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Evaluation of antioxidant, anti-arthritic and anthelmintic activities of methanolic leaves extract of *Chaetocarpus castanocarpus* (Roxb.)

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Article info

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Abstract

The studies were conducted to asses phytoconstituent, quantitative and qualitative research to ensure scopes of pharmacological properties of methanol extracts of Chaetocarpus castanocarpus *(Roxb.)* leaves (MECCL). Phytochemical screenings were performed by using standard protocols. MECCL demonstrated solid free radical scavenging activity in DPPH (1, 1-diphenyl-2-picrylhydrazyl) test, as well as total phenol and total flavonoid content were found in a quantitative assay. The topmost value of antioxidant potency (87.38%) of MECCL has exposed at 500 µg/ml concentration, whereas standard ascorbic acid reveals a 97.49% outcome at an identical concentration. However, the flavonoid and phenolic potentials of MECCL were found at 149.14± 0.084 mg QE/gm and 184.66 ± 0.064 mg GAE/gm, correspondingly. The in vitro anti-arthritic activity was appraised by protein denaturation method, whereas plant extracts proclaimed dose-dependent response working on protein denaturation method and the peak inhibition was found (60.32 ± 1.72) at 500 µg/mL concentration. MECCL also proclaimed the dose-related anthelmintic activity on aquarium worm (Tubifex tubifex) as revealed by the decreased paralyzing time and death time. So, the current research propose that *Chaetocarpus castanocarpus* (Roxb.) could be a better choice for managing cardiovascular disease, arthritis and helminthiasis .

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1. Introduction

The consumption of herbal medicines in treatment of different disorders has expanded considerably in today's world. The popularity of herbal drugs and food supplements in the world is due to more efficacy and less toxicity

than synthetic drugs. Secondary metabolites remaining in raw products can play a vital part in the therapy of various ailment like neurological disorders, heart disease, diarrhea, stroke, and various worm infection. (Mishra & Tiwari, 2011). Though natural products have been a prime origin of therapeutic agents for several sprinkle of decades, but based on their traditional customs, natural products also a source of extent of latest synthetic drugs that have been secluded from raw sources. Reactive oxygen species are commonly known to be a critical factor in the pathophysiology of ischemia, arthritis, reperfusion injury of various tissues and atherosclerosis (Camkurt, Fındıklı, İzci, Kurutaş, & Tuman, 2016; Vaváková, Ďuračková, & Trebatická, 2015). One of the most momentous pathways for the production of free radicals in food, medication, and even biological process is the oxidation process (da Silveira et al., 2016). Several commercially accessible synthetic antioxidants, including gallic acid esters, tertiary butylated hydroquinone, butylated hydroxy anisole (BHA), butylated hydroxytoluene (BHT), and have been linked to detrimental clinical outcomes. The therapeutic possibilities of medicinal plants as antioxidants in preventing various free radical generated tissue damage have recently gained considerable attention (Gbolade & Adeyemi, 2008).

Chaetocarpus castanocarpus (Roxb.) (Synonym: *C. pungens*; Family: Peraceae) is a large evergreen tree which is able to grow with a height of about 45 m and indigenous to Myanmar, Indonesia, Cambodia, India, Srilanka, Thailand, Bangladesh, Andaman Islands, Laos and Vietnam. The leaf extract of this plant is being used conventionally in tribe's area to treat swollen joints (Azwanida, 2015; Santiago, Lim, Loh, & Ting, 2015). Beside this, the leaf of this plant also used as food (Tsukiyama et al., 2010). Thus, the investigation was planned to scrutinize the antioxidant, anti-arthritic and anthelmintic effects of the methanol extract of *Chaetocarpus castanocarpus* (Roxb.) leaves.

2. Materials & methods

2.1. Chemicals and reagents

Methanol, Folin-Ciocalteu reagent, Gallic acid, 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) were bought from (Sigma Chemical Company, St. Louis, MO, USA). All the other analytical grade chemicals were procured from Taj Scientific Ltd., Bangladesh.

