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Antioxidant, Cytotoxic, Thrombolytic and Antimicrobial Activity of *Zanthoxylum rhetsa* Root Bark with Two Isolated Quinolone Alkaloids

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Abstract

The background of this study was to investigate the antioxidant, cytotoxic, thrombolytic and antimicrobial activity of the petroleum ether (PE), carbon tetrachloride (CTC), chloroform (CF) and aqueous (AQ) soluble fractions of crude methanolic *Zanthoxylum rhetsa* root bark with two isolated quinolone alkaloids, 8-methoxy-n-methylflindersine (**1**) and zanthodioline (**2**). Structures were characterized by 1D NMR analyses. Antioxidant activity was assessed by using DPPH assay and antimicrobial activity was screened by disc diffusion method. An *in vitro* thrombolytic model was used to evaluate the clot lysis effect of different extracts of root bark of *Z. rhetsa* along with streptokinase as a positive control and distilled water as a negative control and the cytotoxic activity of different extracts of *Z. rhetsa* root bark was evaluated by Brine Shrimp Lethality Bioassay. AQ fraction exhibited strongest antioxidant, cytotoxic and thrombolytic activity among four fractions. The CTC and CF soluble fractions exhibited significant antioxidant, cytotoxic and thrombolytic activity. CTC and AQ fractions gave highest anti-bacterial activity against *Vibrio cholera* and *Klebsiella pneumonia* respectively. Compound **1** showed significant activity at a concentration of 100 µgm/disc against *Sarcina lutea*, *Staphylococcus aureus and Salmonella paratyphi-A*, *Shigella dysenteriae*, *Shigella boydii and Shigella sonnei* with high antioxidant activity. The antioxidant, thrombolytic and antimicrobial activity of 8-methoxy-n-methylflindersine and zanthodioline are the first record from root bark of this plant.

Keywords

Zanthoxylum rhetsa, Antioxidant, Thrombolytic, Antimicrobial,

8-Methoxy-n-Methylflindersine, Zanthodioline

1. Introduction

A great number of medicinal plants contain chemical compounds that exhibit antioxidant properties [1] [2]. The brine shrimp lethality assay is considered a useful tool for preliminary assessment of toxicity. It has also been suggested for screening pharmacological activities in plant extracts [3].

Though the currently available thrombolytic [4] are wonderful clot lytics, they have still significant shortcomings, including the need for large doses, limited fibrin specificity, bleeding tendency and allergic reactions [5] and in some cases the thrombi have been proven to be resistant to intravenous t-PA6. In recent years, there has been a growing interest in researching and developing new antimicrobial agents from various sources to combat microbial resistance. The fruits and stem bark of *Z. rhetsa* are used in the treatment of asthma, bronchitis, heart complaints and rheumatism [6]. Chemical analysis of *Zanthoxylum rhetsa* allows the isolation of two pyranoquinoline alkaloids, 8-methoxy-n-methylflindersine (**1**) and zanthodioline (**2**), which has been previously reported from *Zanthoxylum rhetsa* and *Zanthoxylum simulans* respectively [7]. The present paper also describes antioxidant, thrombolytic, cytotoxicity and antimicrobial activity of the PE, CTC, CF and AQ soluble fractions of *Zanthoxylum rhetsa* root bark along with two isolated quinolone alkaloids.

2. Materials and Method

2.1 General

Preparative TLC was conducted over glass plates coated with silica gel 60 PF254 (0.5 mm thickness), Merck. Gel permeation chromatography was performed using Sephadex LH-20. TLC was also carried out using Merck precoated TLC plates (Silica gel 60, F254), eluting with suitable solvent system. Spots on the TLC plates were visualized under UV light at 254 and 366 nm as well as by spraying with vanillin sulphuric acid followed by heating for 5 min at 110˚C. NMR spectra were recorded in $CDCl_3$ on a Bruker Advance100 and 400 MHz Ultra shield NMR Spectrophotometer.

2.2. Plant Material

The root barks of *Zanthoxylum rhetsa* were collected from Narsingdi district, Bangladesh in the month of August, 2013. The plant part was identified by a taxonomist (Ms. Nasrin Aktar), Bangladesh National Herbarium where a voucher specimen was deposited for future reference (DACB Accession No. 42528).

