Volume 21 Issue 2: KUS: 1259: 20082024: 2024

KHULNA UNIVERSITY
STUDIES

journal homepage: www.kus.ku.ac.bd

Research article

Comparison in Nutrient and Bioactivities of Carbohydrate and Ethanol-methanol Extracts of *Aegiceras corniculatum* (L.) Fruit

Marina Aziz Jyoti, M. Rabiul Islam, G. M. Shamim Ahmed and Sheikh Julfikar Hossain*

Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna-9208, Bangladesh

ABSTRACT

Carbohydrate and carbohydrate-based therapeutics are used in treating various diseases. In this study, carbohydrate-enriched (Cah) and ethanol-methanol (1:1; Eth-Met) extracts were prepared from the mangrove fruits of *Aegiceras corniculatum* in the Sundarbans composed of high content of carbohydrate. The amounts of total carbohydrate, protein, and lipid in Cah extract were 70.4, 7.8, and 2.2% whereas those for Eth-Met extract were 43.9, 8.1, and 2%, respectively. Cah extract had the higher antioxidant activity as it showed significantly ($P < 0.05$) high total polyphenol content (73.5 mg gallic acid equivalent (GAE)/g extract), 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging (at 50 µg extract/mL 85.2%), and total antioxidant capacity (155.5 mg GAE or 120.4 mg ascorbic acid equivalent (AAE)/g extract) than Eth-Met extract. Eth-Met extract showed significantly higher analgesic activity than Cah extract. When mice were orally treated with 250 mg Eth-Met extract/kg body weight showed acetic acid-induced writhing inhibition of 43.1% and needed 14.8 s for response time on a hot plate (55°C). Both Cah and Eth-Met extracts exhibited strong anthelmintic activity with similar concentrations for death time 50 (CDT_{50}) of 11.5 and 10.6 mg/mL respectively for the parasite, *Paramphistomum cervi*. Therefore, Cah extract can be exploited as a potential source of functional carbohydrates and polyphenols with high antioxidant and anthelmintic activities.

ARTICLE INFO

Article timeline:
Date of Submission:
20 August, 2024
Date of Acceptance:
28 November, 2024
Article available online:
05 December, 2024

Keywords:
Anthelmintic
Antioxidant
Aegiceras corniculatum
Carbohydrate
Fruit
The Sundarbans

Introduction

The mangrove tree, *Aegiceras corniculatum* belongs to the Myrsinaceae family. It is known as khalshi in Bengali and black mangrove or river mangrove in English and is widely distributed in coastal Bangladesh, Myanmar, Malaysia, Philippines, South China, Papua New Guinea, Indonesia, northern Australia, Sri Lanka, and India. *A. corniculatum* certainly produces various secondary metabolites to adapt to stressful coastal environments. Polyphenols are a major group of secondary metabolites and generally involved in plant defense systems. They have attracted the scientific community because of their role in health promotion and applications in food, nutraceutical, pharmaceutical, and cosmeceutical industries. Traditionally, coastal dwellers usage the genus, *Aegiceras* to treat ulcers, liver injuries, diabetes, inflammation, and rheumatism (Bandaranayake, 1998, 2002). The different parts of this plant are also used in treating arthritis, asthma, cancer, inflammation, and hepatic diseases (Roome et al., 2011; Sarkar et al., 2024).

Nutrient composition, antioxidant, antidiabetic, antibacterial, antidiarrheal, analgesic, and iron chelation activities have been described from the fruit of *Sonneratia apetala* (Hossain et al., 2013, 2016, 2017; Mithila et al., 2023). In addition, the polyphenol content and health-promoting bioactivities of various mangrove fruits from the Sundarbans have also been reported (Hosen et al., 2020, 2021; Khatun et al., 2022; Biswas et al., 2023).

Report showed that the fruit of *A. corniculatum* is composed of high amount of carbohydrates (Ray et al., 2015) but no research indicated its therapeutic uses. Recently carbohydrate-based drugs have been approved for treating viral, bacterial, parasitic, diabetes, cancer, and cardiovascular diseases (Cao et al., 2022). However, pure carbohydrates such as a disaccharide, lactulose is used to treat chronic constipation (Bae & Kim, 2020); a sulfated polysaccharide, heparin for intravenous antithrombotic drug (Qiu et al., 2021); and GV-971, an oligosaccharide originated from seaweed approved in treating Alzheimer (Wang et al., 2019). Therefore, in this study, a carbohydrate-enriched extract of *A. corniculatum* fruit was prepared and compared its nutrient contents, antioxidant, analgesic, and anthelmintic activities with that of ethanol-methanol (1:1) extract.

