction was 6.3%.

Animals

Four to six weeks aged, young Swiss-albino mice (*Mus musculus*) possessing average weights 20–25 gm were obtained from Jahangirnagar University, Bangladesh. They were placed in standard environmental condition for 2-3 weeks in the animal house of Pharmacology laboratory of Pharmacy Discipline, Khulna University, Bangladesh for adaptation after their purchase. All the experiments were conducted in an attenuated, separated and noiseless condition. All experimental procedures performed in current study involving animals were in accordance with the ethical standards of Animal Ethics Committee (AEC), Khulna University, Khulna-9208, Bangladesh [Ref: KUAEC-2020/02/01].

Phytochemical screening

The extract was tested for the presence of reducing sugars, polyphenols, flavonoids, tannins, glycoside, steroids, terpenoids, alkaloids etc. and different phytoconstituents were identified following the method of Ayoola *et al*.[9]

Test for in vitro antioxidant activity

Qualitative antioxidant activity test

The test was performed using pre-coated Silica gel TLC plates and developed in polar, medium polar and nonpolar solvent systems. A suitable diluted plant stock solution was spotted on TLC plates and then 0.02% DPPH solution of ethanol was sprayed. Any hydrogen donating molecule from the extract will react with and bleach DPPH.[10]

DPPH free radical scavenging assay

DPPH free radical scavenging activity of sample extract was measured by the method of Biswas *et al*.[5] Plant extract solutions of different concentrations (1, 2, 4, 8, 16, 32, 64, 128, 256, 512, 1024, 2048, 3072 and 4096 µg/mL) were prepared and then 0.007886% w/v methanol DPPH solution were added to each. The absorbance was measured at 517 nm. Thus free radical scavenging capacity was calculated from log concentration-percent scavenged curve.[10]

Hydrogen peroxide scavenging assay

Hydrogen peroxide radical scavenging assay was tested according to the method of Keser *et al.* and Okolie *et al.* with minor modifications.[11,12] In the assay, plant extract solutions of varying concentrations (800, 400, 200, 100, 50, 25, 12.5 and 6.25 µg/mL) were prepared and then hydrogen peroxide (40 mM) was added into each. The absorbance was measured at 230 nm. The hydrogen peroxide scavenging effect by the sample extract and standard compounds were calculated.

Superoxide radical scavenging assay

In the superoxide radical scavenging activity test, superoxide anion (O^{2-}), derived from dissolved PMS-NADH coupling reaction, reduces NBT to a purple form and hence NBT is the probe to quantify its (O^{2-}) concentration.[13] Superoxide radical scavenging assay was determined by the method of Nishikimi *et al.*[14] Here, plant extract of varying concentrations (800, 400, 200, 100, 50, 25, 12.5 and 6.25 µg/mL) as well as NBT (312 µM), NADH (936 µM) and PMS (120 µM) were used. The superoxide scavenging effect by the sample extract and standard compounds were calculated from the curve of log concentration vs. percent inhibition using LDP line software.

Reducing power assay

The ferric reducing power of plant extract was measured according the method used by Okolie *et al.* with slight modifications.[15] In this assay, 1 mL of different concentrations of sample solution (800, 400, 200, 100, 50, 25, 12.5 and 6.25 µg/mL) was mixed with 2.5 mL of 0.2 M phosphate buffer and 2.5 mL of 1% potassium ferricyanide and incubated at 50ºC. Then 2.5 mL of 10% trichloroacetic acid (TCA) was added and centrifuged. After that 2.5 mL of this solution was added to 2.5 mL of distilled water and 0.5 mL of ferric chloride (0.1%). Absorbance was taken at 700 nm.

Determination of secondary metabolites

Total phenolic content assay

Total phenolic content (TPC) of the extract was determined by using FC reagent along with analytical grade gallic acid as the standard. TPC was ascertained as mg of Gallic acid equivalent per gram of dry extract by using the calibration curve.[5]

Total flavonoid content assay

Aluminum chloride colorimetric assay was used to determine total flavonoid content (TFC) of the extract.[16] TFC was expressed as mg of quercetin equivalent per gram of dry extract using the calibration curve.

