The Journal of Phytopharmacology 2021; 10(5):345-349
Online at: www.phytopharmajournal.com


Research Article

ISSN 2320-480X
JPHYTO 2021; 10(5): 345-349
September- October
Received: 16-08-2021
Accepted: 17-09-2021

doi: 10.31254/phyto.2021.10511

**Adeeba Anjum**
Department of Pharmacy, Faculty of Science, University of Rajshahi, Rajshahi-6205, Bangladesh

**Rifat Ara Rima**
Department of Pharmacy, Faculty of Science, University of Rajshahi, Rajshahi-6205, Bangladesh

**Shoriful Islam Sagor**
Department of Pharmacy, Faculty of Science, University of Rajshahi, Rajshahi-6205, Bangladesh

**Correspondence:**
**Dr. Adeeba Anjum**
Department of Pharmacy, Faculty of Science, University of Rajshahi, Rajshahi-6205, Bangladesh
Email: adeeba.sumi@gmail.com

# Evaluation of *in vitro* antibacterial and *in vivo* cytotoxic activities of Bangladeshi *Coffea benghalensis* B Heyne ex Schult. roots

Adeeba Anjum*, Rifat Ara Rima, Shoriful Islam Sagor

## ABSTRACT

This study was designed to evaluate the *in vitro* antibacterial and *in vivo* cytotoxic activity of the roots of Bangladeshi *Coffea benghalensis* B Heyne ex Schult. (Family: Rubiaceae), locally known as bonnyo kofee native to the regions of Sylhet and Chittagong in Bangladesh. The finely crushed coarse powder was extracted with methanol and solvent-solvent partitioning was done to obtain *n*-hexane, chloroform, ethyl acetate and aqueous soluble fractions. The crude methanol extract along with it's four soluble fractions were then evaluated for their *in vitro* antibacterial and *in vivo* cytotoxic activities. The disc diffusion method was used to evaluate the antibacterial activity at a concentration of 200 and 400 µg/disc. All the soluble fractions and the crude methanolic extract did not demonstrate any zone of inhibition. In case of brine shrimp lethality bioassay, all the test samples exhibited cytotoxic activity against brine shrimp nauplii having remarkable $LC_{50}$ and $LC_{90}$ values. The *n*-hexane soluble materials demonstrated highest lethality with $LC_{50}$ value of 6.84 ± 0.87 µg/ml and $LC_{90}$ 59.86 ± 0.83 µg/ml in contrast to the standard vincristine sulphate. Further investigations are necessary to evaluate the antibacterial activity. These findings indicates that the roots of the plant contains cytotoxic bioactive compounds but advanced research is necessary to elucidate the cytotoxic bioactive compounds. Other techniques are also required to assess the antibacterial activity of the plant.

**Keywords:** *Coffea benghalensis*, Antibacterial, Brine shrimp nauplii, Cytotoxicity.

## INTRODUCTION

Today, one of the most augmentative public health issue is the universal microorganism resistance to man-made artificial antibiotics [1]. Researchers stipulated that the bacterial strains which are resistant arises due to several reasons like misuse and patient non-compliance to antibiotic therapy, extensive use of antibiotics in animal feed as promoters of growth and the trans-bounding spreading of the strains that are resistant to antibiotics [2]. Thus, the treatment with recently used antibiotics against various pathogenic bacteria has become very much complicated. Additionally, in the developing countries of the Asian and African continents, the lack of novel, safe, inexpensive and available antibiotics urgently needs intercedetion, because over 50% of the worldwide deaths due to infectious diseases occur in this region [3, 4]. Besides, the currently used antimicrobial agents exhibits untoward effects like gastrointestinal complications, hepatotoxicity and nephrotoxicity [5]. This is slowly losing interest in orthodox medicine and continuously increasing the demand for plant sourced drugs. This revival of interest in plant sourced drugs is mainly due to the belief that "green medicine" is safe and dependable more than the synthetic ones, many of which have adverse side effects [6]. Since time immemorial the practice of herbal plants in traditional medicine and their medicinal values have been docmented [7]. The biologically active phytochemicals also known as the secondary metabolites are profoundly found in the medicinal plants; which are a rich and diverse sources of such. Promising antimicrobial activity can be induced by some of these bioactive phytochemicals [8]. According to the published literature, the susceptibility of micro-to antimicrobial agents can be measured *in vitro* by a number of techniques but there is no one method followed by majority of the investigators and there is no definite study to determine which is the best method [9] but most widely used techniques are: Disc diffusion (also known as zone of inhibition method), broth dilution/microdilution and agar dilution method. Of these three, the disc diffusion method is most widely used for testing antibacterial and antifungal activity using different concentration of the agents absorbed on sterile filter paper discs and for the preliminary evaluation of antimicrobial activity this method is widely acceptable [10]. To identify the antibacterial and antifungal activities of the plant extracts [11], pure bioactive compounds isolated from plants [12] and also to find out antimicrobial resistant strains [13, 14] various investigators have used this method. For expressing the results of antimicrobial screening there is no standardized method [15]. Either the minimum weight of extract that inhibits the growth of a micro-organism or the diameter of the zone of inhibition is used by some investigators. Disc diffusion is essentially a qualitative or semi quantitative test which indicates the

