AUTHOR	Khairuzzaman M., Hasan Md Mehedi, Ali Mohammad Tuhin, Mamun Abdullah Al, Akter Sheuly, Nasrin Papia, Islam Md Khirul, Nahar Akhlak Un, Sarker Dipto Kumer, Hamdi Omer Abdalla Ahmed, Uddin Shaikh Jamal, Seidel Veronique, Shilpi Jamil A.
TITLE	Anthelmintic screening of Bangladeshi medicinal plants and related phytochemicals using in vitro and in silico methods : An ethnobotanical perspective
YEAR	2024
DOI	https://doi.org/10.1016/j.jep.2024.118132
VERSION	Final draft
CITATION	M. Khairuzzaman, Md Mehedi Hasan, Mohammad Tuhin Ali, Abdullah Al Mamun, Sheuly Akter, Papia Nasrin, Md Khirul Islam, Akhlak Un Nahar, Dipto Kumer Sarker, Omer Abdalla Ahmed Hamdi, Shaikh Jamal Uddin, Veronique Seidel, Jamil A. Shilpi, Anthelmintic screening of Bangladeshi medicinal plants and related phytochemicals using in vitro and in silico methods: An ethnobotanical perspective, Journal of Ethnopharmacology,Volume 328,2024,118132,ISSN 0378-8741, https://doi.org/10.1016/j.jep.2024.118132
LICENSE	CC-BY-NC-ND

UNIVERSITY
OF TURKU

In vitro and in silico anthelmintic activity screening of Bangladeshi medicinal plants and their phytochemicals: an ethnobotanical approach

M Khairuzzaman[1], Md Mehedi Hasan[1], Mohammad Tuhin Ali[2], Abdullah Al Mamun[1], Papia Nasrin[1], Sheuly Akter[1], Md Khirul Islam[3], Omer Abdalla Ahmed Hamdi[4], Shaikh Jamal Uddin[1], Jamil A Shilpi[1*]

[1]Pharmacy Discipline, Life Science School, Khulna University, Khulna, Bangladesh

2

[3]Department of Life Technologies, Division of Biotechnology, University of Turku, Finland.

[4]Department of Chemistry, Faculty of Science and Technology, Al-Neelain University, Khartoum, 11121, Sudan

ORCID author IDs:

Md Khirul Islam: 0000-0001-8963-1965
Shaikh Jamal Uddin: 0000-0003-3163-2118
Jamil A Shilpi: 0000-0001-8938-9240

***Corresponding author**

Jamil A Shilpi
E-mail: jamilshilpi@yahoo.com
Phone: +8801910010054

Abstract

Ethnopharmacological relevance: Helminthiasis poses a serious health and economic problem mainly in tropical and subtropical regions of the world. Use of medicinal plants for the control and management of helminthiasis is commonly practiced among the native people in Bangladesh. Identification of such plants through ethnobotanical survey, compare the bioactivity of the plants by in vitro method, analyzing the secondary metabolite content of such plants by in silico method can contribute in finding novel molecular scaffolds to combat helminthiasis.

Aim of the study: This is an ethnobotanical survey guided in vitro anthelmintic study of Bangladeshi medicinal plants to find and compare plants used in helminthiasis. This was followed by in silico screening of secondary metabolite content of the most active plant to find chemical scaffolds that might have contributed in the observed activity.

Methods: Ethnomedicinal survey was conducted in three upazilas of Bangladesh, namely Mathbaria, Phultala and Khan Jahan Ali to collect data on plants used to treat helminthiasis. Plants with highest use values were subjected to adult motility test using adult worms of *Haemonchus contortus*. A library was created with reported natural products from most active plants for virtual screening for colchicine binding site of β-tubulin.

Results: A total of 32 plants were reported by the respondents for their use in helminthiasis. On the basis of their use values, eight plants, namely *Ananas comosus, Azadirachta indica, Carica papaya, Citrus grandis, Curcuma longa, Momordica charantia, Nigella sativa* and *Syzygium cumini* were found to be the most popular choices. In vitro anthelmintic test revealed both the *A. indica* leaves and bark as the most active with LC_{50} value of 60 mg/ml. Other plant extracts also showed good anthelmintic activity with LC_{50} values in the range of 66-87 mg/ml while that of albendazole was 9.33 mg/ml. Both nimbolide and 28-deoxonimbolide, satisfying the parameters of drug-likeness showed binding affinity of -8.9 kcal/mol while that of control molecule *N*-deacetyl-*N*-(2-mercaptoacetyl)colchicine was -6.9 kcal/mol.

Conclusion: A strong correlation was observed between the popularity of the plants with their in vitro and in silico results. Present investigation dictates ethnobotanical survey based new drug discovery to treat helminthiasis.

Keywords: *Azadirachta indica*; *Ananas comosus*; *Carica papaya*; *Haemonchus contortus*; 1SA0; Nimbolide; 28-Deoxonimbolide

1. Introduction

Helminthiasis is a serious problem in tropical and subtropical regions of the world resulting in increased mortality rate, blasting of weight caused by reduced appetite, retarded growth and impaired digestive efficiency both in human and livestock (Mondal et al., 2015). They pose serious threat to public health and contribute to the prevalence of anemia, malnutrition, eosinophilia and pneumonia in developing countries. School-going children are often affected with heavy contamination of helminths. Although these worms prevalently cause infections in tropical countries, travelers who have visited those areas and those who are living in temperate climates have the risk of being infected. The resulting diseases cause severe morbidity including lymphatic filariasis, onchocerciasis, and schistosomiasis. These can affect most populations in endemic areas with major economic and social consequences. Millions of livestock are also affected resulting in serious economic losses in domestic and farm animals (Ogedengbe-Olowofoyeku et al., 2021).

