Phytochemical Screening and Antimicrobial activity of leaves of *Ardisia colorata*

By

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor Of Pharmacy
[B.Pharm]

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Declaration

It is hereby declared that

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3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
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The thesis/project titled “Phytochemical Screening and Antimicrobial activity of leaves of *Ardisia colorata*” submitted by

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Ethics Statement

The study doesn’t include any kind of animal or human trial.

Abstract/ Executive Summary

Ardisia colorata Roxb. is a traditional medicinal plant of long standing in use in South and South East Asian countries, especially for treating inflammation, digestive ailments and infections. Even though there has been a great ethnomedicinal tradition for the plant, there has been little formal scientific research done on the plant. The aim of this study was to evaluate the chemical composition, antioxidant activity and antimicrobial activity of the methanol extract of this species.

The preliminary phytochemical screening revealed the presence of various classes of bioactive compounds such as alkaloids, flavonoids, tannins, terpenoids, saponins and reducing sugars. Interestingly, glycosides, steroids and proteins were not detected. The antioxidant activity of the extract was then assessed by DPPH radical scavenging assay, where it exhibited moderate activity in comparison to the standard ascorbic acid. The disk diffusion method was used to evaluate the antimicrobial activity of the extracts against Staphylococcus aureus, where the concentrations of the extracts showed an increasing trend in inhibitory zones with the highest extract concentration (80µL) showing the highest antibacterial activity.

Altogether, these findings provide scientific evidence that supports the therapeutic uses of this plant historically identified and also highlight its possible use as a source of biologically active molecules suitable for the development of new pharmaceutical products. The authors suggest that further studies should be directed towards the isolation of the active compounds, toxicity testing as well as clinical trials to establish the plant's therapeutic value and safety in full

Keywords : *Ardisia colorata*, Phytochemical Screening, Antimicrobial Activity, Antioxidant Activity, DPPH Assay, Methanolic Extract, Bioactive Compounds

Dedication

I dedicate this thesis to my beloved parents, whose unconditional love, endless sacrifices, and constant prayers have been my greatest strength throughout every step of my life. Without their unwavering support and encouragement, none of this would have been possible. This achievement is as much theirs as it is mine.

Acknowledgement

First and foremost, I want to express my gratitude to Almighty Allah for his blessings and guidance to me.

Following that, I want to express my heartfelt gratitude to my supervisor, ***Mehrin Haque Tanisha*** Ma'am, for her invaluable guidance, patience, and expertise throughout this research. Her thoughtful feedback and continuous support have been instrumental in shaping this work. During difficulties or uncertainty related to my work, she was always there to offer valuable advice and motivate me to push forward. Her dedication toward my progress and her genuine interest in my research made this entire experience truly meaningful. I am deeply grateful for the time and dedication she has devoted to my academic development.

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Lastly, I want to thank my family and my friends for their support and encouragement throughout this entire process. Their love and belief in me kept me going during the most challenging moments of this journey.

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List of Acronyms

Acronym	Full Form
DPPH	2,2-Diphenyl-1-picrylhydrazyl
IC_{50}	Inhibitory Concentration 50%
MIC	Minimum Inhibitory Concentration
WHO	World Health Organisation
CLSI	Clinical and Laboratory Standards Institute
EUCAST	European Committee on Antimicrobial Susceptibility Testing
MHA	Mueller-Hinton Agar

Chapter 1

Introduction

1.1 Herbal Medicines as a Source of Drugs

Botanical resources have played a major role in the pharmacopoeia of mankind for millennia. Plants continue to be a significant source of chemical diversity in drug discovery even in modern medicine. Natural products offer structurally complex scaffolds, which have been evolved to interact with biological targets effectively, which many purely synthetic molecules do not possess (Atanasov et al., 2021). The long-standing and current relevance of plant-derived compounds in clinical practice can be exemplified by classic agents (morphine and quinine) and by modern agents (paclitaxel and artemisinin)

1.2 Genus Ardisia and *Ardisia colorata*

The Ardisia genus is composed of over 700 species of evergreen shrubs and trees that are found predominantly in the tropical and subtropical areas. These plants are characterized by a high phytochemical content, such as alkylphenols, benzoquinones, flavonoids, terpenoids, and others that play a role in different biological activities, including antimicrobial, anti-inflammatory, and cytotoxic effects (Atanasov et al., 2021).

Ardisia colorata is a bush or a small tree that is located in Southeast Asia countries such as Bangladesh, Thailand and Malaysia. It is commonly used to treat liver diseases, chronic cough and gastrointestinal disorders and reaches a height of 3-10 meters. It has leathery leaves, terminal flowers that are pink to purple and glossy black globose fruits.

Phytochemical studies of A. colorata have revealed various secondary metabolites, especially phenolic compounds and flavonoids, which are linked to antioxidant and cytotoxic effects. Such bioactive compounds justify its conventional medicinal purpose and emphasize its role in pharmacological applications in the future (Newman and Cragg, 2020).

1.3 Medicinal Plants Around the Globe: A Comprehensive Overview

Mediational plants use is one of the pillars of healthcare systems across the globe with their therapeutic effects scientifically proven and by means of millennia long evolution. These plant resources are still critical in the practice of traditional medicine. The global population is estimated by the World Health Organization to have some 80 % population depending on plant based medications

to meet their primary healthcare requirement. It has been shown that more than 35000 types of plants have been documented to have medicinal value and new forms of therapeutic uses still continue to be discovered. Combination with modern science has confirmed the efficacy and complexity of the biochemical mechanisms of action of many traditional plant based treatments as well as demonstrated that most are efficacious.

Medicinal plants are rich in bioactive compounds such as alkaloids, flavonoids, saponins and essential oils which make them useful in treatment. The active constituents are concentrated and distributed in different parts of the plant's components so that various parts of the plant are used in leaves, roots, bark, flowers, seeds, and fruits. Medicinal plants have been the most available and cheap means of health care to many millions of people in various parts of the world especially in Asia, Africa and South America. Plant chemistry remains a source of great inspiration to the pharmaceutical industry and some 25 %of prescription medications today include a substance originally found in plants or a synthetic version.

