Bioactivity analysis of *Sarcolobus globosus* Wall., a mangrove plant of the Sundarbans

Afiya Aunjum¹, Rana Biswas¹, Mohammad Shaef Ullah³, Md. Morsaline Billah¹,², Md. Emdadul Islam¹, Kazi Didarul Islam¹✉

¹Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna 9208, Bangladesh
²Interdisciplinary Chair in Biobusiness, University of Lincoln, UK
³Department of Entomology, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh

**ARTICLE INFO**

| Article history: |
| --- |
| Received: 25 July 2019 |
| Accepted: 04 November 2019 |
| Published: 31 December 2019 |

**Keywords:** Sarcolobus globosus, Antioxidant activity, Antibacterial activity, Cytotoxicity

**Correspondence:** Kazi Didarul Islam
✉: didar950718@yahoo.com

**ABSTRACT**

The aspiring prospect for bioactive metabolites has guided this very study to screen for antioxidant, antibacterial and cytotoxic activities of leaf, and bark extracts of Sundarbans mangrove plant, *Sarcolobus globosus* that might lead to novel drug, agrochemicals and nutraceuticals. Methanolic bark extract of *S. globosus* revealed the highest antioxidant properties among all the extracts in DPPH free radical scavenging activity (IC₅₀: 26.04 µg/ml), reducing efficiency (EC₅₀: 77.72 µg/ml), total phenolic content (47.25 ± 0.002 mg GAE/g of extract), total flavonoid content (101 ± 0.003 mg GAE/g of extract) and total tannin content (93.5 ± 0.007 mg GAE/g of extract). Free radical scavenging activity and reducing power are significantly correlated with total phenol, flavonoid and tannin content. For evaluation of antibacterial activity, Gram positive bacteria (*Bacillus megaterium, Staphylococcus aureus*, and *Micrococcus sp.*) and Gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Vibrio cholerae, Salmonella paratyphi*, *Pseudomonas sp.*, and *Proteus sp.*) were used in disc diffusion bioassay. Both extracts possessed significant (P < 0.05) inhibition competency against most of the bacteria. Test for cytotoxic activity was carried out by means of brine shrimp lethality bioassay and the chloroformic bark extract demonstrated greater cytotoxicity (LC₅₀: 19.487 µg/ml) than standard vincristine sulphate (LC₅₀: 26.68 µg/ml). Further exploration is required focusing especially the bark extract that could be utilized as source and template for the synthesis of new potential pharma and agrochemicals.

**Copyright** ©2019 by authors and BAURES. This work is licensed under the Creative Commons Attribution International License (CC By 4.0).

**Introduction**

As part of an attempt to discover novel lead compounds for pharma and agrochemicals, plant extracts have become the target to spot secondary metabolites with respective biological activities. Therefore, a number of simple bioassays have been established for screening purposes of such bioactive compounds from extracts (Hostettmann, 1991). Oxidative injury plays the vital role in the initiation of numerous neurodegenerative diseases such as stroke, Alzheimer’s disease, etc. (Senol et al., 2010). Antioxidants provide protection by inhibiting lipid peroxidation and scavenging radicals and thus terminate the progress of many chronic diseases. Natural antioxidants including phenolic acids, flavonoids, vitamins and carotenoids found in higher plants are being used for pharmaceutical as well as food and feed formulations as active compounds (Qi et al., 2005; Athukorala et al., 2006; López et al., 2007). In parallel, the extensive emergence of multidrug resistant bacteria (MDR) is making the researchers interested to find unique entities to eliminate these bacteria. In this case, plant can exhibit the path by being a crucial source of diversified chemical compounds against MDR (Tchinda et al., 2017).

However, due to excessive use and lack of adequate knowledge of other detrimental by-products possessing by some plants, harmful impacts have been observed through the use of local medicinal plants and that’s why, it is necessary to determine the toxicity of medicinal plants (Olowa and Nuñez, 2013). Moreover, general bioassay regarding toxicity is considered a useful tool for preliminary assessment as well as detection of cytotoxic (Siqueira et al., 1998), antimalarial (Pérez et al., 1997), insecticidal (Oberlies et al., 1998), antitumor (Meyer et al., 1982), anti-parasitic (Ziegler et al., 2002) and anti-rodent compounds (Lyousse et al., 2018).