sensitivity or resistance of micro-organisms to the test material. However no distinction between bacteriostatic and bactericidal activity can be made by this method [16]. *It is also important to take into account that the activity exhibited by the disc diffusion method in vitro does not always translate to the in vivo activity* [9].

Due to observed adverse effects related to their use in traditional medicine practice it has become obligatory to investigate plant's secondary metabolites [17]. If a substance on exposure to any organism inhibits important metabolic processes or causes disorders in living organisms or anomalies in the organ system resulting in contortion of behavior or death of the organism it is said to be cytotoxic [17, 18].

The most simple and uncomplicated method of screening crude plant extracts for cytotoxicity is the brine shrimp lethality bioassay [19, 20]. This assay is an indicant of presumptive antitumor, fungicidal and insecticidal activity [21, 22, 23]. The mechanism of action that is responsible for the toxicity is not known, but the outcomes are usually correlated with more specific bioactivity assays. For the isolation of bioactive components, detection of fungal toxins and testing of water quality the brine shrimp bioassay has always been used as a guideline24,25,26]. The $LC_{50}$ concentrations in µg/ml are calculated in this assay which is considered toxic when below 1000 µg/ml but when the value is above 1000 µg/ml it is considered to be safe [19, 20].

As part of our ongoing research on the medicinal plants of Bangladesh, our present study was done on the roots of a deciduous shrub (2-3 m tall) *Coffea benghalensis* B. Heyne ex Schult. (Family: Rubiaceae). Locally this plant is known as bonnyo kofee, which is native to Bangladesh (Sylhet and Chittagong), India (Arunachal Pradesh, Assam, Bengal, Meghalaya, Orissa, Rajasthan and Sikkim), East Himalaya, Bhutan, Myanmar, Nepal, Thailand and Vietnam [27]. Our previously reported studies on this plant [28, 29] and as per other reports, near about nine polyphenolic compounds of which four are flavonols (quercetin, isoquercitrin, rutin and kaempferol) and five are phenolic acids (caffeic acid, chlorogenic acid, ferulic acid, *p*-coumaric acid and sinapic acid) were isolated from the leaf, pericarp and seed of *C. benghalensis* [30]. Again, from the leaves of the plant a cafestol, bengalensol was identified [31]. In Nepal, the flowers are used for excessive bleeding during menstruation [32]. The fruits showed antibacterial activities against *Streptococcus faecalis, S. aureus, Proteus vulgaris, Escherichia coli, Vibrio cholerae* and *Salmonella typhi* [33, 34]. Thus, we evaluated the preliminary *in vitro* antimicrobial and *in vivo* cytotoxic activities of the roots of the plant for the first time and we, herein, report the outcomes of the study.