*Corresponding author: <sjhossainbgeku@gmail.com>

DOI: https://doi.org/10.53808/KUS.2024.21.02.1259-ls

Materials and Methods

Chemicals

Arthrone, chloroform, ammonium molybdate, ethanol, hydrochloric acid, methanol, and sulfuric acid were procured from Merck Ltd., India. Ascorbic acid, (+)-catechin, 3,5-dinitrosalicylic acid, gallic acid, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were acquired from Sigma-Aldrich. Morphine was collected from Gonoshasthaya Pharmaceuticals Limited, Bangladesh.

Collection and preparation of fruits

From the different saline zones of the Sundarbans mangrove forest of Bangladesh, *Aegiceras corniculatum* (L.) Blanco. fruits were collected in August and September 2022. After washing, they were shade-dried, and ground into a fine powder. Then, the powder was preserved in a clean air-tight container.

Preparation of extracts

Defatted powder (10 g) of *A. corniculatum* fruits was mixed with 80% ethanol (400 mL) for 2 days. Then in a water bath at 90°C, the mixture was kept for 10 min. After centrifugation at 8000 rpm for 6 min, the supernatant was collected from the mixture. Then the supernatant was dried at 100°C as a carbohydrate extract (Cah). For the preparation of ethanol-methanol (1:1, Eth-Met) extract, defatted powder (10 g) was extracted in ethanol and methanol (1:1; 400 mL) mixture for 2 days with frequent shaking. Then after filtration (Whatman no. 1) the filtrate was evaporated and dried at 100°C

Determination of total carbohydrate, protein, and lipid contents

Total carbohydrate and reducing sugar contents in Cah and Eth-Met extracts were determined following the methods of Dreywood (1946) and Miller (1959), respectively. Non-reducing sugar was determined by the deduction of reducing sugar from total carbohydrate. The methods of Lowry et al. (1951) and Bligh & Dyer (1959) were followed to estimate total protein and lipid contents, respectively.

Determination of total polyphenol content and antioxidant activity

Ough & Amerine (1988) was followed to assess total polyphenol contents in Cah and Eth-Met extracts with a little modification. Folin-Ciocalteu solution (1 mL, 50% v/v) was mixed with the diluted extract, and then after 3 min, the mixture was mixed with 10% Na_2CO_3 solution (1 mL) followed by 1h incubation at room temperature. The absorbance (OD 700 nm) was recorded using a spectrophotometer. The result was designated in mg GAE (gallic acid equivalent)/g extract.

The antioxidant activity in Cah and Eth-Met extracts was evaluated following two assays. To investigate DPPH free radical scavenging (Blois, 1958), the diluted extract was mixed with ethanol (50%), and sodium acetate buffer solution (0.5 M, pH 5.5, 0.5 mL). Then, 0.2 mM DPPH solution (1 mL) was added and mixed well by vortex. The mixture was incubated (40 min) in the dark at room temperature. Spectrophotometrically measured the absorbance (OD 517 nm). Ascorbic acid (AA) was used as a standard.

The total antioxidant capacity in the extracts was assayed following Prieto et al. (1999). The diluted extract was added in 2.5 mL of reagent solution (4 mM $(NH_4)_6Mo_7O_{24}.4H_2O$, 0.6 M H_2SO_4, and 28 mM NaH_2PO_4) and mixed well. The mixture was then incubated in a water bath (90°C, 90 min). Spectrophotometric reading was recorded at OD 695 nm. The results were specified as mg GAE, and mg ascorbic acid equivalent (AAE)/g extract.

Evaluation of analgesic activity

Young Swiss-Albino mice (6-8 weeks old; 18-20 g) were collected from the International Centre for Diarrheal Disease Research, Bangladesh (ICDDR'B). The assay was conducted according to the guidelines of Khulna University (Research ref. no. KUAEC-2022/09/17). Mice were supplied water and standard food *ad labitum* and maintained in a noiseless room with 12h day-night cycle at 25°C. To investigate the analgesic activity of the extracts, Koster et al. (1959) and Eddy & Leimbach (1953) were followed for writhing induced by acetic acid and a hot plate (55°C) stimulated response time (second, s) in mice (n = 6-8, each group), respectively.