However, drug resistance to currently available drugs is making treatment of helminthiasis more challenging both in humans and livestock (Demeler et al., 2013). Many anthelmintic drugs are also not suitable for use in humans due to their serious side effects leaving with very few therapeutic options. One of the treatment options proposed is the use of synergistic agents that could reduce the therapeutic dose of anthelmintic drug (Burns et al., 2017). Use of medicinal plants for the treatment of helminthiasis is a common practice among native people in different parts of the world (Zarza-Albarrán et al., 2020). Medicinal plants with a good history of their use in helminthiasis can be handy in finding novel anthelmintic agents in addition to their use in tandem with currently available anthelmintics either to reduce their therapeutic dose or to reverse drug resistance.

Being a subtropical country, infection by helminthiasis is widespread in rural areas of Bangladesh which is further deteriorated by the habitation of cattle in close proximity of human abode and use of compost fertilizer (Penakalapati et al., 2017). Because of poverty and lack of access to modern medical facilities, a huge number of people frequently depend on traditional knowledge of folk medicinal remedies for treatment of various ailments including helminthic disorders. Identification of plant secondary metabolites with anthelmintic activity in recent years suggests that plants represent a potential source to search for natural products with anthelmintic activity (Zarza-Albarrán et al., 2020).

The objective of the present study was to conduct an ethnobotanical survey among the different classes of people of three historically rich upazilas of Bangladesh to collect ethnobotanical knowledge of native people on the medicinal use of plants or plant parts that exists in the local communities to treat helminthiasis in human. The three upazilas, namely Mathbaria (Pirojpur district), Phultala (Khulna district) and Khan Jahan Ali (Khulna district) were chosen for the survey. Plants were ranked according to their use values and plants with highest use values were further subjected to in vitro anthelmintic activity study by motility test using adult *Haemonchus contortus*. Plant with the best in vitro result was chosen for in silico study to find whether any of the secondary metabolites can interact with colchicine binding site of β-tubulin.

2. Materials and Methods

2.1. Ethnobotanical survey

2.1.1. Study area

Mathbaria upazila (area: 353.25 km^2, coordinate: 89°52' to 90°03' E, 22°09' to 22°24' N) of Pirojpur district, Phultala upazila (area: 87.41 km^2, coordinate: 89°23' to 89°29' E, 22°54' to 23°01' N) and Khan Jahan Ali upazila (area: 33.07 km^2, coordinate: 89°29' to 89°31' E, 22°53' to 22°55' N) of Khulna district were selected to collect the information of medicinal plants used for the treatment of helminthiasis.

2.1.2. Sampling of informants

The survey period was conducted following the standard protocols of the ethnobotanical data collection (Alexiades and Sheldon, 1996). Information was collected from all sorts of people having practical knowledge on the use of different medicinal plants. A total of 150 people were interviewed during the fieldwork whom include local traditional health practitioners (Kabiraj/ Ayurved/Hakim/Unani/independent healer), farmers, housewives, service holders and others.

2.1.3. Ethnobotanical data collection

Informed consent was obtained from the informants explaining that the only purpose of the survey was documentation of their medicinal plant usage. All interviews were conducted in Bangla language spoken by both the informants and the interviewers. A semi-structured questionnaire and guided field-walk method was used to conduct the interviews as described by Martin (1995) and Maundu (1995). Respondents from various categories including traditional healers, farmers, housewives were chosen to encompass all types of knowledgeable users. The questionnaire form was designed to gather the following information of plants: local name, habitat, source, used plant part(s), method of preparation, other use(s) and the availability of the plant in study area (Islam et al., 2014).

2.1.4. Data analysis

Botanical names of the plants were arranged alphabetically in tabulated form for each of the upazilas with information on plant part used, mode of use, other uses and habitat. The plants were further analyzed and made a comparison on the basis of the use values for individual plants to give a quantitative measure of its relative significance to the informants objectively (Phillips et al., 1994). It was calculated by following equation where use value (UV_s) of a plant is equal to the sum of use reports of that plant cited by informants divided by total number of informants:

$$UV_s = \Sigma_i UV_{is}/ns$$

where 'UV_s' is the use value of a plant, 'UV_{is}' is the total number of uses of that plant reported by the informants and 'ns' is the total number of informants. Thus, a high use for any given plant will refer that the plant is widely used for the treatment of helminthiasis (Islam et al., 2014).

2.2. In vitro anthelmintic activity investigation

2.2.1. Collection and identification of the listed plant

Plant/plant parts were collected for their identification while selected plants for in vitro analysis were collected in bulk amount. The plants were identified by Dr Md Azharul Islam, Professor, Forestry and Wood Technology Discipline, Khulna University.

2.2.2. Extraction

Selected plant or plant parts were washed with water, dried in the shed with continuous air flow and ground into a coarse powder. Around 150 g of the dried powdered plant material was macerated in 600 ml of ethanol for three days with occasional shaking and stirring. The filtrate obtained was evaporated using rotary vacuum evaporator at 45ºC under reduced pressure to get the crude extract. Percent yield of the extracts is given in supplementary file (**Table S1**). The extracts were stored in freezer (-20ºC) until experiments commenced.

2.2.3. Experimental worms

Cattle nematode *H. contortus* was collected from Gollamari Slaughter House, Khulna. Adult worms with optically same length and width were separated, properly cleaned and kept in 0.9% phosphate-buffered saline (PBS) of pH 7.4 at the temperature of 37±1ºC until the commencement of the experiment.

2.2.4. Adult motility test

Eight medicinal plants with highest use values were selected for in vitro anthelmintic activity study by adult motility test. As reported by the respondents, both the leaves and bark of *A. indica* were included in the study. Extracts or standard drug prepared in 0.9% saline solution with the assistance of 0.5% tween 80 were such added 24-well plate to attain extract concentrations of 20, 40, 60, 80, 100 and 120 mg/ml. Concentrations for albendazole, used as the standard in this study consisted of 5, 10, 15, 20, 25 and 30 mg/ml. Ten helminths were transferred in each well with incubation for 3 h at ambient temperature (26-30°C), and the whole experiment was done in duplicate. At the end of the experiment, helminths were removed and washed with distilled water to remove residual extracts and then placed in a Petri dish containing Lukewarm saline solution for 10 min. The number of dead parasites were noted. Parasites those failed to show any sort of motility or did not respond to pricking were considered as dead (Aderibigbe et al., 2021; Ogedengbe-Olowofoyeku et al., 2021).

