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ORIGINAL ARTICLE

Neuroprotective and Anti-inflammatory Activities of *Aegiceras corniculatum* (L.) Blanco

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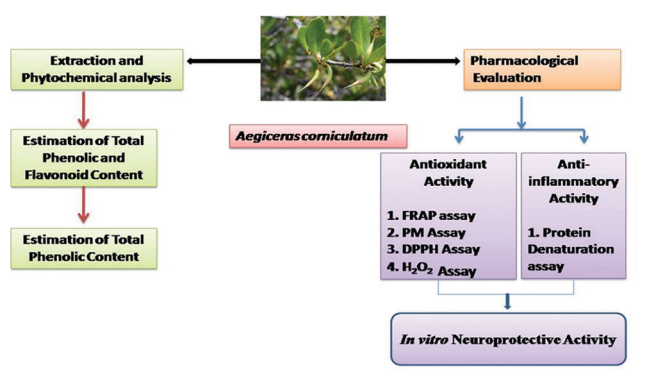
ABSTRACT

Objectives: The objective of the study is to evaluate the antioxidant, anti-inflammatory, and neuroprotective potential of *Aegiceras corniculatum* (L.) Blanco. **Materials and Methods:** Sequential Soxhlet extraction of titled plant material was carried out. Phytochemical analysis was performed using standard procedures. The total phenolic and flavonoid content was estimated along with *in vitro* antioxidant property of the extracts which was assessed by ferric ion-reducing power assay, phosphomolybdenum assay, 2,2-diphenyl-2-picrylhydrazyl assay, and hydrogen peroxide scavenging assay. Further, the extracts were assessed for anti-inflammatory property by *in vitro* protein denaturation assay, whereas neuroprotective activity was performed by pretreatment 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. **Results:** Qualitative phytochemical analysis revealed the presence of alkaloids, phenols, flavonoids, tannins, anthraquinones, glycosides, lignins, and sterols. Quantitative phytochemical analysis revealed that the aqueous extract possessed high phenolic content and methanol extract possessed high flavonoid content when compared to other extracts. All the extracts showed antioxidant activity revealed through different assays. Among all the extracts, methanol extract showed significant anti-inflammatory activity with 85.32 ± 1.63 percentage inhibition of protein degradation. Methanol extract at the concentration of 5 µg showed comparatively significant neuroprotection against trimethyltin (TMT)-induced toxicity with 70.02% SK-S-NH cell survival against 56.30% cell survival TMT-intoxicated group. **Conclusion:** The leaf extracts of *A. corniculatum* possess antioxidant, anti-inflammatory, and neuroprotective properties.

Key words: *Aegiceras corniculatum,* anti-inflammatory activity, antioxidant activity, mangrove plants, neuroprotective activity

SUMMARY

- In the current investigation, the mangrove plant *Aegiceras corniculatum* L. Blanco was subjected to extraction using Soxhlet apparatus. The extracts were analyzed for the presence of secondary metabolites. The total phenolic and flavonoid content was analyzed using standard methods. These extracts were subjected for antioxidant, anti-inflammatory, and *in vitro* neuroprotective activities. The extracts showed potent activities with all the activities by comparing with the respective standard drugs. This investigation can be further extrapolated to isolate pure phytoconstituents with potent pharmacological capabilities to treat various dreadful diseases.



Abbreviations Used: DPPH-2: 2,2-diphenyl-2-picrylhydrazyl, WHO: World Health Organization, NSAIDs: Nonsteroidal anti-inflammatory drugs, TMT: Trimethyltin, FRAP: Ferric ion-reducing antioxidant power, PM: Phosphomolybdenum, ROS: Reactive oxygen species, MTT: 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide.

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INTRODUCTION

Plants are the rich sources of various phytochemicals which act as defense mechanism from any plant predator. These secondary metabolites are very useful in treatment of large number of human ailments with negligible any side effect. Plant-based drugs are being utilized since ancient times to cure many human and animal diseases. According to the WHO reports, roughly 80% of the world population is reliant on the traditional medicine for the treatment of human ailments.[1] The herbal medicine systems such as Ayurveda, Homeopathy, Unani, and Siddha have taken over modern medicines because modern medicines are highly expensive and pose a high risk of side effects which are difficult to be afforded by the morbid people in the developing and overpopulated countries such as India. Most of the plants are still unexplored for their medicinal properties, so there is a great need to explore therapeutic plants. Thus, the present investigation was a focus on the same. Mangrove vegetation is a wetland ecosystem formed by a unique association of animals and plants which multiply lavishly in the coastal areas and river estuaries throughout the lower tropical and subtropical latitudes.[2] The term mangrove is also used to describe halophytic and salt-resistant marine forests consisting of trees, shrubs, palms, epiphytes, ground ferns, and grasses.[3] Many mangrove plants possess medicinal properties and were used to treat many diseases (reference). *A. corniculatum* (L.) Blanco belongs to family *Myrsinaceae*, and this is a mangrove shrub which grows in

