Asian Journal of Chemistry; Vol. 33, No. 9 (2021), 2162-2166

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2021.23167

Evaluation of Antimicrobial, Thrombolytic and CNS-Depressant Activities of Three Medicinal Plants Available in Bangladesh

FATEMA TABASSUM*, AMENA AKTHER AKHI, MUKTA AKTER CHUMKI, LIOYAJA RAHMAN, FURHATUN-NOOR and MD. IMRAN NUR MANIK

Department of Pharmacy, Northern University Bangladesh, Dhaka-1205, Bangladesh

*Corresponding author: E-mail: fatematabassum23@gmail.com

Received: 13 February 2021; *Accepted*: 12 July 2021; *Published online*: 20 August 2021; AJC-20479

The aim of the present study was to explore antimicrobial, thrombolytic and CNS-depressant activity of three medicinal plants *Plumbago zeylanica*, *Trewia nudiflora* and *Aphanamixis polystachya* available in Bangladesh. At room temperature, the plant parts were subjected to cold extraction with methanol, giving rise to concentrated methanolic extracts (MEF) followed by fractionation applying revised Kupchan partitioning procedure to obtain different soluble fractions namely to hexene fraction (HXF), ethyl acetate fraction (EAF), chloroform fraction (CLF) and aqueous fraction (AQF). To study the antimicrobial activity of these fractions, the disc diffusion method was used, where kenamycin was used as standard. Thrombolytic potential was determined by investigating clot rupture (% clot lysis) for this purpose streptokinase was employed as the positive control whereas water was the negative control. For the evaluation of CNS depressant activity, the open-field method was utilized and diazepam was chosen as the reference standard. Among the three plants, the crude methanolic fraction of *P. zeylanica* demonstrated good antimicrobial action over the majority of the bacterial strains assayed and the crude methanolic extract exhibited the maximum antimicrobial activity against *S. aeruginosa* (zone of inhibition was 23.46 ± 2.19 mm). The chloroform fraction of *T. nudiflora* showed the highest thrombolytic activity (43.45 ± 2.12% clot lysis). It was observed that the four extracts from the plants under investigation had CNS depressant activity. Particularly, the aqueous fraction of *P. zeylanica* (12.00 ± 0.913); *n*-hexane fraction of *T. nudiflora* (09.75±0.854) and *A. polystachya* (08.50 ± 0.645) demonstrated consistently significant CNS depressant activity, in terms of the number of squares crossed at 120 min; producing a prominent decrease in the measurement of movement.

Keywords: *Plumbago zeylanica*, *Trewia nudiflora*, *Aphanamixis polystachya*, Thrombolytic activity, Antimicrobial, CNS depressant.

INTRODUCTION

Secondary metabolites of the medicinal plants are an important source of both preventive and curative medical therapy preparations for men and animals. Around 80% of the world's total population mainly in third world countries, depends on traditional medicine and products for its healthcare needs. Globally more specifically the Asian traditional medicinal system commonly used about 50,000 plant species [1]. Valuable ethnobotanical information may be comprises by the medicinal plants that could navigate new drug discoveries. The only natural remedies available and accessible in the remote rural communities in developing countries are the traditional medicine, which are usually cheaper than modern medicine [2]. One of the important sources of modern drug discoveries are the medicinal plant source. The long history of clinical uses of the medicinal plants increases the success ratio of a new drug lead candidate. Fabricant & Farnsworth [3] reported that 80% of 122 plants derived drugs were related to their original ethnopharmacological uses between 1981 and 2001. In this study, three medicinal plants available in Bangladesh were selected and used for their different pharmacological properties.