Evaluation of anthelmintic activity

Tandon et al. (1997) were followed for performing the anthelmintic activity. Phosphate buffer (PBS, pH 7.4) was used to collect the parasites, *Paramphistomum cervi* from the bovine small intestines of a slaughterhouse located in Gallamari, Khulna. Ten (10) parasites in each plate (three replicates) with or without treatment were incubated at 37°C. They were frequently examined to recognize the paralysis time (min) at first, and then the death time (min). The equation (Biswas et al., 2023) was followed to calculate the concentration of treatments for death time 50 (CDT_{50}) of the parasites.

Statistical analysis

The results were presented in mean ± SD. Statistical differences among several groups were accomplished by the analysis of variance (ANOVA) where $P \leq 0.05$ indicated significance.

Results and Discussion

Total carbohydrate, protein, and lipid contents

The amounts of total carbohydrate, reducing and non-reducing sugars, protein, and lipid in Cah and Eth-Met extracts of the fruits, *A. corniculatum* were determined. The contents of total carbohydrate, reducing, and non-reducing sugars in Cah extract were 70.4, 56, and 14% whereas those for Eth-Met extract were 43.9, 34.9, and 7.3% respectively (Figure 1). Cah and Eth-Met extracts showed similar amounts of protein (~8%) and lipid (~2%) contents. Though carbohydrates, proteins, and lipids are prime needs for life processes, recent research trend emphasizes recognizing bioactive molecules in them since they possess various pharmacological properties. It is reported that carbohydrate and carbohydrate-based therapeutics are applied in treating cardiovascular, inflammatory, and hematological diseases (Wang et al., 2021; Cao et al., 2022)

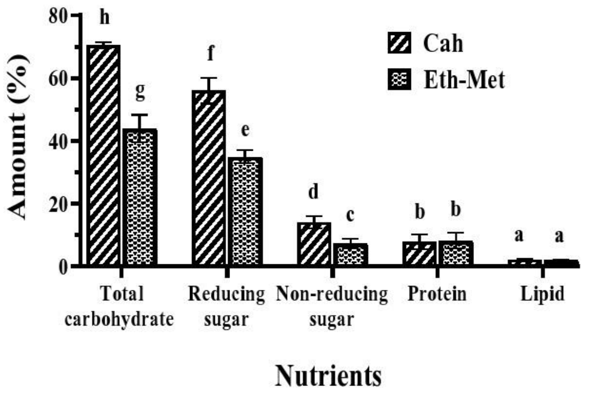

Figure 1. Total carbohydrate, reducing and non-reducing sugars, protein, and lipid contents (%) in carbohydrate (Cah) and ethanol-methanol (Eth-Met) extracts of *Aegiceras corniculatum* fruit. Data (n = 3-5) are expressed as mean ± SD (bar). Different letters (a-h) for the same test indicated significant ($P < 0.05$) differences.

Total polyphenol content and antioxidant activity

Figure 2(A) showed total polyphenol content in Cah was 73.5 mg GAE/g extract whereas 59.6 mg GAE/g extract in Eth-Met ($P < 0.05$). Total polyphenol contents and the reducing power of fruits and medicinal plants showed a strong correlation (Basar et al., 2013; Hosen et al., 2020; Alam et al., 2021), and therefore, it can be said that polyphenols are reducing agents. Dietary polyphenols are inversely associated with the manifestation of cancer, diabetes, inflammation, and cardiovascular diseases (Aravind et al., 2021). A large amount of phenolic compounds in *A. corniculatum* fruits probably conjugated with carbohydrates that required a more aqueous environment to be extracted than ethanol-methanol (1:1) since a significantly high amount of total polyphenol was detected in Cah extract. The DPPH scavenging activities of Cah and Eth-Met extracts were 85.2 and 62.2% at 50 µg extract/mL, respectively. It was found that the DPPH scavenging was dependent on the concentration of extracts (Figure 2(B)). From the dose-dependent curve, the IC_{50} (inhibitory concentration 50) values for both extracts were calculated. The lowest IC_{50} value indicated the highest potential in scavenging of DPPH free radicals, and it was observed for Cah extract (12.7 µg/mL) whereas that for Eth-Met extract was 17.8 µg/mL (Figure 2(C)). Total antioxidant capacity was determined by the reduction of phosphate-molybdenum (VI) to phosphate-molybdenum (V) and presented mg GAE and mg AAE/g extract. Figure 2(D) showed that Cah extract had a significantly higher amount of total antioxidant capacity (155.5 mg GAE or 120.4 mg AAE/g extract) than Eth-Met extract (95.3 mg GAE or 73.8 mg AAE/g extract). Considering the results, Cah extract showed higher antioxidant activity, and it was probably due to the presence of more polyphenols than Eth-Met extract. In previous, polyphenol content, DPPH scavenging, and total antioxidant capacity of various fruits and vegetables in Bangladesh were also reported (Hossain et al., 2014, 2015; Hosen et al., 2020; Alam et al., 2021).