Mangroves inhabit the intertidal forest wetlands at the interface between land and sea with numerous physical stress conditions including high salinity, extreme tides, variation in moisture, or biological stress factors. Therefore, to cope with these extreme environments, it is
assumed that they would produce exceptional natural products on their own (Salini, 2015). Many mangrove plant species have their uses in folk or traditional medicine as cures for various ailments. As a consequence, traditional uses of mangroves draw the attention of the scientific communities to find out the pharmaceutical products to combat several serious diseases (Lin et al., 1999; Bandaranayake, 2002). One important medicinal plant, S. globosus Wall. (Asclepiadaceae), known as Baoa lilata or Caw phal in Bengali, is a prostrate or climbing shrub growing in the mangrove forest of the Sundarbans estuary, situated in the southwest of Bangladesh (Naskar, 2004; Hossain, 2014). In traditional medicine, the plant has been used to treat rheumatism, dengue and fever (Kuddus et al., 2011).

The present study was designed to enrich the scientific data on S. globosus as a potential source providing potent bioactivity. In the light of above context, the goal of this study was to evaluate antioxidant, antibacterial and cytotoxic activities possessing by bark and leaf of this plant. For better understanding of such biological activities, polar solvent methanol and non-polar solvent chloroform were used to extract the bioactive metabolites from S. globosus.

Materials and Methods

Collection of plant material

The plant sample of S. globosus was collected from Dhangmaree, Chadpai range, East zone of the Sundarbans East Division, Khulna, Bangladesh. Collected plant samples were identified and representative specimens (AA-KU-2018014) were deposited at the herbarium of the Forestry and Wood Technology Discipline, Khulna University, Khulna, Bangladesh.

Extraction

Leaf and bark of the plant were separated and cut into small pieces followed by gentle washing with distilled water. After completion of sun drying, the plant materials were ground into powdered form with a grinder and kept in a dry, cool and dark place in a suitable airtight container until further analysis. The powder of plant parts was transferred into different clean, flat-bottomed glass jars and soaked into chloroform (Merck, Germany). They were then sealed and kept for a period of 5 days in a dark room. Individual mixtures were filtered using white cotton material. After filtration, sample was re-extracted using methanol (Merck, Germany) and filtered in the similar manner. Filtrates were evaporated yielding the chloroformic and methanolic extracts, respectively and stored in a refrigerator for experimental uses.

Determination of antioxidant activity

Measurement of DPPH free radical scavenging activity

The free radical scavenging property of extracts was evaluated by DPPH assay established by Brand-Williams et al. (1995). Different concentrations (1.75, 3.13, 6.25, 12.5, 25, 50, 100, 200 and 400µg/ml) of the extracts and the positive control of Quercetin (Sigma Aldrich, Germany) in ethanol (Merck, Germany) were prepared by serial dilution. Then, 2 mL of 0.004% DPPH (Sigma Aldrich, Germany) solution was added in each test tube of different concentrations. DPPH was also applied on the blank test tubes at the same time where only ethanol was taken as blank. The test tubes were kept for 30 minutes at dark to complete the reaction and after this the absorbance of each sample was measured at 517nm and recorded (Gupta et al., 2003). The experiment was carried out in triplicate.

Percent scavenging activity was calculated using the formula:

\[
\% \text{ inhibition} = \frac{\text{absorbance of blank} - \text{absorbance of sample}}{\text{absorbance of blank}} \times 100
\]

Determination of reducing power

The reducing power assay of extracts was conducted following Afrin et al. (2016). Various concentrations of extracts (25, 50, 100, 200, 400 µg/ml) in 1 ml of distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide [K₃Fe(CN)₆] (purchased from UNICHEM, China). After 20 minutes incubation at 50°C, 2.5 ml of trichloroacetic acid (10%) was added to the mixture and centrifuged at 3000 rpm (605 × g) for 10 minutes. Before the absorbance was measured at 700 nm, the 2.5 ml upper layer of the solution was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% FeCl₃ (Merck, Germany). Blank was prepared in the same way as the sample without addition of extract or standard. Increased absorbance of the reaction mixture indicated increased reducing power. Reducing power with absorbance 1.0 is equal to 100% of reductivity. The effective concentration obtaining 0.5 of absorbance or 50% reductivity, is assumed to be EC₅₀ (µg/ml) (Sugahara et al., 2015).