The first selected medicinal plant for present study was *Plumbago zeylanica* (Plumbaginaceae) has several therapeutic properties. It is used to treat diseases of the spleen, dysentery, fevers, liver diseases, diarrhoea, leprosy, bacterial, microbial and helminth infections [4,5]. Root is the major portion of this plant, which contains different bioactive constituents like naphthoquinones, binaphthoquinones, anthroquinone and coumarin [5]. The main bioactive component is a naphthoquinone known

as plumbagin, which claimed to possess anticoagulant, anticarcinogenic, antitumor, cardiotonic, hypolipidaemic, antiatherosclerotic, antimutagenic, wound healing, antifungal and antibacterial properties [6].

Trewia nudiflora L. (Euphorbiaceae), a potential herbal medicinal plant is mainly distributed in Malaysia, India and Southern China [7]. Decoction of root is used as stomachic treatment of flatulence, gout, rheumatism, malignancy especially leukemia and hepatobiliary affections, *etc*. A decoction of shoots and leaves of *T. nudiflora* is used to relieve swelling and to treat flatulence, excessive bile and sputum [8]. A pyridine alkaloid namely *N*-methyl-5-carboxamide-2-pyridone was isolated from this plant. Leaves of this plant contain an alkaloid, nudiflorine, whereas bark yields taraxerone and β-sitosterol. Seeds contain an alkaloid ricinidine and a maytansinoid compound, trewiasine [9].

Aphanamixis polystachya Wall. Parker (Meliaceae) is distributed in evergreen forests particularly in India. The stem bark and seeds contains different bioactive components which are used in splenomegaly, liver disorders and treatment of tumors [10,11]. A study reported that the alcoholic extract of the stem bark showed anticancer activity against Friend's leukemia and Ehrlich ascites carcinoma in mice [12]. The radiation-induced chromosome damage was found to be protected by the ethyl acetate fraction of this plant [13]. The stem bark extract contain some alkaloids like amoorastatin and 12-hydroxyamoorastatin, which have been reported to possess cytotoxic and growth inhibitory activities in murine P388 lymphocytic leukaemia cells [14].

EXPERIMENTAL

Leaves of *Trewia nudiflora*, *Aphanamixis polystachya* and *Plumbago zeylanica* were collected from Narayangang, Bangladesh in the month of February 2019 and washed with distilled water for several times to remove dirt material, dried under shade with casual sun drying, milled into coarse powder and preserved in a well-sealed container at 25 ºC for further use.

Preparation, extraction and fractionation of plant material: Cold maceration technique was employed for the extraction of the plant materials. Powder portions of different plants (700 g) were soaked in 3000 mL of distilled methanol for about 10 days at room temperature with occasional stirring. For successful filtration, cotton followed by filter paper were used and the filtrate obtained was then concentrated by a rotary evaporator. The concentrated methanolic extracts (MEF) was partially separately by modified Kupchan method [15] using *n*-hexene, ethyl acetate and chloroform to yield hexene fraction (HXF), ethyl acetate fraction (EAF), chloroform fraction (CLF). Aqueous portion was retained as aqueous fraction (AQF). By evaporation method, different fractions of the plants were dried for 7-8 days.

Antimicrobial activity

Microorganisms: For the microbial assay, six bacteria (two Gram-positive, four Gram-negative) were collected from Institute of Nutrition and Food Science (INFS), University of Dhaka, Bangladesh.

Antimicrobial assay: Disc diffusion method [16] was employed to test the antimicrobal activity of different extractives against six selected bacteria. Measured amount of test substances were dissolved in methanol (40 µg/mL) and impregnated into dried, sterilized filter paper discs (6 mm diameter) and the finally residual solvents were evaporated completely. Nutrient agar medium was selected for this study, which is uniformly seeded with test microorganisms and discs containing the test material (400 µg/disc) were placed on the medium. For positive control, standard disc of kanamycin (30 µg/disc) and for negative control, blank discs (impregnated with solvents followed by evaporation) were used. The plates were then kept at low temperature (4 ºC) for 24 h to allow maximum diffusion of test samples and after that, the plates were then incubated at 37 ºC for 24 h for the maximum growth of organisms. The growth of the microorganisms was inhibited by the test materials having antimicrobial, distinct zone of inhibition was visualized surrounding the disc. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition. To obtain better results, the experiment was done in triplicate and the average zone of inhibition was calculated.