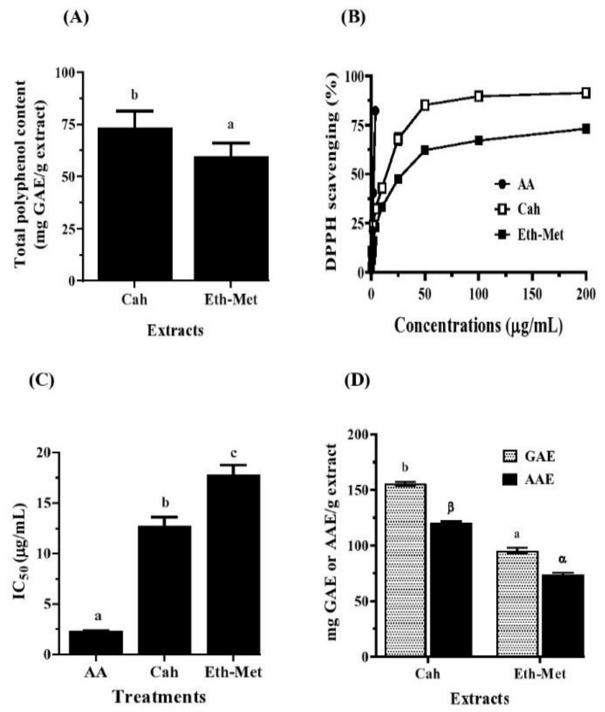

Figure 2. Total polyphenol content (A) and antioxidant activity (B, C, D) in carbohydrate (Cah) and ethanol-methanol (Eth-Met) extracts of *Aegiceras corniculatum* fruit. (B) Dose-dependent DPPH free radicals scavenging (%); (C) inhibitory concentration 50 (IC_{50}) for DPPH scavenging; and (D) total antioxidant capacity of the extracts. Data (n = 3-5) are represented as mean ± SD (bar). Significant ($P < 0.05$) differences are indicated by different letters (a-c; α, β). AA: ascorbic acid, positive control; GAE: gallic acid equivalent; AAE: ascorbic acid equivalent.

Analgesic activity

Mice treated with Cah, and Eth-Met extracts orally at 250 mg/kg b.w. (body weight), significantly ($P < 0.05$) prevented the writhing induced by acetic acid. Eth-Met extract strongly ($P \leq 0.05$) inhibited writhing in mice (43.2%) than Cah extract (25.7%) whereas diclofenac sodium (positive control) at 25 mg/kg b.w. showed the highest inhibition (47.6%) (Figure 3(A). Figure 3(B) showed a significant increase in response time (paw licking/jumping) starting from 30 min until 240 min of mice when treated with the extracts at 250 mg/kg b.w. than that of control. The highest response time of Cah (13.9 s) and Eth-Met (14.8 s) extracts exhibited at 120 and 60 min, respectively whereas that for morphine (positive control; 10 mg/kg b.w.; 17 s) at 60 min. Both the extracts had peripheral (inhibition of writhing induced by acetic acid) and central (increase in response time on a hot plate) analgesic activities. A strong correlation was reported (Biswas et al., 2023) between analgesic activity and polyphenol or flavonoid content. Though Cah extract had a high content of polyphenols, it showed a smaller analgesic activity than Eth-Met extract. It was probably due to a little entry of carbohydrate-conjugated polyphenols into the central nervous system. Islam et al. (2019a) reported that (+)-catechin inhibits histamine, bradykinin, and serotonin release and opens the ATP-sensitive K^+ channel resulting in antinociceptive effects. Various studies on fruits and medicinal plants showed analgesic, anti-inflammatory, and anti-histamine activities (Hossain et al., 2008, 2009; Mubassara et al., 2011; Tsujiyama et al., 2013; Hossain et al., 2017).

