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Original Article

In vitro Screening of *Litsea monopetala* (Roxb.) Pers. (*Lauraceae*) Extract: Potential Antioxidant, Cytotoxic, Thrombolytic, and Anti-Inflammatory Properties

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Abstract: *Litsea monopetala* (Roxb.) Pers. is a plant of significant ethnobotanical importance, and in this study, we conducted a comprehensive investigation into its potential bioactive properties. Different fractions of the ethyl acetate (EtOAc) crude extract of the plant leaves were evaluated for antioxidant, cytotoxicity, thrombolytic, and anti-inflammatory activities, *in vitro*. The hexane-soluble fraction (LMH) exhibited notable antioxidant activity with IC_{50} value of 5.44 µg/ml and cytotoxicity with LC_{50} value of 1.10 µg/ml in DPPH radical scavenging assay and Brine shrimp lethality bioassay, respectively. Moreover, Dichloromethane soluble fraction (LMD) exhibited the highest thrombolytic activity, achieving an impressive rate of 31 %, compared to the standard Streptokinase (45 %). Additionally, LMD demonstrated a significant inhibition of 36 % of hemolysis, while LMH exhibited a slightly lower inhibition rate of 33 %, while the standard drug acetyl salicylic acid achieved a 47 % inhibition of hemolysis, suggesting their relevance in the context of inflammation-related disorders. These results underscore the multifaceted pharmacological properties of *Litsea monopetala* and provide a solid foundation for further research, including the isolation and characterization of bioactive compounds, elucidation of underlying mechanisms, and potential applications in drug development and healthcare.

Keywords: *Litsea monopetala*; Cytotoxicity; DPPH; Antioxidant; Thrombolytic; Anti-inflammatory;

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1. Introduction

Oxidative stress arises when there is an imbalance between the protective functions provided by the body's antioxidant system and the generation of reactive

oxygen species (ROS). Reactive oxygen species are produced as a natural part of cellular processes. However, when their production surpasses the body's ability to effectively counteract them through antioxidants, they can lead to the oxidative damage of various components, including blood vessel walls, carbohydrates, DNA, lipids, and other molecules [1]. The utilization of synthetic antioxidants for medical treatments has been restricted due to their significant adverse reactions. As a result, contemporary scientists have shifted their focus to natural antioxidants, with a particular emphasis on phenolic compounds, which have garnered substantial attention. These natural antioxidants exhibit promise in the advancement of therapeutic approaches that do not produce harmful side effects [2,3].

Cancer represents a significant global public health challenge, with an approximate 18.1 million new cases and 9.6 million cancer-related deaths reported in the year 2018 [4]. According to a World Health Organization (WHO) report, there are presently 14 million individuals worldwide who have been diagnosed with cancer, and the number of fatal cases totals 8 million [5]. Many cancer patients begin exploring alternative approaches, such as herbal remedies, due to the elevated mortality rates associated with cancer and the potentially harmful side effects of conventional anticancer treatments [6].

The development of blood clots (thrombi) in arteries, stemming from disruptions in the body's natural equilibrium, can lead to blockages in coronary arteries, resulting in severe consequences like acute heart attacks and strokes. Thrombolytic drugs are employed to dissolve these blood clots, a process known as thrombolysis [7]. There is a growing need for natural sources of thrombolytic agents because they hold the potential to offer safer, more cost-effective alternatives to synthetic drugs, potentially with fewer side effects. Additionally, natural sources can provide a sustainable and renewable supply of therapeutic agents.

Inflammation is the complex biological response of body tissues, often involving a cascade of events that includes the activation of various cellular enzymes, subsequent membrane damage, and overall tissue dysfunction. Consequently, focusing on the stabilization of cell membranes may be a vital strategy in addressing inflammation and its related consequences [8]. Numerous drugs, including steroids, nonsteroidal anti-inflammatory drugs, and immunosuppressants, are available to manage and reduce inflammatory crises. However, these medications often come with adverse effects. Our objective is to use the lowest effective dose that provides maximum efficacy with minimal side effects. To achieve this, incorporating natural anti-inflammatory agents into treatment can enhance the drug's effectiveness while minimizing unwanted side effects [9].