The list of medicinally important plants in different geographical areas, which represent various plant families and uses in therapy, is provided below in a detailed manner:

Scientific Name	**Family**
Azadirachta indica A. Juss.	Meliaceae
Withania somnifera (L.) Dunal	Solanaceae
Ocimum sanctum L.	Lamiaceae
Aloe vera (L.) Burm.f.	Xanthorrhoeaceae
Catharanthus roseus (L.) G.Don	Apocynaceae
Centella asiatica (L.) Urban	Apiaceae
Phyllanthus emblica L.	Phyllanthaceae
Andrographis paniculata (Burm.f.) Nees	Acanthaceae
Tinospora cordifolia (Willd.) Miers	Menispermaceae
Gymnema sylvestre (Retz.) R.Br.	Apocynaceae

Bacopa monnieri (L.) Wettst.	Plantaginaceae
Asparagus racemosus Willd.	Asparagaceae
Tribulus terrestris L.	Zygophyllaceae
Piper betle L.	Piperaceae
Camellia sinensis (L.) Kuntze	Theaceae
Zingiber officinale Roscoe	Zingiberaceae
Allium sativum L.	Amaryllidaceae
Cinnamomum verum J.Presl	Lauraceae
Mentha piperita L.	Lamiaceae

Table 1 : List of Few Medicinal Plants Around the Globe

1.4 Medicinal Plants of Bangladesh: A Rich Botanical Heritage

Bangladesh is a nation located in the northeastern region of South Asia that has a rich biodiversity because of its geographical location, fertile deltaic plains and tropical monsoon climate. This biodiversity offers a perfect habitat where a large number of medicinal plant species can thrive and be grown naturally that have long been part of the healthcare practice in the country.

The Bangladesh traditional systems of medicine (Ayurveda, Unani, and the indigenous folk medicine) are based upon plant-based medicines to cure a variety of ailments. Medicinal plants remain as the main source of healthcare in the rural regions where there is a low access to modern healthcare facilities. The local healers, also referred to as kavirajes, are well versed with empirical backgrounds with respect to plant identification, preparation methods and therapeutic uses. Their use is both with acute conditions, including digestive and respiratory disorders, and chronic diseases, including arthritis, hypertension, cardiovascular diseases, and metabolic disorders (WHO, 2019).

The ethnobotanical knowledge is very diverse among the indigenous people like the Garo, Chakma, Marma, and Tripura in the Chittagong Hill Tracts. These societies make use of the forest-based medicinal plants, and these communities tend to use species which are less frequently used in mainstream traditional medicine. As an illustration, Centella asiatica is popular among these populations to heal wounds and improve thinking abilities, whereas Azadirachta indica (neem) is

popular due to its powerful antimicrobial and anti-inflammatory effects. They also possess more species-specific, localized and forest-related knowledge systems compared to the larger Ayurvedic or Unani systems (Rahman et al., 2017).

Phytochemical research has revealed that the Bangladeshi medicinal plants are endowed with a plethora of bioactive compounds such as alkaloids, glycosides, phenolic compounds, terpenoids, and essential oils. These substances are directly related to certain pharmacological processes. As an example, alkaloids have analgesic and antimicrobial effects, phenolic compounds are thought to have antioxidant actions, terpenoids have anti-inflammatory effects, and glycosides regulate cardiovascular actions. Azadirachta indica has terpenoids and flavonoids that cause antimicrobial effect, and Centella asiatica has triterpenoids that enhance wound healing and tissue regeneration (Uddin et al., 2020).

Recent ethnobotanical survey work in coastal areas, especially in the Barisal and Khulna divisions, has reported over 200 plant species used by traditional healers to treat waterborne diseases, skin infections and gastrointestinal diseases that are common in the coastal areas (Uddin et al., 2020). Nevertheless, with this abundant diversity, most of the medicinal plant species are threatened by loss of habitat, climatic changes, and over harvesting. Thus, conservation and scientific authentication in terms of phytochemical and pharmacological research are needed to keep this precious natural resource intact and to facilitate its use in the modern healthcare systems.

The following is a complete list of notable medicine plants within Bangladesh and belonging to a variety of botanical families and uses:

Scientific Name	Bengali Name	Family
Aegle marmelos (L.) Corrêa	Bel	Rutaceae
Azadirachta indica A. Juss.	Neem	Meliaceae
Adhatoda vasica Nees	Basak	Acanthaceae
Terminalia arjuna (Roxb. ex DC.) Wight & Arn.	Arjun	Combretaceae

Table 2 : List of Medicinal Plants of Bangladesh

1.5 Parts of Plants Used in Medicine:

The number of parts that are present in plants and which may be of medicinal use is vast leaves, roots, bark, fruits, seeds, and flowers. The same plant may have different bioactive compounds in different parts and therefore a particular part may be therapeutic whilst the other may be harmful. Medicinal properties have been observed to occur in different parts of the plant including the bark, flowers, bulbs, leaves, gum, fruits, resin, rhizomes, roots, seeds and tubers. The sample material that chosen in the current study was plant leaves to examine the availability of phytochemicals.

1.6 Introduction and Taxonomic Classification of Sample Plant: Ardisia colorata Roxb.

Ardisia colorata Roxb. or the colored ardisia or Christmas berry is a decorative evergreen, a shrub belonging to the family Primulaceae. The locality to which this species belongs is the tropical and subtropical regions of Southeast Asia and the natural range reaches to India, Bangladesh, Myanmar, Thailand, Malaysia, Indonesia and south China. The plant thrives in wet and darker understorey forests and its banks of streams in lowland to montane altitudes, which are typically at sea level to 1,500 meters (Chen & Pipoly, 1996). A. colorata possesses a pleasant shiny foliage, small flower stars in white hue that fade to pale pink, and bright red to purple berries that persist throughout the year making the plant popular in decorative horticulture and traditional landscapes in tropical gardens. The plant is of great significance in the traditional medicine of Asia where various parts of the plant including leaves, roots and fruit have been used in treating inflammatory and gastrointestinal diseases in addition to their aesthetic value (Huang et al., 2011). The geographic range of the species has a high level of morphological diversification, plants in the majority of cases reach a height of 1 to 3 meters. The leaves are alternate, elliptic-lanceolate, simple, having a strong vein and smooth or slightly serrated edges. It is a terminal or axillary panicle containing many small flowers that would further develop into globose drupes that change their colors to bright red or purplish-black when they are mature (Pipoly & Ricketson, 2014).Phytochemical analysis of A. colorata has indicated it possesses a number of bioactive compounds that aid in its pharmacological actions that include antimicrobial, antioxidant, antiinflammatory and cytotoxic activities, including alkylphenols, benzoquinones, flavonoids, triterpenoids and saponins. These chemicals contribute to emphasizing the prospective application of the plant as a source of natural therapeutic agents and its application in the current studies in the sphere of pharmacognostics.