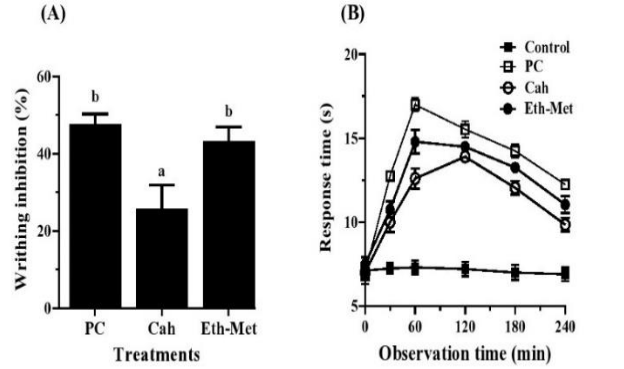

Figure 3. Analgesic activity of *Aegiceras corniculatum* fruit extracts (carbohydrate, Cah and ethanol-methanol, Eth-Met) on mice. (A) Inhibition (%) of acetic acid-induced writhing, and (B) response time (s) increase on a hot plate at different times (min) with 250 mg extract/kg body weight (b.w.). Data (n = 6-8) are presented as mean ± SD (bar). Significant ($P < 0.05$) differences are indicated by different letters (a, b). Control means without treatment; PC means positive control, diclofenac sodium 25 mg/kg b.w. for writhing and morphine 10 mg/kg b.w. for hot pate tests.

Anthelmintic activity

A. corniculatum fruit extracts, Cah and Eth-Met were used to treat *P. cervi* to evaluate the anthelmintic activity by observing the paralysis time and the death time. Both the Cah and the Eth-Met extracts showed strong anthelmintic activity as required a small time for paralysis (Cah 54 min; Eth-Met 43 min) and death (Cah 90 min; Eth-Met 101 min) at 20 mg extract/mL (Figure 4 A, B). The times (min) required for paralysis, and death of control parasites (without treatment) were 284 and 347 min, respectively whereas albendazole (15 mg/mL), used as a positive control (PC) showed the same at 80 and 108 min, respectively. Concentration-dependent paralysis time as well as death time of parasites were also observed for Cah, Eth-Met, and PC. Using the dose-dependent data, CDT_{50} (concentration for death time 50) of Cah, Eth-Met, and PC were calculated as 11.5, 10.6, and 6.9 mg/mL, respectively (Figure 4C).

Anthelmintic activity of various mangrove fruits and honey was also reported (Islam et al. 2019b; Biswas et al., 2023). Though Cah extract was composed of a larger amount of total polyphenol, its anthelmintic activity was similar to Eth-Met extract. Biswas et al. (2023) observed a poor correlation between anthelmintic activity and total polyphenol content. Therefore, the anthelmintic activity of both extracts was probably displayed from other group(s) of compound(s) rather than polyphenols. However, a glycoconjugate was characterized as anthelmintic (Sajid & Azim, 2012).