2.3. Molecular docking

2.3.1. Protein preparation

Crystal structures of the protein β-tublin-colchicine (PDB ID: 1SA0) complex was downloaded in Protein Data Bank (PDB) format from protein data bank website (https://www.rcsb.org/). The structure of the ligand inhibitor *N*-deacetyl-*N*-(2-mercaptoacetyl)colchicine (DMC) was retrieved from its corresponding PDB structure. All the heteroatom and water molecules were removed from the protein using BIOVIA Discovery Studio Visualizer (v.4.5). The protein was further prepared using AutoDock Tools (v.1.5.7) which include addition of Kollman charges, polar hydrogens and missing atoms. The binding site residues for tubulin-colchicine site found was in β-chain and they are Cys241, Leu248, Ala250, Leu255, Asn258, Met259, Ala316, Ala317, Val318, Val238, Lys352 Thr353, Thr376 (Naik et al., 2011).

2.3.2. Ligand preparation

The structure of 415 natural products reported from *A. indica* were retrieved from PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Structural geometry of the compounds were optimized by Chem3D v.15.1 for energy minimization (Allinger, 1977). To perform rigid docking and minimize standard errors, all rotatable bonds of the compounds were considered as non-rotatable bonds (Trott and Olson, 2010). Partial charges were added to the ligands using Gasteiger charge calculation method (Gasteiger and Marsili, 1980). The optimized ligand structures were converted to PDB format using Open Babel v.2.4.1 (O'Boyle et al., 2011).

2.3.3. Grid box preparation and docking

Docking of the ligands at the colchicine binding site of β chain was carried out by PyRx v.0.8 (Trott and Olson, 2010). Docking poses with the lowest value of root-mean-square deviation (RMSD) (threshold < 1.00 Å) were considered as the most stable pose. Ligand-protein interactions were visualized by BIOVIA Discovery Studio Visualizer v.4.5.

3. Results

3.1. Results of ethnobotanical survey

Plant names, plant part used, mode of use and other uses were recorded and presented in alphabetic order for each of the upazials, namely Mothbaria (**Table 1**), Fultala (**Table 2**) and Khan Zahan Ali (**Table 3**). Information regarding the participants such as gender, age, class, experience, occupation and educational background were also recorded (**Table S2**). Calculation of use value revealed eight medicinal plants out of 32 to be very popular among the villagers and traditional practitioners, namely *Ananas comosus, Azadirachta indica, Carica papaya, Citrus grandis, Curcuma longa, Momordica charantia, Nigella sativa* and *Syzygium cumini* (**Table 4**). Among these plants, highest use value was found for *C. papaya* seeds (UV=2.01), *A. comosus* peel (UV=1.79) and *A. indica* (UV=1.67).

3.2. Results of in vitro anthelmintic study

All the plant extracts showed strong anthelmintic action against the test parasites in a concentration dependent manner with the death of all parasites at the highest concentration tested. Results of in vitro anthelmintic test is presented in **Fig. S1** and calculated LC_{50} values presented in **Table 5**. Among the plants, both the leaves and bark extract of *A. indica* showed highest in vitro anthelmintic activity with LC_{50} value 60.26 mg/ml. LC_{50} of albendazole was observed at 9.33 mg/ml with the death of all parasites at 25 mg/ml.

3.3. Results of in silico docking of natural products

With 233 compounds showing better binding affinity (≥-7.0 kcal/mol) than that of colchicine (-6.9 kcal/mol) or DMC (-6.9 kcal/mol) clearly dictates that *A. indica* contains secondary metabolites that have good affinity for colchicine binding site of β-tubulin. Best docking scores was obtained for gramisterol, limocinol, nimbolide, 28-deoxonimbolide and 5,7,4'-trihydroxy-3',5'-diprenylflavanone with binding energy of -9.1, -9.0, -8.9, -8.9 and -8.9 kcal/mol, respectively (**Table 6**). Being steroid in nature, both gramisterol and limocinol showed strong hydrophobic interaction at the binding site which probably resulted in good binding affinity. Higher lipophilicity of flavonoid 5,7,4'-trihydroxy-3',5'-diprenylflavanone, which showed one hydrogen bond with Asn350 (2.1 Å) with some hydrophobic interaction through the two isoprene units. Nimbolide and 28-deoxonimbolide, fulfilling all the criteria of drug likeness showed three hydrogen bonds with Cys 241, Ala250 and Leu 255 along with hydrophobic interactions with some other key amino acid residues associated with the binding of colchicine (**Fig. 1**).

4. Discussion

The present work is a systematic approach to identify and compare medicinal plants used in the treatment of helminthiasis through an ethnobotanical survey with further evaluation of the activity by in vitro and in silico methods. Use of medicinal plant for the control and cure of helminthiasis is a common practice in a subtropical country like Bangladesh. Ethnobotanical survey can give an insight of a comparative effectiveness of these plants since users often rely on plants that gives better result. Thus, present study was undertaken to conduct ethnobotanical survey in three historically rich upazilas with relatively old human habitat, namely Mathbaria, Fultala and Khan Jahan Ali. A total of 32 plants were identified that are used in helminthiasis among the local people which include traditional healers, farmers, housewives and others. To further screen most trusted plants among the users, use value was calculated which revealed *C. papaya* seeds (UV=2.01), *A. comosus* peel (UV=1.79), *A. indica* leaves and bark (UV=1.67) as the most highly used plants against helminthiasis. However, *C. papaya* seeds and *A. comosus* peel were found to be used in two different upazilas under investigation while *A. indica* was used in all three upazilas. Although the anthelmintic activity of *A. indica* is widely accepted by the respondents, it is possible that the bitterness of the plant renders it behind *C. papaya* and *A. comosus* in terms of its popularity to use as anthelmintic.