Presently, approximately 88 % of the world's population, roughly 3.5 billion people, are utilizing herbal remedies from medicinal plants. This extensive utilization underscores the importance of finding new compounds with potent healing attributes and few side effects. These compounds could have a significant impact on addressing diverse health issues like oxidative stress, cancer, thrombosis, and inflammation. Consequently, the exploration of novel bioactive substances from medicinal plants remains essential, expanding treatment possibilities and enhancing global healthcare outcomes [10].

Litsea monopetala (Roxb.) Pers., a member of the *Lauraceae* family, is a small tree that can reach heights of up to 18 meters. It is primarily found in Nepal but can also be located in other Asian countries, including India and Bangladesh. In Bangladesh, it is known as "khara jora," while in Malaysia, it goes by "medang busok" and "bangang." Indonesians refer to it as "huru koneng," Thais as "kathang," and in Myanmar, it's called "ondon laukya." In northeast India, it's referred to as "sualu," and in Tamil, it's known as "maidalagadil." It holds significance in Ayurvedic medicine, where in Sanskrit, it's named "maidaa-lakdi." In Ayurveda, the bark is recognized for its stimulant, astringent, spasmolytic, and antidiarrheal properties, while the roots are applied externally to alleviate pains, bruises, and contusions [11].

In Nawalparasi District, Nepal, the seeds are employed to alleviate stomach aches [12]. In Bangladesh, the Naik clan of the Rajbongshi tribe uses the bark to address constipation [13], while the Bongshi tribe utilizes both leaves and bark to combat chronic severe fever [14]. Folk medicinal practitioners in Dinajpur District, Bangladesh, turn to the plant's leaves for treating bone fractures in cattle [15]. Additionally, in Sreemangal, Maulvibazar District, Bangladesh, tribal tea workers rely on the leaves to manage jaundice accompanied by fever [16]. These diverse applications of the plant highlight its significance in folk medicine across different cultures.

In the present investigation, a comprehensive assessment was conducted to elucidate the potential bioactive properties of the plant extract. Specifically, the study encompassed an evaluation of antioxidant, anticancer, thrombolytic, and anti-inflammatory activities. The primary objective was to discern whether the plant harboured latent bioactivity beyond its established traditional usage and previously documented research findings.

2. Materials and Methods

2.1 Sample Collection and Preparation

Whole plant of *L. monopetala* were collected in Gazipur, Bangladesh, and a voucher specimen was preserved. Authentication was conducted by an expert at the Bangladesh National Herbarium (BNH). Leaves were meticulously harvested, cleaned to remove impurities, and then subjected to two weeks of shaded drying with proper ventilation, followed by additional sun drying. Subsequently, a high-capacity grinding machine was used to finely grind the leaves into approximately 1000 grams of coarse powder.

2.2 Drugs and Chemicals

All the chemicals and solvents were sourced from reputable suppliers, including Active Fine Chemicals Ltd. in Bangladesh, Merck in Germany, and DaeJung in Korea. Specific compounds, such as Tert-butyl-1-hydroxytoluene (BHT), vincristine sulfate (VS), streptokinase (SK), and acetylsalicylic acid (AcSA), were acquired from Opsonin Pharma Ltd., a pharmaceutical company located in Bangladesh.

2.3 Experimental Design

2.3.1 Extraction of Plant Material

The coarse plant powder was placed in clean, 3-liter amber bottles. EtOAc was added to fully immerse the powder, and this mixture was soaked for 18 days with regular agitation. Afterward, the mixture underwent filtration through a cotton plug and Whatman No.1 filter paper. The resulting crude extract was then concentrated using low-temperature evaporation, keeping the temperature below 40°C and maintaining proper pressure. The final concentrated crude extract was precisely weighed for measurement.

2.3.2 Preparation of Different Partitions for Biological Tests

The partitioning of the crude EtOAc extract followed a method initially devised by Kupchan and later modified by Van Wagenen et al [17]. It involved fractionation using hexane, dichloromethane (DCM), and ethyl acetate solvents, resulting in three distinct fractions: hexane-soluble (LMH), DCM-soluble (LMD), and ethyl acetate-soluble (LME). Each fraction was separately evaporated using a rotary evaporator.