## MATERIALS AND METHODS

### Collection and identification of plant

In the month of August 2017 the roots of *C. benghalensis* were collected from Modhupur forest, Tangail, Bangladesh and was identified at the Bangladesh National Herbarium, where a voucher specimen has been maintained and authenticated (DACB Accession No. 45789). After proper washing the roots were sun dried for a week. The sun dried roots were cut into small pieces, cleaned, oven dried for two days at considerably low temperature (not more than 30 °C) and then finally crushed to coarse powder and kept in an air tight container.

### Extraction and solvent-solvent partitioning

1 kg of the powdered material was soaked in methanol (4 L) and was accompanied with occasional shaking and stirring for a period of 13 days. Then, firstly fresh cotton plug and finally sterilized Whatman No. 1 filter paper was used to filter the whole mixture. By using rotary evaporator under reduced pressure at 40 °C temperature the volume of the filtrate was then concentrated to obtain the crude methanolic extract (22 gm). The modified Kupchan partitioning method [35] was followed to fractionate an aliquot (10 g) of the crude methanolic extract (CME) into *n*-hexane (HSF), chloroform (CHSF), ethyl acetate (EASF) and aqueous (AQSF) soluble fractions and preserved in the refrigerator at 4 °C until further use. The yields of *n*-hexane (HSF), chloroform (CHSF), ethyl acetate (EASF) and aqueous (AQSF) soluble fractions are summarized in Table 1.

**Table 1:** Kupchan partitioning of 10 gm of the crude methanolic extract (CME) of the roots of *C. benghalensis*

| Soluble fraction | Weight (gm) |
|---|---|
| *n*-Hexane (HSF) | 3.39 |
| Chloroform (CHSF) | 2.29 |
| Ethyl acetate (EASF) | 0.21 |
| Aqueous (AQSF) | 3.11 |

### Antibacterial activity

For the evaluation of antibacterial activity the disc diffusion method [36, 37] was used against five Gram positive bacteria (*Staphylococcus aureus, S. brodie, S. epidermis, Bacillus cereus and B. subtilis* and seven Gram negative bacteria (*Escherichia coli, Klebsilella* sp., *Salmonella typhi, Shigella dysenterae, Proteus mirabilis, P. vulgaris* and *Pseudomonas aeruginosa*). These bacteria were chosen for the study because of their clinical importance and availability at the Department of Pharmacy, University of Rajshahi, Rajshahi, Bangladesh. They were maintained in nutrient agar slants at 4 °C. In definite volume (200 µl) of solvent (methanol) the test samples (4 and 8 mg) were dissolved and carefully impregnated on sterile Whatman paper discs having a diameter of 7 mm to obtain a concentration of 200 and 400 µg/disc, respectively. The residual solvent was then carefully evaporated from the discs. The dried discs containing the test material were very gently pressed on the nutrient agar medium which was homogeneously seeded with the respective test microorganism. Azithromycin, an antibacterial agent (30 µg/disc) and blank disc (infused with solvent) were used as positive and negative control, respectively. For proper diffusion the plates were then kept at low temperature (4 ºC) for 2 hours and then to ensure maximum growth of the organisms, they were incubated for 24 hours at 37 ºC. The diameter of the zone of inhibition (mm) of the microbial growth was then measured and the antimicrobial activity of the test samples were thus determined.

### Brine shrimp lethality bioassay

As mentioned earlier, one of the most simple and convenient *in vivo* method for the evaluation of cytotoxic activity is the brine shrimp lethality bioassay [22, 18]. According to this method *Artemia salina*

Leach was introduced to the DMSO solution of the plant extracts and kept for 24 hours and then allowed to hatch for 48 hours in simulated seawater at 25 ℃ to mature as nauplii (Larvae). For the experiment, serial dilution technique was used and solutions of varying concentrations such as 200, 100, 50, 25, 12.5, 6.25, 3.125 and 1.563 µg/ml was obtained by separately dissolving 4 mg of each of the plant extracts in DMSO. Then each of this test solution was added to the premarked test tubes containing 15 live shrimp nauplii in 5 ml of simulated brine water. Then each vial was checked for the survivor nauplii after 24 hours with the help of a magnifying glass. The vials were observed for 30 seconds and the absence of the controlled forward motility determined the mortality endpoint of the bioassay [38]. The percent of mortality of the brine shrimp nauplii was calculated from the obtained data by plotting the percentage of the shrimp killed (% mortality) against the logarithm of the sample concentration. The % mortality versus log concentration data were then analyzed statistically by using Microsoft Excel and the $LC_{50}$ and $LC_{90}$ values of the plant extract were determined. Vincristine sulphate was used as positive control and as negative control 5 ml simulated seawater was used.