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highly alkaline conditions near the coastal areas of India. The plant is used in various traditional medicinal systems.[4]

Free radicals are highly reactive agents with an unpaired electron produced when cells are exposed to stress(s). They instigate chain reactions, which lead to crumbling of cell membranes and cell components, including lipids, proteins, and nucleic acids. Oxidative stress generated from free radicals is the major implication in the pathogenesis of a wide variety of clinical disorders, such as cancer, cardiovascular disease, Alzheimer's disease, autoimmune disease, diabetes, multiple sclerosis, and arthritis.[5-8]

Inflammation is an extremely complex process involving many systems which are closely associated with the process of repair. Inflammation can be defined as a localized response of the tissues to injury caused due to any agent and exhibit usually in the form of painful swelling associated with some changes in the skin. The typical signs of inflammation are local redness, swelling, pain, heat, and loss of function. Inflammation can be either acute or chronic based on the occurrence. Acute inflammation is the immediate response of the body to disparaging stimuli, wherein the plasma and leukocytes of the blood infiltrate into the site of injury. Chronic inflammation involves shifting of the cells which are present at the site of inflammation characterized by instantaneous destruction and curative effects of the tissue from the inflammatory process.[9] At present, nonsteroidal anti-inflammatory drugs are being administered for the treatment of orthopedic-related injuries, arthritis, and bone fractures. The greatest concern about these drugs is toxicity and reappearance of the symptoms after the discontinuation of the drugs. Hence, it is needed to look into alternative medicines for the treatment of the inflammatory responses.[10]

Neurodegenerative disorders are a major concern in the present population. The principal cause for acute and chronic neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and stroke is age dependent. In a global scenario, the incidence of these diseases is expected to increase drastically in the near future. The effective preventive measures for these disorders are still less known till date. The currently available treatments are mainly symptomatic and are effective for a shorter period of time. Due to lack of effective treatment options, natural and herbal medicines have become an important alternative strategy to prevent or delay the lethal effects of aging.[11,12]

Trimethyltin (TMT) is an organotin compound which is used as a stabilizer in plastics as chemosterilant for the control of fungus, bacteria, and insects.[13] TMT is a well-known neurotoxic agent, which affects majorly the central nervous system and limbic system.[14] In the present study, the neuroprotective effects of *A. corniculatum* were evaluated against TMT-induced neurotoxicity in SK-S-NH neuroblastoma cell lines.

The present study aims at evaluating therapeutic applications of *A. corniculatum* leaf extracts as a potential antioxidant, anti-inflammatory, and neuroprotective agent.

MATERIALS AND METHODS

Collection of plant material

The *A. corniculatum* plant material (leaf) was collected from Karwar, Karnataka, India (14°48'N, 74° 11' E) in the month of February 2018. The plant was authenticated by a taxonomist, Dr. K. Kotresha, Department of Botany, Karnatak Science College, Dharwad, Karnataka, India. The herbarium was prepared, and a voucher specimen was deposited in the Department of Botany, Karnatak Science College, Dharwad, Karnataka, India (No. 6). The plant leaf material was washed under running tap water to remove the dust particles and dried under the shade for 15–20 days, powdered coarsely, and stored at airtight containers at cool and dry place.

Solvent phytoextraction

Around 100 g of leaf material was subjected to sequential Soxhlet extraction using different solvents, namely chloroform, ethyl acetate, methanol, ethanol, and water. The extracts were then concentrated using rotary flash evaporator (BUTCHI Rotavapor R-210, Mumbai, India) to get thick concentrated extracts. The concentrated extracts were kept in airtight containers and kept under refrigeration till further use.

Qualitative phytochemical analysis

Qualitative phytochemical analysis was carried out for all the extracts by the following standard methodology.[15]

Evaluation of total phenolic and flavonoid content

Total phenolic content of the *A. corniculatum* extracts was evaluated by Folin–Ciocalteu (FC) method described by Singleton *et al*. with minor modifications. 0.5 ml of extracts was mixed with 2 ml of FC reagent (1:10 diluted), and the reaction was neutralized by the addition of 8% (w/v) sodium carbonate solution and incubated for 30 min in dark conditions. The reaction mixture was checked for color development and analyzed using ultraviolet-visible (UV-Vis) spectrophotometer at 765 nm. Gallic acid was used as standard for comparison. The total phenolic content was estimated from the linear equation of standard curve prepared with gallic acid. The total phenolic content was expressed as mg/g of gallic acid equivalent (GAE).[16]

