Highly Anticancer and Moderate Thrombolytic Property of Accacia rugata of Mimosaceae Family

Bibi Humayra Khanam, Ahmed Rakib, Farhana Binta Faiz, Md. Giash Uddin, Mohammed Kamrul Hossain, Ramiz Ahmed Sultan

Department of Pharmacy, University of Chittagong, Chittagong, Bangladesh

Email address: humayra.khanam@cu.ac.bd (B. H. Khanam), rakib.pharmacy@cu@gmail.com (A. Rakib), farhanafaiz037@gmail.com (F. B. Faiz), giash.uddin@cu.ac.bd (M. G. Uddin), mhossain73@yahoo.com (M. K. Hossain), ramiz@cu.ac.bd (R. A. Sultan)

*Corresponding author

To cite this article: Bibi Humayra Khanam, Ahmed Rakib, Farhana Binta Faiz, Md. Giash Uddin, Mohammed Kamrul Hossain, Ramiz Ahmed Sultan. Highly Anticancer and Moderate Thrombolytic Property of Accacia rugata of Mimosaceae Family. Journal of Plant Sciences. Vol. 8, No. 1, 2020, pp. 12-16. doi: 10.11648/j.jps.20200801.12

Received: February 2, 2020; Accepted: February 26, 2020; Published: April 7, 2020

Abstract: Medicinal plants containing potent bioactive compound effective in treating many diseases exert different pharmacological action. This study designed to evaluate the cytotoxic and thrombolytic activity of Accacia rugata of Mimosaceae family. The cytotoxic and thrombolytic activity was evaluated by brine-shrimp lethality bioassay and in-vitro clot lysis method. Methanol, petroleum-ether, n-hexane, chloroform and dichloromethane fraction leaves (MEL, PETFL, n-HxFL, CHFL, DCMFL), methanol and n-hexane fraction of fruits (MEF, n-HxFF) and methanol fraction of bark (MEB) were used to evaluate cytotoxicity of the plant. Each extracts showed significant cytotoxic property. The LC50 values of MEL, PETFL, n-HxFL, CHFL, DCMFL, MEF, n-HxFF, MEB were observed 1.436, 0.039, 0.974, 0.626, 0.121, 0.176, 0.865, 0.081 µg/ml when compared to standard vincristine (positive control) which LC50 value was 0.049 µg/ml. In thrombolytic activity evaluation 20 mg/ml, 10 mg/ml, 5 mg/ml and 2.5 mg/ml dose of MEL showed 40.52 ± 2.91, 35.09 ± 2.71, 31.96 ± 2.02 and 24.91 ± 3.05% clot lysis respectively while 0.9% NaCl solution (negative control) and standard streptokinase (positive control) exhibited 7.41 ± 1.73% and 48.91 ± 3.52% of clot lysis. It can be assumed that different solvent extracts of A. rugata have important cytotoxic and thrombolytic activity as compared to standard compounds.

Keywords: Cytotoxic, Thrombolytic, Streptokinase, Vincristine, % of Clot Lysis, Acacia rugata

1. Introduction

Medicinal plants, being a healthy source of life have always been considered to be the mean of recuperation for various diseases [1]. According to world Health Organization (WHO), 80% population of developing countries depends on traditional medicine for their primary health care [2]. These secondary metabolites produced by plants can be used as templates to discover newer drugs [3]. Plants derived active compounds are established to be safer, efficient while synthetic drugs are feared to be used in chronic disease [4]. Approximately 20% of medicinal plants that have been used in pharmaceutical studies are useful in treating cancer, invasive aspergillosis and harmful diseases [5]. Since plant possess significant pharmacological activity, low toxicity profile, economic flexibility play role in exploring new disease, investigation for their medicinal properties have been performed [6].

Following cardiovascular disease cancer is a leading cause of mortality and morbidity which results from uncontrolled cell proliferation because of the inhibition of apoptotic process. Standing as a notorious disease of present world cancer responsible for human mortality in large case and approximately half of mortality occur in Asia [7]. Chemotherapy, radiotherapy and chemically derived drugs are the currently available treatments and cause a lot of strain and further damage to the patient t health. Therefore, researchers look forward to using alternative treatments and therapies against cancer [8]. Over the past 30 years Natural products have received increasing attention for their potential as novel cancer preventive and therapeutic agents [9, 10].
Because of its simplicity, cost effectiveness and small sample requirements [11].