Thrombolytic activity

Streptokinase (SK): Streptokinase (15, 00,000 I.U.) used as a standard and was collected from Beacon Pharmaceuticals Ltd., Bangladesh. A sterile distilled water (5 mL) was added to streptokinase vial and mixed properly. From this suspension 100 µL (30,000 I.U) was used for *in vitro* thrombolysis [17].

Preparation of sample: The thrombolytic activities of all plant extracts were evaluated by a method using streptokinase (SK) as a reference standard. A 100 mg of MEF, HXF, EAF, CLF and AQF of different plants were dissolved, respectively in 10 mL of methanol, hexene, ethyl acetate, chloroform and distilled water and kept overnight. The soluble supernatant was decanted and filtered.

Blood sample: Blood samples were collected from healthy human volunteers (n = 5) by maintaining aseptic condition without a history of oral contraceptive or anticoagulant therapy. A 1 mL of blood was transferred to the previously weighed microcentrifuge tubes to form clots. Study protocol was approved by the ethical committee of Department Pharmacy, Northern University Bangladesh.

Thrombolytic activity assay: From each volunteers, 5 mL of blood (venous) were collected and taken in five different pre-weighed sterile Eppendorf tubes and the tubes were then incubate at 37 ºC for 45 min. The fluid was completely released from each tubes after clot formation and the clot weight was determined by subtracting weight of clot containing tube from weight of tube alone. Streptokinase (100 µL) was used as positive control while 100 µL of distilled water was used as negative non-thrombolytic control. Each samples (100 µL) were separately added to the Eppendorf tubes. All the exterimental tubes as well as the positive and negative control were then incubated at 37 ºC for 90 min. After incubation, the clot lysis occurred and the released fluid was removed. The tubes were again weighed to observe the difference in weight after clot disruption. Finally percentage of clot lysis was determined as follows:

TABLE-1
ANTIBACTERIAL ACTIVITY OF *P. zeylanica, T. nudiflora* AND *A. polystachya*

Experimental plant	Sample	Diameter of zone of inhibition (mm)					
		Gram-negative bacteria				Gram-positive bacteria	
		E. coli	*S. paratyphi*	*S. aeruginosa*	*S. marcescens*	*B. subtilis*	*B. cereus*
P. zeylanica	Crude	17.13 ± 4.00	20.73 ± 2.03	23.46 ± 2.19	16.47 ± 1.12	–	10.86 ± 1.80
	HXF	13.46 ± 2.20	11.93 ± 1.62	–	–	10.10 ± 1.18	–
	EAF	12.62 ± 2.15	–	–	–	–	–
	CLF	–	18.74 ± 1.30	–	10.88 ± 1.47	–	–
	AQF	13.16 ± 0.75	18.15 ± 2.08	–	–	10.29 ± 0.80	–
T. nudiflora	Crude	14.33 ± 1.80	17.86 ± 1.60	–	–	–	–
	HXF	–	16.46 ± 0.96	–	10.00 ± 1.32	12.80 ± 0.36	–
	EAF	–	–	9.20 ± 1.51	–	11.00 ± 0.31	–
	CLF	–	22.23 ± 1.10	–	–	8.61 ± 1.00	–
	AQF	–	17.20 ± 2.60	–	–	–	15.30 ± 1.10
A. polystachya	Crude	–	–	–	–	11.46 ± 0.90	12.66 ± 0.72
	HXF	12.86 ± 1.60	–	–	8.10 ± 0.55	20.30 ± 1.30	12.40 ± 1.90
	EAF	–	–	–	–	10.13 ± 2.15	14.16 ± 1.30
	CLF	–	12.13 ± 1.10	10.16 ± 0.12	–	–	–
	AQF	14.26 ± 2.15	–	–	–	–	15.90 ± 0.65
	Control	29.00 ± 2.31	26.00 ± 1.63	33.00 ± 1.35	28.00 ± 0.81	25.00 ± 2.44	28.00 ± 1.62

$$\text{Clot lysis (\%)} = \frac{\text{Weight of released clot}}{\text{Clot weight}} \times 100$$