Total flavonoid content was estimated by the method of Chang *et. al*. (2002). 0.5 ml of the plant extracts was mixed with 1.5 ml of methanol, 0.1 ml of 10% (w/v) anhydrous aluminum chloride ($AlCl_3$), 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water in separate test tubes. The whole reaction mixture was incubated at room temperature for 30 min. The absorbance of the color complex formed was read at 415 nm using UV-Vis spectrophotometer with quercetin as a reference standard. 12.5–100 µg/ml of quercetin in methanol as standard was used for generation the calibration curve.[17]

In vitro antioxidant activity of *Aegiceras corniculatum*

Ferric ion-reducing power assay

Ferric ion-reducing power was measured according to the method proposed by Oyaizu with minimal modifications.[18] The extracts of *A. corniculatum* were pipetted in different concentrations ranging from 100 µl to 500 µl and were mixed with 2.5 ml of 20 mM phosphate buffer and 2.5 ml (1% w/v) potassium ferricyanide, and then, the mixture was incubated at 50°C for 30 min. To this mixture, 2.5 ml of (10% w/v) trichloroacetic acid and 0.5 ml of (0.1% w/v) ferric chloride were added to the mixture and kept for 10 min at room temperature to produce green-colored complex. The absorbance of the color developed was measured at 700 nm using UV-Vis spectrophotometer. Ascorbic acid was used as a positive reference standard.

Phosphomolybdenum assay

Total antioxidant activity was estimated by phosphomolybdenum (PM) assay using standard procedure of Prieto *et al*.[19] Ascorbic acid was used as a standard reference. The extracts of *A. corniculatum* in various concentrations ranging from 100 µl to 500 µl were added to each test tube individually containing 3 ml of distilled water and 1 ml of ammonium molybdate reagent. The tubes were incubated in water bath at 95°C for 90 min. After incubation, these tubes were cooled at room temperature for 20–30 min, and the absorbance of the reaction mixture was measured at 695 nm.

2,2-diphenyl-2-picrylhydrazyl radical scavenging assay

The 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay was carried out with all the extracts of *A. corniculatum* according to the method proposed by Rice-Evans *et. al*.[20] About 100 µl of a DPPH solution in prepared in ethanol (60 µmol/l) was mixed with 100 µl of the test samples in various concentrations. The blend was incubated for ½ h in dark at room temperature, and the resultant absorbance was measured at 517 nm. Ascorbic acid was used as a standard. The DPPH scavenging activity of each sample was calculated using the following equation:

DPPH scavenging activity (%) = $A_c - A_t / A_c \times 100$

Where A_c is the absorbance of the control reaction (100 µl of ethanol with 100 µl of the DPPH solution) and A_t is the absorbance of the test sample. The whole experimental setup was performed in triplicates.

Hydrogen peroxide scavenging assay

In vitro hydrogen peroxide (H_2O_2) scavenging activity was performed in accordance with the method developed by Gulcin *et al*.[21] using ascorbic acid as the standard reference. 0.5 ml test extracts were mixed with 0.5 ml of phosphate buffer (pH-6.4) and 0.6 ml of 4 mM H_2O_2. Then, the absorbance was measured immediately at 230 nm using phosphate buffer as blank. The percentage inhibition was calculated by the formula:

% inhibition = $A_c - A_t / A_c \times 100$

Where A_c is the absorbance of the control containing phosphate buffer and H_2O_2 and A_t is the absorbance of the test samples.

In vitro anti-inflammatory assay

In vitro anti-inflammatory activity was performed by protein denaturation assay. This activity was performed in accordance with the method developed by Padmanabhan and Jangle.[22] Diclofenac sodium was used as a standard reference (Reactin-100, Cipla). In this method, 0.5 ml of each extract of *A. corniculatum* was mixed with 2.5 ml of phosphate-buffered saline (PBS) and 2 ml of egg albumin. The reaction mixture was preincubated at 27°C for 15 min. Then, the tubes were transferred to water bath and incubated at 70°C for 10 min and cooled. The absorbance of each tube was measured at 660 nm using UV-Vis spectrophotometer against double-distilled water as blank. PBS and egg albumin were used to take the control readings. The percentage inhibition was measured by the formula:

% inhibition = $A_t - A_c / A_c \times 100$

Where A_c is the absorbance of the control and A_t is the absorbance of the test.

In vitro neuroprotective activity

In vitro neuroprotective activity was evaluated by "MTT pretreatment method." SK-S-NH neuroblastoma cell lines were used for the neuroprotective activity. The cells were trypsinized and aspirated into a 50 ml centrifuge tube. Cell pellet was obtained by centrifugation at 300 ×g. The cell count was adjusted, using Minimum Essential Medium containing 10% FBS, such that 200 µl of suspension contained approximately 15,000 cells. The cells were seeded on 96-well plate and incubated for 24 h in CO_2 incubator at 37°C and 5% CO_2. The test concentration of the extract under the study was added in different concentrations and incubated for 24 h under the same conditions. The media containing extract was removed by aspiration, and the cells were then challenged with TMT (1 mM) and incubated for 3 h. After the incubation, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added and observed for the formation of formazan using microplate reader.