2.4 Antioxidant Assay

2.4.1 DPPH free radical scavenging assay

To assess the free radical scavenging activity of plant extracts, we prepared a mixture by combining 3.0 ml of a 20 g/ml DPPH methanol solution with 2.0 ml of a plant extract solution at various concentrations, ranging from 500 g/mL to 0.977 g/mL. This experiment aimed to gauge the plant extracts' capacity to counteract 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals. We compared the fading of the purple DPPH methanol solution induced by the plant extract with the fading caused by BHT, which served as a reference compound [18].

$$\%\ Inhibition\ of\ free\ radical\ DPPH = \left(1 - \frac{Absorbance\ of sample}{Absorbance\ of the\ control\ reaction}\right) x\ 100$$

2.4.2 Total phenolic content

To determine the total phenolic content, we employed the Folin-Ciocalteu Reagent (FCR) as an oxidizing agent and used gallic acid as the reference standard, following the protocol outlined by Harbertson et al. Specifically, 2.5 ml of FCR was mixed with 2 ml of Na_2CO_3 with 0.5 ml of an extract solution (2 mg/ml). After a 20-minute incubation at room temperature, absorbance values were recorded at 760 nm using a UV spectrophotometer. By analyzing a standard curve generated with gallic acid at various concentrations, we calculated the total phenol content, expressed in milligrams of gallic acid equivalent (GAE) per gram of extract [19].

2.5 Cytotoxicity Assay

2.5.1 Brine Shrimp Lethality Bioassay

To assess potential cytotoxicity, we conducted a brine shrimp lethality test. An artificial saltwater solution was prepared by dissolving 38 g of NaCl in 1000 mL of distilled water, with the pH adjusted to 8.0 using NaOH. Brine shrimp eggs were incubated in this solution to hatch into nauplii. Test samples and the reference standard (vincristine sulfate) were prepared in diluted dimethylsulphoxide (DMSO) at various concentrations (ranging from 400 µg/mL to 0.78125 µg/mL). After visually counting the nauplii, each vial received 5 ml of simulated saltwater, exposing the nauplii to the test compounds. Cytotoxic effects were assessed based on their impact on the brine shrimp nauplii [20].

2.6 In vitro Thrombolytic Assay

The study followed the methodology outlined by Bhowmick et al [21]. 10 ml of venous blood was collected from healthy volunteers and distributed it into

pre-weighed, sterile Eppendorf tubes (0.5 ml per tube). These tubes were incubated at 37°C for 45 minutes, allowing the blood to clot. Following incubation, all the serum was meticulously removed that had separated from the clots, leaving only the clots in each tube. Subsequently, we re-weighed the tubes to determine the clot weights.

$$Clot\ weight = weight\ of\ clot\ containing\ tube - weight\ of\ the\ tube\ alone$$

In the tubes containing the clots, 100 µl of the extract solutions were added. As a positive control, a separate tube received 100 µl of streptokinase, while each individual tube received 100 µl of distilled water. Subsequently, all the tubes, including both control and test samples, were incubated at 37°C for 90 minutes. The difference in weight before and after clot lysis was calculated, expressing this difference as a percentage of clot lysis. This percentage indicated the extent to which the clots were dissolved or lysed by the respective samples.

2.7 In vitro Anti-inflammatory Assay (Membrane Stabilizing Activity)

2.7.1 Heat-induced Haemolysis

Two groups of centrifuge tubes were prepared, each containing 5 ml of isotonic buffer with a plant extract concentration of 2.0 mg/ml. In a separate tube, an equal volume of a vehicle was added as a control. To each tube, 30 µl of erythrocyte suspension was added and gently mixed by inverting the tubes. One group of tubes was incubated at 54°C for 20 minutes in a water bath, while the other group was maintained at a temperature between 0 and 5°C using an ice bath. After the incubation period, the reaction mixture was centrifuged at 1300 g for 3 minutes. The resulting supernatant was then analyzed by measuring its absorbance at 540 nm using a UV spectrometer. The equation provided was utilized to determine the percentage inhibition of hemolysis or membrane stabilization [22].

$$\%\ inhibition\ of\ hemolysis\ = (1 - \frac{OD2 - OD1}{OD3 - OD1}) \times 100$$