2.3. Extraction and Isolation

The air dried and powdered root barks of *Z. rhetsa* (3.5 kg) were extracted with

methanol over the period of 15 days. The crude methanol extract (40 g) was then fractionated sequentially by petroleum ether (9 g), ethyl acetate (6 g), chloroform (12 g) and methanol (12 g) fractions with continuous stirring. Chloroform (12 g) soluble fraction was subjected to silica gel column and was fractionated with a gradient of petroleum ether-dichloromethane-ethyl acetate-methanol which was given total of 295 fractions each with 20 ml. Similar fractions were mixed together and which showed mixture of several compounds were then subjected to Gel permeation chromatography (GPC) over Sephadex (LH-20) using a mixed solvent system of PE: CF (2:8) followed by mixtures of methanol and chloroform of increasing polarity and finally only with methanol. Depending upon the TLC behavior fractions were mixed and compound **1** (51.7 mg) and **2** (34.6 mg) were isolated followed by eluting with toluene/EtOAc (95:5 and 87:13 respectively) (**Figure 1**).

8-methoxy-n-methylflindersine(**1**): Yellow gum; 1H-NMR (400 MHz; $CDCl_3$): d 7.62 (1H, *dd*, *J* = 8, 1.6 Hz, H-5), 7.15 (1H, *dd*, *J* = 8, 8 Hz, H-6), 7.07 (1H, *d*, *J* = 8 Hz, H-7), 5.55 (1H, *d*, *J* = 9.6 Hz, H-3'), 6.77 (1H, *d*, *J* = 10 Hz, H-4'), 3.95 (3H, *s*, OMe-8), 3.91 (3H, *s*, N-Me), 1.52 (6 H, *s*, Me-2' *cis*, Me-2' *trans*); 13C-NMR (100 MHz; CDCl3: 162.2 (C-2), 106.0 (C-3), 154.9 (C-4), 115.6 (C-5), 122.2 (C-6), 114.2 (C-7), 148.5 (C-8), 131.0 (C-9), 18.3 (C-10), 77.2 (C-29), 126.5 (C-39), 118.0 (C-49), 28.2 (2Me-29), 35.1 (N-Me), 56.7 (OMe-8)).

Zanthodioline (**2**): White crystals; 1H-NMR (500 MHz, CDCl3): δ 7.64 (dd, *J* = 8.0, 1.2 Hz, H-5), 7.22 (1H, t, *J* = 8.0 Hz, H-6), 7.12 (1H, dd, *J* = 8.0, 1.2 Hz, H-7), 4.76 (1H, d, *J* = 8 Hz, H-4'), 3.97 (3H, s, N-Me), 3.93 (3H, s, OMe-8), 3.84 (1H, d, *J* = 8 Hz, H-3'), 1.64 (3H s, H-2' Me), 1.33 (3H, s, H-2' Me). 13C-NMR (125 MHz, CDCl3): δ 164.6 (C-2), 154.8 (C-4), 148.8 (C-8), 130.8 (C-9), 122.8 (C-6), 118.2 (C-10), 116.2 (C-5), 114.4 (C-7), 106.0 (C-3), 80.9 (C-2'), 75.3 (C-3'), 67.7 (C-4'), 56.7 (OMe-8), 34.8 (N-Me), 26.1 (C-2' Me), 19.3 (C-2' Me).

2.4. Preparation of Sample for Biological Investigation

Solvent-solvent fractionation of the crude methanolic extract was conducted by using the protocol intended by Kupchan [8] and altered by Van Wagenen *et al.* [9] 5 g of the obtained methanolic crude extract. All the four fractions were kept in beakers for analysis (PE 830 mg, CTC 560 mg, CF 675 mg and AQ 400 mg).

3. Results and Discussion

Comparison of above 1D NMR data with known physical constants of compound **1** [10] and compound **2** [11] definitely confirmed the structures determination (**Figure 2**).