2.2. Plant collection and identification

Collection of leaves of *Chaetocarpus castanocarpus* was done from Sita Pahar area of Kaptai, rangamati district, Chittagong, Bangladesh in March 2019

and authenticated by a taxonomist Prof. Dr. Shaikh Bakhtear Uddin, Department of Botany, University of Chittagong, Bangladesh (Specimen No#201022-157). The unwanted adulterants were separated by hand, then the the plant materials were washed by using fresh water. Finally cleaned plant materials were shade dried at a low temperature of (15-50) °, coarsely powdered by a suitable grinder and reserved in an airtight vessel.

2.3. Preparation of plant extract

About 400 gm rough pulverized leaves were solvated in 2000 mL of 80% methanol at a room temperature of (25±2) ° C for 10 days with random shaking and then stirred on a shaker device and also manually. The blended materials was filtered by using cotton plug following by Whatman #1 filter paper. To conclude, filtrate was then subjected to water bath at 40 °C to evaporate the solvent and the raw methanol extract was obtained which preserved at 4 °C (Haque, Jantan, Harikrishnan, & Ghazalee, 2019).

2.4. Phytochemical analysis

Initially qualitative phytochemical screening for the recognition of bioactive compounds existing in crude methanol extract of *Chaetocarpus castanocarpus (Roxb.)* leaves were performed according to standard phytochemical methods (Harborne, 1998; Hossain et al., 2018).

2.5. Antioxidant activity

2.5.1. Determination of DPPH free radical scavenging activity

The DPPH free radical scavenging activity of MECC was assessed according to the protocol stated earlier (Bursal & Gülçin, 2011). 3 mL, 0.004% DPPH solution (4 mg DPPH was in 100 mL, 95% methanol) was diluted in 1mL of either extract or standard of different concentration (15.625 to 500 µg/mL). After thirty min incubation at 25°C, absorbance was taken at 517 nm by the use of UV-visible spectrophotometer. Here, the percentage (%) of inhibition then done by the using the following equation,

$$[A_0-A_1/A_0]\times 100$$

Here, A_0= control absorbance, A_1=sample/standard absorbance

Using a non-linear regression algorithm, the IC_{50} was measured from the percentage inhibition against log concentration plot.

2.5.2. Determination of total phenolic content

The total phenolic content (TPC) was performed using Folin-Ciocalteu reagent (FCR) according to Reza et al. (2018) modified method. In brief, 1000 µg/mL of test sample/ standard (15.625 to 500 µg/mL) were assorted with 2.5 mL of FCR (10 times diluted with the water). Subsequently, 2.5mL 7.5% Na_2CO_3 was add up with the solution after five min of incubation (25 °C)

followed by the inclusion of 10mL of distilled water. The admixture was incubated for 20 minutes at room temparature and absorbance was evaluated at 765 nm against blank. The estimation of TPC was conducted triplicate. Graph of standard gallic acid was used to quantify TPC. The outcomes were revealed as mg of gallic acid equivalents (GAE) per g of dried extract.

2.5.3. Determination of total flavonoid content

The total flavonoid content (TFC) was estimated by aluminium colorimetric method described by Oyedemi and Afolayan (2011). 1mL of test sample (1mg/mL) or standard (100-12.5 μg/mL) was added with 3mL of methanol and followed by inclusion of 0.2 mL 10% $AlCl_3$, 0.2 mL of 1M CH_3CO_2Na and 5.6 mL of distilled water. The blend was then incubated for 30 min at RT and absorbance was calculated at 420 nm against blank. The estimation of TFC was lead triplicate. A graph of standard quercetin was used to figure out TFC and the outcome was revealed as of mg of quercetin equivalents (QE) per gm of dried extract.