## RESULTS

In the antibacterial screening, the crude methanolic extract (CME) along with its four soluble fractions (HSF, CSF, EASF and AQSF) demonstrated no sensitivity to microbial growth as shown in Table 2 against any of the five Gram positive and seven Gram negative bacteria in both the concentrations (200 and 400 µg/disc).

**Table 2:** Zone of inhibition in mm of the crude methanolic extract and it's various soluble fractions of the roots of *C. benghalensis*

| **Gram positive** | **Zone of inhibition (mm)** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **CME** | | **HSF** | | **CSF** | | **EASF** | | **AQSF** | | **Azithro-mycin** |
| | **200 (µg/disc)** | **400 (µg/disc)** | **200 (µg/disc)** | **400 (µg/disc)** | **200 (µg/disc)** | **400 (µg/disc)** | **200 (µg/disc)** | **400 (µg/disc)** | **200 (µg/disc)** | **400 (µg/disc)** | **30 µg/disc** |
| *Staphylococcus aureus* | NZI | NZI | NZI | NZI | NZI | NZI | NZI | NZI | NZI | NZI | 24 |
| *S. brodie* | NZI | NZI | NZI | NZI | NZI | NZI | NZI | NZI | NZI | NZI | 35 |
| *S. epidermis* | NZI | NZI | NZI | NZI | NZI | NZI | NZI | NZI | NZI | NZI | 36 |
| *Bacillus cereus* | NZI | NZI | NZI | NZI | NZI | NZI | NZI | NZI | NZI | NZI | 37 |
| *B. subtilis* | NZI | NZI | NZI | NZI | NZI | NZI | NZI | NZI | NZI | NZI | 38 |
| **Gram negative** | | | | | | | | | | | |
| *Escherichia coli* | NZI | NZI | NZI | NZI | NZI | NZI | NZI | NZI | NZI | NZI | 29 |
| *Klebsiella sp.* | NZI | NZI | NZI | NZI | NZI | NZI | NZI | NZI | NZI | NZI | 21 |
| *Salmonella typhi* | NZI | NZI | NZI | NZI | NZI | NZI | NZI | NZI | NZI | NZI | 22 |
| *Shigella dysenterae* | NZI | NZI | NZI | NZI | NZI | NZI | NZI | NZI | NZI | NZI | 27 |
| *Proteus mirabilis* | NZI | NZI | NZI | NZI | NZI | NZI | NZI | NZI | NZI | NZI | 22 |
| *P. vulgaris* | NZI | NZI | NZI | NZI | NZI | NZI | NZI | NZI | NZI | NZI | 23 |
| *Pseudomonas aeruginosa* | NZI | NZI | NZI | NZI | NZI | NZI | NZI | NZI | NZI | NZI | 22 |

NZI = No zone of inhibition

In case of brine shrimp lethality bioassay, the lethality of the crude methanolic extract (CME) along with it's *n*-hexane (HSF), chloroform (CSF), ethyl acetate (EASF) and aqueous soluble fractions (AQSF) to brine shrimp was ascertained on *A. salina*. Table 3, Figure 1 displays the results obtained after a day of exposure to the positive control vincristine sulphate (VS) and the test samples. The $LC_{50}$ obtained from the best-fit line slope were measured to be 9.27, 6.84, 12.33, 9.77, 11.94 and 0.45 µg/ml and $LC_{90}$ were calculated to be 84.57, 59.86, 106.53, 74.45, 78.13 and 10.00 for CME, HSF CSF, EASF, AQSF and vincristine sulfate, respectively. The preliminary cytotoxic effect exhibited by the crude methanolic extract along with it's four soluble fractions were significant when compared to vincristine sulphate (positive control). The *n*-hexane soluble fraction of the crude methanolic extract demonstrated strong cytotoxic activity, while the other three partitionates along with the crude methanolic extract showed moderate to high cytotoxic activity.