Thrombosis in portal vein is one kind of venous thrombosis leading to hypertension and reduced blood supply to liver [12]. Cardiovascular diseases associated with thrombus formation are increasing in recent years at an alarming rate [13]. In UK, the rate annual death was reported 25000 because of thrombosis [14]. Thrombolytic therapy recognized as a treatment to alleviate dangerous clots in blood vessels, improve blood flow, and prevent damage to tissues and many organs. In general, Thrombolysis is applied as an emergency treatment which vanish blood clots, the underlying reason of heart attacks and ischemic strokes by feeding the heart and brain with clots [15]. Thrombolysis also may increase the risk of complications in pregnancy or aging, and in people with other conditions. A tiny risk of infection and a slight risk of an allergic reaction to the antithetical dye may arise in patient who sustains thrombosis [16]. Tissue plasminogen activator, streptokinase, urokinase, anti-streptokinase etc. are the commonly known thrombolytic drugs which exert their effect by dissolving the blood clot [17]. Though these thrombolytic drugs are wonderful clot lytics, they still some limitations such as need of large dose, limited fibrin specificity, tendency of bleeding, allergic reactions and resistance to intravenous t-Pa6 [18]. Because of these complications scientists are focusing on plants based active compounds to treat thrombus associated disease.

Acacia rugata (Lam.) is a species belonging to Mimosaceae family and its local name is Banritha. It is a large stragling shrub with more or less hooked prickles. Acacia rugata is used as medicinal plant in hill tracts of Bangladesh available in hill tracts of Bangladesh having purgative, anthelmintic, anti-diarrhoeal, emetic and diuretic activities. Seeds are usually used in childbirth to facilitate delivery. For leprous patches, prurigo, abscesses, eczema and bubos they are applied externally [19]. A literature overview of A. rugata showed that this plant is not studied for its biological activity still. Considering the traditional and local use of A. rugata, we designed the study to evaluate cytotoxic and thrombolytic activity by brine-shrimp lethality bioassay and in-vitro clot lysis method.

2. Material and Methods

2.1. Crude Plant Parts Collection and Identification

After evaluating the history of traditional use Acacia rugata was selected because of its wide use in the tribal communities for different purposes. Leaves, fruits and barks of Acacia rugata were collected from Bandarban Hill Districts, Bangladesh in April 2019. Each part of the plant then identified by Dr. Sheikh Bokhtear Uddin, Associate professor, Department of Botany, University of Chittagong, Chittagong, Bangladesh.

2.2. Processing for Powdered Plant Material

The collected plant parts were thoroughly cleaned with tap water and cut into small pieces and dried in the sun. The sun dried materials then processed with grinder to have coarse powder. The dried powdered material then kept air tight pot in a moist free place.

2.3. Test Reagents and Chemicals

All the chemicals and drugs used in these tests were of analytical grade. Vincristine sulphate and Streptokinase were used as standard drugs for brine shrimp cytotoxic activity and thrombolytic activity respectively and are collected from Beximco Pharmaceuticals Ltd, Bangladesh. Tween-80, Methanol, pet-ether, n-hexane, chloroform, dichloromethane, sea salt (NaCl), DMSO all solvents and reagents were analytical grade and obtained from local suppliers.

2.4. Preparation of Extract

Having the dried plant materials 400 mg of leaves powder, 500 mg of fruits powder and 800 gm of barks powder were soaked in 1.8 liters, 2 liters and 3 liters of methanol respectively and these bottles containing the soaked material were kept for 10 days at normal room temperature with 2-3 times shaking in a day. The crude extract of leaves, fruits and barks were filtered using fresh cotton plug and then by using Whatman no. 1 filter paper. The filtrated extract of each part were evaporated to have concentrated extracts using vacuum rotary evaporator. Total percentage yield of each plant part extract was calculated using the following equation [20].

\[
\% \text{ of yield of extract} = \left( \frac{W_1}{W_2} \right) \times 100
\]

Here, \( W_1 = \) Weight of extracted material and \( W_2 = \) Weight of original plant material used.

The resultant methanol extract of leaves was then partitioned by n-hexane, pet-ether, chloroform and dichloromethane and fruits extract partitioned by n-hexane respectively.