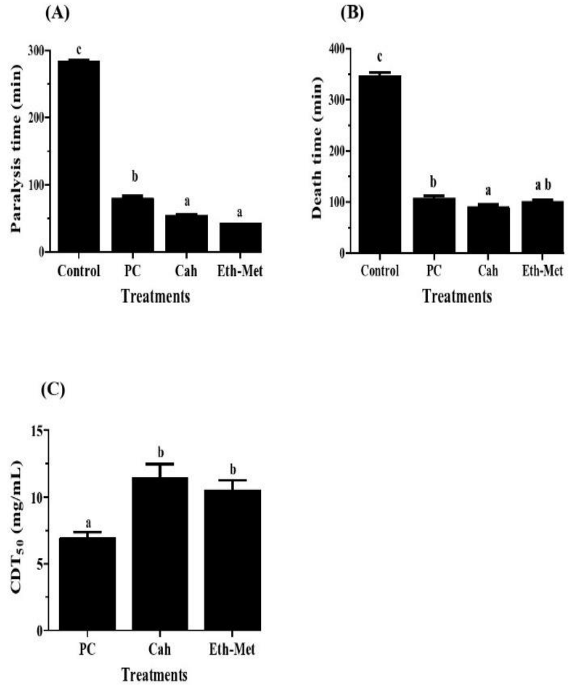

Figure 4. Anthelmintic activity of *Aegiceras corniculatum* fruit extracts (carbohydrate, Cah, and ethanol-methanol, Eth-Met) on *Paramphistomum cervi*. (A) Paralysis time (min) and (B) death time (min) of parasites at 20 mg extract/mL; (C) concentration for death time 50 (CDT_{50}) values of the treatments. Data (n = ~30) are presented as mean ± SD (bar). Significant (P < 0.05) differences are indicated by different letters (a-c). Control means without treatment; PC means positive control, albendazole 15 mg/mL.

Conclusion

The results revealed that *A. corniculatum* fruits have potential antioxidant, analgesic, and anthelmintic activities. Cah extract of *A. corniculatum* fruits showed high carbohydrate and polyphenol contents, antioxidant, and anthelmintic activities. Therefore, Cah extract can be treated as a source of functional carbohydrate(s). However, future research should be concentrated on isolating and identifying antioxidant and anthelmintic compound(s) from both the Cah and Eth-Met extracts whereas analgesic only from Eth-Met extract.

Acknowledgement

The facilities, provided by the Biotechnology and Genetic Engineering Discipline of Khulna University, are gratefully acknowledged.

Conflict of Interest

There is no conflict of interest among the authors.

References

Alam, H. M. I., Biswas, A., Hosen, M. Z., Islam, M. R., Hossain, S. J. (2021). Antioxidant properties and potentiality of silver nanoparticles biosynthesis of thirty-five edible Bangladeshi fruits. *Bangladesh Journal of Botany*, *50*(3), 445-451. https://doi.org/10.3329/bjb.v50i3.55822

Aravind, S. M., Wichienchot, S., Tsao, R., Ramakrishnan, S., Chakkaravarthi, S. (2021). Role of dietary polyphenols on gut microbiota, their metabolites and health benefits. *Food Research International*, 142, 110189. https://doi.org/10.1016/j.foodres.2021.110189

Bae, S. H., Kim, M. R. (2020). Subtype classification of functional constipation in children: polyethyleen glycol versus lactulose. *Pediatrics International*, *62*(7), 816–819. https://doi.org/10.1111/ped.14235

Bandaranayake, W. M. (1998). Traditional and medicinal uses of mangroves. *Mangroves and Salt Marshes*, 2, 133-148. https://doi.org/10.1023/A:1009988607044

Bandaranayake, W. M. (2002). Bioactivities, bioactive compounds and chemical constituents of mangrove plants. *Wetlands Ecology and Management*, 10, 421-452. https://doi.org/10.1023/A:1021397624349

Basar, M. H., Hossain, S. J., Sadhu, S. K., Rahman, M. H. (2013). A comparative study of antioxidant potential of commonly used antidiabetic plants in Bangladesh. *Oriental Pharmacy and Experimental Medicine*, *13*(1), 21-28. https://doi.org/10.1007/s13596-013-0102-x

Biswas, A., Islam, M. R., Hosen, M. Z., Ahmed, K. S., Hossain, H., Hossain, S. J. (2023). Analgesic and anthelmintic activities in common fruits of the Sundarbans mangrove forest, Bangladesh. *Bangladesh Journal of Botany*, *52*(1), 79-86. https://doi.org/10.3329/bjb.v52i1.65237

Bligh, E. G., Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, *37*(8), 911-917. https://doi.org/10.1139/o59-099

Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, 181, 1199-1200. https://doi.org/10.1038/1811199a0

Cao, X., Du, X., Jiao, H., An, Q., Chen, R., Fang, P., Wang, J., Yu, B. (2022). Carbohydrate-based drugs launched during 2000-2021. *Acta Pharmaceutica Sinica B*, *12*(10), 3783-3821. https://doi.org/10.1016/j.apsb.2022.05.020

Dreywood, R. (1946). Qualitative test for carbohydrate material. *Industrial & Engineering Chemistry Analytical Edition*, *18*(8), 499. https://doi.org/10.1021/i560156a015

Eddy, N. B., Leimbach, D. (1953). Synthetic analgesics. II. Dithienylbutenyl-and dithienylbutylamines. *The Journal of Pharmacology and Experimental Therapeutics*, *107*(3), 385-393.