Where, OD1 = test sample unheated; OD2 = test sample heated; and OD3 = control sample heated

3. Results

3.1 Effect of L. monopetala extracts on DPPH Free Radical Scavenging Activity

The study aimed to evaluate the antioxidant potential of various extracts from *L. monopetala* in neutralizing free radicals. The results indicated that the extracts demonstrated increased free radical scavenging ability with rising concentrations, displaying a dose-dependent trend. Notably, at a concentration of 500 µg/mL, LMH exhibited the highest scavenging activity (93.85 %) with IC_{50} value of 5.44 µg/mL, followed by LMD (92.92 %) with IC_{50} value of 12.69 µg/mL.

The percentage inhibition of the standard was calculated at 92.96 %. The determination of IC_{50} values for both BHT and the fractions utilized linear regression equations, as depicted in Figure **1**.

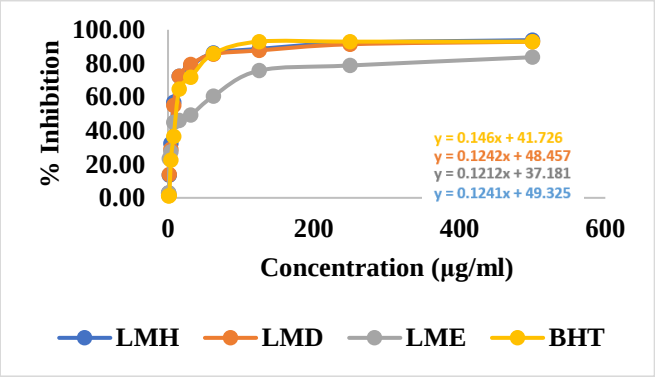

Figure 1: Percentage inhibition and the regression line prediction of BHT and various extracts of *L. monopetala*.

3.2 Total Phenolic Content

To determine the total phenolic content of different fractions of *L. monopetala*, we conducted a test using Folin-Ciocalteu reagent. The absorbance values of the various fraction solutions were utilized for colorimetric analysis, comparing them to the standard curve of gallic acid equivalents. The total phenolic content of each sample was expressed in milligrams of gallic acid equivalent (GAE) per gram of extract, as presented in **Table 1**. Among all the extracts, LMH displayed the highest phenolic content (13.45 mg of GAE/g of extract), followed by LME (3.5 mg of GAE/g of extract). LMD exhibited the lowest phenolic content (0.16 mg of GAE/g of extract).

Table 1: Total Phenolic Content (mg of GAE/gm Extract) of Different Fractions of *Litsea Monopetala*

Plant Part	Sample Code	Absorbance	Total phenolic content (mg of GAE/ gm of extract)
Leaves	LMH	1.03	13.45
	LMD	0.375	0.16
	LME	0.5175	3.05

3.3 Effect of L. monopetala extracts on Brine Shrimp Lethality Bioassay

In the brine shrimp lethality test, it was noted that different extracts of *L. monopetala* displayed a dose-dependent escalation in mortality rates compared to the standard substance. Notably, the LMD extract showed the highest mortality percentage, with an IC_{50} value of 1.10 compared to that of standard (0.88) **(Figure 2)**

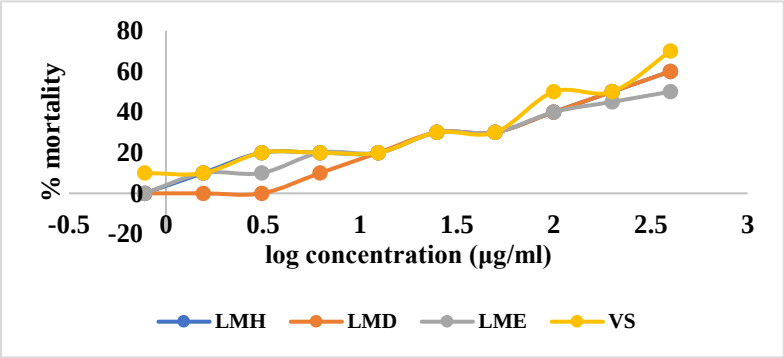

Figure 2: % Mortality and predicted regression line of vincristine sulphate and different extracts of *L. monopetala.*