2.6. Anti-arthritic activity

The anti-arthritic activity of MECCL was conducted according to method designated by Uddin et al. (Ansari et al., 2017) with minor modifications. Serial dilution of different concentrations (31.25-500 μg/ml) of MECC extract and as standard diclofenac-Na were procured. For each concentration 0.5 mL of test sample or standard from prepared dilutions was mixed with the aqueous albumin (0.45 mL, 5% w/v). To adjust the pH at 6.3, 1N HCl was added. The admixtures were first incubated at 37ºC for 20 min and then we increased the temperature to retain the mixtures at 57ºC for 30 min. After the completion of incubation, the samples were cooled and then phosphate buffer (2.5 mL) was added. The blank solution contain all reagents excluding extract. The absorbance taken at 416 nm by the use of UV-Visible spectrophotometer. The percent inhibition of protein denaturation was done from "$[A_0\text{-}A_1/A_0] \times 100$", where A_0=control absorbance and A_1=sample/standard absorbance.

2.7. Anthelmintic activity

The anthelmintic activity of MECCL extract was examined using the mentioned protocol with minor modifications (Adnan et al., 2019; Sujavanthi, Thayalini, & Mikunthan, 2023). The aquarium ringed worms (*Tubifex tubifex*; size: 2-2.5 cm long) were used owing to their relevance with intestinal worms both anatomically and physiologically. 50 worms (7-10 days old) were divided into five groups comprising of ten (n=10) in each group and randomly placed in a cell-culture dish. In the control group (group I)

water was added, group-II was then served with standard drug levamisole (1 mg/mL), and test groups (III, IV, and V) were treated with 3 mL of MECCL extract at three concentrations (5,10 and 20 mg/mL). The experiment was performed in triplicate for all worms group. Anthelmintic activity was recorded by determining both the time of the paralysis and death of worms. Paralysis periods were reported when worms lost all movement unless shaken strongly. Death time was famed when the worms were neither shaken as strongly nor drenched in hot water (50℃) followed by vanishing of their body colors.

3. Results

3.1. Phytochemical screening

The qualitative phytochemical screening of methanol extract of *Chaetocarpus castanocarpus leaves (Roxb.)* (MECCL) evident the appearance of many secondary metabolites like alkaloid, glycosides, phenol, flavonoid, tannin etc. The result of this demonstration encapsulated in Table I.

Table I

Outcome of phytochemical screening of methanol extract of Chaetocarpus castanocarpus (Roxb.) leaves (MECCL)

Phytochemicals	Test Type	Appearance	Result
Alkaloid	Wagner test	A reddish-brown color	++
	Mayer's test	Yellow color	++
Glycosides	Shinoda test	Deep brown ring	+
Cardiac Glycosides	Legal test	Brown color	+
"Flavonoids"	Lead acetate test	Fluorescence yellow	+
Phenols	$FeCl_3$ test	Violet color	+
Coumarins	Ammonia test	Green color	++
Tannin	Lead acetate test	Yellow color	+
Phlobatannins	HCl test	No reddish precipitation form	-
Xanthoproteins	Xanthoprotein test	No reddish-brown precipitation form	-
Cholesterols	General test	No red rose color	-
Triterpenoids	Salkowski's test	Reddish-brown color form	+
Resins	FeCl3 test	No precipitation	-
Quinones	HCl test	Yellow color not form	-

++: Hugely present; +: moderately present; −: absent.

3.2. Antioxidant effect

3.2.1. DPPH radical scavenging assay

The antioxidant activity of MECCL was examined by DPPH free radical scavenging assay. The raw extract demonstrates potential antioxidant properties which were obtainable in Figure 1. The topmost value of antioxidant potency (87.38%) of MECCL has exposed at 500 µg/ml

concentration, whereas standard ascorbic acid reveals a 97.49% outcome at a identical concentration. Here, the capability of scavenging properties was increased when we increased the concentration. The IC_{50} values of MECCL and ascorbic acid were 146.44% and 19.72%, subsequently, that was assessed using linear regression formula.