Determination of total phenol, flavonoid, tannin content

Total phenol content of the extracts was measured by applying the Folin-Ciocalteu (FC) assay (Petros and UY, 2010). FC reagent was purchased from Merck, India. In this assay, 1 ml of extract was added to 9 ml of distilled water and then 1 mL of FC reagent (10-fold diluted with distilled water) was mixed with it. After 5 min, 10 ml of 7% Na₂CO₃ (Merck, India) was added to the mixture, kept for 30 minutes and then the absorbance was measured at 750 nm using UV spectrophotometer. The total phenolics was calculated from the calibration curve.
Bioactivity of *S. globosus*

of gallic acid (Merck, India) and expressed as mg gallic acid equivalent (GAE)/g of plant extract.

By using an aluminium chloride colorometric assay (Petros and UY, 2010), total flavonoid content of the extracts was determined, where 1 ml of extract was added to distilled water (5 ml); 0.3 ml 5% NaNO₂ (Merck, India) was then added to the mixture followed by the addition of 0.6 ml 10% AlCl₃ (Merck, India) and 2 ml 1M NaOH (Merck, India). Standard (quercetin) solution (25, 50, 100, 200, 400 µg/ml) was prepared for creating standard calibration curve. The absorbance was measured at 510 nm; the total flavonoid was calculated from the calibration curve of quercetin and expressed as mg quercetin equivalent (QE)/g of plant extract.

In accordance with the Folin-Denis method as described by Polshettiwar and Ganjiwale (2007), total tannin content was determined. One ml of extract solution (100 µg/ml) was mixed with 7.5 ml of distilled water and 0.5 ml of FC reagent (Merck, India). After 5 minutes, 1ml of 35% Na₂CO₃ was added and the final volume was adjusted to 10 ml with distilled water. The mixture was kept at room temperature for 30 minutes and absorbance was recorded at 725 nm. For calibration curve, gallic acid was used as standard.

**Determination of antibacterial activity**

Antibacterial activity of *S. globosus* extracts was evaluated by disc diffusion method (Bauer et al., 1966). Three Gram-positive (*Micrococcus* sp., *Staphylococcus aureus* (ATCC 25923), *Bacillus megaterium* (ATCC 14581)) as well as seven Gram-negative bacteria (*Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 27833), *Salmonella typhi* (ATCC 13311), *Vibrio cholerae* (ATCC 9458), *Salmonella paratyphi* (ATCC 9150), *Pseudomonas* sp. and *Proteus* sp.) used in this study were collected as pure cultures from the Biochemistry Laboratory, Biotechnology and Genetic Engineering Discipline, Khulna University, Bangladesh. The bacterial isolates were cultured in nutrient broth at 37 °C for 24 hours. The sterile filter paper discs were prepared by adding desired concentration (250 and 500 µg/disc) of extracts on the disc with the help of a micropipette. Bacterial broth culture was spread over the nutrient agar medium. Sample impregnated discs, standard Erythromycin disc (10 µg/disc) and negative control discs were placed gently on the solidified agar plates, freshly seeded with the test organisms with the help of sterile forceps. Finally, the plates were incubated overnight at 37°C and then checked for the zone of inhibitions.

**Screening of cytotoxic activity**

Brine shrimp lethality bioassay was carried out for the cytotoxicity test and vincristine sulphate was used as positive control (Meyer et al., 1982; Afrin et al., 2016). The eggs of the brine shrimp, *Artemia salina* and sea water were collected from BRAC Prawn Hatchery, Bagerhat, Bangladesh. *S. globosus* extracts were dissolved in DMSO and each test tube contained 4 mL of sea water with different concentrations of extracts (5, 10, 20, 40, 80, 160, 320 µg/ml). The final volume for each test tube was adjusted to 10 mL with artificial sea water and 10 living nauplii were transferred into each tube. After 24 hours, the number of survived nauplii was recorded. The lethal concentration (LC₅₀) values of the plant extracts were obtained by a plot of percentage of the dead nauplii against the concentrations of the extracts.