The second phase of the study was to investigate these plants through in vitro studies to find some rationale of their use in helminthiasis. Depending on the high use values, eight plants were chosen for anthelmintic study by adult motility test using cattle nematode *H. contortus*. Ethanol extract of all the selected plants showed significant anthelmintic activity against test parasites and results were comparable to that of albendazole, used as standard drug in this assay. Most reported plants found in the ethnobotanical study also appeared to perform good in the in vitro investigation with the lowest LC_{50} value of 60 mg/ml observed for both the leaves and bark of *A. indica*, Thus, the use value of the plants was reasonably reflected by their in vitro anthelmintic activity. This proves that these plants are relatively more effective in controlling helminths making them popular among people for the control and cure of helminthiasis.

Benzimidazoles, the most commonly used broad spectrum anthelmintic drugs bind with β-tubulin at the colchicine binding site causing conformational change of the protein which prevents further polymerization of α- and β-tubulin subunits. This inhibition of microtubule formation leads to death in rapidly dividing cells, impairment of several physiological processes of parasites including inhibition of fumarate reductase (Page, 2008). It is possible that some of the secondary metabolites present in the tested extract would bind at colchicine binding site of β-tubulin protein to exert observed anthelmintic activity. With a view to identify such compounds, a library created with 415 compounds reported from *A. indica* was used for virtual screening for the colchicine binding site of β-tubulin protein. Although highest binding affinity was observed for two steroidal compounds namely, gramisterol and limocinol, protein-ligand interaction was exclusively hydrophobic in nature which arises from the hydrophobic cyclopentanoperhyrdophenanthrene ring of the steroid nucleus. These two compounds also failed to meet the Lipnski's rule of five due to their high lipophilicity. Nimbolide and 28-deoxonimbolide, two limonoids appeared to be the best docked ligands with binding affinity of -8.9 kcal/mol for both molecules. Both molecules showed similar interactions since the part of the molecules that differ does not participate in the interaction at the binding site. Nimbolide and 28-deoxonimbolide formed hydrogen bonds with three key amino acid residues, namely Cys241, Ala250 and Leu255 along with hydrophobic interactions with three more key amino acid residues. i.e., Ala316, Ala317 and Val318. Colchicine and DMC used as control molecules for in silico study also showed similar pattern of interaction with hydrogen bond with Cys241 and hydrophobic interactions with Leu248, Ala250, Leu 255 and Lys352.

Limonoids are often credited for the biological activities shown by extract of *A. indica*. The plant *A. indica* itself is metabolically very rich with more than 400 secondary metabolites reported so far. Majority of these compounds are limonoids. A previous study with sheep treated with *A. indica* leaves for 18 months reduced the parasitic burden but was not able to eradicate parasites completely (Chagas et al., 2008). Similar results were found when leaves of *A. indica* was given to *H. contortus* infected goats for two months. Same study also found anthelmintic effect of azadirachtin in adult motility test only with higher concentrations (800 mg/ml and above) (Radhakrishnan et al., 2010). Azadirachtin was also found to reduce motility of *Cotylophoron cotylophorum* and a potent inhibitor of acetylcholinesterase enzyme (Veerakumari and Priya, 2006). One study also found the extract of *A. indica* aerial parts (98% inhibition at 3 mg/ml) to be more potent than azadirachtin (68% inhibition at 10 mg/ml) when tested by egg hatch assay of *H. contortus* (Costa et al., 2008). Virtual screening conducted in this study showed a weak binding affinity of azadirachtin (-5.4 kcal/mol) for the colchicine binding site of β-tubulin. Thus, it is possible that other limonoids including nimbolide and 28-deoxonimbolide have good chance as inhibitors of β-tubulin while azadirachtin might work through other pathways with mild inhibitory activity. Nimbolide has been studied extensively for its anticancer property and considered as potential lead molecule for anticancer drug development (Nagini et al., 2021; Spradlin et al., 2019; Wang et al., 2016). However, nimbolide or 28-deoxonimbolide has not be studied for their anthelmintic activity and results of the virtual screening shed light to the promising anthelmintic activity of these molecules.

5. Conclusion

Development of new anthelmintic agent is becoming more important with ever increasing globalization making helminthiasis a problem for all countries irrespective of its geographical location. Present investigation helped us to identify medicinal plants used in traditional medicine for the treatment of helminthiasis. In vitro test results of most popular plants were in agreement with the rank of the plants prepared on the basis of their use values making the following plants as the most promising ones: *A. indica*, *A. comosus*, *C. papaya* and *C. longa*. Virtual screening of the reported secondary metabolites of the most active plant of in vitro test, nimbolide or 28-deoxonimbolide of *A. indica* was found to be the best docked ligands at the colchicine binding site of β-tubulin. Present investigation dictates that enthobotanical survey can be a powerful tool to find plants with anthelmintic activity to serve in the new drug discovery process.

Funding This research was supported by the Ministry of Science and Technology, Bangladesh.

Acknowledgements M Khairuzzaman thanks Khulna University for postgraduate research fund.

Declaration of competing interests The authors declare no competing interest.

List of Abbreviations

UV: use value

PBS: phosphate-buffered saline

PDB: protein data bank

DMC: *N*-deacetyl-*N*-(2-mercaptoacetyl)colchicine

Table 1: Plants used against helminthic disorder in Mathbaria upazila.

Table 2: Plants used against helminthic disorder in Phultala upazila.

Table 3: Plants used against helminthic disorder in Khan Jahan Ali upazila.

Table 4: Comparative analysis of medicinal plants and their families used for treatment of helminthic disorders.