Taxonomic Classification:

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Ericales

Family: Primulaceae (formerly Myrsinaceae)

Genus: Ardisia

Species: *Ardisia colorata* Roxb.

This genus has been moved from the ancient Myrsinaceae family to the new and enlarged family of Primulaceae based on molecular phylogenetic evidence. This change highlights improvements in the understanding of evolutionary patterns among flowering plants using DNA sequencing (Ståhl & Anderberg, 2004).

Figure 1 : ***Ardisia colorata***

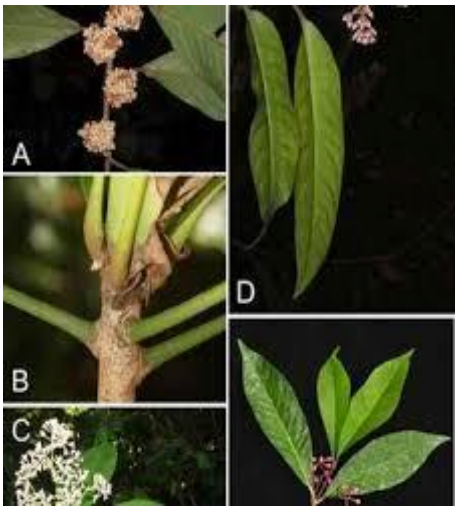


Figure 2 : Parts of ***Ardisia colorata***

1.7 Literature Review

Ardisia colorata Roxb. is an ethnomedicinal herb that belongs to the family Primulaceae and is widely used in South and South-East Asia. Various parts of the plant, such as the leaves, roots, bark, and fruits, have been reported to have medicinal properties and are commonly used in traditional medicine. Traditional practitioners frequently use leaf and root extracts, decoctions and infusions to treat inflammatory skin ailments, fever, digestive disorders, and infectious diseases.
Ethnobotanical surveys have reported use of *A. colorata* in the management of diarrhea and dysentery, pain in the abdomen, rheumatism and skin infections (Uddin, Wood, & Hassan, 2014). Moreover, paste of the leaves is used topically in dressing wounds and ulcers, and decoctions of the roots are

used for the treatment of abdominal colic. The fruits have also been used as an anthelmintic for the treatment of childhood intestinal helminth infections (Uddin et al., 2014).
Phytochemical analysis has identified a range of active ingredients in A. colorata, such as flavonoids, saponins, tannins, alkaloids, glycosides and phenolics. These compounds are believed to be responsible for its various pharmacological effects including antioxidant and anti-inflammatory effects. Research has shown that A. colorata extracts have dose-dependent antioxidant properties, suggesting they may potentially neutralise free radicals and participate in the antioxidant defence mechanism (Islam et al., 2021). Additionally, other pharmacological studies have indicated the potent antibacterial and antimicrobial activity of A. colorata against Gram-positive and Gram-negative bacteria, implying broad spectrum activity (Islam et al., 2021). Laboratory studies also suggested anti-inflammatory and analgesic properties, further confirming its traditional applications for inflammatory diseases and pain relief.
In conclusion, the literature supports the proposition of Ardisia colorata being a valuable potentially ethnopharmacologically active medicinal plant. But further research on the extraction and identification of active constituents, toxicity testing and clinical trials is needed to verify its safety and efficacy.

1.8 Morphological Description of *Ardisia colorata*

Ardisia colorata is an evergreen shrub or small tree that grows 3-8 meters tall in forests, 3-10 meters in optimum conditions. The species has an erect and slender trunk with glossy grayish-brown bark, not as conspicuous as its dense and glossy foliage.

Leaves: Alternate, coriaceous and elliptic-lanceolate, the leaves are around 10-25 cm long and 3-7 cm wide. The upper side is dark and shiny, with the lower side paler green. They have prominent lateral venation, with veins running towards the margins.

Flowers: The flowers grow in large terminal, pyramidal panicles ranging from 20-30 cm in length. The flowers are small, pink to purplish in colour. The petals are ovate, 4-5 mm in length and may have glandular spots.

Fruits and Seeds: The fruit is a globose drupe, 5–8 mm in diameter. It is green when immature, turning bright red and eventually shiny black upon maturation. Each fruit typically contains a single hard, spherical seed.

1.9 Ecology of *Ardisia colorata*

Ardisia colorata is native to the moist tropical regions of Southeast Asia, including Thailand, Malaysia, Indonesia (Sumatra and Java), Vietnam, and parts of the eastern Himalayas. The species is a shade-tolerant plant and typically occurs in the understory of primary and secondary evergreen forests.

It thrives in humid tropical climates characterized by high and well-distributed rainfall, typically ranging from 1500 to 3500 mm annually, with a short or absent dry season (Flora of China, 1996). The species prefers moist, well-drained soil and is commonly found in nutrient-rich forest habitats, particularly along riverbanks and in valley depressions.

The optimal growth temperature ranges between 18°C and 28°C. Although relatively more tolerant to cooler conditions compared to many tropical species, it remains restricted to frost-free environments.

Chapter 2

Methodology

2.1 Preparation of plant extracts (*Ardisia colorata* leaf*)*

Leaves of *Ardisia colorata* were collected in dried powder form in August 2025 from Shantimoychara, Bagaichhari, Rangamati, Bangladesh (Accession No.: 75885).

A total of 700 g of dried *Ardisia colorata* leaf powder was used for the extraction process.

The dried leaf powder (700 g) was transferred into a clean 1000 millilitre glass beaker and 1000 millilitres of methanol was used to extract 700g of leaf powder of the sample plant . The beaker was covered with aluminium foil to prevent solvent evaporation and contamination. It was mixed and shaken manually on a daily basis while being left for two weeks to ensure maximum extraction of phytochemical constituents.

After 14 days , the crude extract was separated from the plant residue by straining through a clean cotton cloth. The filtrate was collected in a clean, dry container for further processing.

The methanolic extract (300 ml of crude extract per batch) was concentrated using a rotary evaporator at 33° C and pressure 200 millibar for 2 hours to obtain a semi-solid extract.

Approximately 60 mL of semi-solid, jelly-like concentrated extract per 300 mL of crude extract was obtained .

The concentrated extract was collected and stored at room temperature with foil cover in a container until further analysis.

2.2 Antioxidant Activity Evaluation

- **DPPH Free Radical Scavenging Assay**

The antioxidant potential of *Ardisia colorata* leaf extract was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. The assay was performed 24 hours after the concentration of the extract

A stock solution of the plant extract was prepared by dissolving 115 mg of the concentrated extract in 115 mL of methanol to obtain a final concentration of 1 mg/mL (1000 µg/mL).