3.4 Effect of L. monopetala extracts on Thrombolytic Assay

The study aimed to explore the potential cardio-protective properties of extracts obtained from *L. monopetala* by assessing their thrombolytic activity. The findings, as detailed in **Table 2**, revealed that among the tested extracts, LMD derived from the methanolic extract of the plant demonstrated the highest thrombolytic activity, achieving a rate of 31 %. In contrast, LMH exhibited the lowest thrombolytic activity, with a rate of 17 %. **Figure 3** provides a visual comparison of the thrombolytic activities of these various extracts.

Table 2: Effects of different fractions of *L. monopetala* crude extract on thrombolytic and membrane stabilizing assay.

Sample	% clot lysis	% inhibition of heat-induced hemolysis
LMH	17 %	33 %
LMD	31 %	36 %
LME	27 %	7 %
SK	45 %	-
AcSA	-	47 %

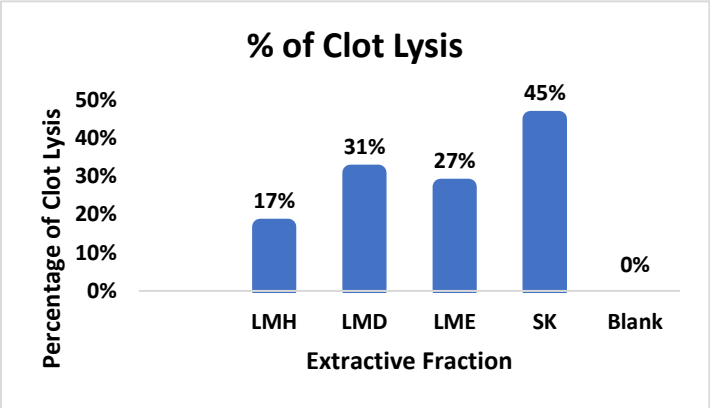

Figure 3: Percentage clot lysis of different fractions of methanolic crude extract of *L. monopetala*.

3.5 Effect of L. monopetala extracts on Membrane Stabilizing Assay

The methanolic extract and its various fractions from *L. monopetala* were found to possess effective membrane-stabilizing activity, preventing the lysis of erythrocytes induced by heat. Among these fractions, LMD inhibited 36 % of hemolysis, and LMH inhibited 33 % inhibition **(Table 2)**. As a point of reference, Acetyl Salicylic Acid, used as the standard drug for assessing membrane stabilizing activity, displayed a 47 % inhibition of hemolysis under normal conditions.

4. Discussion

Synthetic drugs frequently come with a higher likelihood of side effects, making them intolerable at elevated doses for certain individuals. In contrast, traditional medicinal plants have played a vital role in treating numerous ailments, even though the precise mechanisms behind their effectiveness remain elusive. Throughout history, plant extracts have been employed to address various infections, and herbal remedies continue to enjoy popularity due to their cost-effectiveness and milder nature in comparison to synthetic medications [23].

Our study was conducted to investigate the antioxidant, cytotoxic, thrombolytic, and anti-inflammatory properties of different fractions of the EtOAc crude extract of the plant through *in vitro* experiments. Our quantitative phytochemical analysis revealed that LMH has a total phenolic content (TPC) of 13.45 milligrams of gallic acid equivalents per gram (mg GAE/g). Additionally, various fractions of the plant exhibited varying degrees of potential in terms of antioxidant, cytotoxic, thrombolytic, and anti-inflammatory activities.

Phenolic compounds have been directly linked to antioxidant, and anti-inflammatory activities. These compounds have been demonstrated to possess a wide range of beneficial properties, including analgesic, antibacterial, antioxidant, anticancer, anti-inflammatory, and antimicrobial effects [24,25]. The *Litsea* genus

is known for being a rich source of biologically-active compounds, including butanolides found in the leaves of *Litsea acutivena* [26], flavonoids present in the leaves of *Litsea coreana* and *Litsea japonica* [27], sesquiterpenes found in the leaves and twigs of *Litsea verticillate* [28], and essential oils extracted from the leaves of *Litsea cubeba*, as well as from the fruits, flowers, and bark of *Litsea monopetala*, and the fruits of *Litsea glutinosa* [29]. This suggests that *L. monopetala* might possess a significant reducing capacity and the capability to interrupt free radical chain reactions due to presence of the flavonoids.