**Table 3:** $LC_{50}$ and $LC_{90}$ value of *C. benghalensis* root extractives and vincristine sulphate (Positive control)

| **Samples** | **$LC_{50}$ (µg/ml)** | **$LC_{90}$ (µg/ml)** |
|---|---|---|
| VS | 0.45 ± 0.04 | 10.00 ± 0.02 |
| CME | 9.27 ± 0.13 | 84.57 ± 0.72 |
| HSF | 6.84 ± 0.87 | 59.86 ± 0.83 |
| CSF | 12.33 ± 0.20 | 106.53 ± 0.57 |
| EASF | 9.77 ± 0.22 | 74.45 ± 0.01 |
| AQSF | 11.94 ± 1.01 | 78.13 ± 0.15 |

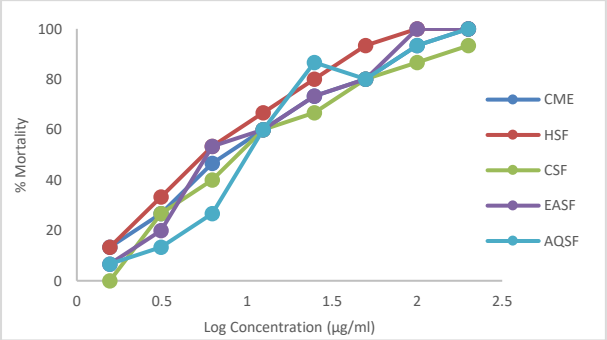


**Figure 1:** Effect of crude methanolic extract along with it's four soluble fractions of the roots of *C. benghalensis* on brine shrimp nauplii

### Statistical analysis

The experiments were conducted in triplicates and results were taken as means ± SD. Microsoft Excel 2010 was used to statistically analyze the % mortality versus log concentration data.

## DISCUSSION

The crude methanolic extract along with it's four soluble fractions did not demonstrate any zone of inhibition against any of the five Gram positive (*Staphylococcus* and *Bacillus* species) and seven Gram negative bacteria like *Escherichia coli*, *Salmonella typhi*, *Shigella dysenterae*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *P. vulgaris* and *Pseudomonas aeruginosa*, even at the concentration of 400 µg/disc. These results were not accordant with the previous reports describing antibacterial activities of *C. benghalensis* fruit extract [33, 34]. It is known that a lot of factors effect antibacterial activity. The plant part that is used, the extraction solvent and the tested organism used plays a key role in bacterial inhibition [39]. The usage of cold extraction method and use of crude extract may be another possibility for the limited antibacterial potency of the plant [40]. So, this necessarily does not determine that the test samples are resistant to all the bacteria used in the antibacterial assay but may also be due to the interruption of general cellular functions or disruption of bacterial membrane potential [41, 42].

The National Cancer Institute (NCI, USA) exhibited that there is a very significant correlation between the brine shrimp lethality bioassay and *in vitro* growth inhibition of human solid tumor cell lines. It is significant since it illustrates the value of this bioassay as a pre-screening tool for antitumor drug research [43]. Several phenomenons like pesticidal effects, teratogenic effects, toxicity to environment, antifungal effects and many more are indicated by the brine shrimp lethality bioassay [44]. The concentration of the extractives ranging from the lowest concentration (1.563 µg/ml) to the highest concentration (200 µg/ml) was found to be directly proportional to the degree of lethality shown by the extractives. Thus the mortality percentage of brine shrimp nauplii produced by the *C. benghalensis* is concentration dependent which indicates the presence of cytotoxic principles in these extractives. Again, former studies have revealed a plant extract or chemical with an $LC_{50}$ of less than 30 µg/ml is very cytotoxic, those with $LC_{50}$ values of 30-100 µg/ml are toxic, and those having $LC_{50}$ values of more than 100 µg/ml have a low toxicity profile hence safe [45]. Taking these criteria under consideration, the crude methanolic extract along with it's four soluble fractions of the roots of *C. benghalensis* proved to be very cytotoxic to brine shrimp nauplii.