To prepare a 0.004% (w/v) DPPH solution, at first 40 mg of DPPH was dissolved in methanol in a 1000ml volumetric flask .

- **Assay Procedure**

Concentration (µg/mL)	**Volume of Stock Extract (µL)**	**Volume of Methanol (µL)**	**Total Volume (mL)**
80	160	1840	2.0
60	120	1880	2.0
40	80	1920	2.0
20	40	1960	2.0

Table 3 : DPPH Assay Volumes

The DPPH assay was performed in test tubes with varying concentrations of the plant extract. The dilution series was prepared using the dilution formula ($C_1V_1 = C_2V_2$), where the stock solution concentration was 1000 µg/mL.

Each test tube was prepared to contain a final volume of 2 mL before the addition of DPPH solution. The dilution series was prepared as follows:

Calculation example for 80 µg/mL concentration:

Using $C_1V_1 = C_2V_2$

(1000 µg/mL) × V_1 = (80 µg/mL) × (2 mL)

V_1 = 0.16 mL = 160 µL of stock solution

Volume of methanol = 2000 µL - 160 µL = 1840 µL

- **Control and Blank Preparation:**

Blank: 4 mL of methanol only

Control: 2 mL of methanol + 2 mL of DPPH solution

To each test tube containing the extract-methanol mixture, 2 mL of DPPH solution was added, making the final volume 4 mL. The test tubes were gently mixed and kept in dark for 30 minutes at room temperature. The incubation was carried out in the dark since DPPH is light sensitive.

Ascorbic Acid Standard

100 mg of Vitamin C (ascorbic acid) was used as a positive control for comparison of antioxidant activity of the plant extract. Ascorbic acid was dissolved in methanol to make a 1000 µg/mL stock solution. The solution was diluted to prepare different concentrations of 20, 40, 60, 80 and 100 µg/mL, using the same dilution procedure as mentioned for the plant extract. These were mixed with 2 mL of DPPH and incubated as described in the plant extract.

Absorbance Measurement

After 30 minutes of incubation in the dark, the absorbance of each sample was measured at 517 nm using a UV-Visible spectrophotometer against the blank (methanol). The following absorbance values were recorded:

- **Ascorbic Acid (Standard) Absorbance Values:**

Concentration (µg/mL)	**Absorbance at 517 nm**
Blank	0.041
20	0.084

40	0.056
60	0.053
80	0.049
100	0.047

Table 4 : Ascorbic Acid (Standard) Absorbance Values

- **Plant Extract (*Ardisia colorata*) Absorbance Values:**

Concentration (µg/mL)	**Absorbance at 517 nm**
Blank	0.036
20	0.564
40	0.097
60	0.095
80	0.121
100	0.101

Table 5 : Plant Extract (*Ardisia colorata*) Absorbance Values

Control (DPPH + Methanol): 0.855 nm

After incubation, the absorbance of each sample was measured at 517 nm using a UV-Visible spectrophotometer against the blank (methanol). Ascorbic acid was used as the positive control

Calculation of DPPH Scavenging Activity

The percentage of DPPH radical scavenging activity was calculated using the following formula:

% Inhibition = $[(A_0 - A_1) / A_0] \times 100$

Where:

A_0 = Absorbance of DPPH control (DPPH + methanol, without extract)

A_1 = Absorbance of test sample (DPPH + extract) or standard (DPPH + ascorbic acid)

The IC_{50} value (concentration of extract or standard required to scavenge 50% of DPPH radicals) was determined by plotting percentage inhibition against extract/standard concentration and calculating the concentration corresponding to 50% inhibition using linear regression analysis control/standard.

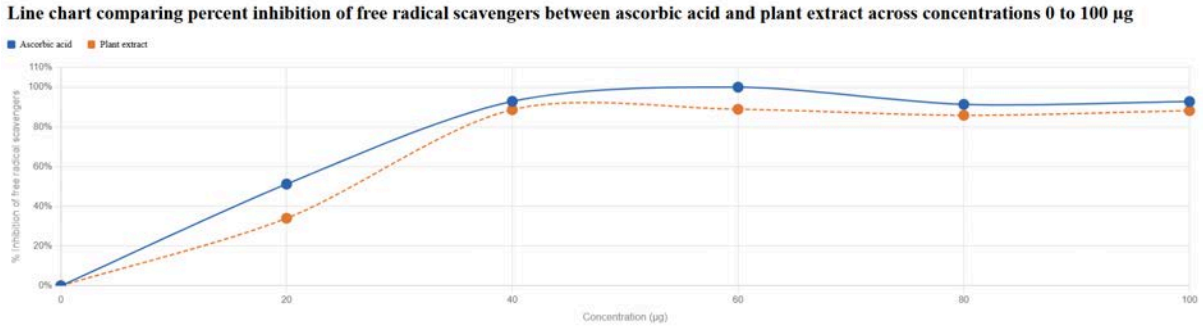
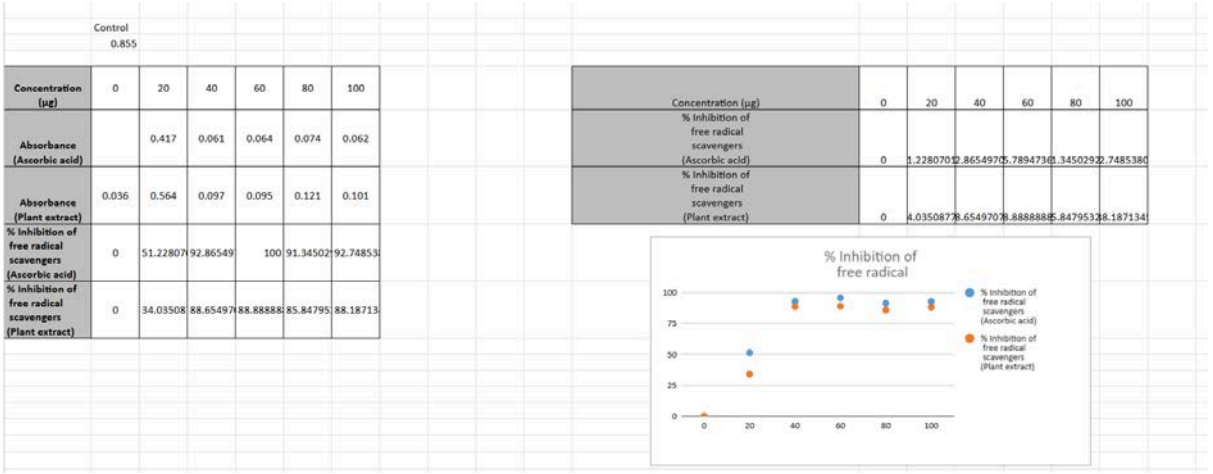