CNS-depressant activity: Open field test was employed for the CNS-depressant activity assay in accordance to the method as described by Gupta *et al*. [18]. This test observes and measures the movement of mice by the numbers of squares crossed. The test animals were categorized into control, positive control and extract based test groups each comprising of four mice.

Animals: The study protocol was permitted by the Ethics Committee of the Research Center, Department of Pharmacy, Northern University of Bangladesh (Approval number: DoP/RC/EC/2018/11/). Around 6 to 8 weeks of Swiss albino mice of age with 22 ± 2 g of either sex were accustomed for week days to the appropriate laboratory conditions. The experimental animals were kept in 12 h light/dark cycles at 22 ± 2 ºC with 60 to 70 % relative humidity. Studies were executed randomly using 6 mice of either sex in each group.

The doses of extracts namely *n*-hexene soluble fraction (HXF), ethyl acetate soluble fraction fraction (EAF), chloroform soluble fraction (CLF) and aqueous fraction (AQF) provided to the test groups contained the oral doses of 200 mg/kg body weight. On the other hand, the control group obtained distilled water, (0.1 mL/mouse, p.o.). For the positive control diazepam (1 mg/kg, i.p.) was used. The open field was separated into a succession of squares, separately coloured black and white. The animals came by the squares and the number of visits was counted at the intervals of 3 min at 0, 30, 60, 90 and 120 min, respectively.

RESULTS AND DISCUSSION

Antimicrobial activity: Selected three medicinal plants of different fractionates were evaluated for the antimicrobial activity. Antibiotic disc, Kenamycin was used as standard. All experiments were done in triplicate and the mean of triplicate taken as a final result. Among all the fractions, the crude methanoic fraction of *P. zeylanica* possessed prominent antimicrobial activities and the highest activity was found against *P. aeruginosa* (23.46 ± 2.19 mm) of crude fraction of *P. zeylanica* and chloroform fraction of *T. nudiflora*, also showed very good antimicrobial activity against *S. paratyphi*, *n*-hexene fraction of *A. polystachya* showed good antibacterial activity against *B. subtilis* (Table-1).

Thrombolytic activity: The thrombolytic activity of *P. zeylanica*, *T. nudiflora* and *A. polystachya* extracts were determined. The result showed (73.43 ± 4.21)% lysis clot in a process of subsequent incubation for 90 min at 37 ºC, where 100 µL streptokinase was added as a positive control (30,000 I.U.). On the other side, it showed negligible percentages of lysis of clot (5.33 ± 3.29)%, when distilled water was used as a negative control. The highest thrombolytic activity was exhibited by the chloroform fraction of *T. nudiflora* (43.45 ± 2.12)% and crude methanolic fraction of *P. zeylanica* (40.51 ± 5.017)%, however crude methanolic fraction of *A. polystachya* (29.21 ± 2.61)% showed moderate thrombolytic activity. Other fractions showed very weak thrombolytic activity (Table-2).

TABLE-2
THROMBOLYTIC ACTIVITY (% CLOT LYSIS)
P. zeylanica, T. nudiflora AND *A. polystachya*

Fractions	*P. zeylanica*	*T. nudiflora*	*A. polystachya*
Crude	35.53 ± 5.09	16.65 ± 4.44	29.21 ± 2.61
HXF	17.11 ± 3.85	8.49 ± 2.74	9.92 ± 6.03
EAF	12.38 ± 2.22	23.98 ± 0.30	10.57 ± 3.17
CLF	11.14 ± 2.84	43.45 ± 2.12	8.37 ± 3.19
AQF	14.55 ± 5.50	13.47 ± 2.11	20.43 ± 3.91