Hosen, M .Z., Biswas, A., Islam, M. R., Bhuiyan, M. N. I., Hossain, S. J. (2020). Comparison of physicochemical and antioxidant properties of edible fruits in the Sundarbans mangrove forest, Bangladesh. *Bangladesh Journal of Botany*, *49*(3), 671-678. https://doi.org/10.3329/bjb.v49i3.50009

Hosen, M. Z., Biswas, A., Islam, M. R., Hossain, S. J. (2021). Antibacterial, antidiarrheal, and cytotoxic activities of edible fruits in the Sundarbans mangrove forest of Bangladesh. *Preventive Nutrition and Food Science*, *26*(2), 192-199. https://doi.org/10.3746/pnf.2021.26.2.192

Hossain, S. J., Aoshima, H., El-Sayed, M., Ahmed, F. (2009). Antioxidative and anti-histamine-release activities of *Excoecaria agallocha* L. *PharmacologyOnline*, 2, 927-936.

Hossain, S. J., Bashar, M. H., Rokeya, B., Arif, K. M. T., Sultana, M. S., Rahman, M. H. (2013). Evaluation of antioxidant, antidiabetic and antibacterial activities of the fruit of *Sonneratia apetala* (Buch.-Ham.). *Oriental Pharmacy and Experimental Medicine*, *13*(2), 95-102. https://doi.org/10.1007/s13596-012-0064-4

Hossain, S. J., Iftekharuzzaman, M., Haque, M. A., Saha, B., Moniruzzaman, M., Rahman, M. M., Hossain, H. (2016). Nutrient compositions, antioxidant activity, and common phenolics of *Sonneratia apetala* (Buch.-Ham.) fruit. *International Journal of Food Properties*, *19*(5), 1080-1092. https://doi.org/10.1080/10942912.2015.1055361

Hossain, S. J., Islam, M. R., Pervin, T., Iftekharuzzaman, M., Hamdi, O. A. A., Mubassara, S., Saifuzzaman, M., Shilpi, J. A. (2017). Antibacterial, antidiarrhoeal, analgesic, cytotoxic activities, and GC-MS profiling of *Sonneratia apetala* (Buch.-Ham.) seed. *Preventive Nutrition and Food Science*, *22*(3), 157-165. https://doi.org/10.3746/pnf.2017.22.3.157

Hossain, S. J., Sultana, Mst. S., Iftekharuzzaman, M., Hossain, S. A., Taleb, M. A. (2015). Antioxidant potential of common leafy vegetables in Bangladesh. *Bangladesh Journal of Botany*, *44*(1), 51-57. https://doi.org/10.3329/bjb.v44i1.22723

Hossain, S. J., Sultana, Mst. S., Taleb, M. A., Basar, M. H., Sarower, M.G., Hossain, Sk. A. (2014). Antioxidant activity of ethanol and lipophilic extracts of common fruity vegetables in Bangladesh. *International Journal of Food Properties*, *17*(9), 2089-2099. https://doi.org/10.1080/10942912.2013.790052

Hossain, S. J., Tsujiyama, I., Takasugi, M., Islam, Md. A., Biswas, R. S., Aoshima, H. (2008). Total phenolic content, antioxidative, anti-amylase, anti-glucosidase, and antihistamine release activities of Bangladeshi fruits. *Food Science and Technology Research*, *14*(3), 261-268. https://doi.org/10.3136/fstr.14.261

Islam, M. R., Islam, M. R., Anisuzzaman, Md., Hossain, S. J. (2019b). Antidiarrheal, analgesic, and anthelmintic activities of honeys in the Sundarbans mangrove forest, Bangladesh. *Preventive Nutrition and Food Science*, *24*(1), 49-55. https://doi.org/10.3746/pnf.2019.24.1.49

Islam, S., Shajib, Md. S., Rashid, R. B., Khan, M. F., Al-Mansur, M. A., Datta, B. K., Rashid, M. A. (2019a). Antinociceptive activities of *Artocarpus lacucha* Buch.-Ham. (Moraceae) and its isolated phenolic compound, catechin, in mice. *BMC Complementary and Alternative Medicine*, *19*(1), 214. https://doi.org/10.1186/s12906-019-2565-x

Khatun, Mst. R., Luna, N. I., Akter, S., Islam, M. R., Hossain, S. J. (2022). Antioxidant activity and capacity of silver nanoparticles biosynthesis of common fruits aqueous extracts of the Sundarban forest. *Khulna University Studies*, *19*(1), 66-73. https://doi.org/10.53808/KUS.2022.19.01.2208-ls

Koster, R., Anderson, M., De Beer, E. J. (1959). Acetic acid for analgesic screening. *Federation Proceedings*, 18, 412-417.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, *193*(1), 265-275.