Total tannin content assay

Total tannin content (TTC) of the sample extract was also determined by using FC reagent and analytical grade gallic acid standard.[17] TTC was ascertained as mg of Gallic acid equivalent per gram of dry extract using the calibration curve.

Evaluation of antidiabetic activity

Oral glucose tolerance test

In the present study, oral glucose tolerance test of plant extract was done and compared with the standard and control group according to the method of Golder *et al*.[18] Positive control group was treated with glibenclamide (standard drug) at the dose of 5 mg/kg body weight (b.w.) and control group were administered with 1% Tween 80 in water at the dose of 10 mL/kg. 30 minutes before the oral administration of glucose solution at 2 g/kg b.w., mice were treated with sample extract at 250 and 500 mg/kg b.w. doses. Blood glucose level (mmol/L) of each mouse were then measured at the 0, 30, 60, 90, 120 and 150 minutes with the help of a glucometer.

α-Glucosidase enzyme inhibitory activity

α-Glucosidase inhibitory activity was tested according to the method of Lawag *et al*.[19] and Qaisar *et al.*[20] with minor modifications. The enzymatic action of *α*-glucosidase is assessed by measuring the cleavage of the part of *α*-D-glucopyranoside. In this assay, mixture of 10 µL acarbose (standard drug) or plant extract solutions of different concentrations (0.5, 0.4, 0.3, 0.2, 0.1 mg/mL for acarbose and 16, 8, 4, 2, 1, 0.5, 0.25, 0.125 and 0.0625 mg/mL for plant extract), 112 µL phosphate buffer (PBS, pH 6.8), 20 µL *α*-glucosidase enzyme solution (1 unit/mL) was incubated for 15 minutes at 37ºC. 20 µL substrate (p-nitropheynyl glucopyranoside, pNPG) was added to it and again incubated for 15 minutes at 37ºC. 80 µL terminator (Na_2CO_3) solution was then added to terminate the enzyme-substrate reaction. Finally,

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the absorbance was measured at 405 nm with Thermo Scientific Multiskan Go spectrophotometer and IC_{50} value was calculated.[18]

Evaluation of analgesic activity

Peripheral analgesic activity of *A. corniculatum* extract was tested using acetic acid-induced writhing method.[18] Diclofenac Na (standard drug) at the dose of 25 mg/kg b.w. was the standard. After 30 minutes of oral administration of plant sample at 250 and 500 mg/kg b.w. doses, pain or writhing (constriction of abdomen, turning of trunk and extension of hind legs) was induced by administering 0.7% acetic acid intraperitoneally. After an interval of 15 minutes, writhing was counted for 5 minutes.

Evaluation of anti-inflammatory activity

Anti-inflammatory activity of *A. corniculatum* was tested using formaldehyde-induced paw edema method in mice.[21] Among six groups of animal, positive control group was treated with Ibuprofen (standard drug) at dose 100 mg/kg b.w. The plant sample was administered orally at 250 and 500 mg/kg b.w. doses. After 30 minutes, the linear circumference of the right hind paw was measured by slide calipers. Then 0.2% of 0.1 mL formaldehyde solution was injected into the right hind paw of the mice. After injecting, the linear circumference of the injected paw was measured at 1 hr, 2 hr, 3 hr and 4 hr, after formaldehyde injection and then change in paw size was calculated.

Evaluation of antidiarrhoeal activity

Antidiarrhoeal test was performed by using castor oil-induced diarrhoeal in mice.[18] Castor oil increases the intestinal motility and it reduces the absorption of fluid content. Positive control group was given loperamide (standard drug) at dose 3 mg/kg and test groups were given sample extract at 250 and 500 mg/kg b.w. doses. 30 minutes later, 0.5 mL castor oil was administered orally to induce diarrhoea. By placing each mouse in separate cage with blotting paper, the latent period and number of stools were counted for 4 hours.