CNS depressant activity: The depressive effect on CNS by the extracts from the studied plants using the open field test was conducted. Three plants *viz*. *P. zeylanica*, *A. polystachya* and *T. nudiflora* with different extracts namely HXF, EAF, CLF, AQF demonstrated a moderate to evident reduction in the movement at dose level (200 mg/kg bodyweight). In most cases, the effect showed an increasing trend with time and a perce-

ptible result was observed at 120 min after the administration of test sample (Fig. 1). Tested animals depict significant decline in the number of movements for the dosage of 200 mg/kg for *P. zeylanica* (HXF: 06.25 ± 1.109, AQF: 12.00 ± 0.913, EAF: 6.50 ± 0.957 and CLF: 16.75 ± 1.181); *A. polystachya* (HXF: 8.50 ± 0.645, AQF: 16.75 ± 1.109 and EAF: 15.75 ± 1.250); *T. nudiflora* (9.75 ± 0.854, AQF: 8.50 ± 0.645 and CLF: 19.00 ± 0.913), respectively as compared to control & standard group for *P. zeylanica* (9.00 ± 1.915; 2.00 ± 0.408); *A. polystachya*: (19.75 ± 1.377; 04.50 ± 1.443) and *T. nudiflora*: (15.00 ± 2.415; 02.00 ± 0.707), respectively at 120 min of administration of the extract (Table-3).

Conclusion

Various fractions of the plant extracts as well as the crude methnolic extract of three medicinal plants (*Plumbago zeylanica*, *Trewia nudiflora* and *Aphanamixis polystachya*) possesed moderate antibacterial, thrombolytic and CNS-depressant activities. The plant extracts should be further studied extensively to isolate the secondary metabolites from those plants which are responsible for their potential bioactivities.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

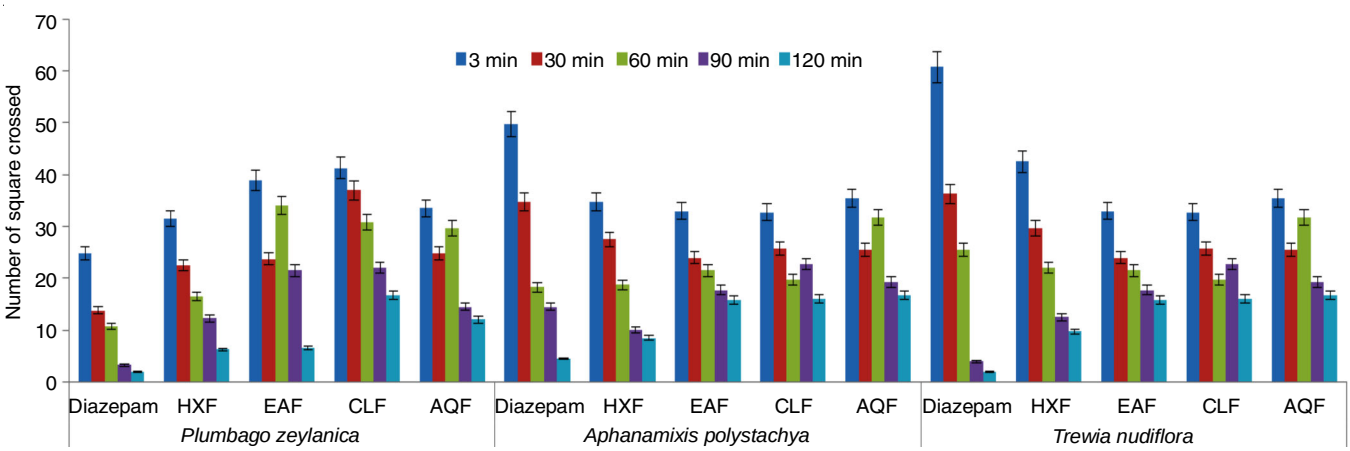

Fig. 1. CNS depression effect by open filed method

TABLE-3
CNS DEPRESSANT ACTIVITY OF DIFFERENT FRACTIONS OF *Plumbago zeylanica*, *Aphanamixis polystachya* AND *Trewia nudiflora* EXTRACTS BY OPEN FIELD TEST IN RATS