Statistical analysis

All the results (triplicates) were represented as mean ± standard deviation. One-way ANOVA was carried out to check the variation between the samples using IBM SPSS Statistics 20. Ink, India. The levels of significance were considered as $P < 0.01$ and $P < 0.05$ for the comparison.

RESULTS

Phytochemical analysis

The preliminary phytochemical analysis revealed the presence of alkaloids, phenols, flavonoids, tannins, anthraquinones, glycosides, lignins, sterols, and volatile oils in various extracts such as chloroform, ethyl acetate, methanol, ethanol, and aqueous. The chloroform extract showed the presence of flavonoids, glycosides, and sterols. Ethyl acetate extract showed the presence of alkaloids, phenols, glycosides, and sterols. Methanol extract revealed the presence of flavonoids, glycosides, lignins, sterols, and tannins. Ethanol extract showed the presence of glycosides, phenols, sterols, and tannins. The aqueous extract unveiled the presence of alkaloids, flavonoids, glycosides, phenols, sterols, tannins, anthraquinones, and volatile oils. The results are depicted in Table 1.

Estimation of total phenolic and flavonoid content

The total phenolic content of the selected extracts was estimated with gallic acid as a reference standard. The aqueous extract showed high phenolic content (27.0090 ± 0.04129 mg) when compared to ethanol extract (10.0991 ± 0.09487 mg) and ethyl acetate

Table 1: Preliminary phytochemical analysis of the extracts of *Aegiceras corniculatum*

	Inference			
	Chloroform	Ethyl acetate	Methanol	Aqueous
Alkaloids				
Iodine	–	–	–	–
Wagner's test	–	+	–	+
Dragendroff's test	–	–	–	+
Flavonoids				
Pew's test	+	–	–	+
Shinoda's test	++	–	+	–
NaOH test	+	–	+	–
Glycosides				
K-K test	+	+	+	+
Glycoside test	–	+	–	–
Molisch's test	+	+	++	
Phenols				
Ellagic acid test	–	+	–	+
Phenol test	–	+	–	+
Lignin				
Lobat test	–	–	++	-
Saponins				
Foam test	–	–	+	+
Sterols				
L-B test	–	++	+	++
Salkowski test	++	++	+	++
Tannins				
Gelatin test	–	–	+	+
Lead acetate test	–	–	+	+
Anthraquinone				
Borntrager's test	–	–	–	+
Phlobatannins	–	–	–	–
Reducing sugar	–	–	–	–
Volatile oil	–	–	–	+
Terpenoids	–	–	++	–

–: Absence; +: Presence; ++: Present in high amount

extract (8.40 ± 0.05000 mg). The results were expressed as mg/g GAE per gram of plant extract [Table 2]. Total flavonoid content was performed using $AlCl_3$ method using quercetin as a standard. The chloroform extract showed high flavonoid content (63.47 ± 0.88059 mg), whereas methanol extract showed moderate flavonoid content (51.9221 ± 0.07300 mg) and aqueous extract (20.3568 ± 0.05064 mg). The results were expressed as mg/g of quercetin equivalent [Table 3].

In vitro antioxidant activity of *Aegiceras corniculatum*

Ferric ion-reducing power assay

Ferric ion-reducing power assay (FRAP) was performed with chloroform, ethyl acetate, methanol, and aqueous extracts using ascorbic acid as standard. In this assay, aqueous extract (1.2407 ± 0.00702) showed the highest antioxidant activity among the extracts used for the assay which was comparable to standard [Table 4 and Figure 1].

Phosphomolybdenum assay

PM assay was performed for four different extracts with standard ascorbic acid. The results revealed that the chloroform extract showed higher antioxidant capacity (0.8983 ± 0.00351) than the other extracts and was compared to the standard ascorbic acid. The results are depicted in Table 5 and Figure 2.

2,2-diphenyl-2-picrylhydrazyl assay

DPPH assay was performed with four different extracts and ascorbic acid standard. Results of this assay showed potent antioxidant activity with ethyl acetate extract being the highly potent (86.24 ± 0.35), aqueous extract (83.4033 ± 0.17786), and methanol extract (82.5333 ± 0.23714) and also showed better activity which was comparable with the standard ascorbic acid [Table 6 and Figure 3].

Hydrogen peroxide scavenging assay

H_2O_2 radical scavenging assay was performed with four extracts and ascorbic acid standard. The chloroform extract showed potent scavenging activity (76.8233 ± 0.09074), and also, the aqueous extract showed better scavenging activity (67.7133 ± 0.07506) which was comparable to the standard [Table 7 and Figure 4].

In vitro anti-inflammatory activity

In vitro anti-inflammatory activity of four different extracts of *A. corniculatum* was performed using protein denaturation assay. Diclofenac sodium was used as a standard reference. The methanol extract showed high anti-inflammatory activity (85.3200 ± 1.63000) which was comparable with standard. The aqueous extract also showed potential anti-inflammatory activity (71.9167 ± 1.12970) compared to the remaining extracts [Table 8 and Figure 5].