**Statistical analysis**

The results were expressed as means ± standard deviation (SD). P values < 0.05 were considered as the threshold level of significance. Experimental results of antioxidant activity evaluation were analyzed for Pearson’s correlation coefficient. Statistical analysis for disc diffusion method was evaluated by two-way ANOVA followed by Tukey’s multiple comparisons test. Regression analysis was conducted for analyzing the data obtained from brine shrimp lethality bioassay to observe the relationship between different samples and vincristine sulphate as standard. The statistical analysis was carried out using Graph Pad Prism 6.

**Results and Discussion**

**Antioxidant related activity**

As shown in Figure 1, with the increase of concentration, both extracts and standard provided enhanced free radical scavenging activities. Figure 2 shows the reducing activities of various extracts in comparison to quercetin as standard. The higher the absorbance of the reaction mixture, the higher would be the reducing power. Hence, *S. globosus* extracts exhibited concentration-dependent reducing power.
Table 1 depicts the summarized results of antioxidant activities as IC$_{50}$ value of DPPH free radical scavenging activity, EC$_{50}$ value of reducing power as well as total phenolic content (mg GAE/g), total flavonoid content (mg QE/g) and total tannin content (mg GAE/g) of all extracts. Methanolic bark extract has maximum inhibitory activity (IC$_{50}$~26.04 μg/ml) against the DPPH free radical among all extracts. As can be seen in Table 1, 50% effective concentration of standard was 37.87μg/ml. Although the reducing power of methanolic bark (EC$_{50}$~77.72μg/ml) was lower than the standard, it gave higher activity than other extracts.

The amount of total content of phenolics varies in different extracts ranging from 10.75 to 47.25 mg GAE/g (Table 1). The total phenolic content of extracts is in descending order of MBE > CBE > MLE > CLE while total flavonoid content of different extract varies from 59 to 121.25 mg GAE/g with a decreasing order of MBE > MLE > CBE > CLE. Phenolic compounds of plants fall into several categories; flavonoids are the most dominant class of phenolic compounds among these categories which have potent antioxidant activities (Nunes et al., 2012). Moreover, antioxidant activity of flavonoids depends on the structure and substitution pattern of hydroxyl groups (Sharififar et al., 2009). Consequently, variation in reaction pattern and structure of flavonoids might be the reason of no DPPH scavenging activities in MLE and CLE, in spite of possessing moderate amount of flavonoid.

In the case of total tannin content, highest total tannin content (93.5 mg GAE/g) belonged to MBE (Table 1). Additionally, the ranking order for total tannin content of the extracts is MBE > MLE > CBE > CLE. Li et al. (2009) and Kumar et al. (2014) found significant correlation between DPPH activity and total phenolic content (correlation coefficient $r = 0.760$ and $r = 0.994$, respectively). As can be observed in Table 2, DPPH scavenging activity and reducing power of S. globosus extracts are significantly correlated with total phenolic, flavonoid and tannin contents.
Bioactivity of *S. globosus*

Table 1. Antioxidant properties of different types of extracts from *S. globosus*

| Extracts   | DPPH IC₅₀ (µg/ml) | Reducing power EC₅₀ (µg/ml) | TPC (mg GAE/g) | TFC (mg QE/g) | TTC (mg GAE/g) |
|------------|------------------|-----------------------------|----------------|---------------|----------------|
| CLE        | 3162.28          | 4859.54                     | 10.75 ± 0.005  | 59 ± 0.001    | 3.5 ± 0.003    |
| CBE        | 498              | 503.58                      | 17 ± 0.001     | 63.25 ± 0.004 | 7.25 ± 0.001   |
| MLE        | 2344.22          | 2320.34                     | 13 ± 0.002     | 79.5 ± 0.009  | 10.125 ± 0.005 |
| MBE        | 26.04            | 77.72                       | 47.25 ± 0.002  | 121.25 ± 0.013| 93.5 ± 0.007   |
| Standard   | 7.65             | 37.87                       | -              | -             | -              |

CLE=Chloroformic leaf extract, MLE=Methanolic leaf extract, CBE=Chloroformic bark extract, MBE=Methanolic bark extract, TPC=Total phenolic content, TFC=Total flavonoids content, TTC=Total tannin content, GAE=Gallic acid equivalent, QE=Quercetin equivalent, IC₅₀=50% inhibition concentration, EC₅₀=50% effective concentration

Therefore, these phytochemicals might be some of the major contributors responsible for the antioxidant efficacy of *S. globosus*. DPPH scavenging activity is significantly correlated with phenol (*p* ≤ 0.001, *r* = 0.9822) (Table 2). Reports have suggested that there is a correlation between the total phenolic content and antioxidant activity of plant extracts (Zhang and Wang, 2009). However, low level of correlation was found between the flavonoid content and the antioxidant activities of the extracts in this study. Different extraction methods and antioxidant assays may be responsible for this lower correlation with flavonoid content.