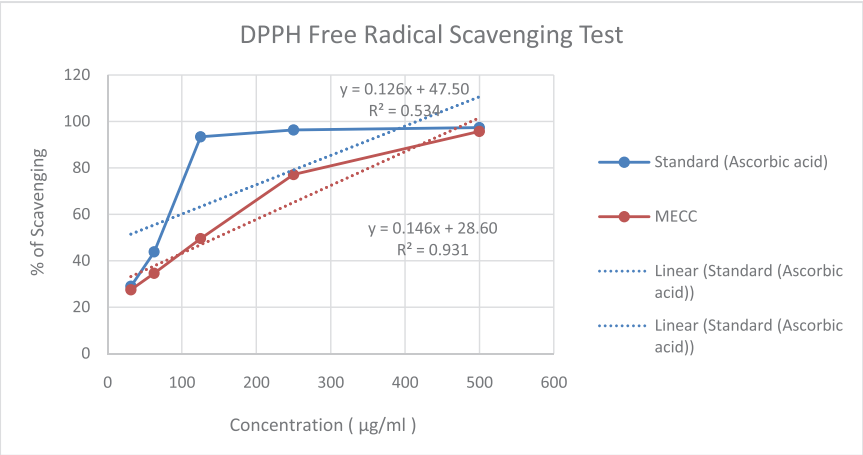

Figure 1

Radical scavenging activity: by the DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay of MECCL and ascorbic acid (standard drug) at different concentrations.

3.2.2. Total flavonoid and phenolic contents

The total flavonoid and phenol content of our rough extract was assessed quantitatively. The outcome is exhibited in Table II. The flavonoid and phenolic potentials of MECCL were found at 149.14± 0.084 mg QE/gm and 184.66 ± 0.064 mg GAE/gm, correspondingly. Here, MECCL was carried out through linear regression equation (As for flavonoid activity, equation stands for y = 0.003x + 0.032; for phenolic inspection, it was y = 0.003x + 0.004).

Table II

Total phenol and flavonoid contents of methanol extract of *Chaetocarpus castanocarpus (Roxb.)* leaves (MECCL).

Extract	Total phenol content (mg GAE/gm dried extract)	Total Flavonoid content (mg GAE/gm dried extract)
MECCL	184.66 ± 0.064	149.14± 0.084

MECCL: Methanol extract of *Chaetocarpuscastanocarpus (Roxb.)* leaves

3.3. Antiarthritic activity

The antiarthritic activity of MECCL on protein denaturation is offered in Figure 2. The extract manifested dose-dependent inhibitory potency when compared to diclofenac sodium. Percentage of inhibition was 26.71 ± 2.53, 37.62± 1.88, 42.58 ± 2.03, 48.82 ± 3.25, and 60.32 ± 1.71 for MECCL; and 50.29± 1.09, 58.95 ± 0.89, 70.57 ± 0.82, 81.19 ± 0.73, and 86.78 ± 0.21 was for diclofenac sodium at the various concentration of 31.25, 62.5, 125, 250 and 500 μg/mL, subsequently.

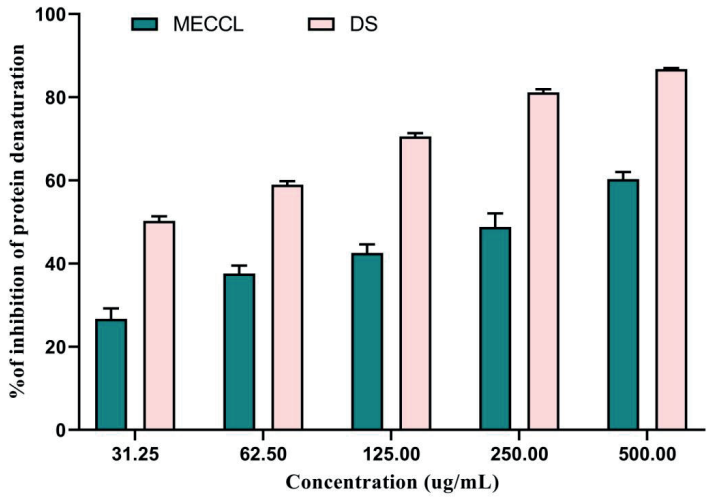

Figure 2
Antiarthritic activity of MECCL at different concentrations.