3.1. Evaluation of Antioxidant Activity

The free radical scavenging activity of different soluble extracts of root bark of *Z. rhetsa* and the pure compound was measured by using DPPH• method of McCune and Johns [12]. Lower absorbance of the reaction mixture indicated

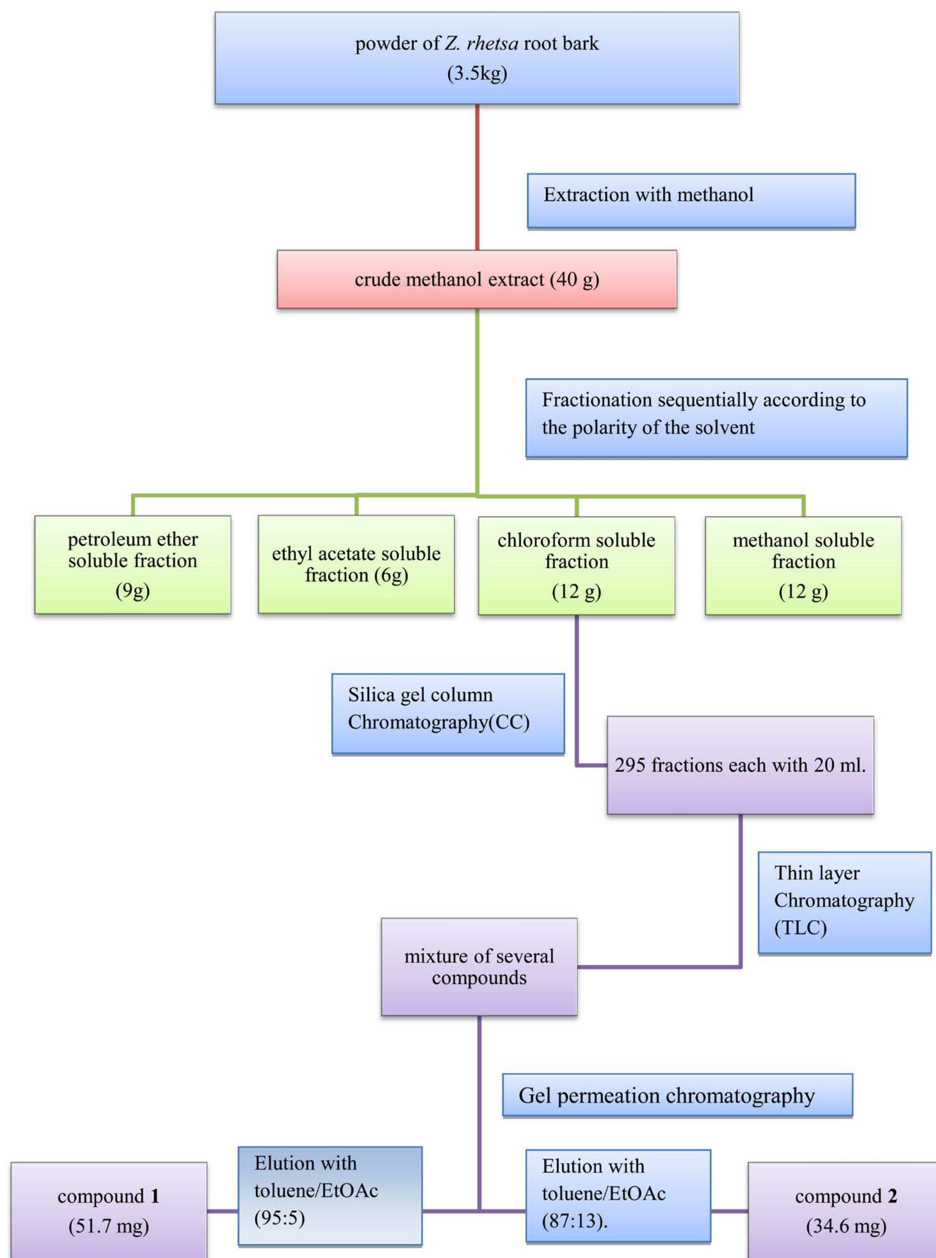

Figure 1. Schematic sequences of the isolation experiment.