**Antibacterial activity**

The data pertaining to the antibacterial potential of the plant extracts are presented in Table 3. For the interpretation of antibacterial assay results, we have implemented the scale of measurement according to Carović-Stanko et al. (2010) which considers zone of inhibition value of >15 mm as strongly inhibitory, 10-15 mm as moderately inhibitory and <10 mm as not inhibitory (Carović-Stanko et al., 2010). The antibacterial activity was detected at 25 µg/µl and 50 µg/µl concentrations, where highest activities were found at 50 µg/ml concentrations.

Table 2. Correlation among antioxidant activities and total phenolic, flavonoid and tannin contents of *S. globosus* extracts

| Parameter  | Phenol | Flavonoid | Tannin |
|------------|--------|-----------|--------|
| DPPH assay | 0.987**| 0.895*    | 0.995**|
| Reducing power | 0.998***| 0.872* | 0.982**|
* indicates significance at *p* ≤ 0.05, ** indicates significance at *p* ≤ 0.01, *** indicates significance at *p* ≤ 0.001.

Table 3. Mean values of inhibition zone diameter (mm) of *S. globosus* extracts and standard.

| Bacteria  | Conc. (µg/ml) | CLE | MLE | CBE | MBE | Erythromycin |
|-----------|---------------|-----|-----|-----|-----|--------------|
| B. megaterium | 25             | 7±0 | 9.25±0.5 | 7.5±0.58 | 8.75±0.5 | 28.75±0.5 |
|            | 50             | 10.5±0.5 | 13.25±0.95 | 11.5±0.58 | 12.25±0.95 | - |
| S. aureus  | 25             | -   | -   | 7.75±0.5 | -   | 28±1.41     |
|            | 50             | -   | -   | 10.75±0.95 | -   | -           |
| Micrococcus | 25             | 7.25±0.5 | 10.5±0.58 | 8.75±0.5 | - | 21.75±0.98 |
|            | 50             | 9.25±0.58 | 13.75±0.5 | 13.75±0.5 | - | -           |
| E. coli    | 25             | 8.75±0.5 | 9.75±0.95 | 7.5±0.58 | 7.25±0.5 | 15±0.82     |
|            | 50             | 11.75±0.5 | 12.5±0.58 | 10±0.81 | 10.5±0.58 | - |
| P. aeruginosa | 25             | 7.75±0.5 | 11.25±0.5 | 8.5±0.58 | 11.75±0.5 | 27.75±0.96 |
|            | 50             | 13.75 | 14.75±0.5 | 14.25±0.95 | 15.5±0.58 | - |
| S. typhi   | 25             | -   | 11.5±0.58 | 8.75±0.5 | 8.75±0.5 | 29.5±1.29   |
|            | 50             | 7.5±0.58 | 16.25±0.5 | 14.75±0.5 | 12.75±0.5 | - |
| V. cholerae| 25             | 8.75±0.5 | 9.25±0.5 | 13.75±0.5 | 7.5±0.75 | 32.25±0.96 |
|            | 50             | 10.75 | 13.25±0.5 | 21.5±0.58 | 10.75±0.5 | - |
| S. paratyphi| 25             | -   | 8.75±0.5 | 11±0.82 | 8.5±0.57 | 18±0.82     |
|            | 50             | 7.5±0.58 | 12.75±0.5 | 15.5±0.58 | 11.75±0.5 | - |
| Pseudomonas| 25             | 7.25±0.5 | 8.25±0.5 | 8.5±0.58 | 9.75±0.5 | 28.5±1.29   |
|            | 50             | 12.75±0.5 | 10.5 | 10.75±0.5 | 11.5±0.58 | - |
| Proteus    | 25             | -   | 7.25±0.5 | 10.5±0.58 | - | 32.75±0.98 |
|            | 50             | 8.75±0.5 | 9.75±0.5 | 14.5±0.58 | 7±0 | - |

MBE-methanolic bark extract, MLE-methanolic leaf extract, CBE-chloroformic bark extract, CLE-chloroformic leaf extract; Significant at *p* < 0.05.