Miller, G. L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, *31*(3), 426-428.

Mithila, M., Islam, M. R., Khatun, Mst. R., Gazi, M. S., Hossain, S. J. (2023). *Sonneratia apetala* (Buch.-Ham.) fruit extracts ameliorate iron overload and iron-induced oxidative stress in mice. *Preventive Nutrition and Food Science*, *28*(3), 278-284. https://doi.org/10.3746/pnf.2023.28.3.278

Mubassara, S., Takasugi, M., Iga, R., Hossain, S. J., Aoshima, H. (2011). Inhibition of the histamine and leukotriene B4 release from rat peritoneal exudates cells by six Bangladeshi plants. *PharmacologyOnline*, 2, 76-85.

Ough, C. S., Amerine, M. A. (1988). Methods analysis of musts and wines. John Wiley & Sons, New York, pp. 196-221.

Prieto, P., Pineda, M., Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical Biochemistry*, *269*(2), 337-341. https://doi.org/10.1006/abio.1999.4019

Qiu, M., Huang, S., Luo, C., Wu, Z., Liang, B., Huang, H., Ci, Z., Zhang, D., Han, L., Lin, J. (2021). Pharmacological and clinical application of heparin progress: an essential drug for modern medicine. *Biomedicine & Pharmacotherapy*, 139, 111561. https://doi.org/10.1016/j.biopha.2021.111561

Ray, R., Banerjee, A., Mullick, J., Jana, T.K. (2015). Nutritional composition of some selected wild mangrove fruits of Sundarbans. *Indian Journal of Geo-Marine Sciences*, *44*(7), 1059-1066.

Roome, T., Dar, A., Naqvi, S., Choudhary, M. I. (2011). Evaluation of antinociceptive effect of *Aegiceras corniculatum* stems extracts and its possible mechanism of action in rodents. *Journal of Ethnopharmacology*, *135*(2), 351-358. https://doi.org/10.1016/j.jep.2011.03.025

Sajid, M., Azim, M. K. (2012). Characterization of the nematicidal activity of natural honey. *Journal of Agricultural and Food Chemistry*, *60*(30), 7428-7434. https://doi.org/10.1021/jf301653n

Sarkar, P., Ahnaf, T. R., Rouf, R., Shilpi, J. A., Uddin, S. J. (2024). A review on bioactive phytochemical constituents and pharmacological activities of *Aegiceras corniculatum*: a pharmaceutically important mangrove plant. *Journal of Chemistry*, ID 9992568. https://doi.org/10.1155/2024/9992568

Tandon, V., Pal, P., Roy, B., Rao, H. S., Reddy, K. S. (1997). *In vitro* anthelmintic activity of root-tuber extract of *Flemingia vestita*, an indigenous plant in Shillong, India. *Parasitology Research*, *83*(5), 492- 498. https://doi.org/10.1007/s004360050286

Tsujiyama, I., Mubassara, S., Aoshima, H., Hossain, S. J. (2013). Anti-histamine release and anti-inflammatory activities of aqueous extracts of citrus fruits peels. *Oriental Pharmacy and Experimental Medicine*, *13*(3), 175-180. https://doi.org/10.1007/s13596-012-0093-z

Wang, J., Zhang, Y., Lu, Q., Xing, D., Zhang, R. (2021). Exploring carbohydrates for therapeutics: a review on future directions. *Frontiers in Pharmacology*, 12, 756724. https://doi.org/10.3389/fphar.2021.756724

Wang, X., Sun, G., Feng, T., Zhang, J., Huang, X., Wang, T., et al. (2019). Sodium oligomannate therapeutically remodels gut microbiota and suppresses gut bacterial amino acids-shaped neuroinflammation to inhibit Alzheimer's disease progression. *Cell Research*, *29*(10), 787–803. https://doi.org/10.1038/s41422-019-0216-x