Treatment	Dose (kg/mg)	Number of square crossed				
		3 min	30 min	60 min	90 min	120 min
Plumbago zeylanica						
Control	0.1 mL/mouse	29.50 ± 1.041	22.00 ± 2.646	21.25 ± 1.377	15.75 ± 1.493	09.00 ± 1.915
Diazepam	1	24.75 ± 0.946	13.75 ± 1.315	10.75 ± 1.548	03.25 ± 0.479	02.00 ± 0.408
Crude	200	31.75 ± 1.702	23.00 ± 1.472	19.50 ± 1.323	16.75 ± 1.652	11.25 ± 1.493
HXF	200	31.50 ± 1.258	22.50 ± 1.190	16.50 ± 0.645	12.25 ± 0.854	06.25 ± 1.109
EAF	200	39.00 ± 0.707	23.75 ± 1.493	34.00 ± 0.913	21.50 ± 1.709	06.50 ± 0.957
CLF	200	41.25 ± 1.548	37.00 ± 1.581	30.75 ± 1.493	22.00 ± 1.291	16.75 ± 1.181
AQF	200	33.50 ± 1.936	24.75 ± 0.946	29.75 ± 1.652	14.50 ± 0.645	12.00 ± 0.913
Aphanamixis polystachya						
Control	0.1 mL/mouse	41.50 ± 1.756	31.00 ± 2.160	25.25 ± 1.931	24.75 ± 1.797	19.75 ± 1.377
Diazepam	1	49.75 ± 2.056	34.75 ± 1.315	18.25 ± 2.016	14.50 ± 1.658	04.50 ± 1.443
Crude	200	62.50 ± 1.323	32.00 ± 1.080	39.00 ± 0.913	21.75 ± 1.315	38.50 ± 0.645
HXF	200	34.75 ± 0.629	27.50 ± 1.041	18.75 ± 1.315	10.00 ± 0.913	08.50 ± 0.645
EAF	200	33.00 ± 2.309	24.00 ± 1.225	21.50 ± 1.708	17.75 ± 0.854	15.75 ± 1.250
CLF	200	32.75 ± 1.797	25.75 ± 1.109	19.75 ± 2.955	22.75 ± 1.377	16.00 ± 1.472
AQF	200	35.50 ± 1.041	25.50 ± 0.645	31.75 ± 1.315	19.25 ± 0.629	16.75 ± 1.109
Trewia nudiflora						
Control	0.1 mL/mouse	43.00 ± 2.646	33.25 ± 3.146	31.00 ± 2.799	22.50 ± 2.327	15.00 ± 2.415
Diazepam	1	60.75 ± 3.683	36.25 ± 1.702	25.50 ± 0.645	04.00 ± 1.080	02.00 ± 0.707
Crude	200	48.50 ± 0.957	34.00 ± 2.739	22.00 ± 0.816	19.50 ± 1.708	13.50 ± 1.323
HXF	200	42.50 ± 1.041	29.75 ± 1.931	22.00 ± 1.080	12.50 ± 0.645	09.75 ± 0.854
EAF	200	30.50 ± 1.258	26.75 ± 2.287	20.50 ± 1.848	18.00 ± 1.581	15.25 ± 2.462
CLF	200	39.00 ± 1.292	18.50 ± 0.645	20.75 ± 0.750	28.75 ± 1.109	19.00 ± 0.913
AQF	200	40.00 ± 0.816	21.25 ± 0.946	16.00 ± 0.913	11.75 ± 0.479	08.50 ± 0.645

Values are presented as mean ± SEM (n = 4).