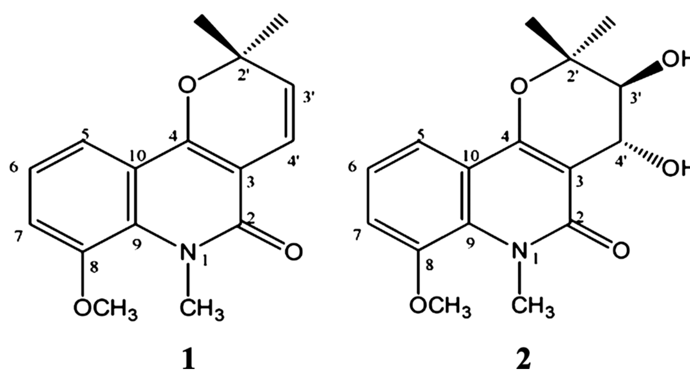

Figure 2. Structure of 8-methoxy-n-methylflindersine (1) and zanthodioline (2).

higher free radical scavenging activity [13]. **Table 1** shows DPPH radical scavenging activity of the different fractions of the plant and the pure compounds **1** and **2** compared with butylated hydroxyl toluene (BHT). It was observed that the aqueous soluble fraction and 8-methoxy-n-methylflindersine have more proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants. The antioxidant activity of the chloroform and aqueous soluble fractions validated the traditional claims of this plant for the treatment of various ailments (**Figure 3**) [14].

3.2. Brine Shrimp Lethality Bioassay

Compared to positive control (vincristine sulphate, VS, LC_{50} 0.42 µg/ml), all the fractions tested showed good brine shrimp larvicidal activity (**Table 2**). The cytotoxic activity exhibited by the solvent fractions was promising and this clearly indicates the presence of potent bioactive compounds [15] [16].

Table 1. Antioxidant activity of different fractions and pure compounds of *Z. rhetsa.*

SL no	Sample	Free radical scavenging activity (IC_{50} in µg/ml)
1	BHT	29.23 ± 5.38
3	PE	263.66 ± 19.87
2	CTC	138.38 ± 4.80
4	CF	96.00 ± 4.13
5	AQ	55.25 ± 2.78
6	8-methoxy-n-methylflindersine	71.18 ± 1.74
7	Zanthodioline	101.90 ± 5.24

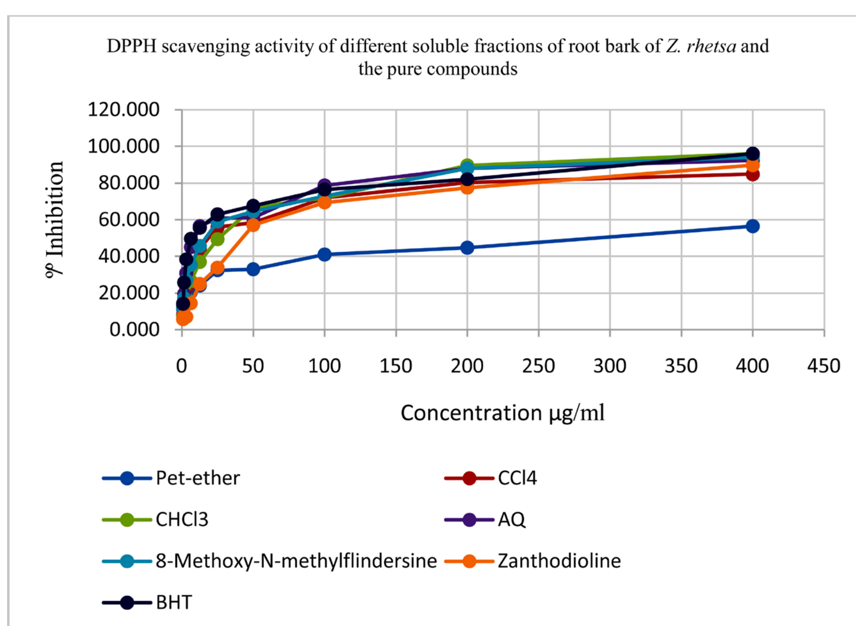

Figure 3. DPPH scavenging activity of different fractions of root bark of *Z. rhetsa* and the pure compounds.