Antibacterial activity revealed that chloroformic extract of bark strongly inhibited the growth of *V. cholerae* (ZOI=21.5 mm) and *S. paratyphi* (ZOI=15.5 mm). Moreover, *P. aeruginosa* (ZOI=15.5 mm) and *S. typhi*...
(ZOI=16.25 mm) were strongly sensitive to methanolic bark and leaf, respectively. In the case of leaf, methanolic extract exhibited larger zone of inhibition than chloroformic extract at respective concentrations. From analyzing the results, it can be stated that there were moderate to strong activities against all the test bacteria in the chloroformic extract from bark. Based on statistical analyses, the antimicrobial action of the extracts for concentrations 250 µg/disc and 500 µg/disc (Table 3) differed significantly for any of the strains tested except the cases where there is no zone of inhibition at both concentrations. Moreover, the diameter of the inhibition at 250 µg/disc and 500 µg/disc was significantly different from the diameter of zone for standard erythromycin.

Cytotoxic activity

Brine shrimp lethality activity of *S. globosus* extracts are listed in Table 4. The bark chloroform extract is the most active extract among all extracts tested, presenting an LC₅₀ of 19.487 µg/ml which is less than the standard (LC₅₀ 26.68 µg/ml). In addition, chloroformic leaf extract exhibits LC₅₀ of 28.872 µg/ml, which is very close to the standard value. These extracts can be regarded as a promising candidate for plant-derived antitumor, anti-parasitic and anti-rodent compounds.

Table 4. Brine-shrimp bioassay of different extracts of *S. globosus*

| Extracts      | LC₅₀ (µg/ml) | Regression equation | R² |
|---------------|--------------|---------------------|----|
| CBE           | 19.487       | y = 40.57x – 2.325  | 0.988 |
| MBE           | 495.709      | y = 16.34x + 5.960  | 0.987 |
| CLE           | 28.872       | y = 39.16x – 7.192  | 0.933 |
| MLE           | 288.4        | y = 24.62x – 9.439  | 0.984 |
| VS            | 26.68        | y = 52.79x – 25.255 | 0.972 |

CLE=Chloroformic leaf extract, MLE=Methanolic leaf extract, CBE=Chloroform bark extract, MBE=Methanolic bark extract, VS=Vincristine sulphate.

Conclusion

Since a moderately polar and a non-polar solvent system were employed for extraction, bioactivity namely antioxidant, antibacterial and cytotoxicity, were found at different levels. All the extracts showed varying degrees of antimicrobial activity on test microorganisms. The promising and novel finding of this study was that bark extracts either methanolic or chloroformic showed noticeable activity in all the tests, in some cases highest activities were observed. In antioxidant evaluation, methanolic bark demonstrated the highest results, whereas chloroformic bark extracts gave strong activity than the standard in cytotoxic assay. Furthermore, the distinctive result of this study provides striking baseline information for the potential and constructive use of this plant and generates our anticipation that detailed investigation for pharmacological activity may lead to the isolation of interesting pharma and agrochemicals of plant origin through elucidation of the identity of compounds responsible for respective activity.

Acknowledgements

We would like to thank Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna, Bangladesh.

References

Afrin, S., Pervin, R., Sabrin, F., Sohrob, M., Rony, S. and Billah, M. M., 2016. Assessment of antioxidant, antibacterial and preliminary cytotoxic activity of chloroform and methanol extracts of *Caesalpinia crista* L. leaf. Bangladesh Journal of Botany, 45: 1061-1068.

Athukorala, Y., Kim, K.-N. and Jeon, Y.-J., 2006. Antiproliferative and antioxidant properties of an enzymatic hydrolysate from brown alga, *Ecklonia cava*. Food and Chemical Toxicology, 44(7): 1065-1074. https://doi.org/10.1016/j.fct.2006.01.011

Bandaranayake, W. M., 2002. Bioactivities, bioactive compounds and chemical constituents of mangrove plants. *Wetlands Ecology and Management*, 10(6): 421-452.