Table 5: Adult motility test of selected plant extracts against *H. contortus.*

Table 6: Ligands with best docking results

Fig. 1: Molecular interaction of nimbolide at the colchicine binding site of β-tubulin.

References

Aderibigbe, S.A., Idowu, S.O., Olaniyi, A.A., Wright, C.W., Fatokun, A.A., 2021. Bioactivity and cytotoxicity profiling of vincosamide and strictosamide, anthelmintic epimers from Sarcocephalus latifolius (Smith) Bruce leaf. Journal of Ethnopharmacology 265, 113142.

Alexiades, M.N., Sheldon, J.W., 1996. Selected guidelines for ethnobotanical research: a field manual.

Allinger, N.L., 1977. Conformational analysis. 130. MM2. A hydrocarbon force field utilizing V1 and V2 torsional terms. Journal of the American Chemical Society 99(25), 8127-8134.

Burns, A.R., Bagg, R., Yeo, M., Luciani, G.M., Schertzberg, M., Fraser, A.G., Roy, P.J., 2017. The novel nematicide wact-86 interacts with aldicarb to kill nematodes. PLoS Negl Trop Dis 11(4), e0005502-e0005502.

Chagas, A.C.S., Vieira, L.S., Freitas, A.R., Araújo, M.R.A., Araújo-Filho, J.A., Araguão, W.R., Navarro, A.M.C., 2008. Anthelmintic efficacy of neem (Azadirachta indica A. Juss) and the homeopathic product Fator Vermes® in Morada Nova sheep. Veterinary Parasitology 151(1), 68-73.

Costa, C.T.C., Bevilaqua, C.M.L., Camurça-Vasconcelos, A.L.F., Maciel, M.V., Morais, S.M., Castro, C.M.S., Braga, R.R., Oliveira, L.M.B., 2008. In vitro ovicidal and larvicidal activity of Azadirachta indica extracts on Haemonchus contortus. Small Ruminant Research 74(1), 284-287.

Demeler, J., Krüger, N., Krücken, J., von der Heyden, V.C., Ramünke, S., Küttler, U., Miltsch, S., López Cepeda, M., Knox, M., Vercruysse, J., Geldhof, P., Harder, A., von Samson-Himmelstjerna, G., 2013. Phylogenetic characterization of β-tubulins and development of pyrosequencing assays for benzimidazole resistance in cattle nematodes. PloS one 8(8), e70212.

Gasteiger, J., Marsili, M., 1980. Iterative partial equalization of orbital electronegativity—a rapid access to atomic charges. Tetrahedron 36(22), 3219-3228.

Islam, M.K., Saha, S., Mahmud, I., Mohamad, K., Awang, K., Uddin, S.J., Rahman, M.M., Shilpi, J.A., 2014. An ethnobotanical study of medicinal plants used by tribal and native people of Madhupur forest area, Bangladesh. Journal of ethnopharmacology 151(2), 921-930.

Martin, G.J., 1995. Ethnobotany.'People and Plants' Conservation Manuals Series. Chapman and Hall, London.

Maundu, P., 1995. Methodology for collecting and sharing indigenous knowledge: a case study. Indigenous Knowledge and Development Monitor 3(2), 3-5.

Mondal, H., Hossain, H., Awang, K., Saha, S., Mamun-Ur-Rashid, S., Islam, M.K., Rahman, M., Jahan, I.A., Rahman, M.M., Shilpi, J.A., 2015. Anthelmintic Activity of Ellagic Acid, a Major Constituent of Alternanthera sessilis Against Haemonchus contortus. Pakistan Veterinary Journal 35(1).

Nagini, S., Nivetha, R., Palrasu, M., Mishra, R., 2021. Nimbolide, a Neem Limonoid, Is a Promising Candidate for the Anticancer Drug Arsenal. Journal of Medicinal Chemistry 64(7), 3560-3577.

Naik, P.K., Santoshi, S., Rai, A., Joshi, H.C., 2011. Molecular modelling and competition binding study of Br-noscapine and colchicine provide insight into noscapinoid–tubulin binding site. Journal of Molecular Graphics and Modelling 29(7), 947-955.

O'Boyle, N.M., Banck, M., James, C.A., Morley, C., Vandermeersch, T., Hutchison, G.R., 2011. Open Babel: An open chemical toolbox. Journal of cheminformatics 3(1), 33.

Ogedengbe-Olowofoyeku, A.N., Ademola, I.O., Wright, C.W., Idowu, S.O., Fatokun, A.A., 2021. Anthelmintic activity and non-cytotoxicity of phaeophorbide-a isolated from the leaf of Spondias mombin L. Journal of Ethnopharmacology 280, 114392.
Page, S.W., 2008. Chapter 10 - Antiparasitic drugs, in: Maddison, J.E., Page, S.W., Church, D.B. (Eds.), Small Animal Clinical Pharmacology (Second Edition). W.B. Saunders, Edinburgh, pp. 198-260.
Penakalapati, G., Swarthout, J., Delahoy, M.J., McAliley, L., Wodnik, B., Levy, K., Freeman, M.C., 2017. Exposure to Animal Feces and Human Health: A Systematic Review and Proposed Research Priorities. Environmental science & technology 51(20), 11537-11552.
Phillips, O., Gentry, A.H., Reynel, C., Wilkin, P., Gálvez-Durand B, C., 1994. Quantitative ethnobotany and Amazonian conservation. Conservation biology 8(1), 225-248.
Radhakrishnan, L., Gomathinayagam, S., Balakrishnan, V., 2010. Evaluation of anthelmintic effect of neem (Azadirachta indica) leaves on Haemonchus contortus in goats. Research Journal of parasitology 5(4), 292-297.
Spradlin, J.N., Hu, X., Ward, C.C., Brittain, S.M., Jones, M.D., Ou, L., To, M., Proudfoot, A., Ornelas, E., Woldegiorgis, M., Olzmann, J.A., Bussiere, D.E., Thomas, J.R., Tallarico, J.A., McKenna, J.M., Schirle, M., Maimone, T.J., Nomura, D.K., 2019. Harnessing the anti-cancer natural product nimbolide for targeted protein degradation. Nature Chemical Biology 15(7), 747-755.
Trott, O., Olson, A.J., 2010. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of computational chemistry 31(2), 455-461.
Veerakumari, L., Priya, P., 2006. In vitro effect of azadirachtin on the motility and acetylcholinesterase activity of Cotylophoron cotylophorum (Fischoeder, 1901). Journal of Veterinary Parasitology 20(1), 1-5.
Wang, L., Phan, D.D., Zhang, J., Ong, P.S., Thuya, W.L., Soo, R., Wong, A.L., Yong, W.P., Lee, S.C., Ho, P.C., Sethi, G., Goh, B.C., 2016. Anticancer properties of nimbolide and pharmacokinetic considerations to accelerate its development. Oncotarget 7(28), 44790-44802.
Zarza-Albarrán, M.A., Olmedo-Juárez, A., Rojo-Rubio, R., Mendoza-de Gives, P., González-Cortazar, M., Tapia-Maruri, D., Mondragón-Ancelmo, J., García-Hernández, C., Blé-González, E.A., Zamilpa, A., 2020. Galloyl flavonoids from Acacia farnesiana pods possess potent anthelmintic activity against Haemonchus contortus eggs and infective larvae. J Ethnopharmacol 249, 112402.