3.3. Evaluation of Thrombolytic Activity

In vitro clot lysis activity carried out according to the method of Prasad *et al* [17]. The fractions of the root bark of *Z. rhetsa* showed an adequate amount of thrombolytic activity except the pet-ether soluble fractions and the pure compounds. Among all the fractions and pure compounds, the aqueous fraction showed highest clot lysis activity (50.5%), whereas standard streptokinase at 37˚C showed 70.6% lysis of the clot as compared to distilled water showing a negligible lysis of clot (2.4%) (**Table 3**). This evaluation is previously studied from the leaves of the plant.Further study is required to investigate the *in vivo* thrombolytic activity and the causative component(s), and mechanism for clot lysis by *Z. rhetsa* (**Figure 4**) [18].

3.4. Antibacterial Activity

The agar disc diffusion method was employed for the determination of antimicrobial activities [19]. The methanol crude root extracts of *Z. rhetsa* and its Carbon Tetra Chloride, Chloroform, aqueous soluble fractions and 8-methoxy-n-methylflindersine exhibited significant antibacterial activity against microbial growth which indicated that these extracts contain cheimical substances having antibacterial property [20]. Compound **1** showed significant activity at a concentration of 100 µgm/disc against *Sarcina lutea*, *Staphylococcus aureus* and *Salmonella paratyphi-A*, *Shigella dysenteriae*, *Shigella boydii* and *Shigella sonnei* with high antioxidant activity

Table 2. Cytotoxic activity of different fractions and pure compound of *Z. rhetsa.*

SL No.	Test samples	LC_{50} in µg/ml
1	VS	0.42 ± 0.01
3	PE	1.36 ± 0.39
2	CTC	1.27 ± 0.07
4	CF	1.52 ± 0.01
5	AQ	0.52 ± 0.11
6	8-methoxy-n-methylflindersine	1.45 ± 0.54

Table 3. Thrombolytic activity of different fractions and pure compounds of *Z. rhetsa.*

SL No.	Sample name	% of clot lysis
1	PE	24.80% ± 3.6%
3	CTC	41.10% ± 4.3%
2	CF	43.8% ± 2.3%
4	AQ	50.5% ± 3.3%
5	8-methoxy-n-methylflindersine	17.5% ± 1.6%
6	Zanthodioline	32.0% ± 9.0%
7	Streptokinase	70.6% ± 7.9%
8	Water	2.4% ± 0.2%

has not yet been reported. Pet-ether fraction showed very small zone of inhibition against all other bacteria (Table 4).

3.5. Statistical Analysis

The experimental results were expressed as mean ± standard deviation (SD) of three replicates. Statistical significance was determined was considered as significant.

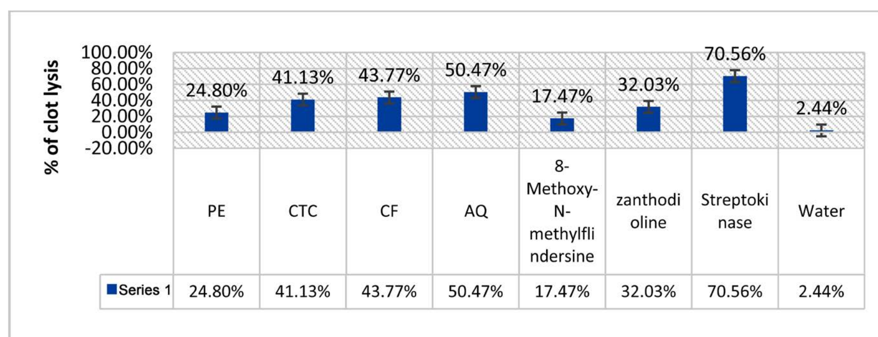

Figure 4. Comparison of percentage of clot lysis of different fractions and pure compounds of *Z. rhetsa*.

Table 4. Screening of antimicrobial activity of different fractions and pure compounds of *Z. rhetsa*.