Brine shrimp lethality bioassay

Brine shrimp lethality bioassay was performed on *Artemia salina* nauplii in order to investigate the cytotoxic activity of the *A. corniculatum* extract according to the method of Sarkar *et al*.[22] At first, the eggs of *A. salina* were hatched for nauplii. Solution of different concentrations of plant extract (5, 10, 20, 40, 80, 160, 320, 640 µg/mL) and standard (10, 5, 2.5, 1.25, 0.625, 0.3125, 0.1562 µg/mL) were prepared by serial dilution. In every test tube, 10 live nauplii of *A. salina* and different concentrations of samples were put and kept for 24 hours. After that time, the number of live nauplii were counted and percentage mortality was calculated to determine the LC_{50} value.

Statistical analysis

Statistical evaluations of test results were carried out using Dunnet's test for one-way ANOVA analysis ($p < 0.05$, versus control). Within the mean values, pair wise comparisons were carried out with Post-hoc Tukey test ($p < 0.05$, versus standard/extract). Data analysis was done with SPSS software of IBM Corporation, New York, USA (version 16.0).[23]

Results and Discussion

In the phytochemical assay, we observed that the ethanol extract of *A. corniculatum* leaves contain different types of secondary metabolites which include; reducing sugar, tannins, flavonoids, saponins, steroids, alkaloids, glycosides, terpenoids, etc.

In the qualitative antioxidant assay, TLC plates were kept under UV light at 254 and 366 nm where several UV positive and fluorescent components were found. Later, the plates were sprayed with DPPH solution that resulted in development of yellow colours which indicated the presence of antioxidant compounds. In quantitative DPPH free radical scavenging assay, percent inhibition of DPPH free radical by the extract and ascorbic acid was calculated at respective concentrations and from these values, the SC_{50} i.e., concentration required to scavenging 50% radical, was calculated. The SC_{50} values were 159.7 µg/mL and 14.8 µg/mL for *A. corniculatum* and ascorbic acid, respectively (Table 1). Another study on the leaves of the plant indicated a slightly better effect where a different DPPH scavenging method was used.[24] In the hydrogen peroxide scavenging assay, percent inhibition of hydrogen peroxide radical by the extract and ascorbic acid was calculated at respective concentrations. The SC_{50} values for *A. corniculatum* and ascorbic acid standard were 151.8 µg/mL and 11.1 µg/mL, respectively (Table 1). In the superoxide radical scavenging assay, the amount of percent inhibition of superoxide radical by the extract and ascorbic acid were also calculated at respective concentrations and the SC_{50} values of *A. corniculatum* and ascorbic acid were found to be 346.76 µg/mL and 114.36 µg/mL, respectively (Table 1). Again, in reducing power assay, the absorbance was measured at different concentrations and the RC_{50} (concentration required to reduce 50% radical) value of *A. corniculatum* was 233.04 µg/mL and for ascorbic acid, it was 28.09 µg/mL (Table 1). Moreover, total phenolic content of *A. corniculatum* was found to be 71 mg gallic acid equivalent (mg GAE)/g, total flavonoid content was 151 mg quercetin equivalent (QE)/g and total tannin content was 32 mg GAE/g (Table 1).

Secondary metabolites of mangrove plants like flavonoids, tannins, polyphenols, etc. protect them from harsh environmental conditions like; low oxygen, high salinity, water logging, high wind and temperature, etc.[24] These compounds also reduce the risk of developing chronic diseases like cancer, diabetes, cardiovascular diseases, etc. as well as can lessen the lipid peroxidation and DNA mutation in human.[25-27] In the present antioxidant activity evaluations, leaves of *A. corniculatum* demonstrated substantial radical scavenging potential as well as high content of secondary metabolites. Previous studies on *A. corniculatum* manifested the presence of different phytoconstituents like gallocatechin, epi-gallocatechin, epi-gallocatechin-3-O-gallote, epi-catechin-3-O-gallate, epigallocatechin benzylthioether, benzylmercaptan, isorhamnetin and so on. These secondary metabolites may be responsible for scavenging free radicals and protect oxidative damage.[28]

Traditionally, this plant has also been reported for its cytotoxic, analgesic and antidiabetic activities.[29,30] For this reasons we have further evaluated this plant for antihyperglycemic, analgesic, anti-inflammatory, antidiarrhoeal and cytotoxic potential.