3.4. Anthelmintic activity

Anthelmintic activity of MECCL was demonstrated on Tubifex tubifex worms which findings were encapsulated in Table III. In this examination, at the 5, 10, 20 µg/mL concentrations the plant extract demonstrated paralysis time 38.12 ±2.60, 21.22 ±2.21 and 11.15 ±5.33 min; and death time 43.10 ±7.79, 29.08 ±6.72 and 17.22 ±4.55 min, subsequently, however the standard drug levamisole presented paralysis time 15.15±9.29, 7.4±4.02 and 4.5±5.33 min, and the death time 20.10±3.14, 12.26±7.47 and 8.55±2.42 respectively. The results indicated that the anthelmintic effect was in dose dependent manner.

Table III
Anthelmintic activity of methanol extract of Chaetocarpus castanocarpus (Roxb.) leaves (MECCL).

Group	Concentration, mg/mL	Paralysis time(min)	Dead time(min)
MECCL	5	38.12±2.60	43.10±7.793
	10	21.22±2.215	29.08±6.72
	20	11.15±5.33	17.22±4.553
Standard (Levamisole)	5	15.15±9.295	20.10±3.149
	10	7.4±4.028	12.26±7.479
	20	4.5±5.331	8.55±2.428

MECCL: Methanol extract of *Chaetocarpus castanocarpus (Roxb.)* leaves

4. Discussion

Since the down of human civilization, plants have been one of the dominant origins of medicines and are still available as one of the crucial sources of drugs in modern as well as in traditional systems of medicine (Akter et al., 2021; Jahan et al., 2022). Divergent classes of bioactive compounds have been secluded and distinguished from plants since the middle of the nineteenth century and a great number of these are used as the active ingredients of modern medicines, or as the lead compounds for new drugs uncovering. In this investigation, we directed numerous tests to estimate variety of health interest like antioxidant, antiarthritic, and anthelmintic potential of the methanolic extract of *Chaetocarpus castanocarpus (Roxb.)* leaves. Phytochemical screening of methanol extract of *Chaetocarpus castanocarpus (Roxb.)* leaves authenticated the existence of variety of secondary metabolites such as alkaloids, phenols, flavonoid, triterpenoids, coumarins, glycosides, tannin and cardiac glycoside etc. The presence of secondary metabolites are responsible for their antioxidant, antiarthritic, and anthelmintic activity (Akter et al., 2021).

An overabundance of free radical put up oxidative stress leads to destruction of proteins, DNA and lipids that is connected with chronic declinatory diseases (Hossen et al., 2021). Essential antioxidants in the form of raw extracts or their chemical components are very fruitful to put a stop to the declinatory processes brought about by oxidative stress (Hoque et al., 2021). Methanol extract of *Chaetocarpus castanocarpus (Roxb.)* leaves (MECCL) was found to possess better ROS (Reactive oxygen species) counteracting capacity. Our present study manifested dose dependent antioxidant activity that is when concentration increased, the antioxidant potency also increased and a handful of studies confirmed dose dependent activity of plant extracts. MECCL also exhibit dose dependent antiarthritic activity. Antiarthritic potency was conducted by inhibition of protein denaturation method

considering that denaturation of protein is one of the causes of rheumatoid arthritis. The standard drug and the plant extract both showed dose dependent activity but standard drug was noticed to give superior results.

The in vitro anthelmentic activity of MECCL revealed moderate potency when we compared with standard levamisole. People prefer medicinal plant as an possible sources of anthelmintic drugs, despite the availability of a number of commercial drugs. It is evident through a large number of studies that plants possessing strong anthelmintic activity (Osei Akoto, Acheampong, Boakye, Naazo, & Adomah, 2020).

5. Conclusion

The incidence of phytoconstituents such as total phenolics, alkaloids, flavonoids, triterpinoid, glycosides, and tannins in *Chaetocarpus castanocarpus (Roxb.)* offers a few scientific mark for biological activity and also responsible for pharmacological usage of the plants. The existence of high flavonoid, phenolic compounds could be accredited to its pharmacological action related with free radicals. In this investigation, MECCL has been shown to have favourable radical scavenging properties. Additionally, MECCL holds notable 'anti-inflammatory, arthritis, and anthelmintic' activity. However, *Chaetocarpus castanocarpus (Roxb.)* can be contemplated as a latent origin for uncovering the secondary metabolites that can be used for several pharmacological utilizations. Additional analysis are essential to disclose the procedure behind its potency.