Table 1: Plants used against helminthic disorder in Mathbaria upazila.

Botanical name	Part used†	Mode of use	Other uses	Habitat‡
Allium sativum	Bu	Paste with water taken orally	Hypertension, asthma, hair loss	C
Ananas comosus	L, Fr	Leaf paste or decoction with water & salt. Raw fruit eaten	Labor induction, constipation, GI disorder	W, C
Anthocephalus indicus	L, Fl	Leaf & flower are warmed and paste with water	wounds, conjunctivitis	W, O
Azadirachata indica	L, B	Decoction or paste with water	Sedative, fever, dental treatments, cough	W, O
Capsicum frutescens	F, Se	Paste or decoction with water	Analgesia, stomach upset, toothache.	C
Carica papaya	Fr, Se	Fruit eaten and juice taken orally. Seed decoction with vinegar	Psoriasis, piles, constipation, blood dysentery	W, C
Cocos nucifera	Se	Cut into small pieces, make paste with water & taken orally	Pox, influenza, diabetes	W, C
Curcuma longa	Rh	Cut & soaked overnight in water & drunk the juice	Acne, cough, blood purifier, skin care	W, C
Cucurbita moschata	Se	Fried & crudely eaten	Diabetes, nutrient	C
Daucus carota	R	Directly eaten or as salad	Skin care, nutrition	C
Delonix regia	L, Fl	Dried leaf or flower soaked overnight and taken orally	Anti-inflammatory, piles, carminative	W, O
Erythrina variegata	B	Mixed with leaf juice of *Ananas comosus* and taken with lime water	Fever, diabetes	W, E
Momordica charantia	L, Fr	Leaf juice taken orally. Fruit cooked as vegetable	Diabetes, arthritis, dysmenorrhea	C
Tinospora cordifolia	St	Juice of crushed stem is drunk in morning	Diabetes, fever, cough, jaundice, asthma	W, E

†L: leaf, Fl: flower, Fr: fruit, Se: seed, St: stem, B: bark, Bu: bulb, R: root, Rh: rhizome.
‡C: cultivation, W: wild, O: ornamental, E: extinct.

Table 2: Plants used against helminthic disorder in Phultala upazila.

Botanical name	Part used†	Mode of use	Other uses	Habitat‡
Adhatoda vasica	R, L	Juice of these parts drunk	Cold, cough, asthma, heart disorder, Jaundice	W, C
Andrographis paniculata	L	Leaf decoction with salty water	Liver complaints, fever, anti-inflammatory	W
Averrhoa carambola	Fr	Crude fruit is eaten	Laxative, coughs, asthma, jaundice	W, C
Azadirachata indica	L, B	Decoction or paste with water	Sedative, fever, cough	O
Benincasa hispida	Se, L	Dried powder and crushed seed are taken with water. Juice of leaves is taken in empty stomach	Diabetes, baldness, dandruff, brain tonic	W, C
Carica papaya	L, Se	Leaf juice with crushed seed is taken orally	Piles, constipation, blood dysentery	C
Citrus grandis	Fr	Juice is taken with salt	Cough, asthma, diarrhea	W, C
Cucurbita moschata	Se	Decoction is taken with honey	Diabetes	C
Curcuma longa	Rh	Soaked in water overnight & drunk with lime water	Acne, blood purifier, skin care	W, C
Cuscuta reflexa	WP	Plant juice is eaten	Carminative, alterative, jaundice	W
Enhydra flactuans	WP	Boiled in water to make syrup and eaten	Laxative, fever, typhoid, bronchitis	W
Erythrina variegata	L, B	Leaf warmed with salt water & decoction. Bark soaked overnight and sap is taken at morning	Fever, astringent, expectorant, obesity	W, E
Eugenia caryophyllus	Fb	The bud is boiled in salty water or tea and then drunk	Digestive problem, gastric irritability, tooth pain	C
Momordica charantia	L, Fr	Leaf juice with zinger taken orally. Fruit eaten as vegetable	Diabetes, blood purifier, arthritis	C
Nigella sativa	Se	Seed dried & paste with saline water	Prevention of hair loss, diabetes	C
Phoenix sylvestris	Fr, L	Mixture of fruit and leaf juice is taken in empty stomach at morning	Digestive disorder, nutritive, laxative	W, C
Psidium guajava	Fr, L	Decoction, juice	Dysmenorrhea, hypertension, diabetes	C
Syzygium cumini	Se, Fr	Juice is taken after meal	Treat vomiting, hyperglycemia, dysentery, acne	W

†WP: whole plant, L: leaf, Fb: flower buds, Fr: fruit, Se: seed, B: bark, Bu: bulb, R: root, Rh: rhizome.
‡C: cultivation, W: wild, O: ornamental, E: extinct.