REFERENCES

1. F. Chassagne, T. Samarakoon, G. Porras, J.T. Lyles, M. Dettweiler, L. Marquez, A.M. Salam, S. Shabih, D.R. Farrokhi and C.L. Quave, *Front. Pharmacol*., **11**, 586548 (2021); https://doi.org/10.3389/fphar.2020.586548
2. Z. Popovic, R. Matic, S. Bojovic, M. Stefanovic and V. Vidakovic, *J. Ethnopharmacol.*, **181**, 182 (2013); https://doi.org/10.1016/j.jep.2016.01.034
3. D.S. Fabricant and N.R. Farnsworth, *Environ. Health Perspect.*, **109**, 69 (2001); https://doi.org/10.1289/ehp.01109s169
4. K.R. Kirtikar and B.D. Basu, eds., E. Blatter, J.R. Cauis, K.S. Mhaskar and L.M. Basu, Indian Medicinal Plants, vols I & II (1984).
5. R.S. Thakur, H.S. Puri and A. Husain, Major Medicinal Plants of India, Central Institute of Medicinal and Aromatic Plants: Lucknow, India (1989).
6. J.C. Tilak, S. Adhikari and T.P. Devasagayam, *Redox Rep.*, **9**, 219 (2004); https://doi.org/10.1179/135100004225005976
7. B.J. Li, C. Wan, X.K. Xu, X.F. Yue, Z.M. Sheng, J.X. Han, L.-J. Lu, C.-H. Xu and W.-Y. Yang, *Acta Bot. Yunn.*, **13**, 432 (1991).
8. N. Balakrishn, M. Srivastava and P. Tiwari, *Pak. J. Biol. Sci.*, **16**, 1403 (2013); https://doi.org/10.3923/pjbs.2013.1403.1406
9. N. Balakrishnan, M. Srivastava and P. Tiwari, A Comprehensive Review on Tumari (*Trewia nudiflora* Linn.) (2012); http://www.pharmatutor.org/articles/comprehensive-review-tumari-trewia-nudiflora-linn.
10. R.N. Chopra, S.L. Nayar and I.C. Chopra, Glossary of Indian Medicinal Plants, Council of Scientific & Industrial Research, New Delhi, India (1956).
11. T. Rabi and R.C. Gupta, *Int. J. Pharmacol.*, **33**, 359 (1995); https://doi.org/10.3109/13880209509065396
12. G.C. Jagetia and V.A. Venkatesha, *Int. J. Complem. Alt. Med.*, **3**, 00083 (2016); https://doi.org/10.15406/ijcam.2016.03.00083
13. G.C. Jagetia, *J. Clin. Biochem. Nutr.*, **40**, 74 (2007); https://doi.org/10.3164/jcbn.40.74
14. J. Polonsky, Z. Varon, C. Marazano, B. Arnoux, G.R. Pettit, J.M. Schmid, M. Ochi and H. Kotsuki, *Experientia*, **35**, 987 (1979); https://doi.org/10.1007/BF01949897
15. S.M. Kupchan, *Pure Appl. Chem.*, **21**, 227 (1970); https://doi.org/10.1351/pac197021020227
16. A.W. Bauer, W.M. Kirby, J.C. Sherris and M. Turck, *Am. J. Clin. Pathol.*, **45(4_ts)**, 493 (1966); https://doi.org/10.1093/ajcp/45.4_ts.493
17. S. Prasad, R.S. Kashyap, J.Y. Deopujari, H.J. Purohit, G.M. Taori and H.F. Daginawala, *BMC Complement. Altern. Med.*, **7**, 36 (2007); https://doi.org/10.1186/1472-6882-7-36
18. B.D. Gupta, P.C. Dandiya and M.L. Gupta, *Jpn. J. Pharmacol.*, **21**, 293 (1971); https://doi.org/10.1016/S0021-5198(19)36218-3