2.5. Assessment of Cytotoxic Activity

The cytotoxic activities of all the extracts of Acacia rugata were performed using brine shrimp nauplii following Mayer’s method [21-23]. Brine shrimp eggs (Artemia salina Leach) were collected and hatched in a tank containing 1 L of simulated seawater at 37°C and pH 8.4 supplying constant oxygen. The nauplii could hatch and mature for 2 days. 4 mg of MEL, PETFL, n-HxFL, CHFL, DCMFL, MEF, n-HxFF and MEB of A. rugata were taken and dissolved in 200 µl of pure dimethyl sulfoxide (DMSO) in separate vials to get stock solutions and concentration of these prepared solutions was 400 µg/mL. 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.781 µg/mL solutions prepared from stock solution for each extract by serial dilution. A vial containing 50 µL DMSO diluted to 5 mL was used as a measurements control.
Vincristine sulphate was used as positive control [11]. The formulated test solutions were applied to pre-marked vials with 10 live brine shrimp nauplii in 5 mL of simulated seawater, and incubated for 24 h. After 24 hours the number of survival of nauplii was counted and percentage of mortality was determined using the equation: \[ \text{mortality} = \frac{\text{no. of dead nauplii}}{\text{initial no. of live nauplii}} \times 100 \]

The criterion of toxicity for fractions were established according to Déciga-Campos et al. [24]; LC\(_{50}\) values > 1000 µg/mL (non-toxic), 500 ≤ 1000 µg/mL (weak toxicity) and <500 µg/mL (toxic).

### 2.6. Thrombolytic Activity

The thrombolytic activity of methanol leaves extract of A. rugata was evaluated with the method developed by Dagingawala [7]. 600 mg crude methanol leaves extract was taken and dissolved in 30 mL of 0.9% NaCl solution to make a stock solution of 20 mg/mL. This stock solution further used to prepare 10 mg/mL, 5 mg/mL, and 2.5 mg/mL solution by serial dilution. 5 mL of phosphate buffer was added to commercially available lyophilized streptokinase (1,500,000 I.U.) vial. So concentration of streptokinase became 30,000 I.U. and used as reference for standard for thrombolytic activity. Venous blood (n=10) was drawn from healthy human volunteers ensuring no history taking oral contraceptive or anticoagulant therapy and citrated with 3.1% sodium citrate solution. Then 500 µl was transferred in different pre weighed sterile micro centrifuge tube and incubated at 37 °C for 45 minutes for clotting to occur. After clot formation the serum was aspirated out and each tube containing clot was again weighed to calculate clot weight.

About 500 µl of different extract concentration, 0.9% NaCl (negative control) and streptokinase 30,000, I.U (positive control) was added to clot containing tubes (n=10). These tubes are then incubated for 90 minutes at 37 °C. The released fluid was then removed and tubes were again weighed. The weight difference between before and after clot lysis expressed as % of clot lysis.

\[
\text{Clot lysis} = \frac{\text{wt of released clot}}{\text{clot wt}} \times 100
\]

#### 2.7. Statistical Analysis

% of clot lysis expressed as mean ± SEM. One way ANOVA following Dunnett’s multiple comparison was used for statistical analysis. The statistical analysis was carried out in SPSS (version 20.0).

### 3. Results

#### 3.1. Assessment of Cytotoxic Activity

After 24 hour incubation with test and control samples, LC\(_{50}\) value was measured. The LC\(_{50}\) value for methanolic leaves extract (MEL), pet ether fraction of leaves (PEFL), n-hexane fraction of leaves (n-HxFF), chloroform fraction of leaves (CHFL), dichloromethane fraction of leaves (DCMFL), methanolic fruits extract (MEF), n-hexane fraction of fruits (n-HxFF), methanolic extract of bark (MEB), dichloromethane fraction of fruits (m-HxFF), dichloromethane fraction of leaves (n-HxFL), and methanol extract of bark (MEB) were found 1.436, 0.039, 0.974, 0.626, 0.121, 0.176, 0.865, 0.081 µg/mL respectively. The standard vincristine (positive control) showed cytotoxicity at 0.049 µg/mL and no mortality was found for the negative control did LC\(_{50}\) (µg/mL) less than 500 µg/mL consider as toxic for brine shrimp larvae. Each extract exhibited significant cytotoxic property where PEFL showed more toxicity than vincristine. The LC\(_{50}\) after treating the nauplii with standard, plants extracts is shown in Table 1.