Oral glucose tolerance test is a measurement of cell's capacity to clear off glucose after consumption of a specific amount of sugar possibly by inhibiting α-glucosidase (enzyme responsible for breakdown carbohydrate).[31] In the oral glucose tolerance test, *A. corniculatum* extract showed reduction in the blood glucose level within the observed time in a dose-dependent manner. The average values of blood glucose level (mmol/L) are presented in Figure 1.

Table 1: Approximate SC_{50} values of different radicals scavenging assays and total content of secondary metabolites (phenolics, flavonoids and tannins) of *A. corniculatum* leaves

Sample Extract	DRSA (SC_{50} µg/mL)	HPSA (SC_{50} µg/mL)	SRSA (SC_{50} µg/mL)	RPA (RC_{50} µg/mL)	TPC (mg GAE/g)	TFC (mg QE/g)	TTC (mg GAE/g)
A. corniculatum	160	152	347	233	71	151	32

DRSA (DPPH Radical Scavenging Activity), HPSA (Hydrogen Peroxide Scavenging Activity), SRSA (Superoxide Radical Scavenging Activity), RPA (Reducing Power Assay), TPC (Total Phenolic Content), TFC (Total Flavonoid Content), Total Tannin Content (TTC).

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Hence, we investigated the α-glucosidase inhibitory potential of the extract and percent inhibition of α-glucosidase by the extract and acarbose (standard) were calculated at respective concentrations and from these, the IC_{50} (concentration required to inhibit 50% enzyme) values were obtained using LdP Line software. The values were 0.342 mg/mL and 7.042 mg/mL for acarbose and *A. corniculatum*, respectively (Figure 2). Earlier reports stated that *A. corniculatum* leaves are rich in alkaloids, steroids, triterpenoids and flavonoids, polyphenols and tannins which may be responsible for the antihyperglycemic potential as well as the inhibition of α-glucosidase.[32]

Algesia or pain is an unpleasant sensation, usually evoked by external or internal noxious stimulus that causes excessive pain and other effects – sinking sensation, apprehension, sweating, nausea, palpitation, rise or fall in BP, etc.[33] Analgesic activity was measured by intraperitoneal administration of acetic acid which is responsible for synthesis of inflammatory mediators. This mediators are known to be accountable for pain sensation.[22] In the analgesic activity evaluation, *A. corniculatum* extract showed significant inhibition of writhing impulse, by 54.78% and 75.60% at 250 mg/kg and 500 mg/kg b.w. doses, respectively. The standard drug (Diclofenac Na) showed 78.25% inhibition of writhing at the dose of 25 mg/kg. The values are expressed in Table 2. Our findings were also supported by a previous work on the analgesic activity of the plant.[7] Significant inhibition of writhing impulse showed by the *A. corniculatum* extract may be due to the presence of aforementioned phytochemical groups like polyphenols, terpenoids, reducing sugar, flavonoids and tannins.

Inflammation, pain and pyrexia produce similar compulsive environments in our body.[34] So, following the evaluation of analgesic activity of the plant, we have tested its anti-inflammatory potential. In the anti-inflammatory test, the *A. corniculatum* extract showed significant inhibition in the paw edema in mice within the observation period at both the doses of 250 mg/kg and 500 mg/kg b.w. The values are shown in Figure 3. Substantial inhibition of paw edema demonstrated by the extract can be due to the presence of anti-inflammatory compounds like 4H-Pyran-4–one, catechin, gallocatechin, etc.[32] The study also justifies the use of the plant parts as an anti-inflammatory agent in folklore medicine.[35]