Bacterial strain	Crude Extract 400 µgm/disc	Pet Ether fraction 400 µgm/disc	Carbon Tetra Chloride fraction 400 µgm/disc	Chloroform fraction 400 µgm/disc	Aqueous fraction 400 µgm/disc	8-Methoxy-N-methylflindersine 100 µgm/disc	Kanamycin 30 µgm/disc
Gram Positive Bacteria							
Bacillus subtilis	14.33 ± 1.53	NA	9.67 ± 0.58	11 ± 1.00	8 ± 1.00	10.67 ± 1.53	19.33 ± 2.08
Bacillus aerius	8.67 ± 1.53	-	10 ± 1.00	8 ±	7 ± 1.00	-	18 ± 1.00
Bacillus megaterium	NA	-	NA	NA	-	-	17 ± 1.00
Sarcina Lutea	11 ± 2.00	5.33 ± 2.31	7 ± 1.00	NA	7 ± 1.00	10.33 ± 2.08	NA
Staphylococcus aureus	NA	NA	-	-	-	10 ± 1.73	NA
Pseudomonas aeruginosa	NA	-	-	-	-	NA	NA
Gram Negative Bacteria							
Salmonella typhi	9 ± 1.73	5.67 ± 2.52	8.67 ± 0.58	NA	NA	NA	16.67 ± 2.52
Salmonella paratyphi-A	10.33 ± 0.58	5.33 ± 1.53	6.33 ± 1.53	7 ± 1.00	NA	10 ± 0	12.67 ± 0.58
Shigella dysenteriae	9 ± 1	NA	5 ± 1.73	8 ± 1.00	NA	8.33 ± 1.15	13.67 ± 1.15
Shigella boydii	11 ± 1	NA	NA	NA	NA	8 ± 1.00	NA
Shigella sonnei	8.33 ± 0.58	NA	-	NA	-	9.67 ± 0.58	NA
Shigella fexneri	8 ± 2.65	NA	NA	NA	NA	NA	NA
Escherichia coli	12 ± 0	NA	8 ± 2.65	NA	NA	NA	17 ± 2.65
Vibrio cholerae	11.67 ± 1.53	NA	18.67 ± 3.21	NA	8.33 ± 3.06	NA	24.67 ± 0.58
Klebsiella pneumonia	8 ± 0	7.67 ± 2.52	8 ± 2.65	12 ± 1.00	14 ± 3.61	NA	25.33 ± 1.53

NA = not active; - = absence of growth of microorganism. Diameter of inhibition zone (mm) including well diameter of 8 mm.

4. Conclusion

The biological activity found in this investigation justifies the traditional medicinal uses of this plant for treating different dieses. The antioxidant and cytotoxic activity unveiled by the aqueous solvent fraction is promising and this clearly specifies the existence of potent bioactive compounds and will be a good source of herbal medicine. In context of the above discussion it would be interesting to investigate to find out the causative components from the aqueous extract which are responsible for this biological activity.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Pratt, D.E. and Hudson, B.J.F. (1990) Natural Antioxidants Not Exploited Commercially. In: Hudson, B.J.F., Ed., *Food Antioxidants*, Elsevier, Amsterdam, 171-192. https://doi.org/10.1007/978-94-009-0753-9_5

[2] Mathew, S. and Abraham, E.T. (2006) *In Vitro* Antioxidant Activity and Scavenging Effects of *Cinnamomum verum* Leaf Extract Assayed by Different Methodologies. *Food and Chemical Toxicology*, **44**, 198-206. https://doi.org/10.1016/j.fct.2005.06.013

[3] Ferro, M. and Doyle, A. (2001) Standardization for *in Vitro* Toxicity Tests. *Cell Biology and Toxicology*, **17**, 205-212.

[4] Shapiro, S.S. (2003) Treating Thrombosis in the 21st Century. *The New England Journal of Medicine*, **349**, 1762-1764. https://doi.org/10.1056/NEJMe038152

[5] Califf, R.M., Topol, E.J., George, B.S., Boswick, J.M., Abbottsmith, C., Sigmon, K.N., *et al*. (1988) Hemorrhagic Complications Associated with the Use of Intravenous Tissue Plasminogen Activator in Treatment of Acute Myocardial Infarction. *The American Journal of Medicine*, **85**, 353-362. https://doi.org/10.1016/0002-9343(88)90586-4

[6] Yusuf, M., Chowdhury, J.U., Wahab, M.A. and Begum, J. (1994) The Essential Oil Is Used to Treat Cholera and as an Antiseptic and Disinfectant. Medicinal Plants of Bangladesh. BCSIR, Dhaka.