Table 3: Plants used against helminthic disorder in Khan Jahan Ali upazila.

Botanical name	Part used†	Mode of use	Other uses	Habitat‡
Acalypha indica	L	Powder juice with water	Gum problems, stomach aches, rheumatism, skin diseases	W
Ananas comosus	L, Fr	Leave or fruit juice taken orally	Constipation, fever	C
Anthocephalus chinesis	L, B	Powder of dried leaf and bark is taken after making juice	Mouth ulcers, diarrhea	W, O
Azadirachata indica	Se, L	Pills made from dried seed are swallowed with water. Juice of leaves drunk	Diabetes, Contraceptive, fever, dental treatments	C
Citrus grandis	Fr	Raw fruit is eaten	Coughs, fevers and gastric disorders	W, C
Cocos nucifera	Se	Paste with salt	Pox, diabetes	C
Musa sapientum	R, Fr	Root juice or ripe fruit directly eaten	Constipation, having nutritional value	C
Nigella sativa	Se	Paste taken with honey	Prevention of hair loss, diabetes	C
Phoenix sylvestris	L, Fr	Juice is taken in empty stomach at morning	Nutritive, laxative	W, C
Piper betle	L	Paste or directly eaten	Carminative, astringent	C
Syzygium cumini	B	Decoction taken with water	Treat acne, hyperglycemia, dysentery	W
Tagetes patula	L	Juice drunk with ginger	Digestive, diuretic, sedative, severe constipation	O
Terminalia bellerica	Fr	Taken as a component of trifala	Reduce cholesterol, blood pressure, diarrhea	W, C
Vitex negundo	L	Decoction, paste	Muscle relaxant, anti-mosquito, anti-anxiety	W

†L: leaf, Fr: fruit, Se: seed, B: bark, R: root.
‡C: cultivation, W: wild, O: ornamental.

Table 4: Comparative analysis of medicinal plants and their families used for treatment of helminthic disorders.

Botanical name	Family	Upazila(s) where used*			Use Value
		Mathbaria	Fultala	Khan Jahan Ali	
Acalypha indica	Euphorbiaceae			+	0.26
Adhatoda vasica	Acanthaceae		+		0.73
Allium sativum	Amaryllidaceae	+			0.55
Ananas comosus	Bromeliaceae	+		+	1.79
Andrographis paniculata	Acanthaceae		+		0.58
Anthocephalus indicus	Rubiaceae	+		+	0.96
Averrhoa carambola	Oxalidaceae		+		0.78
Azadirachta indica	Meliaceae	+	+	+	1.67
Benincasa hispida	Cucurbitaceae		+		0.46
Capsicum frutescens	Solanaceae	+			0.35
Carica papaya	Caricaceae	+	+		2.01
Citrus grandis	Rutaceae		+	+	1.25
Cocos nucifera	Arecaceae	+		+	1.17
Cucurbita moschata	Cucurbitaceae	+	+		0.71
Curcuma longa	Zingiberaceae	+	+		1.29
Cuscuta reflexa	Convolvulaceae		+		0.37
Daucus carota	Apiaceae	+			0.51
Delonix regia	Fabaceae	+			0.63
Enhydra flactuans	Asteraceae		+		0.36
Erythrina variegata	Fabaceae	+	+		0.91
Eugenia caryophyllus	Myrtaceae		+		0.18
Momordica charantia	Cucurbitaceae	+	+		1.27
Musa sapientum	Musaceae			+	0.39
Nigella sativa	Ranunculaceae		+	+	1.29
Phoenix sylvestris	Arecaceae		+	+	1.08
Piper betle	Piperaceae			+	0.60
Psidium guajava	Myrtaceae		+		0.46
Syzygium cumini	Myrtaceae		+	+	1.22
Tagetes patula	Asteraceae			+	0.30
Terminalia bellerica	Combretaceae			+	0.68
Tinospora cordifolia	Menispermaceae	+			0.46
Vitex negundo	Lamiaceae			+	0.77

*'+' Sign indicates use of that plant reported by respondents.

Table 5: Adult motility test of selected plant extracts against *Haemonchus contortus*.

Plant extract	*A. comosus*	*A. indica* (leaves)	*A. indica* (bark)	*C. papaya* (seeds)	*C. grandis*	*C. longa*	*M. charantia*	*N. sativa*	*S. cumini*
LC_{50} (mg/ml)	72.44	60.26	60.26	72.44	83.18	66.07	77.62	87.01	70.80