## CONCLUSION

Better antibacterial activity might be exhibited, if soxhlet extraction is used instead of cold percolation method, or may be if a sub-fraction of the extracts or a semi-pure compound, or a pure compound isolated from this plant is used for the antimicrobial assay. Additionally, to evaluate the plant as a potential antimicrobial agent other parts of the plant needs to be studied also. Further investigations and other are methods are also necessary to evaluate the antibacterial activity. The results of the brine shrimp lethality bioassay indicate the existence of cytotoxic bioactive compounds in the roots of the plant but more advanced methods are required to elucidate the cytotoxic bioactive compounds.

### Acknowledgement

The authors wish to thank Bangladesh National Herbarium, Ministry of Environment and Forest, Dhaka, Bangladesh for identifying the plant material. The authors are also pleased to acknowledge Department of Pharmacy, Faculty of Science, University of Rajshahi, Rajshahi, Bangladesh for providing laboratory facilities to carry out the experiments.

### Conflicts of interest

The authors declare no conflict of interest.

### Financial Support

None declared.

## REFERENCES

1. Ogidi OI, Oguma LMO, Adigwe PC, Anthony BB. Phytochemical properties and *in vitro* antimicrobial potency of wild edible mushrooms (*Pleurotus ostreatus*) obtained from Yenagoa, Nigeria. The Journal of Phytopharmacology, 2021; 10(3):180-184.
2. Andersson DI and Hughes D. Persistence of antibiotic resistance in bacterial populations. FEMS Microbiology Reviews, 2011; 35(5):901-911.
3. Srivastava J, Chandra H, Nautiyal AR, and Kalra SJS. Antimicrobial resistance (AMR) and plant-derived antimicrobials ($PDA_m$s) as an alternative drug line to control infections. 3 Biotech, 2014; 4(5):451-460.
4. Awouafack MD, McGaw LJ, Gottfried S, Mbouangouere R, Tane P, Spiteller M and Eloff JN. Antimicrobial activity and cytotoxicity of the ethanol extract, fractions and eight compounds isolated from *Eriosema robustum* (Fabaceae). BMC Complementary and Alternative Medicine, 2013; 13:289.
5. Lasemi E, Navi F, Lasemi R, and Lasemi N. Complications of antibiotic therapy and introduction of nanoantibiotics. A Textbook Advanced and Oral Maxillofacial Surgery, 2016; 3:27-52.
6. Trease GE and Evans MC. Pharmacognosy. 14th ed. Elsevier: New Delhi, India, 2005.
7. Abeysinghe PD. Antibacterial activity of some medicinal mangroves against antibiotic resistant pathogenic bacteria. Indian Journal of Pharmaceutical Sciences, 2010; 72(2):167-172.
8. Khanam Z, Wen CS and Bhat, IUH. Phytochemical screening and antimicrobial activity of root and stem extracts of wild *Eurycoma longifolia* Jack (Tongkat Ali). Journal of King Saud University - Science, 2015; 27(1):23-30.
9. Wilkinson JM. Methods for testing the antimicrobial activity of extracts. In: Ahmad I, Aqil F, Owais M, editors. Modern phytomedicine: Turning medicinal plants into drugs. Germany: Wiley-VCH; 2007; 157-69.
10. Bauer AW, Kirby WM, Sherris JC and Turck M. Antibiotic susceptibility testing by a standardized single disc method. American Journal of Clinical Pathology, 1966; 45(4):493-496.