Medicinal plants are valuable resources for discovering new chemotherapeutic agents. The brine shrimp lethality assay is a method used to assess the lethality of compounds, making it a quick, straightforward, and readily accessible bioassay technique. It is particularly useful for evaluating the potential anticancer and cytotoxic properties of compounds and can serve as a reference for assessing the toxicity of pesticides and substances with antiviral, antibacterial, antimalarial, and antitumor properties [30]. We observed notable toxicity for our plant extract when compared to the standard drug vincristine sulfate. These toxic effects may be attributed to the secondary metabolites generated by the bioactive compounds within our plant extract, some of which have previously been linked to cytotoxicity [31].

Ischemic stroke, which is often the leading cause of death in individuals over 60 worldwide, is typically the result of a cerebral artery blockage caused by an embolus or local thrombus. Streptokinase was the first thrombolytic agent introduced for clinical use and has demonstrated significant effectiveness. However, it also comes with limitations, notably an increased risk of hemorrhagic complications due to the degradation of circulating fibrinogen and factors V and VII.

Thrombolytic agents function by activating the plasminogen enzyme, which in turn breaks down cross-linked fibrin networks, leading to the formation of soluble clots. Additionally, these agents initiate various proteolysis activities involving several enzymes, ultimately aiming to restore blood flow that has been obstructed by occlusions [32]. Different fractions of the extract demonstrated a moderate level of thrombolytic activity when compared to the positive control, streptokinase. This observation could be attributed to the presence of bioactive secondary metabolites within the extract.

Medications like corticosteroids (steroids) and non-steroidal anti-inflammatory drugs (NSAIDs) exert their anti-inflammatory effects by stabilizing cell membranes. It's crucial to recognize, though, that these drugs can come with significant side effects [33]. Given the context, investigating natural products that possess the capacity to stabilize cell membranes may be a more

attractive option when seeking anti-inflammatory treatments. LMD and LMH fractions of the plant studied in this research exhibited remarkable membrane-stabilizing abilities against both heat-induced hemolysis. This makes the plant a highly promising candidate for further exploration and investigation as a potential source of anti-inflammatory compounds.

5. Conclusion

In summary, our bioactivity analysis of *Litsea monopetala* has led to the demonstration of remarkable antioxidant, cytotoxic, thrombolytic, and anti-inflammatory properties. These findings underscore the significant potential of the plant as a valuable source of bioactive terpenoids with diverse pharmacological activities. This study paves the way for exciting future research endeavors. Firstly, further investigations are needed to uncover the phytochemicals along with their precise molecular mechanisms responsible for the observed bioactivities.

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References

1. Hayes, J.D., Dinkova-Kostova, A.T., Tew, K.D. Oxidative stress in cancer. *Cancer Cell* 2020; 38: 167–197.
2. Chang, X., Zhang, T., Zhang, W., Zhao, Z., Sun, J. Natural drugs as a treatment strategy for cardiovascular disease through the regulation of oxidative stress. *Oxid. Med. Cell. Longev.* 2020; 5430407.
3. Forman, H.J., Zhang, H. Targeting oxidative stress in disease: promise and limitations of antioxidant therapy. *Nat. Rev. Drug Discov.* 2021; *20:* 689–709.
4. Luo, H., Vong, C.T., Chen, H., Gao, Y., Lyu, P., Qiu, L., Zhao, M., Liu, Q., Cheng, Z., Zou, J. Naturally occurring anti-cancer compounds: shining from chinese herbal medicine. *Chin. Med.* 2019; 14: 48.
5. Kooti, W., Servatyari, K., Behzadifar, M., Asadi-Samani, M., Sadeghi, F., Nouri, B., Zare

Marzouni, H. Effective medicinal plant in cancer treatment, Part 2: Review Study. *J. Evid. Based. Complementary Altern. Med.* 2017; *22*: 982–995.