Figure 3 : Line chart comparing percent inhibition of free radical scavengers between ascorbic acid and plant extract across concentrations 0 to 100 µg



Control
0.855

Concentration (µg)	0	20	40	60	80	100
Absorbance (Ascorbic acid)		0.417	0.061	0.064	0.074	0.062
Absorbance (Plant extract)	0.036	0.564	0.097	0.095	0.121	0.101
% Inhibition of free radical scavengers (Ascorbic acid)	0	51.22807	92.86549	100	91.34502	92.74853
% Inhibition of free radical scavengers (Plant extract)	0	34.03508	88.65497	88.88888	85.84795	88.18713

Concentration (µg)	0	20	40	60	80	100
% Inhibition of free radical scavengers (Ascorbic acid)	0	51.22807	92.86549	100	91.34502	92.74853
% Inhibition of free radical scavengers (Plant extract)	0	34.03508	88.65497	88.88888	85.84795	88.18713

Figure 4 : DPPH Final Assay

2.3 Antimicrobial Activity assessing

Antimicrobial Activity Testing Using the Disk Diffusion Method

The Disk Diffusion Method was used to assess the antibacterial properties of *Ardisia colorata* methanolic leaf extracts.

Microorganisms used for the test: *Staphylococcus aureus* (Gram Positive Bacteria)

One of the most commonly and widely used antimicrobial susceptibility methods used in clinical and research laboratories is the disk diffusion method. This is a phenotypic methodology that allowed the assessment of various antimicrobial agents to bacterial pathogens using a simple but rigorous process. The procedure is to put antimicrobial impregnated paper disks on agar plates which have already been inoculated with a standardized bacterial suspension. After an overnight period of controlling incubation, the numbers of growth inhibition zones around each disk are measured.

The core principle of this method is based on the diffusion of antimicrobial substances of the disk into the adjacent agar medium, defining a concentration gradient which declines with the distance to the disk. A distinct zone of inhibition appears around the disk when the concentration of the antimicrobial at any one point is more than the minimum concentration needed to prevent the growth of bacteria. The disk diffusion test offers an experimentally feasible approximation of the antimicrobial efficacy, the bigger the inhibition zone, the higher is the susceptibility of the tested organism to the antimicrobial agent (Balouiri et al., 2016).

The standardized procedure begins by a suspension of the test bacterium, Staphylococcus aureus (Gram-positive bacteria) adjusted to the turbidity of a 0.5 McFarland standard, which corresponds to about 1-2 x 108 colony-forming units per milliliter. By placing a sterile swab on the surface of MuellerHinton agar plates, uniform inoculation is performed in order to achieve confluent growth. The impregnated disks or the disks that are treated with the plant extract are then positioned on top of the inoculation surface so that several agents can be tested at a time on a single plate. The plates are incubated at 35-37 o C, 16-18 hours after which the diameters of the zone of inhibition are measured precisely with the help of a caliper or automated zone readers (Humphries et al., 2018).

Staphylococcus aureus was chosen to be the test organism since it is a clinically significant Gram-positive pathogen, and is commonly utilized in antimicrobial susceptibility studies as it is known to be sensitive to a variety of antimicrobial agents.

The method was chosen because it is appropriate for preliminary screening of plant extracts as it is easy, cheap and can visualize the antimicrobial activity clearly by zones of inhibition. It proves particularly helpful when it is necessary to determine the presence of bioactive compounds in crude plant extracts prior to the additional quantitative analysis.

The measurements of zone diameter should be compared to the existing clinical breakpoints which should be regularly revised by special organizations like the Clinical and Laboratory Standards Institute and the European Committee on Antimicrobial Susceptibility Testing (Mathers, A. J., MD. (n.d.). Such breakpoints can be used to classify bacterial isolates as susceptible, intermediate, or resistant in relation to the correlation of zone diameters and minimum inhibitory concentrations obtained via extensive clinical and pharmacokinetic investigations. This is a classification system that allows clinicians to make actionable information to make therapeutic decisions.

Various technical variables affect the measurements of zone diameter and should be handled with care to enable reproducible and accurate measurements. The zone size varies because of the composition and depth of agar medium, temperature and atmosphere during incubation, inoculum density and the characteristics of bacterial growth. Also, the antimicrobial concentration of disks, storage medium, and diffusion characteristics of various compounds in agar matrices influence the size of the resulting inhibition zones. These sources of variation are reduced by the compliance with the standardized procedures and improve inter-laboratory comparability.

The disk diffusion technique is still essential in microbiology laboratories around the globe despite the ongoing progress in automated susceptibility testing systems and molecular resistance detection systems. Its advantages are that it is easy, cost-effective, and flexible in the selection of antimicrobials and can be performed to test combinations of agents at the same time. It, however, has its drawbacks: it cannot be used with non-diffusible compounds and does not give quantitative data, such as the Minimum Inhibitory Concentration (MIC). The technique is especially useful in the identification of antimicrobial resistance processes and in informing empirical treatment where resources are limited (Patel et al., 2020).

Procedure of antimicrobial activity test by Disk Diffusion Method.

1.The antibacterial activity of the plant extract was evaluated using the disk diffusion method against Staphylococcus aureus. Mueller–Hinton agar was prepared by dissolving 19 g of agar powder in 500 mL of distilled water in a volumetric flask.

2. The medium was sterilized in an autoclave at 121 °C from 9:30 AM to 11:30 AM. .Before using, ensure that the medium's surface is dry and let it come to room temperature.The microbe that will be tested should be labelled on the agar plates.

3.After cooling, the sterile agar was poured into Petri dishes and left to solidify. A freshly prepared bacterial suspension of S. aureus was evenly spread over the agar surface to obtain uniform growth.

4. Sterile filter paper discs (8 mm diameter) were placed on the inoculated plates. Each plate contained *five discs: four standard antibiotic discs (Amoxicillin, Kanamycin, Vancomycin, and Penicillin) and one disc containing the plant extract. The plant extract was dissolved in methanol and different volumes (20 µL, 40 µL, 60 µL, and 80 µL) were loaded onto the fifth disc. Four plates were prepared, each with a different extract volume.