11. Belboukhari N and Cheriti A. Antibacterial and antifungal activities of crude extracts from *Launeae arborescens*. Pakistan Journal of Biological Sciences, 2006; 9(1):1-2.
12. Khan A, Rahman M and Islam MS. Antibacterial, antifungal and cytotoxic activities of amblyone isolated from *Amorphophallus campanulatus*. Indian Journal of Pharmacology, 2008; 40(1):41-44.
13. Hallander HO and Laurell G. Identification of cephalosporin - resistant *Staphylococcus aureus* with the disc diffusion method. Antimicrobial Agents and Chemotherapy, 1972; 1:422-426.
14. Vedel G. Simple method to determine β-lactam resistance phenotypes in *Pseudomonas aeruginosa* using the disc agar diffusion test. Journal of Antimicrobial Chemotherapy, 2005; 56(4):657-664.
15. Ayafor JF, Sondengam BL and Ngadjui BT. Veprisinium Salt, A Novel Antibacterial Quaternary Alkaloid from *Vepris louisii*. Planta medica, 1982; 44(3):139-142.
16. Roland R. Antibiotics - An Introduction. F.Hoffman La roche and Co.: Basle, Switzerland, 1982; 70-71.
17. Fatope MO. Phytocompounds. Their Bioassay and Diversity. Discovery and Innovations, 1995; 7(3):229-236.
18. Ameen OM, Usman LA, Oladosu IA, Olawore NO and Ogunwande IA. Bioactivity of rhizome essential oils from two varieties of *Cyperus articulatus* L. grown in Nigeria, using brine shrimp (*Artemia salina*) lethality tests. Journal of Medical Plant Research, 2011; 5(6):1031-1033.
19. Meyer BN, Ferrigini RN, Putnam JE, Jacobsen LB, Nichols DE and McLaughlin JL. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Medica, 1982; 45:31-35.
20. McLaughlin JL, Chang CJ and Smith DL. "Bench-top" bioassays for the discovery of bioactive natural products: an update. In: Rahman A (ed) Studies in natural product chemistry, Elsevier, Amsterdam, 1991; 9:383-409.
21. Michael AS, Thompson CG and Abramovitz M. *Artemia salina* as a test organism for bioassay. Science, 1956; 123:464-464.
22. Harwig J and Scott PM. Brine shrimp (*Artemia salina* L.) larvae as a screening system for fungal toxins. Applied Microbiology, 1971; 21(6):1011-1016.
23. McLaughlin JL, Rogers LL and Anderson JE. The use of biological assays to evaluate botanicals. Drug Information Journal, 1998; 32:513-524.
24. Nguta JM, Mbaria JM, Gakuya DW, Gathumbi PK, Kabasa JD and Kiama SG. Biological screening of Kenyan medicial plants using *A. salina* L. (Artemiidae). Pharmacologyonline, 2011; 2:458-478.
25. Arcanjo DDR, Albuquerque ACM, Melo-Neto B, Santana LCLR, Medeiros MGF, Citó AMGL. Bioactivity evaluation against *Artemia salina* L. each of medicinal plants used in Brazilian northeastern folk medicine. Brazilian Journal of Biology, 2012; 72:505-509.
26. Gadir SA. Assessment of bioactivity of some Sudanese medicinal plants using brine shrimp (*Artemia salina*) lethality assay. Journal of Chemical and Pharmaceutical Research, 2012; 4:4148-5145.
27. 27Sivaranjan VV, Biju SD and Mathew P. Revision of the genus *Psilanthus* Hook.f. (Rubiaceae tribe Coffeeae) in India. Botanical Bulletin of Academia Sinica, 1992; 33:214-216.
28. Rima RR, Sagor SI and Anjum A. *In vitro* antioxidant activities of the roots of *Coffea benghalensis* B Heyne ex Schult. Growing in Bangladesh. Journal of Pharmacognosy and Phytochemistry, 2021; 10(2): 40-45.
29. Sagor SI, Sajol GM, Alam AHMK and Anjum A. *In vitro* evaluation of antioxidant potentiality and estimation of total phenolic and flavonoid content of the whole stem of *Coffea benghalensis* B. Heyne ex Schult. The Pharma Innovation Journal, 2021; 10(3): 01-08.