6. Samouh, Y., Lemrani, A., Mimouni, H., Mohamad, J., Said, A.A.H. Ethnopharmacological study of herbal medicines used to treat cancer in Morocco. *J. Phytopharm.* 2019; 8: 135–141.
7. Leite, P.M., Martins, M.A.P., das Graças Carvalho, M., Castilho, R.O. Mechanisms and interactions in concomitant use of herbs and warfarin therapy: an updated review. *Biomed. Pharmacother.* 2021; 143: 112103.
8. Greten, F.R., Grivennikov, S.I. Inflammation and cancer: triggers, mechanisms, and consequences. *Immunity* 2019; 51: 27–41.
9. Ghasemian, M., Owlia, S., Owlia, M.B. Review of anti-inflammatory herbal medicines. *Adv. Pharmacol. Pharm. Sci.* 2016; 2016: 9130979.
10. Shahidullah, A.K.M., Haque, C.E. Linking medicinal plant production with livelihood enhancement in Bangladesh: implications of a vertically integrated value chain. *J. Transdiscipl. Environ. Stud.* 2010; 9: 1-18.
11. Hanif Hasan, M., Islam, M.Z., Rahman, S.M., Islam, M.R., Rahman, S., Rahmatullah, M. Antihyperglycemic activity of methanolic extract of *litsea monopetala* (roxb.) pers. leaves. *Adv. Nat. Appl. Sci.* 2014; 8: 51–55.
12. Bhattarai, S., Chaudhary, R.P., Taylor, R.S.L. Ethno-medicinal plants used by the people of Nawalparasi district, central Nepal. *Our Nat.* 2009; 7: 82–99.
13. Mou, S.M., Mahal, M.J., Bhuiyan, P., Zakaria, A.S.M., Datta, B., Rana, M., Islam, A., Khatun, Z., Rahmatullah, M. Medicinal plants and formulations of small tribes of Bangladesh: a case study of the naik clan of the Rajbongshi tribe. *Am-Eur J Sustain Agr* 2012; *6*: 248–253.
14. Rahmatullah, M., Ferdausi, D., Mollik, A., Jahan, R., Chowdhury, M.H., Haque, W.M. A survey of medicinal plants used by Kavirajes of Chalna area, Khulna district, Bangladesh. *African J. Tradit. Complement. Altern. Med.* 2010; 7(2): 91–97.
15. Islam, M.A., Yeasmin, M., Rahmatullah, M. Ethnoveterinary practices among folk medicinal practitioners of three randomly selected villages of Dinajpur district, Bangladesh. *Am. J. Sustain. Agric.* 2013; 7: 75–84.
16. Kabir, M.H., Hasan, N., Rahman, M.M., Rahman, M.A., Khan, J.A., Hoque, N.T., Bhuiyan, M.R.Q., Mou, S.M., Rahmatullah, M. Tribal medicine in tribes who have lost their identities: medicinal plants of tea garden workers in Sreemangal, Maulvibazar district, Bangladesh. *Am-Eur. J. Sustain. Agr.* 2013; 7: 251–258.
17. VanWagenen, B.C., Larsen, R., Cardellina, J.H., Randazzo, D., Lidert, Z.C., Swithenbank, C. Ulosantoin, a potent insecticide from the sponge ulosa ruetzleri. *J. Org. Chem.* 1993; 58: 335–337.
18. Singh, T.P., Singh, O.M. Recent progress in biological activities of indole and indole alkaloids. *Mini Rev. Med. Chem.* 2018; 18: 9–25.
19. Harbertson, J., Viticulture, Spayd S Measuring phenolics in the winery. *Am. J. Enol. Vitic.* 2006; 57: 280–288.
20. Jasiewicz, B., Kozanecka-Okupnik, W., Przygodzki, M., Warżajtis, B., Rychlewska, U.,

Pospieszny, T., Mrówczyńska, L. Synthesis, antioxidant and cytoprotective activity evaluation of c-3 substituted indole derivatives. *Sci. Reports* 2021; 11: 1–14.