5.Within 15 minutes of the discs being inserted, flip the plates over and put them in an incubator that is set to 35°C.The plates were incubated for 16 to 18 hours.

6. After incubation, the zones of inhibition were measured in millimeters using a Vernier caliper and the results were recorded to assess antibacterial activity.

2.4 Phytochemical screening

Phytochemical screening is a noteworthy analysis tool used to determine and identify biologically active compounds in plants. This is done to establish the nature of phytoconstituents contained in the plant extracts and their relative concentrations. Medicinal plants are rich in natural bioactive compounds that are applicable in the prevention and treatment of different human ailments. Most medicinal plants contain phytochemicals, which are major pharmacological agents. These are naturally found in plants and they consist of alkaloids, flavonoids, phenols, tannins, saponins, terpenoids and glycosides. They have a wide range of biological activities such as antioxidant, anti-inflammatory, antimicrobial and anticancer effects. As a result of these positive impacts, phytochemicals are significant in drug discovery, the formulation of functional foods and nutraceuticals (Evans, 2020).

Phytochemical screening is mainly aimed at determining the presence of bioactive compounds in plant extracts, and the potential therapeutic value of these compounds. It gives initial knowledge on the medicinal worth of plants and assists in identifying promising candidates to undergo further pharmacological and biochemical research. Phytochemicals can be broadly categorized into various groups such as alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenoids and glycosides. The classes have unique chemical properties and biological activities which make plants have medicinal properties.

Phytochemical screening process is a systematic step and usually follows the following steps: collection and identification of plant materials, drying and grinding, extraction in appropriate solvents, and qualitative analysis to identify various classes of phytoconstituents based on particular chemical tests. The detection of these compounds is useful in indicating the therapeutic potential of medicinal plants and justifying their use in the traditional application.The process of phytochemical screening

was conducted in this research to determine the key bioactive compounds in the chosen plant extract as well as to determine the possible medicinal potential of the extract.

The general approach of Harborne (1998) was used to perform a phytochemical analysis of the organic extract. Simple chemical assays were used for basic phytochemical screening in order to find secondary plant elements such alkaloids, tannins, flavonoids, saponins, steroids, phenols, glycoside, reducing sugar, and soluble carbohydrates in the sample.

Test for glycosides:

Keller-kilani Test : Two millilitres of glacial acetic acid containing one or two drops of a 2% FeCl3 solution were combined with crude extract. After that, the mixture was transferred to a second test tube that included two millilitres of concentrated H2SO4 . The presence of cardiac glycosides was indicated by a brown ring at the interphase (Yadav et al. , 2011).

Test for alkaloids: Two millilitres of 1% HCl were combined with crude extract and heated gradually. The mixture was then supplemented with Mayer's and Wagner's reagents. The ensuing precipitate turbidity was seen as proof that alkaloids were present (Yadav et al. , 2011).

Test for terpenoids: Two millilitres of chloroform were used to dissolve the crude extract, which was then dried by evaporation. Two millilitres of concentrated H2SO4 were added and the mixture was heated for approximately two minutes. Terpenoids were characterised by a greyish hue (Yadav et al. , 2011).

Test for steroid: Concentrated H2SO4 was incorporated sidewise to a mixture of crude extract and 2 millilitres of chloroform. Steroids were detected by the production of a red hue in the bottom chloroform layer. Crude extract was combined with two millilitres of chloroform for an additional test. Next, two millilitres of strong acetic acid and H2SO4 . were added to the mixture. Steroids were present because of their formation of a greenish tint (Yadav et al. , 2011).

Test for tannins: Crude extract was combined with two millilitres of a 2% FeCl3 solution.Tannin was indicated by a blue-green or black colouring (Yadav et al. , 2011).

Test for flavonoids: Crude extract was combined with two millilitres of a 2% NaOH solution. When just a few drops of diluted acid were added, the strong yellow hue that had formed went colourless, indicating the presence of flavonoids (Yadav et al. , 2011).

Test for phenols: Crude extract was combined with two millilitres of a 2% FeCl3 solution. Phenol was indicated by a blue-green or black colouring (Yadav et al. , 2011).

Test for proteins: Crude extract when mixed with 2ml of Millon's reagent, white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein (Yadav et al. , 2011).

Test for resin: 10 mL of distilled water was added to each plant extract, to which a few drops of 4% HCL were added. Appearance of turbidity in solution indicates presence of resin (Yadav et al. , 2011).

Chapter 3

Result

3.1 Result of phytochemical screening

Class of compounds	Name of the test	Present (+)/ Absent (-)
Alkaloids	Wagner's Test Mayer's Test	+
Terpenoids	Salkowski test	+
Reducing Sugar	Fehling test	+
Tannins	Ferric Chloride Test	+
Flavonoids	Alkaline reagent Test	+
Glycoside	Keller-kilani Test	-
Protein	Biuret Test	-
Saponin	Frothing Test	+
Steroid	Lieberman-Burchard	-
Anthraquinones	Borntrager's test	-

Table 6 : Result of phytochemical screening

Here, Present (+) and Absent(-).