30. Patay EB, Bencsik T and Papp N. Phytochemical overview and medicinal importance of *Coffea* species from the past until now. Asian Pacific Journal of Tropical Medicine, 2016; 9(12):1127-1135.
31. Patay EB, Nemeth T, Nemeth TS, Filep R, Vlase L, Papp N. Histological and phytochemical studies of *Coffea benghalensis* B. Heyne Ex Schult. compared with *Coffea arabica* L. Farmacia, 2016; 64(1):125-130.
32. Hasan CM, Huda Q, Lavaud C, Connolly JD and Haque ME. Bengalensol, a new 16-epicafestol derivative from the leaves of *Coffea benghalensis*. Natural Product Letters, 1994; 5(1):55-60.
33. Ghimire K and Bastakoti RR. Ethnomedicinal knowledge and healthcare practices among the Tharus of Nawalparasi district in central Nepal. Forest Ecology and Management, 2009; 257:2066-2072.
34. Kiran B, Baruah R, Ojha R, Lalitha V and Raveesha KA. Antibacterial and Antioxidant Activity of *Coffea benghalensis* Roxb.Ex Schult. fruit against human bacteria. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2011; 2(3):856-865.
35. VanWagenen AC, Larsen R, Cardellina JH, Ran dazzo D, Lidert ZC and Swithenbank C. Ulosantoin, a potent insecticide from the sponge *Ulosa ruetzleri*.Journal of Organic Chemistry, 1993; 58:335-337.
36. Bauer AW, Kirby WM, Sherris JC and Turck M. Antibiotic susceptibility testing by a standardized single disc method. American Journal of Clinical Pathology, 1966; 45:493-496.
37. Rahman MS and Rashid MA. Antimicrobial activity and cytotoxicity of *Eclipta prostrata.* Oriental Pharmacy and Experimental Medicine, 2008; 8(1):47-52.
38. Middletona P, Stewarta F, Al-Qahtania S, Egana P, O'Rourkea C, Abdulrahmana A, Byresa M, Middletona M, Kumarasamy Y, Shoeba M, Nahar L, Delazar A and Sarker SD. Antioxidant, antibacterial activities and general toxicity of *Alnus glutinosa*, *Fraxinus excelsior* and *Papaver rhoeas*. Iranian Journal of Pharmaceutical Research, 2005; 2:81-86.
39. Dogruoz N, Zeybek Z and Karagoz A. Antibacterial Activity of Some Plant Extracts. IUFS Journal of Biology, 2008; 67(1):17-21.
40. Manandhar S, Luitel S and Dahal RK. *In vitro* Antimicrobial activity of some medicinal plants against human pathogenic bacteria. Hindawi Journal of Tropical Medicine, 2019; 1895340:1-5.
41. Patra JK and Baek KH. Antibacterial activity and action mechanism of the essential oil from *Enteromorpha linza* L. against foodborne pathogenic bacteria. Molecules, 2016; 21(3):388-398.
42. Khan R, Islam B, Akram M, Shakil S, Ahmad AA, Ali SM, Siddiqui M and Khan AU. Antimicrobial activity of five herbal extracts against multi drug resistant (MDR) strains of bacteria and fungus of clinical origin. Molecules, 2009; 14(2): 586-597.
43. Anderson JE, Goetz CM, McLaughlin JL and Suffness M. A blind comparison of simple bench-top bioassays and human tumour cell cytotoxicities as antitumor prescreens. Phytochemical Analysis, 1991; 2:107-111.
44. Vanhaecke P, Persoone G, Claus C and P. Sorgeloos. Proposal for a short-term toxicity test with *Artemia* nauplii. Ecotoxicology and Environmental Safety, 1981; 5(3): 382-387.
45. Moshi MJ, Innocent E, Magadula JJ, Otieno DF, Weisheit A, Mbabazi PK and Nondo RSO. Brine shrimp toxicity of some plants used as traditional medicines in Kagera Region, northwestern Tanzania. Tanzania Journal of Health Research, 2010; 12:63-67.



**HOW TO CITE THIS ARTICLE**

Anjum A, Rima RA, Sagor SI. Evaluation of *in vitro* antibacterial and *in vivo* cytotoxic activities of Bangladeshi *Coffea benghalensis* B Heyne ex Schult. roots. J Phytopharmacol 2021; 10(5):345-349. doi: 10.31254/phyto.2021.10511