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References

Adnan, M., Chy, M. N. U., Kamal, A. T. M. M., Azad, M. O. K., Paul, A., Uddin, S. B., ... Cho, D. H. (2019). Investigation of the biological activities and characterization of bioactive constituents of Ophiorrhiza rugosa var. prostrata (D. Don) & Mondal leaves through in vivo, in vitro, and in silico approaches. *Molecules*, *24*(7), 1367. https://doi.org/10.3390/molecules24071367

Akter, S., Jahan, I., Khatun, M. R., Khan, M. F., Arshad, L., Jakaria, M., & Haque, M. A. (2021). Pharmacological insights into Merremia vitifolia (Burm. f.) Hallier f. leaf for its antioxidant, thrombolytic, anti-arthritic and anti-nociceptive potential. *Bioscience Reports, 41*(1), BSR20203022.

https://doi.org/10.1042/BSR20203022

Ansari, P., Uddin, M. J., Rahman, M. M., Abdullah-Al-Mamun, M., Islam, M. R., Ali, M. H., & Reza, A. S. M. A. (2017). Anti-inflammatory, anti-diarrheal, thrombolytic and cytotoxic activities of an ornamental medicinal plant: Persicaria orientalis. *Journal of Basic and Clinical Physiology and Pharmacology*, *28*(1), 51-58. https://doi.org/10.1515/jbcpp-2016-0023

Azwanida, N. N. (2015). A review on the extraction methods use in medicinal plants, principle, strength and limitation. *Medicinal and Aromatic Plants, 4*(3), 1-6. http://dx.doi.org/10.4172/2167-0412.1000196

Bursal, E., & Gülçin, İ. (2011). Polyphenol contents and in vitro antioxidant activities of lyophilised aqueous extract of kiwifruit (Actinidia deliciosa). *Food Research International, 44*(5), 1482-1489. https://doi.org/10.1016/j.foodres.2011.03.031

Camkurt, M. A., Fındıklı, E., İzci, F., Kurutaş, E. B., & Tuman, T. C. (2016). Evaluation of malondialdehyde, superoxide dismutase and catalase activity and their diagnostic value in drug naïve, first episode, non-smoker major depression patients and healthy controls. *Psychiatry Research*, *238*, 81-85. https://doi.org/10.1016/j.psychres.2016.01.075

da Silveira, C. C. S. d. M., Fernandes, L. M. P., Silva, M. L., Luz, D. A., Gomes, A. R. Q., Monteiro, M. C., . . . Ferreira, A. G. (2016). Neurobehavioral and antioxidant effects of ethanolic extract of yellow propolis. *Oxidative Medicine and Cellular Longevity, 2016*. https://doi.org/10.1155/2016/2906953

Gbolade, A., & Adeyemi, A. (2008). Investigation of in vitro anthelmintic activities of Pycnanthus angolensis and Sphenocentrum jollyanum. *Fitoterapia, 79*(3), 220-222. https://doi.org/10.1016/j.fitote.2007.11.024

Haque, M. A., Jantan, I., Harikrishnan, H., & Ghazalee, S. (2019). Standardized extract of Zingiber zerumbet suppresses LPS-induced pro-inflammatory responses through NF-κB, MAPK and PI3K-Akt signaling pathways in U937 macrophages. *Phytomedicine, 54*, 195-205. https://doi.org/10.1016/j.phymed.2018.09.183

Harborne, A. (1998). *Phytochemical methods a guide to modern techniques of plant analysis*: London: Chapman and Hall, Springer science & business media.