Table 6: Ligands with best docking results

Ligands	Docking score (kcal/mol)	Ligand interaction with amino acid residues*
Gramisterol	-9.1	**Val238**[f] (5.45), **Cys241**[ff] (4.83, 4.55), Leu242[f] (5.48), **Leu248**[ff] (5.01, 4.49), **Leu255**[ff] (3.96), **Ala316**[f] (4.58)
Limocinol	-9.0	**Cys241**[f] (4.60), Leu242[f] (4.37), **Leu248**[f] (4.78), Lys254[f] (5.13), **Leu255**[ff] (4.16, 3.80), **Ala316**[f] (4.44)
Nimbolide	-8.9	**Cys241**[ac] (3.47, 4.75) **Ala250**[a] (3.03) **Leu255**[a] (2.88), **Ala316**[b] (3.86), **Ala317**[e] (3.58), **Val318**[c] (4.82), Ala354[c] (4.66)
28-Deoxonimbolide	-8.9	**Cys241**[ac] (3.40, 4.70) **Ala250**[a] (2.99) **Leu255**[a] (2.87), **Ala316**[b] (3.91), **Ala317**[e] (3.66), **Val318**[c] (4.84), Ala354[c] (4.67)
5,7,4'-Trihydroxy-3',5'-diprenylflavanone	-8.9	**Cys241**[f] (4.58), **Leu248**[c] (4.98), **Leu255**[f] (4.82), **Ala316**[ff] (3.68, 4.22), **Val318**[f], (4.23), Asn350[a] (2.10), **Lys352**[c] (3.87)
DMC[†]	-6.9	**Cys 241**[ac] (3.23, 4.52) **Leu 248**[bcf] (3.93, 4.83, 4.64) **Ala 250**[c] (5.10), Lys254[e] (3.45), **Leu 255**[b] (3.63), **Ala316**[cf] (5.49, 5.42), **Lys352**[cff] (4.98, 4.89, 5.13), Ala354[f] (4.67)
Colchicine	-6.9	**Cys241**[afc] (3.75, 3.79, 5.26) **Leu248**[bcf] (3.72, 5.33, 5.40), **Ala250**[cf] (5.09, 3.89) **Leu255**[cf] (5.26, 3.92), **Lys 352**[ec] (3.20, 5.32)
Albendazole	-6.2	**Cys241**[dg] (3.41, 3.85), **Ala250**[c] (5.45), **Leu255**[bb] (3.37, 3.57), **Ala316**[cf] (5.22, 3.90), **Lys352**[f] (5.32), Ala354[f] (4.64)

* Bond types: *a = Conventional hydrogend, b = Pi-sigma, c = Pi-alkyl, d = Pi-sulfur, e = carbon hydrogen, f = alkyl, g = Pi donor hydrogen.
Values in the brackets indicate bond length.
Bold indicates key amino acid residues.
[†]DMC = *N*-deacetyl-*N*-(2-mercaptoacetyl)colchicine.

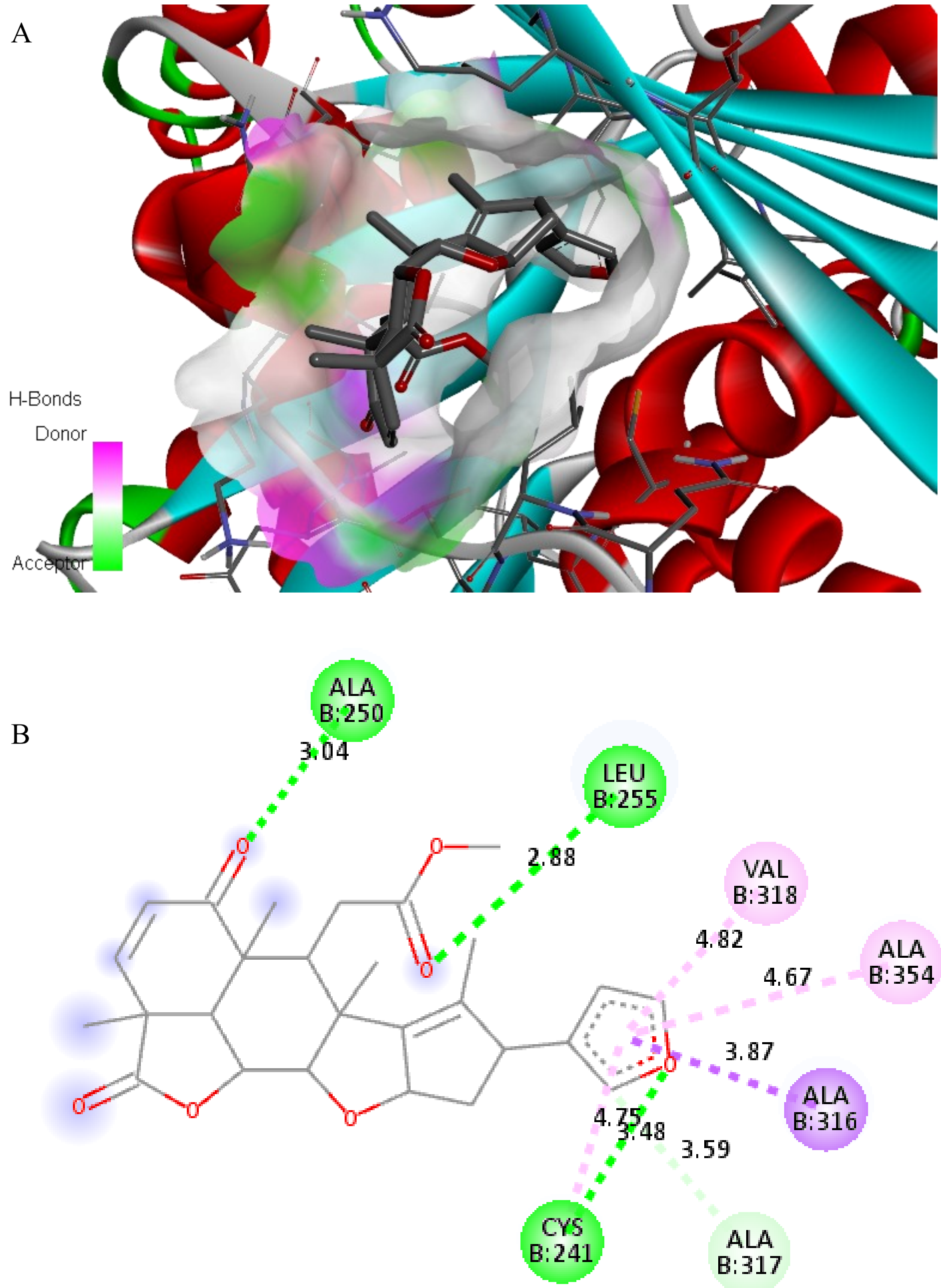

Fig. 1: Docked pose (A) and molecular interaction (B) of nimbolide at the colchicine binding site of β-tubulin. (Interactions shown by dashed lines: hydrogen-bonds in dark green, carbon-hydrogen bonds in light green, pi-alkyl in pink and pi-sigma in purple; images generated by DS v.4.5)