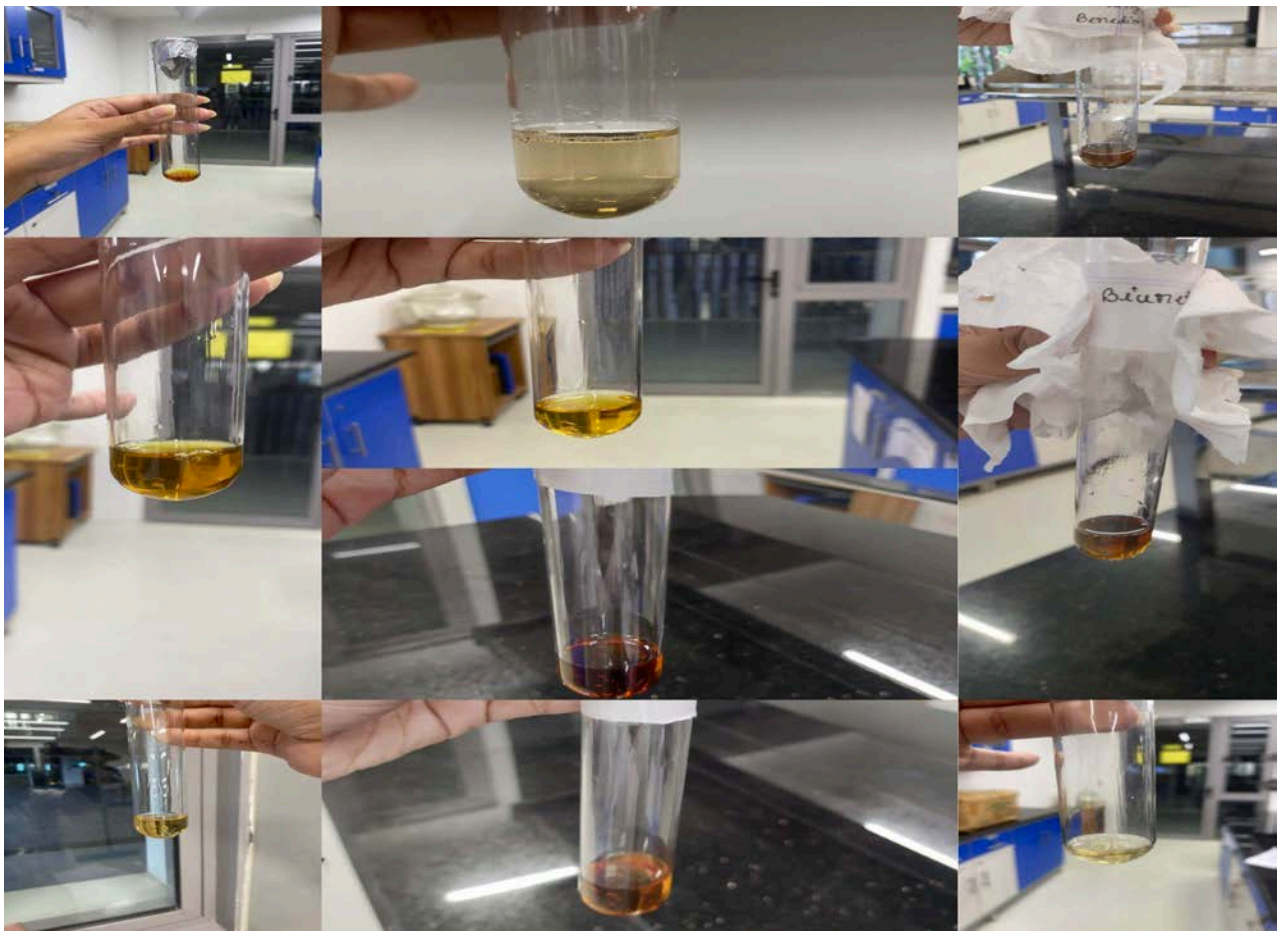


Figure 5 : Results of Phytochemical Screening

3.2 Result of Antimicrobial activity Test

Extract Volume	Amoxicillin	Penicillin	Kanamycin	Vancomycin	Plant Extract
Zone of Inhibition (mm)					
20 µL	38.10	No zone	25.91	2.54	12.70
40 µL	38.10	No zone	25.40	2.54	15.24
60 µL	38.10	No zone	25.40	2.79	17.78
80 µL	27.94	No zone	25.91	25.40	20.54

Table 7 : Result of Antimicrobial activity Test

Figure 6: Results of Antimicrobial activity Test

Chapter 4

Discussion :

The pharmacological potential of the plant *Ardisia colorata* was assessed by phytochemical, antioxidant and antimicrobial screening. The phytochemical analysis identified several important bioactive compounds, such as alkaloids, flavonoids, tannins, terpenoids, and saponins, which have a wide range of therapeutic benefits. The lack of glycosides, proteins, and steroids suggests a particular phytochemical composition which could be responsible for the various biological effects detected in the plant extract.

The antioxidant activity measured by DPPH assay showed that the extract has free radical scavenging property but the activity was not comparable with the standard antioxidant ascorbic acid. The discovery indicates that *Ardisia colorata* can have positive effects in reducing oxidative stress and preventing free radical damage to cells. The antioxidant effect could be attributed to the synergic effect of several phytochemicals, especially flavonoids and phenolic compounds, known as antioxidants. Further purification and fractionation of the extract may help to determine the bioactivity of specific compounds in the extract and thus increase its antioxidant activity.

The antimicrobial activity was performed with disk diffusion method with moderate results against Staphylococcus aureus. The zone of inhibition grew with concentration of extracts showing concentration dependent antimicrobial activity. The study results indicate that Ardisia colorata has the potential to be a natural source of antimicrobial agents for controlling bacterial infections, though not as effective as conventional antibiotics.

The results of this study corroborate earlier studies conducted on *Ardisia colorata* (Sarkar et al. ,2023) reported that the aqueous leaf extract of *Ardisia colorata* was found to be rich in tannins, saponins, flavonoids and alkaloids and showed good dose-dependent anthelmintic activity on Pheretima posthuma. The study showed that increase in extract concentration resulted in increased paralysis and mortality of worms, but not as effective as the activity of albendazole. The authors proposed that tannins can hinder the metabolism of parasites by uncoupling oxidative phosphorylation and saponins have an antagonizing effect on membrane integrity due to increased membrane permeability. The results of the present investigation further substantiate the biological significance of the phytochemicals identified and also indicate the wide range of drug potentials of the plant.

Furthermore, compounds like bergenin, rapanone and ardisiphenols have been found from *Ardisia colorata* by previous phytochemical studies. Bergenin is reported to have antioxidant, anti-inflammatory and antidiabetic effects and ardisiphenols exhibited high antioxidant activity. These bioactive components could be behind the antioxidant and antimicrobial effects seen in this study.

Therefore, the results of this research support the traditional medicinal use of *Ardisia colorata* and indicate that it could be a valuable source of natural therapeutic agents. The biological activities that are attained are very significant which include the antioxidant, antimicrobial and recently reported anthelmintic activity indicating the medicinal relevance of the plant. Isolation and characterization of active compounds, toxicity evaluation, mechanism of action and clinical studies should be performed in future to assess their safety, efficacy and pharmaceutical potential.

Chapter 5

Conclusion

The present study reveals that *Ardisia colorata* has significant phytochemical and biological activities, justifying its traditional use. The detection of active ingredients, including alkaloids, flavonoids, tannins and terpenoids suggests it may serve as a source of natural products in medicine. Its extract exhibited antioxidant properties, albeit less than the control which indicates moderate free radical scavenging potential. Moreover, the antimicrobial test showed dose-dependent antibacterial activity against *Staphylococcus aureus*, indicating its potential use for microbial infections. *Ardisia colorata* can be regarded as a potential lead for herbal drug development. But more in-depth studies are needed on isolation, identification and clinical testing of active constituents to substantiate its effectiveness and safe use in clinical practice.