In vitro neuroprotective activity

In vitro neuroprotective activity by "pretreatment MTT assay" showed the protection at lower concentration, whereas the higher doses showed toxicity as the dose increased. The methanolic extract was found to be nontoxic at lower concentrations and showed protection at lower concentrations. The concentration of 5 µg/ml showed 70.02% cell viability, 50 µg/ml showed 67.86% viability, and 100 µg/ml showed 51% viability, respectively, against the percentage of cell survival in

Table 2: Total phenolic content of the leaf extracts of *Aegiceras corniculatum*

Extract	Total phenolic content (mg)
Ethyl acetate	8.40±0.05000
Methanol	10.0991±0.09487
Aqueous	27.0090±0.04129

Table 3: Total flavonoid content of the leaf extracts of *Aegiceras corniculatum*

Extract	Total flavonoid content (mg)
Chloroform	63.47±0.88059
Methanol	51.9221±0.07300
Aqueous	20.3568±0.05064

Table 4: Ferric ion-reducing power assay of *Aegiceras corniculatum*

Concentration	Standard ascorbic acid	Chloroform extract	Ethyl acetate extract	Methanol extract	Aqueous extract
100 µl	0.4650±0.00300	0.2147±0.00351	0.2887±0.00351	0.2720±0.00600	0.5577±0.00351
200 µl	0.7530±0.00458	0.3713±0.00416	0.4437±0.00404	0.3450±0.00436	0.7747±0.00651
300 µl	0.9943±0.00416	0.4243±0.00351	0.6137±0.00306	0.4917±0.00651	0.9313±0.00850
400 µl	1.1353±0.00416	0.6433±0.00416	0.7497±0.00208	0.5150±0.00436	1.0890±0.00700
500 µl	1.4647±0.00306	0.7913±0.00321	0.8947±0.00351	0.8087±0.01007	1.2407±0.00702

The results expressed as mean±SD (n=3) were analyzed using one-way ANOVA. SD: Standard deviation

Table 5: Phosphomolybdenum assay of *Aegiceras corniculatum*

Concentration	Standard ascorbic acid	Chloroform extract	Ethyl acetate extract	Methanol extract	Aqueous extract
100 µl	0.2780±0.00400	0.4513±0.00306	0.1363±0.00208	0.1123±0.00252	0.1393±0.00153
200 µl	0.5020±0.00400	0.4897±0.00208	0.2537±0.00351	0.2043±0.00252	0.2627±0.00153
300 µl	0.6810±0.00458	0.7100±0.00200	0.3523±0.00351	0.3300±0.00458	0.4017±0.00252
400 µl	0.9017±0.00351	0.8187±0.00503	0.4490±0.00265	0.4773±0.00208	0.4570±0.00173
500 µl	1.1313±0.00702	0.8983±0.00351	0.5453±0.00306	0.4947±0.00058	0.6533±0.00379

The results expressed as mean±SD (n=3) were analyzed using one-way ANOVA. SD: Standard deviation

Table 6: 2,2-Diphenyl-2-picrylhydrazyl assay of *Aegiceras corniculatum*

Concentration	Standard ascorbic acid	Chloroform extract	Ethyl acetate extract	Methanol extract	Aqueous extract
10 µg	67.7867±0.17898	43.116±0.41741	79.9867±0.40624	67.3600±0.30806	66.5433±0.41741
20 µg	73.6533±0.23502	54.4233±0.30616	82.4333±0.13279	69.4200±0.29206	70.8167±0.24420
30 µg	78.2000±0.30806	65.0667±0.17786	83.4067±0.29143	75.2867±0.23502	73.1133±0.46972
40 µg	80.8000±0.35679	68.3700±0.35679	84.5700±0.29206	80.8800±0.47000	79.6367±0.48439
50 µg	83.9100±0.35000	72.1400±0.64838	86.2400±0.35000	82.5333±0.23714	83.4033±0.17786

The results expressed as mean±SD (n=3) were analyzed using one-way ANOVA. SD: Standard deviation

TMT-intoxicated group which was 56.30% [Table 9]. The graph was plotted with concentration of the extract on X-axis with percentage viability on Y-axis. The results were compared with standard ascorbic acid. The cell viability decreased as the concentration of the methanol extract was increased. The results are depicted in Figure 6.

DISCUSSION

Medicinal plants besides potent therapeutic agents are also vast collection of chemical agents which are potent drugs in treating many diseases or conditions. These phytochemicals are more promising with lesser side effects. In recent days, the modern medicine systems are using these phytochemicals as clues for designing newer drugs.[23] The present study has noted the isolation of various secondary metabolites which are known to possess different protective mechanisms to dreadful diseases and conditions. In the recent years, there is an increased demand for traditional medicines which are based on these phytochemical principles. The major secondary metabolites which can potentially inhibit the oxidative stress are mainly alkaloids, flavonoids, phenols, tannins, sterols, glycosides, and terpenoids.