#### 3.2. Thrombolytic Activity

In *in vitro* clot lysis method, after 90 minutes incubation at 37°C, minute clot lysis was observed when 500 µl of normal saline (negative control) added to clot tube (7.41±1.73%). Streptokinase (30,000, I.U) showed 48.91±3.52% clot lysis which is significantly (i.e., p<0.001) differ from negative control. Percent of clot lysis after treating the blood clot with streptokinase 30,000, I.U (positive control), control and plant extracts is shown in Table 2, 20 mg/mL, 10 mg/mL, 5 mg/mL, and 2.5 mg/mL of methanol leaves extract of A. rugata showed significant (p<0.001) clot lysis by 40.52±2.91%, 35.09±2.71%, 31.96±2.02%, 24.91±3.05% respectively. Here the methanol extract showed dose dependent significant thrombolytic activity.

#### Table 1. Screening of cytotoxic activity of the different fractions of A. rugata by using brine shrimp lethality bioassay.

| Sample      | Equation | \(R^2\) | LC\(_{50}\) (µg/mL) |
|-------------|----------|---------|----------------------|
| Vincristine  | \(y = 23.365 + 48.863\) | 0.935  | 0.049                |
| MEL         | \(y = 15.907 + 27.157\) | 0.856  | 1.436                |
| PEFL        | \(y = 22.350 + 49.119\) | 0.931  | 0.039                |
| n-HxFL      | \(y = 18.323 + 32.142\) | 0.947  | 0.974                |
| CHFL        | \(y = 12.886 + 41.924\) | 0.912  | 0.626                |
| DCMFL       | \(y = 15.101 + 48.161\) | 0.848  | 0.121                |
| MEF         | \(y = 20.538 + 46.379\) | 0.938  | 0.176                |
| n-HxFL      | \(y = 26.175 + 27.346\) | 0.914  | 0.865                |
| MEB         | \(y = 23.155 + 48.114\) | 0.952  | 0.081                |

#### Table 2. Effect of methanolic leaves extract of A. rugata on blood clot lysis.

| Group       | Concentration | % of clot lysis |
|-------------|---------------|-----------------|
| NaCl solution | 0.9%          | 07.41±1.73      |
| Streptokinase | 30,000 I.U.   | 48.91±3.52***   |
| MEL         | 20 mg/ml      | 40.52±2.91***   |
| MEL         | 10 mg/ml      | 35.09±2.71***   |
| MEL         | 5 mg/ml       | 31.96±2.02***   |
| MEL         | 2.5 mg/ml     | 24.91±3.05***   |

Values are represented as mean ± SEM (n=10). *p<0.05 compared with control done by one way ANOVA followed by Dunnett’s ‘t’-test.

### 4. Discussion

*A. rugata* has wide range of medicinal properties. This study revealed the cytotoxic and thrombolytic attributes of different fractions of *A. rugata* in vitro.

Medicinal plants are regarded as a vital source of phytoconstituents [25]. Moreover, as a result of the biodiversity plants possess different active principles and they produce different pharmacological activities on human body.
[26]. From the ancient time plants had been used for the treatment of many diseases and nowadays drug discovery from plants can be achieved following phytopharmacological investigation, which renewed the attention towards herbal medicines [27]. Though anticancer drugs should not exert anticancer property to the normal cells but they show toxicity towards rapidly growing cells [13]. Cytotoxicity studies are considered as a key factor for the identification of possible cytotoxicity of numerous substances, for instance, chemicals, plant extracts, and biologically active compound. That is why plant cytotoxicity studies have been regarded as a salient feature for research scientist [21]. From previous data, it has been cleared that, several species of the Acacia have possessed cytotoxic activity [28, 29]. The cytotoxic potential of A. rugata is highly potent and further investigation may lead to isolate the compound responsible for this crucial effect.

Failure of hemostasis results in the development of thrombus in the circulatory system which cause vascular blockage leading to serious consequences in atherothrombotic disease like myocardial infarction which at times leading to death [30]. Thrombolytic agents like reteplase alteplase, urokinase, streptokinase etc. are serine proteases which convert plasminogen into plasmin that further break down fibrinogen and fibrin and dissolve the clot. Research works have been undertaken to discover antithrombogenic agents of plant sources to combat and prevention of coronary heart disease and strokes [31]. A. rugata was evaluated for thrombolytic action and found with having moderate thrombolytic activity and such finding may have important implications in cardiovascular health.

5. Conclusion

Cancer is growing in an alarming rate in both developed and developing countries. Alternative treatments with naturally occurring plant derived anticancer agents are increasingly in demand. From this study it can be reached with a satisfactory conclusion that A. rugata has highly effective cytotoxic property and may lead to have bioactive compounds for anticancer drugs. Moreover, the plant possesses moderate dose dependent anti-thrombosis activity which may be future thrombolytic drugs source. This finding may be further explored for the discovery of anticancer lead compound.