Alteration of intestinal motility and increased amount of bowel movement is the main characteristics of diarrhoea which was induced by giving castor oil orally in the antidiarrhoeal test. In castor oil-induced diarrhoeal mice, *A. corniculatum* extract showed increase in the latent period of defecation by 90 and 129.4 minutes at doses of 250 mg/kg and 500 mg/kg b.w., respectively, whereas for the standard (loperamide at 3 mg/kg dose) and control group, the latent period of defecation was 32 and 171 minutes, respectively. The extract showed significant inhibition of defecation, by 52.33% and 66.53% at 250 mg/kg and 500 mg/kg doses, respectively (Table 3). Our results were also supported by a previous small scale study on the antidiarrhoeal activity of the plant.[7] Presence of polyphenols, tannins, flavonoids, sterols, alkaloids, triterpenes, etc. may be responsible for the antidiarrhoeal potential of the plant.

Brine shrimp lethality bioassay is a common laboratory test for evaluation of cytotoxic activity.[36] It is based on the ability to kill laboratory-cultured *Artemia* nauplii brine shrimp.[37] In brine shrimp lethality bioassay, the LC_{50} value for *A. corniculatum* was 11.297 µg/mL and it was 0.577 µg/mL for vincristine sulphate (Figure 4). The values were calculated using LDP Line software. Percent increase in brine shrimp mortality with the increase in concentration may be due to the presence of stated cytotoxic compounds like 2-O-acetyl-5-O-methylembelin, 5-O-methylembelin, etc.[38]

From the above study it can be comprehended that *A. corniculutum* revealed multiple pharmacologic potential namely; antioxidative, antihyperglycemic, analgesic, anti-inflammatory, antidiarrhoeal and cytotoxic potential as well as the presence of several bioactive phytoconstituents. This justifies the plant's usage in traditional medicine system as well as signifies the plant's potential in future studies for isolating therapeutically active constituents and subjecting it to the biological activity to find fruitful results.

Table 2: Representation of effects of *A. corniculatum* extracts on acetic acid induced writhing in mice.

Treatment	Dose (mg/kg)	Mean Writhing	% Inhibition of Writhing
Negative control	--	27 ± 2.0	--
Standard (Diclofenac Na)	25	5.8 ± 0.84 $*^{▲}$	78.25 ± 4.80 $*^{▲}$
A. corniculatum	250	12.2 ± 2.59 $*^{θΔ}$	54.78 ± 9.42 $*^{θΔ}$
A. corniculatum	500	6.6 ± 1.14 $*^{θ▲}$	75.60 ± 3.56 $*^{θ▲}$

Data are means of five replicates ± SD (standard deviation); * P < 0.05 vs. Control (Dunnett's t test); ^{θ} P < 0.05 vs. Diclofenac Na 25 mg/kg; $^{▲}$ P < 0.05 vs *A. corniculatum* 250 mg/kg; ^{Δ} P < 0.05 vs. *A. corniculatum* 500 mg/kg (pair-wise comparison by Post Hoc Tukey test).

Table 3: Representation of effects of *A. corniculatum* extracts on castor oil induced diarrhoeal mice.

Treatment	Dose (mg/kg)	Mean latent period (min)	% Inhibition of defecation
Negative control	--	32 ± 4.53	--
Standard (Loperamide)	3	171 ± 14.19 $*^{▲Δ}$	76.91 ± 5.54 $*^{■▲}$
A. corniculatum	250	90 ± 10.27 $*^{θΔ}$	52.33 ± 9.40 $*^{θ}$
A. corniculatum	500	129.4 ± 16.06 $*^{θ▲}$	66.53 ± 11.56 *

Data are means of five replicates ± SD (standard deviation); * P < 0.05 vs. Control (Dunnett's t test); ^{θ} P < 0.05 vs. Loperamide 3 mg/kg; $^{▲}$ P < 0.05 vs *A. corniculatum* 250 mg/kg; ^{Δ} P < 0.05 vs. *A. corniculatum* 500 mg/kg (pair-wise comparison by Post Hoc Tukey test).