The mangrove plant *A. corniculatum* is used in the treatment of various diseases by traditional practitioners and it is due to these phytochemical principles which play a role in controlling the disease condition.

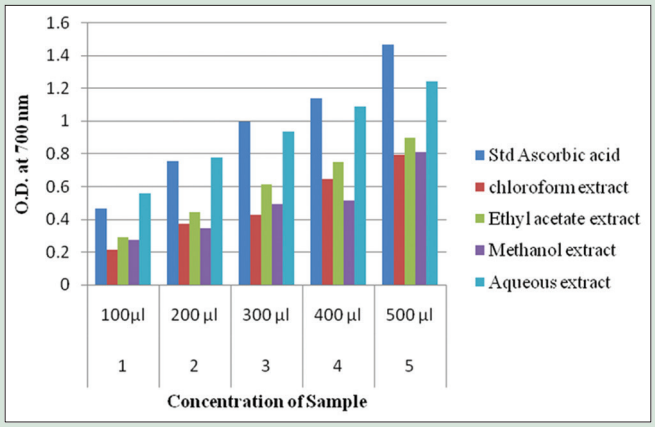

Figure 1: Ferric ion-reducing power assay of *Aegiceras corniculatum* extracts

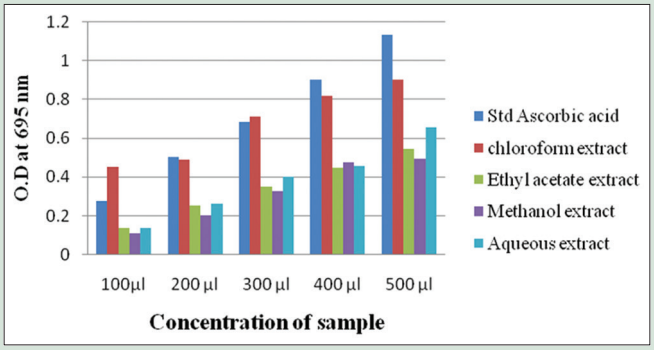

Figure 2: Phosphomolybdenum assay of *Aegiceras corniculatum* extracts

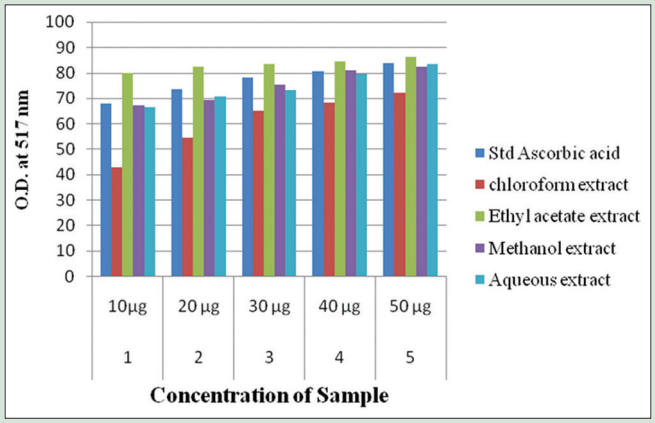

Figure 3: 2,2-Diphenyl-2-picrylhydrazyl assay of *Aegiceras corniculatum* extracts

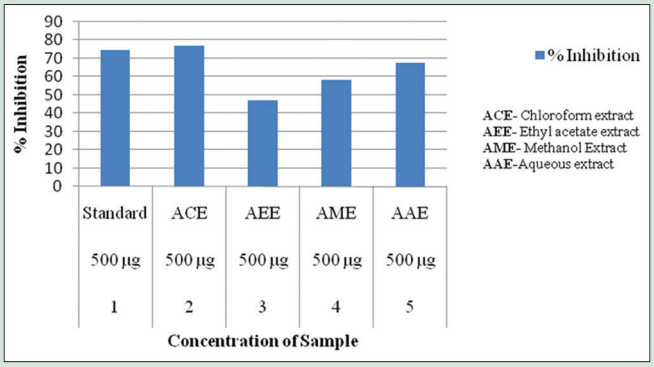

Figure 4: Hydrogen peroxide scavenging activity of *Aegiceras corniculatum* extracts