Conflict of Interest

The authors do not have any conflict of interest. All the blood donors were informed about the blood withdrawal process and ethical rules were maintained before working with human blood.

Acknowledgements

Authors are thankful to Department of Pharmacy, University of Chittagong, for providing the laboratory facilities. Special thanks to the blood donors and Trishola Dutta for helping in this work. The authors are also thankful to the Taxonomist and Professor, Dr. Shaikh Bokhtear Uddin, Department of Botany, University of Chittagong, Bangladesh, for identifying plant.

References

[1] Sen N, Bulbul L, Hussein F, Amin MT. (2016). Assessment of thrombolytic, membrane stabilizing potential and total phenolic content of Typha elephantina Roxb. Journal of Medicinal Plants Research, 10: 609-675.

[2] Prema R, Sekar DS, Sekhar KB, Jeevanandham S. (2012). In vitro cytotoxicity study on combined plants extracts (Cissus quadrangularis and Aegle marmelos). European Journal of Experimental Biology, 2: 882-8.

[3] Wittstock U, Gershenson J. (2002). Constitutive plant toxins and their role in defense against herbivores and pathogens. Current Opinion in Plant Biology, 5 (4): 300-307.

[4] Calixto JB. (2000). Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). Brazilian Journal of Medical and Biological Research, 33 (2): 179-189.

[5] Altemimi A, Lakhssassi N, Baharouei A, Watson DG, Lightfoot DA. (2017). Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. Plants, 6 (4): 42.

[6] Rai PK, Jaiswal D, Singh RK, Gupta RK, Watal G. (2008). Glycemic properties of Trichosanthes dioica leaves. Pharmaceutical Biology, 46 (12): 894-899.

[7] Islam SM, Ahmed KT, Manik MK, Wahid MA, Kamal CS. (2013). A comparative study of the antioxidant, antimicrobial, cytotoxic and thrombolytic potential of the fruits and leaves of Spondias dulcis. Asian Pacific journal of Tropical Biomedicine, 3 (9): 682-91.

[8] Greenwell M, Rahman PK. (2015). Medicinal plants: their use in anticancer treatment. International Journal of Pharmaceutical Sciences and Research, 6 (10): 4103.

[9] Newman DJ. (2008). Natural products as leads to potential drugs: an old process or the new hope for drug discovery?. Journal of Medicinal Chemistry, 51 (9): 2589-2599.

[10] Newman DJ, Cragg GM, Snader KM. Natural products as sources of new drugs over the period 1981–2002. (2003). Journal of Natural Products, 66 (7): 1022-37.

[11] Apu AS, Muhit MA, Tareq SM, Pathan AH, Jamaluddin AT, Ahmed M. (2010). Antimicrobial Activity and Brine Shrimp Lethality Bioassay of the Leaves Extract of Dillenia indica Linn. Journal of Young Pharmacists, 2 (1): 50-3.

[12] Ali MS, Amin MR, Kamal CM, Hossain MA. (2013). In vitro antioxidant, cytotoxic, thrombolytic activities and phytochemical evaluation of methanol extract of the A. philippense L. leaves. Asian Pacific Journal of Tropical Biomedicine, 3 (6): 464-9.

[13] Kamal AM, Chowdhury KA, Rana MM, Islam A, Khan EA, Haque MA, Chy MM. (2015). Study of cytotoxic, thrombolytic and anthelmintic activity of extract of Neolamarckia cadamba (Roxb.) leave. European Journal of Medicinal Plants, 1-9.
Bibi Humayra Khanam et al. (2019). Highly Anticancer and Moderate Thrombolytic Property of Accacia rugata of Mimosaceae Family.

[14] Hunt BJ. (2008). Awareness and politics of venous thromboembolism in the United Kingdom. Arteriosclerosis, Thrombosis, and Vascular Biology, 28 (3): 398-399.

[15] Aasim M, Khawar KM, Ahmed SI, Karataş M. (2019). Multiple uses of some important aquatic and semiaquatic medicinal plants. In Plant and Human Health, 2: 541-577.

[16] Dhiman N, Patial V, Bhattacharya A. (2018). The current status and future applications of hairy root cultures. In Biotechnological Approaches for Medicinal and Aromatic Plants, 87-155.