Bauer, A. W., Kirby, W. M., Sherris, J. C. and Turck, M., 1966. Antibiogram susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, 45(4): 493-496.

Brand-Williams, W., Cuvelier, M.-E. and Berset, C., 1995. Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology*, 28(1): 25-30.

Carovia-Listo, K., Orifie, S., Polito, O., Strik, F., Kolak, L., Milos, M. and Satovic, Z., 2010. Composition and antibacterial activities of essential oils of seven *Ocimum* taxa. *Food Chemistry*, 119(1): 196-201. https://doi.org/10.1016/j.foodchem.2009.06.010

Gupta, M., Mazumdar, U. K., Sivakumar, T., Vamsi, M. L. M., Karki, K., Sambathkumar, R. and Manikandan, 2003. Antioxidant and anti-inflammatory activities of *Acalypha fruticosa*. *Nigeria Journal of Natural Products and Medicine*, 7: 25-29. http://dx.doi.org/10.4314/njnpm.v7i1.11700

Hossain, M., 2014. Rediscovery of a butterfly, *Neptis soma shaniana* Evans 1924 (Lepidoptera: Nymphalidae) in the Sundarbans of Bangladesh. *International Journal of Fauna and Biological Studies*, 1(3): 8-10.

Hostettmann, K., 1991. Methods in Plant Biochemistry: Assays for Bioactivity, Academic Press, London.

Kuddus, M. R., Akbar, F. and Rashid, M. A., 2011. Polyphenols content, cytotoxic, membrane stabilizing and thrombolytic activities of *Sarcocolus globosus*: A medicinal plant from Sundarban forest. *Boletin Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas*, 10(4): 363-368.

Kumar, S., Sandhir, R. and Ohja, S., 2014. Evaluation of antioxidant activity and total phenol in different varieties of *Lantana camara* leaves. *BMC Research Notes*, 7: 560. https://doi.org/10.1186/1756-0500-7-560

Li, X., Wu, X. and Huang, L., 2009. Correlation between antioxidant activities and phenolic contents of radix *Angelicae sinensis* (Danggu). *Molecules*, 14(12): 5349-5361. https://doi.org/10.3390/molecules14125349

Lin, C.-C., Hsu, J.-F. and Lin, T.-C., 1999. Effects of punicalagin and punicalin on carrageenan-induced inflammation in rats. *The American Journal of Chinese Medicine*, 27(3-4): 371-376. https://doi.org/10.1142/S0219455199000422

López, V., Akerreta, S., Casanova, E., García-Mina, J. M., Cavero, R. Y. and Calvo, M. I., 2007. In *vitro* antioxidant and anti-thrombopoeitic activities of Lamiaceae herbal extracts. *Plant Foods for Human Nutrition*, 62(4): 151-155. https://doi.org/10.1007/s11130-007-0056-6

Lyoussi, B., Cherkaoui Tangi, K., Morel, N., Haddad, M. and Quetin-Leclercq, J., 2018. Evaluation of cytotoxic effects and acute...
Bioactivity of *S. globosus*

...and chronic toxicity of aqueous extract of the seeds of *Calycotome villosa* (Poiret) Link (subsp. *intermedia*) in rodents. Avicenna journal of phyomedicine, 8(2): 122-135.

Meyer, B., Ferrigni, N., Putnam, J., Jacobsen, L., Nichols, D. and McLaughlin, J. L., 1982. Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Medica*, 45(5): 31-34. https://doi.org/10.1055/s-2007-971236

Naskar, K., 2004. Manual of Indian Mangroves, Daya Publishing House, India.

Nunes, X. P., Silva, F. S., Almeida, J. R. G. d. S., Barbosa Filho, J. M., de Lima, J. T., de Araújo Ribeiro, L. A. and Júnior, L. J. Q., 2012. Biological oxidations and antioxidant activity of natural products. In Dr Venketeshwer Rao (eds) Phytochemicals as Nutraceuticals – Global Approaches to Their Role in Nutrition and Health, pp. 521-552. InTech Open Access Publisher, Croatia.