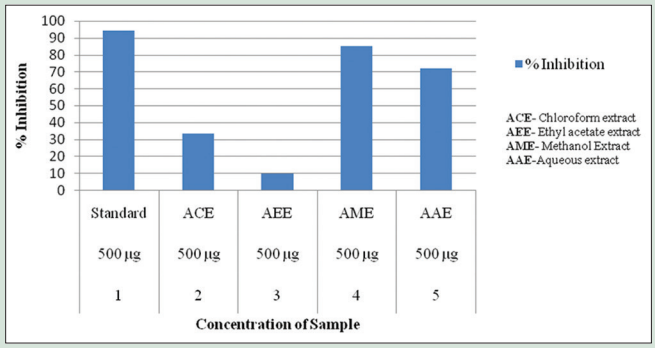

Figure 5: Anti-inflammatory assay of *Aegiceras corniculatum* extracts

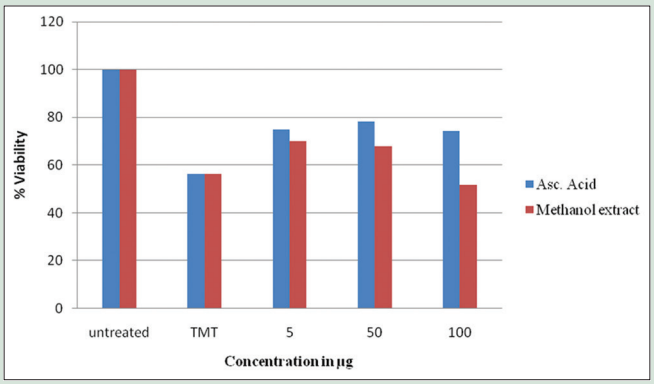

Figure 6: *In vitro* neuroprotective activity of *Aegiceras corniculatum*

Flavonoids are involved in the free radical scavenging, anticancer, and antimicrobial properties. These flavonoids are also involved in improving the blood circulation to the brain in Alzheimer's disease.[24] Alkaloids are mostly used in the treatment of Alzheimer's, Parkinson's disease, and as antimicrobial, antimalarial agents.[25] Phenols are a kind of natural products and antioxidant substances which are competent scavengers of free superoxide radicals, anti-aging, and reducing the risk of cancer.[26] Tannins may be employed medicinally in antidiarrheal, hemostatic, and antihemorrhoidal compounds. The anti-inflammatory effects of tannins help control all indications of gastritis, esophagitis, enteritis, and irritating bowel disorders.[27] Sterols are the biological molecules which are mainly used in the treatment of cardiovascular disease, in lowering the low-density lipoprotein-cholesterol levels, in the treatment of breast cancer and prostate cancer.[28] Terpenoids are having various medicinal properties such as anticarcinogenic, antimalarial, antiulcer, hepaticidal, antimicrobial, or diuretic activity and anticancer activity.[29]

The antioxidant defense mechanism is an inbuilt mechanism found in humans. However, nowadays, due to lifestyle changes and varied food habits, the antioxidant balance to the oxidants produced has been reduced. Hence, there is an immediate need of potent antioxidant molecules which can stabilize the reactive oxygen species (ROS) and free radicals generated during the metabolic processes. To search for potent antioxidant molecules, the present study aimed to evaluate the leaf extracts of *A. corniculatum,* for different *in vitro* antioxidant assays. Four different assays were conducted to check the antioxidant power of the leaf extracts such as FRAP, PM assay, DPPH assay, and H_2O_2 scavenging assay. In FRAP, Fe^{+3} ions donate an electron and converted into Fe^{+2} in the presence of plant extracts as reducing agents. This assay has become a matter of interest due to its ease in performance and its accuracy.[30] In the present study, aqueous extract (1.2407 ± 0.00702) showed the highest antioxidant activity when compared to other extracts.

PM assay is a very useful method to predict the antioxidant activity of crude extracts on the total basis. This assay is based on the principle of reduction of phosphate-molybdenum (VI) to phosphate-molybdenum (V).[31] In the present study, chloroform, ethyl acetate, methanol, and aqueous extracts were subjected to PM assay. Out of all four extracts, the chloroform extract showed potent antioxidant activity (0.8983 ± 0.00351).

DPPH assay is the most widely used method in evaluating *in vitro* antioxidant assay. This method is based on the scavenging of DPPH free radical into a more stable DPPH which can be observed by the change of color from violet to yellow. This change of color is due to the donation of hydrogen atom by antioxidants.[32] In the present investigation, potent antioxidant activity was observed in ethyl acetate extract (86.2400 ± 0.35000), whereas aqueous extract (83.4033 ± 0.17786) and methanol extracts (82.5333 ± 0.23714) also were found to be very potent which were comparable with the standard. Thus, the present study confirms the antioxidant capacity of the different extracts of *A. corniculatum*.

H_2O_2 scavenging assay is a weak oxidizing agent which inactivates certain enzymes directly by oxidation of vital thiol (-SH) groups. It can pass cell membranes quickly; once inside the cell, it is most likely to react with Fe^{2+} and possibly Cu^{2+} ions to form hydroxyl radicals leading to many toxic effects.[33] In the present investigation, the chloroform extract showed higher scavenging activity (76.8233 ± 0.09074) than the standard, and also, the aqueous extract showed scavenging activity (67.7133 ± 0.07506) which was comparable to the standard.