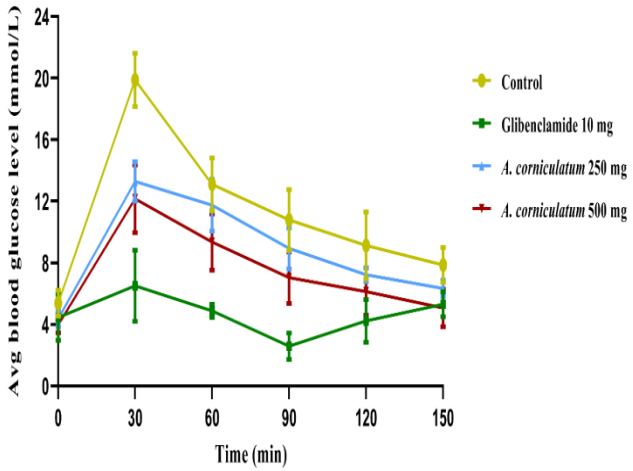

Figure 1: Comparison of blood glucose levels (mmol/L) at different time points for control, standard and *A. corniculatum* oral in the glucose tolerance test.

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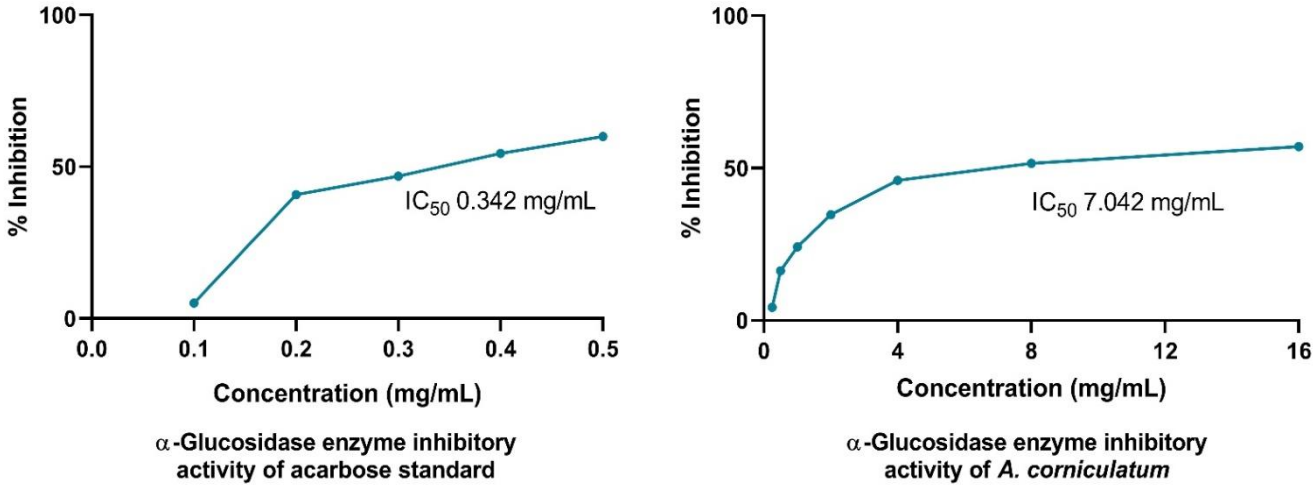

Figure 2: Graphical presentation of α-glucosidase enzyme inhibitory activity and IC_{50} value of standard (acarbose) and *A. corniculatum* leaves extract.