Hoque, M. A., Ahmad, S., Chakrabarty, N., Khan, M. F., Kabir, M. S. H., Brishti, A., . . . Nasrin, M. S. (2021). Antioxidative role of palm grass rhizome ameliorates anxiety and depression in experimental rodents and computer-aided model. *Heliyon, 7*(10), e08199.

https://doi.org/10.1016/j.heliyon.2021.e08199

Hossain, M. S., Reza, A. A., Rahaman, M. M., Nasrin, M. S., Rahat, M. R. U., Islam, M. R., ... Rahman, M. A. (2018). Evaluation of morning glory (Jacquemontia tamnifolia (L.) Griseb) leaves for antioxidant, antinociceptive, anticoagulant and cytotoxic activities. *Journal of Basic and Clinical Physiology and Pharmacology*, *29*(3), 291-299. https://doi.org/10.1515/jbcpp-2017-0042

Hossen, M. A., Reza, A. A., Ahmed, A. A., Islam, M. K., Jahan, I., Hossain, R., . . . Rahman, M. A. (2021). Pretreatment of Blumea lacera leaves ameliorate acute ulcer and oxidative stress in ethanol-induced Long-Evan rat: A combined experimental and chemico-biological interaction. *Biomedicine and Pharmacotherapy, 135*, 111211. https://doi.org/10.1016/j.biopha.2020.111211

Jahan, I., Khan, M. F., Sayeed, M. A., Arshad, L., Hossen, M. A., Jakaria, M., . . . Capasso, R. (2022). Neuropharmacological and Antidiarrheal Potentials of Duabanga grandiflora (DC.) Walp. Stem Bark and Prospective Ligand–Receptor Interactions of Its Bioactive Lead Molecules. *Current Issues in Molecular Biology, 44*(5), 2335-2349. https://doi.org/10.3390/cimb44050159

Mishra, B. B., & Tiwari, V. K. (2011). Natural products: An evolving role in future drug discovery. *European Journal of Medicinal Chemistry, 46*(10), 4769-4807. https://doi.org/10.1016/j.ejmech.2011.07.057

Osei Akoto, C., Acheampong, A., Boakye, Y. D., Naazo, A. A., & Adomah, D. H. (2020). Anti-Inflammatory, Antioxidant, and Anthelmintic Activities of Ocimum basilicum (Sweet Basil) Fruits. *Journal of Chemistry*, *2020*(1), 2153534. https://doi.org/10.1155/2020/2153534

Oyedemi, S. O., & Afolayan, A. J. (2011). Antibacterial and antioxidant activities of hydroalcoholic stem bark extract of Schotia latifolia Jacq. *Asian Pacific Journal of Tropical Medicine*, *4*(12), 952-958. https://doi.org/10.1016/S1995-7645(11)60225-3

Reza, A. S. M. A., Hossain, M. S., Akhter, S., Rahman, M. R., Nasrin, M. S., Uddin, M. J., . . . Alam, A. H. M. K. (2018). In vitro antioxidant and cholinesterase inhibitory activities of Elatostema papillosum leaves and correlation with their phytochemical profiles: A study relevant to the treatment of Alzheimer's disease. *BMC Complementary and Alternative Medicine, 18*(1), 1-8. https://doi.org/10.1186/s12906-018-2182-0

Santiago, C., Lim, K. H., Loh, H. S., & Ting, K. N. (2015). Inhibitory effect of Duabanga grandiflora on MRSA biofilm formation via prevention of

cell-surface attachment and PBP2a production. *Molecules, 20*(3), 4473-4482. https://doi.org/10.3390/molecules20034473

Sujavanthi, L., Thayalini, T., & Mikunthan, G. (2023). Phytochemical screening and anthelmintic activity of four plant extracts on earthworm, Eisenia fetida.

Tsukiyama, M., Sugita, T., Kikuchi, H., Yasuda, Y., Arashima, M., Okumura, H., . . . Shoyama, Y. (2010). Effect of Duabanga grandiflora for human skin cells. *The American Journal of Chinese Medicine, 38*(02), 387-399. https://doi.org/10.1142/S0192415X10007920

Vaváková, M., Ďuračková, Z., & Trebatická, J. (2015). Markers of oxidative stress and neuroprogression in depression disorder. *Oxidative Medicine and Cellular Longevity*, *2015*(1), 898393. https://doi.org/10.1155/2015/898393