Inflammation is a condition in which different immune cells accumulate at the site of the damage. These inflammatory reactions may be acute or chronic. The chronic inflammations lead to severe problems which are to be treated effectively. At present, nonsteroidal drugs are being used in the treatment of such conditions. Because of higher risk of side effects in the form of gastric problems, nowadays, plant-based anti-inflammatory drugs have come up in the mainstream. In the current report, *in vitro* anti-inflammatory activity was performed by protein denaturation assay. In protein denaturation, proteins lose their native structure and secondary structure by the application of heat. Most of the proteins lose their biological function when they are denatured. Denaturation of proteins is a well-documented cause of inflammation. As part of the investigation on the mechanism of the anti-inflammation activity, ability of plant extract to inhibit protein denaturation was studied. It was observed that methanol extract showed high anti-inflammatory activity (85.3200 ± 1.63000) which was comparable with standard. The aqueous extract also showed better anti-inflammatory activity (71.9167 ± 1.12970) compared to the remaining extracts inhibiting the heat-induced albumin denaturation.[34]

In the present study, the methanol extract was subjected for neuroprotective activity by pretreatment MTT assay. The methanol extract showed dose-dependent activity. At lower concentrations, the extract is showing protective action on SK-N-SH cells with cell viability of 70.02%, as the dose is increased to 50 and 100 µg/ml and the cell viability is getting decreased to 67.8% and 51%, respectively. This may be due to the phytochemicals present in the extract. These phytoconstituents are showing protective action at lower dose and get toxic as the concentration increased. This action may be due to synergistic activity of the phytoconstituents in the methanolic extract. This may be due to the reduction in the activity of ROS by the pretreatment of the methanolic extract of *A. corniculatum*.

In the current study, we have evaluated the protective effect of methanolic extract of *A. corniculatum* against TMT challenge by MTT assay on SK-N-SH cells. Some studies indicate that excessive ROS formation

Table 7: H_2O_2 scavenging assay of *Aegiceras corniculatum* extracts

Concentration	Treatment	Percentage inhibition
500 µg	Standard	74.4633±0.13051
500 µg	Chloroform extract	76.8233±0.09074
500 µg	Ethyl acetate extract	47.0000±0.11000
500 µg	Methanol extract	58.1700±0.60225
500 µg	Aqueous extract	67.7133±0.07506

The results expressed as mean±SD, (*n*=3) were analyzed using one-way ANOVA. SD: Standard deviation

Table 8: Anti-inflammatory activity of *Aegiceras corniculatum* extracts

Concentration	Treatment	Percentage inhibition
500 µg	Standard	94.2467±1.90148
500 µg	Chloroform extract	33.6900±1.43816
500 µg	Ethyl acetate extract	10.1367±2.26341
500 µg	Methanol extract	85.3200±1.63000
500 µg	Aqueous extract	71.9167±1.12970

The results expressed as mean±SD (*n*=3) were analyzed using one-way ANOVA. SD: Standard deviation

Table 9: Percentage viability of SK-N-SH cells

Extract/Standard	Untreated	TMT treated	5 µg/ml	50 µg/ml	100 µg/ml
Ascorbic acid	100	56.3	74.9	78.3	74.28
Methanol extract	100	56.30	70.02	67.86	51.66

TMT: Trimethyltin

regarded as a pathogenic factor during TMT induction caused the death of the cells.[35] On the other hand, methanolic extract of *A. corniculatum* can maintain the cell viability by inhibiting lipid peroxidation, DNA damage, and ROS production.[36] Polyphenols, flavonoids, and terpenoids are the main bioactive constituents of herbal extracts, and numerous studies have illustrated the neuroprotective and antistress of these materials.[37]

CONCLUSION

In the study undertaken, phytochemical analysis, antioxidant, and anti-inflammatory activities of a mangrove plant *A. corniculatum* were evaluated. The plants are used as alternative drugs in treating dreadful diseases such as Alzheimer's diseases, Parkinson's diseases, diabetes, and hepatic damage. The phytochemicals observed in *A. corniculatum* can be equally potent as the standard drugs used presently as antioxidants, anti-inflammatory, and neuroprotective agents. The evaluation of total phenolic and flavonoid contents of *A. corniculatum* revealed that this plant possesses a high amount of phenols and flavonoids in different extracts. *In vitro* antioxidant and anti-inflammatory activities of *A. corniculatum* showed the potential of these phytochemicals as novel drugs in the treatment of above-mentioned diseases. Hence, these phytochemical principles may replace the current synthetic drugs as strong antioxidant, anti-inflammatory, and neuroprotective agents. A further detailed study is required to evaluate the efficacy of these compounds *in vivo* using different animal models to elucidate the exact mechanism of action of these bioactive compounds.

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Conflicts of interest

There are no conflicts of interest.

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