References:

1. Evans, W. C. (2020). *Trease and Evans' Pharmacognosy* (17th ed.). Elsevier. https://eopcw.com/assets/stores/Pharmacognosy/lecturenote_89182832(Evans,%20Trease%20and%20Evans%20Pharmacognosy)%20William%20Charles%20Evans-Trease%20and%20Evans%E2%80%99%20Pharmacognosy-Saunders%20Ltd.%20(2009).pdf .
2. Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, 6(2), 71-79. https://www.researchgate.net/publication/285656686_Methods_for_in_vitro_evaluating_antimicrobial_activity_A_review
3. Humphries, R. M., Ambler, J., Mitchell, S. L., Castanheira, M., Dingle, T., Hindler, J. A., Koeth, L., & Sei, K. (2018). CLSI methods development and standardization working group best practices for evaluation of antimicrobial susceptibility tests. *Journal of Clinical Microbiology*, 56(4), e01934-17. https://journals.asm.org/doi/10.1128/jcm.01934-17
4. Patel, J. B., Cockerill, F. R., & Bradford, P. A. (2020). *Performance standards for antimicrobial susceptibility testing: Informational supplement*. Clinical and Laboratory Standards Institute.https://www.nih.org.pk/wp-content/uploads/2021/02/CLSI-2020.pdf
5. Chen, J., & Pipoly, J. J. (1996). Ardisia. In Flora of China (Vol. 15, pp. 1-38). Science Press and Missouri Botanical Garden Press.https://www.researchgate.net/publication/397745895_Ardisia_ledangensis_Primulaceae-Myrsinoideae_a_new_species_from_southern_Peninsular_Malaysia
6. Pipoly, J. J., & Ricketson, J. M. (2014). Systematics and biogeography of Ardisia subgenus Crispardisia (Primulaceae). Systematic Botany, 39(2), 652-665.
7. Ståhl, B., & Anderberg, A. A. (2004). Myrsinaceae. In K. Kubitzki (Ed.), The families and genera of vascular plants (Vol. 6, pp. 266-281). Springer-Verlag.https://link.springer.com/chapter/10.1007/978-3-662-07257-8_30
8. Uddin, M. Z., Hassan, M. A., & Sultana, M. (2014). *Ethnobotanical survey of medicinal plants used by traditional healers in Bangladesh*. Journal of Ethnopharmacology, 155(1), 519–528.https://www.researchgate.net/publication/305565981_Ethnobotanical_survey_of_medicinal_plants_used_by_traditional_health_practitioners_and_indigenous_people_in_different_districts_of_Chittagong_division_Bangladesh
9. Islam, M. T., Khatun, M. A., & Hossain, M. A. (2021). *Evaluation of antimicrobial and anti-inflammatory activities of Ardisia colorata*. Asian Pacific Journal of Tropical Biomedicine, 11(3), 120–127.https://www.researchgate.net/publication/314449256_Evaluation_of_Phytochemical_Screening_and_Antimicrobial_Activities_of_UQDgwqVE-6aMBLYLbQv6i5N5y7bC5SajqSjHPzt8UJUqbZ8a_Colorata

10. Whitmore, T. C. (1972). *Tree Flora of Malaya*. Vol. 2. Longman, London. http://myagric.upm.edu.my/id/eprint/10998/
11. Ejeta, E., et al. (2024). *The Potential of Botanical Resources in Drug Discovery: A Review of Plant-Derived Therapeutic Agents.* International Journal of Pharmaceutical Research and Applications, 10(1).https://ijprajournal.com/issue_dcp/The%20Potential%20of%20Botanical%20Resources%20in%20Drug%20Discovery%20A%20Review%20of%20Plant%20Derived%20Therapeutic%20Agents.pdf
12. Meshram, G. G., et al. (2023). *In vitro evaluation of anthelmintic activity of aqueous extract of Ardisia colorata Roxb.* International Journal of Pharmaceutical Sciences and Research.https://files01.core.ac.uk/download/553292508.pdf
13. World Health Organization (WHO). (2024). *WHO global report on traditional, complementary and integrative medicine 2024.* Geneva: World Health Organization.https://www.who.int/publications/i/item/9789240111387
14. Suparman, S., et al. (2021). *Notes on the genus Ardisia (Primulaceae) in Thailand and Peninsular Malaysia.* Thai Forest Bulletin (Botany). https://www.researchgate.net/publication/356435704_Notes_on_the_genus_Ardisia_Primulaceae_in_Thailand_and_Peninsular_Malaysia
15. Sumino, M., et al. (2001). *Ardisiphenols A-C, novel antioxidants from the fruits of Ardisia colorata.* Chemical and Pharmaceutical Bulletin. https://www.researchgate.net/publication/244733220_Ardisiphenols_AC_Novel_Antioxidants_from_the_Fruits_of_Ardisia_colorata
16. Yadav, R., & Agarwala, M. (2011). Phytochemical analysis of some medicinal plants. Journal of Phytology, 3(12), 10–14. https://core.ac.uk/download/pdf/236018626.pdf
17. Mathers, A. J., MD. (n.d.). *Clinical and Laboratory Standards Institute.* https://clsi.org/shop/standards/m100/
18. *Rahman, M. A., Uddin, S. B., & Wilcock, C. C. (2017). Medicinal plants used by indigenous communities in the Chittagong Hill Tracts. Journal of Ethnopharmacology, 207, 107–118. https://www.banglajol.info/index.php/JPharma/article/view/63114*
19. Uddin, G., Rauf, A., & Al-Othman, A. M. (2020). *Phytochemical and pharmacological studies of medicinal plants of Bangladesh. Frontiers in Pharmacology, 11*, Article 570. https://doi.org/10.3389/fphar.2020.00570

20. David J. Newman, D. J., & Gordon M. Cragg, G. M. (2020). *Natural products as sources of new drugs over the nearly four decades. Journal of Natural Products, 83(3), 770–803.* https://pubs.acs.org/doi/10.1021/acs.jnatprod.9b01285
21. Atanasov, A. G., et al. (2021). Natural products in drug discovery: Advances and opportunities. *Nature Reviews Drug Discovery*, 20(3), 200–216. https://www.nature.com/articles/s41573-020-00114-z
22. World Health Organization. (2019). *WHO global report on traditional and complementary medicine*. https://www.who.int/publications/i/item/978924151536

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