21. Bhowmick, R., Sarwar, M.S., RahmanDewan, S.M., Das, A., Das, B., Nasir Uddin, M.M., Islam, M.S., Islam, M.S. *In vivo* analgesic, antipyretic, and anti-inflammatory potential in swiss albino mice and *in vitro* thrombolytic activity of hydroalcoholic extract from *litsea glutinosa* leaves. *Biol. Res.* 2014; 47: 1–8.
22. Islam, T., Das, A., Shill, K.B., Karmakar, P., Islam, S., Sattar, M.M. Evaluation of membrane stabilizing, anthelmintic, antioxidant activity with phytochemical screening of methanolic extract of *Neolamarckia cadamba* fruits. *J. Med. Plants Res.* 2015; 9: 151–158.
23. Shovo, M.A.R.B., Tona, M.R., Mouah, J., Islam, F., Chowdhury, M.H.U., Das, T., Paul, A., Ağagündüz, D., Rahman, M.M., Emran, T. Bin computational and pharmacological studies on the antioxidant, thrombolytic, anti-inflammatory, and analgesic activity of *Molineria capitulata*. *Curr. Issues Mol. Biol.* 2021; 43: 434–456.
24. Liang, Y.-C., Huang, Y.-T., Tsai, S.-H., Lin-Shiau, S.-Y., Chen, C.-F., Lin, J.-K. Suppression of inducible cyclooxygenase and inducible nitric oxide synthase by apigenin and related flavonoids in mouse macrophages. *Carcinogenesis* 1999; 20: 1945–1952.
25. Uddin, M.M.N., Ahmed, S., Kabir, M.S.H., Rahman, M.S., Sultan, R.A., Emran, T. B. *In vivo* analgesic, anti-inflammatory potential in swiss albino mice and *in vitro* thrombolytic activity of hydroalcoholic fruits extract from *Daemonorops Robusta* Warb. *J. Appl. Pharm. Sci.* 2017; 7: 104–113.
26. Aimaiti, S., Saito, Y., Fukuyoshi, S., Goto, M., Miyake, K., Newman, D.J., O'Keefe, B.R., Lee, K.-H., Nakagawa-Goto, K. Isolation, structure elucidation, and antiproliferative activity of butanolides and lignan glycosides from the fruit of *Hernandia Nymphaeifolia*. *Molecules* 2019; 24: 4005.
27. Ji, S.Y., Bang, E., Hwangbo, H., Kim, M.Y., Kim, D.H., Koo, Y.T., Kim, J.S., Lee, K.W., Park, S.Y., Kwon, C.-Y. Improvement of immune and hematopoietic functions by *Litsea japonica* fruit extract in cyclophosphamide-treated BALB/c mice. *Appl. Sci.* 2022; 13: 145.
28. Hossen, M.F., Hossain, S., Ahamed, M.I., Patwary, M.S., Imtiaz, O., Hasan, M., Al Mahmud, A. Evaluation of *in vivo* analgesic, antiemetic and anxiolytic effect of methanolic extract of *Litsea monopetala* in animal model. *Discov. Phytomedicine* 2019; 6: 126–129.
29. Tripathi, P., Yami, H., Shukla, A.K. Determination of antifungal activity against phytopathogenic fungi by essential oils extracted from some medicinal plants. *Biopestic. Int.* 2021; 17(2): 163-171.
30. Yesmin, S., Paul, A., Naz, T., Rahman, A.B.M., Akhter, S.F., Wahed, M.I.I., Emran, T. Bin, Siddiqui, S.A. Membrane stabilization as a mechanism of the anti-inflammatory activity of ethanolic root extract of choi (*Piper chaba). Clin. Phytoscience* 2020; 6: 1–10.
31. Khanal, S., Bhatt, B.D. Study on biological activity of *Litsea monopetala* from Panchthar district of Nepal. *J. Inst. Sci. Technol.* 2020; 25: 113–118.
32. Emran, T. Bin, Rahman, M.A., Uddin, M.M.N., Rahman, M.M., Uddin, M.Z., Dash, R., Layzu, C. Effects of organic extracts and their different fractions of five bangladeshi plants on *in vitro*

thrombolysis. *BMC Complement. Altern. Med.* 2015; 15: 1–8.

33. Poetker, D.M., Reh, D.D. A comprehensive review of the adverse effects of systemic corticosteroids. *Otolaryngol. Clin. North Am.* 2010; 43: 753–768.