[17] Rahaman MS, Rahaman MS, Bari MA, Barua R, Islam JM, Islam MS, Khan MA. (2019). An approach to evaluate anti-arthritis and thrombolytic activity of different parts of Solanum torvum Sw. (Solanaceae) and Smilax zeylanica L. (Liliaceae). Journal of Drug Delivery and Therapeutics, 9 (4-5): 155-64.

[18] Zohora FT, Islam SN, Khan SA, Hasan CM, Ahsan M. (2019). Antioxidant, cytotoxic, thrombolytic and antimicrobial activity of zanthoxylum rhoetsa root bark with two isolated quinolone alkaloids. Pharmacology & Pharmacy, 10 (3): 137-45.

[19] Ghani A. 2003. Medicinal plants of Bangladesh with chemical constituents and uses. 2nd edition; published by Asiatic Society of Bangladesh, 331-332.

[20] Ezeja MI, Omeh YS, Ezeigbo II, Ekechukwu A. (2011). Evaluation of the analgesic activity of the methanolic stem bark extract of Dialium guineense (Wild). Annals of Medical and Health Sciences Research, 1 (1): 55-62.

[21] Ahmed S, Rakib A, Islam MA, Khanam BH, Faiz FB, Paul A, Chy MN, Bhuiya NM, Uddin MM, Ullah SA, Rahman MA. (2019). In vivo and in vitro pharmacological activities of Taccia integrifolia rhizome and investigation of possible lead compounds against breast cancer through in silico approaches. Clinical Phytoscience, 5 (1): 36.

[22] Islam MA, Sayeed MA, Khan G, Mosaddik MA, Bhuyan MS. (2002). Terpenes from bark of Zanthoxylum budrunga and their cytotoxic activities. Revista Latinoamericana De Quimica, 30: 24-28.

[23] Islam MR, Naima J, Proma NM, Hussain MS, Uddin SN, Hossain MK. (2019). In-vivo and in-vitro evaluation of pharmacological activities of Ardisia solanacea leaf extract. Clinical Phytoscience, 5 (1): 32.

[24] Déciga-Campos M, Rivero-Cruz I, Arriaga-Alba M, Castañeda-Corral G, Angeles-López GE, Navarrete A, Mata R. (2007). Acute toxicity and mutagenic activity of Mexican plants used in traditional medicine. Journal of Ethnopharmacology, 110 (2): 334-42.

[25] Rakib A, Ahmed S, Islam MA, Haye A, Uddin SN, Uddin MM, Hossain MK, Paul A, Emran TB. (2020). Antipyretic and hepatoprotective potential of Tinospora crispa and investigation of possible lead compounds through in silico approaches. Food Science & Nutrition, 8 (1): 547-56.

[26] Kamal AM, Chowdhury KA, Shill LK, Hossain MR, Islam N, Anaytulla IA, Hassan MF. (2015). Phytochemical screening, cytotoxic and thrombolytic activity of extract of Brassica oleracea flower (cauliflower). Global Journal of Pharmacology, 9 (1): 115-20.

[27] Zaman R, Parvez M, Jakaria M, Sayeed MA, Islam M. (2015). In vitro clot lysis activity of different extracts of mangifera sylatica roxb. Leaves. Research Journal of Medicinal Plant, 9 (3): 135-140.

[28] Oukerrou MA, Tilaoui M, Mouse HA, Leouifoudi I, Jaafari A, Zyad A. (2017). Chemical composition and cytotoxic and antibacterial activities of the essential oil of Aloysia citriodora palau grown in Morocco. Advances in Pharmacological Sciences, 2017.

[29] Monga J, Chauhan CS, Sharma M. (2011). Human epithelial carcinoma cytotoxicity and inhibition of DMBA/TPA induced squamous cell carcinoma in Balb/c mice by Acacia catechu heartwood. Journal of Pharmacy and Pharmacology, 63 (11): 1470-1482.

[30] Prasad S, Kashyap R S, Deopujari JY, Purohit HJ, Taori GM, Duginawala HF. (2007). Effect of Fagonia arabica (Dhamasa) on in vitro thrombolysis. BMC Complementary and Alternative Medicine, 7 (1): 36.

[31] Hussain MS, Hossain MS, Amin MT, Millat MS. (2016). In vitro thrombolytic potentials of methanolic extract of Vigna unguiculata Linn (seed). Journal of Pharmacognosy and Phytochemistry, 5 (3): 129.