Oberlies, N. H., Rogers, L. L., Martin, J. M. and McLaughlin, J. L., 1998. Cytotoxic and insecticidal constituents of the unripe fruit of *Persea americana*. *Journal of Natural Products*, 61(6): 781-785. https://doi.org/10.1021/np9800304

Olówa, L. F. and Nuñez, O. M., 2013. Brine shrimp lethality assay of the ethanolic extracts of three selected species of medicinal plants from Iligan City, Philippines. *International Research Journal of Biological Sciences*, 2(11): 74-77.

Pérez, H., Diaz, F. and Medina, J., 1997. Chemical investigation and in vitro antimalarial activity of *Tabebuia ochracea* ssp. neochrysantha. *International journal of pharmacognosy*, 35(4): 227-231. https://doi.org/10.1076/ijphbi.35.4.227.13306

Petros, N. P. and UY, M. M., 2010. Antioxidant and cytotoxic activities and phytochemical screening of four Philippine medicinal plants. *Journal of Medicinal Plant Research*, 4(5): 407-414.

Polshettiwar, S. A. and Ganjiwale, R. O., 2007. Spectrophotometric estimation of Total tannins in some ayurvedic eye drops. *Indian Journal of Pharmaceutical Sciences*, 69(4): 574-576. https://doi.org/10.4103/0250-474X.36049

Qi, H., Zhao, T., Zhang, Q., Li, Z., Zhao, Z. and Xing, R., 2005. Antioxidant activity of different molecular weight sulfated polysaccharides from Ulva pertusa Kjeløm (Chlorophyta). *Journal of Applied Phycology*, 17(6): 527-534. https://doi.org/10.1007/s10811-005-9003-9

Salini, G., 2015. Pharmacological profile of mangrove endophytes-a review. *International Journal of Pharmacy and Pharmaceutical Sciences*, 7(1): 6-15.

Senol, F., Orhan, I., Yılmaz, G., Cicek, M. and Sener, B., 2010. Acetylcholinesterase, butrylcholinesterase, and tyrosinase inhibition studies and antioxidant activities of 33 *Scutellaria* L. taxa from Turkey. *Food and Chemical Toxicology*, 48(3): 781-788. https://doi.org/10.1016/j.fct.2009.12.004

Sharififar, F., Dehghan-Niudeh, G. and Mirtajaldini, M., 2009. Major flavonoids with antioxidant activity from *Teucrium polium* L. *Food Chemistry*, 112(4): 885-888. https://doi.org/10.1016/j.foodchem.2008.06.064

Siqueira, J. M. d., Bommm, M. D., Pereira, N. F. G., Garcez, W. S. and Boaventura, M. A. D., 1998. Estudo fitoquímico de Unonopsis lindmanii-Annonaceae, biomonitorado pelo ensaio de toxicidade sobre a Artemia salina leach. *Química Nova*, 21(5). http://dx.doi.org/10.1590/S0100-40421998000500004

Sugahara, S., Ueda, Y., Fukuhara, K., Kamamuta, Y., Matsuda, Y., Murata, T., Kuroda, Y., Kabata, K., Ono, M. and Igoshi, K., 2015. Antioxidant effects of herbal tea leaves from Yacon (*Smallanthus sonchifolius*) on multiple free radical and reducing power assays, especially on different superoxide anion radical generation systems. *Journal of Food Science*, 80(11): C2420-C2429. https://doi.org/10.1111/1750-3841.13092

Tchinda, C. F., Voukeng, I. K., Beng, V. P. and Kuete, V., 2017. Antibacterial activities of the methanol extracts of *Albizia adianthifolia*, *Alchornea laxiflora*, *Laportea ovalifolia* and three other Cameroonian plants against multi-drug resistant Gram-negative bacteria. *Saudi Journal of Biological Sciences*, 24(4): 950-955. https://doi.org/10.1016/j.sjbs.2016.01.033

Zhang, Y. and Wang, Z.-z., 2009. Phenolic composition and antioxidant activities of two Phlomis species: A correlation study. *Comptes Rendus Biologies*, 332(9): 816-826. https://doi.org/10.1016/j.crvi.2009.05.006

Ziegler, H. L., Staerk, D., Christensen, J., Hviid, L., Hägerstrand, H. and Jaroszewski, J. W., 2002. General bioassay for active plant constituents. *Antimicrobial Agents and Chemotherapy*, 46(5): 1441-1446. https://doi.org/10.1128/AAC.46.5.1441-1446.2002