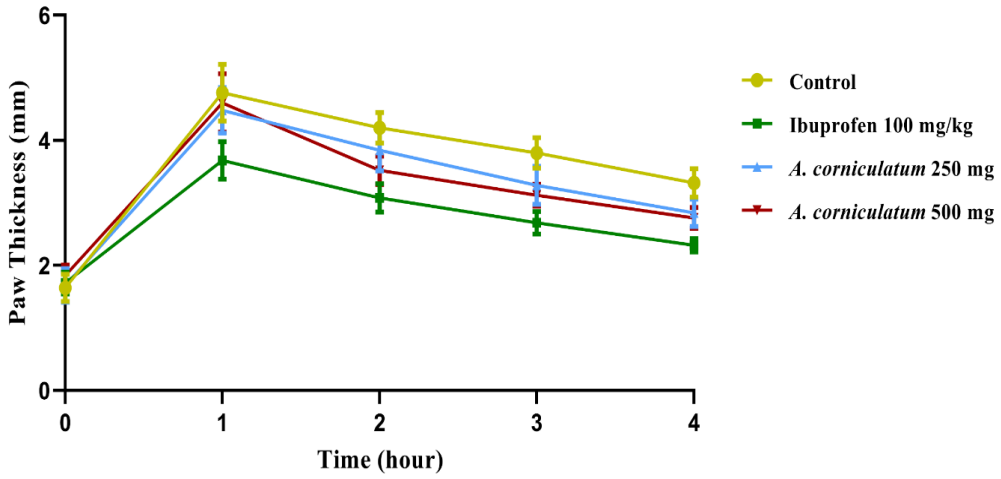

Figure 3: Comparison of paw thickness at different times for control, standard and *A. corniculaum* in the formaldehyde-induced anti-inflammatory activity test.

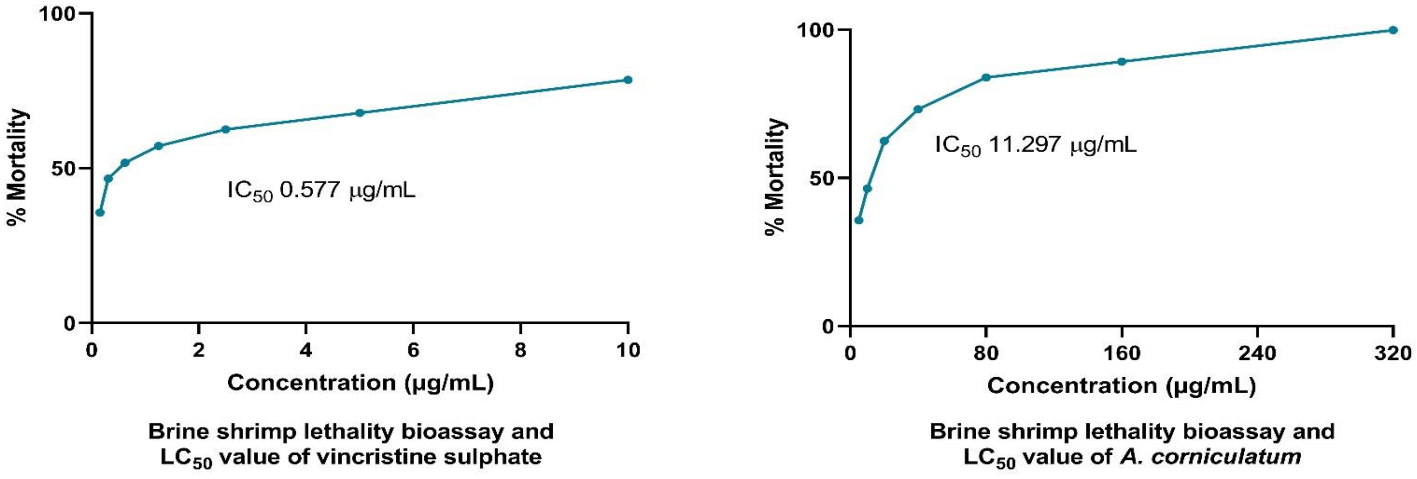

Figure 4: Graphical presentation of brine shrimp lethality bioassay and LC_{50} of vincristine sulphate and *A. corniculatum* leaves extract.

Conclusion

From the current study on the pharmacological activities of ethanol extract of the leaves of a mangrove plant, *A. corniculatum*, it is therefore concluded that the plant extract scavenged free radicals and showed significant biological responses namely, antihyperglycemic, analgesic, anti-inflammatory, antidiarrhoeal and cytotoxic effects. Based on the experimental data, it can be suggested that the leaves of *A. corniculatum* is a potential source of different bioactive medicinal components. Therefore, in future, isolation of pure bioactive compounds from this plant is recommended.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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