[7] Zohora, F.-T., Muhit, A., Hasan, C.M. and Ahsan, M. (2018) Quinolone Alkaloids Along with Other Constituents from *Zanthoxylum rhetsa* and Their Chemotaxonomic Significance. *Records of Natural Products*, **12**, 634-637.

[8] Kupchan, S.M. (1970) Recent Advances in the Chemistry of Terpenoid Tumor Inhibitors. *Pure and Applied Chemistry*, **21**, 227-246. https://doi.org/10.1351/pac197021020227

[9] Van Wagenen, B.C., Larsen, R., Cardellina, J.H.I.I., Ran, D.D., Lidertand, Z.C. and Swithenbank, C. (1993) Ulosantoin, a Potent Insecticide from the Sponge Ulosa Ruetzleri. *The Journal of Organic Chemistry*, **58**, 335-337.

[10] Ahsan, M., Zaman, T.A., Hasan, C.M., Ito, C. and Nazrul Islam, S.K. (2000) Constituents and Cytotoxicity of *Zanthoxylum rhesta* Stem Bark. *Fitoterapia*, **71**, 697-700.

[11] Chen, I.-S., Tsai, I.-W., Teng, C.-M., Chen, J.-J., Chang, Y.-L., Ko, F.-N., Lu, M.C. and

Pezzuto, J.M. (1997) Pyranoquinoline Alkaloids from *Zanthoxylum simulans. Phytochemistry*, **46**, 525-529.

[12] McLaughlin, J.L. and Rogers, L.L. (1999) The Use of Biological Assays to Evaluate Botanicals. *Drug Information Journal*, **32**, 513. https://doi.org/10.1177/009286159803200223

[13] Middleton, P., Stewart, F., Al-Qahtani, S., Egan, P., O'Rourke, C., Sarker, S.D., *et al.* (2005) Antioxidant, Antibacterial Activities and General Toxicity of *Alnus glutinosa*, *Fraxinus excelsior* and *Papaver rhoeas*. *Iranian Journal of Pharmaceutical Research*, **2**, 81-86.

[14] Vidyamadhavi, K., Joshi, C.G., Manjunath Hullikere, M., Nivya, M.T., Anand, D. and Raju, N.G. (2014) Evaluation of *In-Vitro* Antioxidant, Anti-inflammatory Properties of Aerial Parts of *Zanthoxylum rhesta. Research Journal of Pharmaceutical, Biological and Chemical Sciences*, **5**, 997-1003.

[15] Persoone, G., Sorgeloos, P., Roels, O. and Jaspers, E. (Eds.) (1979) The Brine Shrimp *Artemia*. *Proceedings of the International Symposium on the Brine Shrimp Artemia salina*, Corpus Christi, 20-23 August 1979, 3-24.

[16] Ahsan, M., Zaman, T.A., Hasan, C.M., Ito, C. and NazrulIslam, S.K. (2000) Constituents and Cytotoxicity of *Zanthoxylum rhesta* Stem Bark. *Fitoterapia*, **71**, 697-700. https://doi.org/10.1016/S0367-326X(00)00214-8

[17] Prasad, S., Kashyap, R.S., Deopujari, J.Y., Purohit, H.J., Taori, G.M. and Daginawala, H.F. (2007) Effect of *Fagonia arabica* (Dhamasa) on *In-Vitro* Thrombolysis. *BMC Complementary and Alternative Medicine*, **7**, 36. https://doi.org/10.1186/1472-6882-7-36

[18] Azad, A.K., Islam, O., Rima, E., Islam, M., Sultana, C., Nesa, J.-U., Ahmed, F., Azad, A.K., *et al.* (2015) Phytochemical Screening and *In-Vitro* Thrombolytic Activity of Methanolic Leaf Extract of *Zanthoxylum rhetsa. Journal of Pharmaceutical Sciences and Research*, **7**, 302-304.

[19] NCCLS (National Committee for Clinical Laboratory Standards) (1997) Performance Standards for Antimicrobial Disk Susceptibility Test. 6th Edition, Approved Standard, M2-A6, Wayne, PA.

[20] Poornima, K., Krishnakumar, K. and Veena, V. (2018) Phytochemical and Antibacterial Assay of *Zanthoxylum rhetsa* (Roxb). DC. *International Journal of Research in BioSciences*, **7**, 41-45.