Extended Abstract | Clinical Pharmacology & Toxicology Journal | 2019 | Vol. 3 No.2

Research on leaves of Micromelum pubescens and its phytochemicals, in-vitro Thrombolytic and *in-vivo* Antidepressant activity by methanol extract

Mohammad Jamshedur Rahman1, Joy Chowdhury2, Gula Jannat3, Fahmida Haque4, Sadiya Chowdhury5, Shahriyer Mohammad Rafid 6, Md. Mobarak Hossain7 and Md. Shahidul Islam*8

[1] Centre Antipoison et de Toxicovigilance, Service de Pharmacologie Clinique, Hôpital Sainte Marguerite, APHM, Marseille, France
[2] Aix-Marseille University, Marseille, France

Keywords: Micromelum pubescens, Phytochemical screening, Thrombolytic and anti-depressant activity

Abstract

Present study aims to open new avenues for the improvement of medicinal uses of *Micromelum pubescens* leaves under family of *Rutaceae* for the selected area for Phytochemical screening, in-vitro Thrombolytic assay, and in vivo Antidepressant properties of the methanolic extract. In-vitro thrombolytic activity of the plant extract is determined with streptokinase as a positive control & water as negative control. In-vivo antidepressant activity of plant extract is followed by force swim test & tail suspension test. After phytochemical screening of *Micromelum pubescens,* there is presence of carbohydrate, alkaloids, glycosides, tanins, phenol, terpenoids and saponins. In case of thrombolytic activity ,average lysis of the blood clot on the basis of this, % clot lysis = (Weight of the lysis clot / Weight of clot before lysis) × 100 was found 25.03%. In antidepressant test animals treated with two doses of plant extract 200 and 400 mg/kg. In case of tail suspension test, the plant extract showed decreases in their immobility times, which was significant 160second and 142.66second respectively when compared with control (204.33second).Similarly, animals treated with imipramine (10 mg/kg), as expected, showed a significant decrease in the immobility time (85.33second). In case of force swimming test, plant extract showed decreases in their immobility times, which was significant 161.33s(200 mg/kg) and 140s(400 mg/kg) when compared with control (191.33scond).Similarly, animals treated with imipramine (10 mg/kg), as expected, showed a significant decrease in the immobility time (92.66second). The result indicate that, Micromelum pubescens have possess moderate.

Introduction

Nature always stands as a golden mark to exemplify the outstanding phenomenon of symbiosis. The biotic and abiotic elements of nature are all interdependent. The plants are indispensible to man for his life (1). The three important necessities of life – food, clothing and shelter- and a host of other useful products are supplied to him by the plant kingdom (2). Nature has provided a complete store-house of remedies to cure all ailments of mankind. The knowledge of drugs has accumulated over thousands of years as a result of man's inquisitive nature so that today we possess many effective means of ensuring health-care (3).The human being appears to be afflicted with more diseases than any other animal species. There can be little doubt then he, very early, sought to alleviate his sufferings from injury and disease by taking advantage of plants growing around him (4). In the past, almost all the medicines used were from the plants, the plant being man's only chemist for ages. Today, a vast store of knowledge concerning therapeutic properties of different plants has accumulated. All phyla of plants viz. Thallophyta, Bryophyta, Pteridophyta and Spermatophyta contain species that yield official and unofficial products of medicinal importance (5). Many familiar medications of the twentieth century were developed from ancient healing tradition that treated health problems with specific plants. Today, science has isolated the medicinal properties of a large number of botanicals, and their healing components have been extracted and analyzed. Many plant components are now synthesized in large laboratories for use in pharmaceutical preparations (6).A medicinal plant is any plant which, in one or more of its organ, contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs." This definition of Medicinal Plant has been formulated by World Health Organization (7).The plants that possess therapeutic properties or exert beneficial pharmacological effects on the animal body are generally designated as "Medicinal Plants". Although there are no apparent morphological characteristics in the medicinal plants growing with them, yet they possess some special qualities or virtues that make them medicinally important (8). It has now been established that the plants which naturally synthesis and accumulate some secondary metabolites, like alkaloids, glycosides, tannins, volatile oils and contain minerals and vitamins, possess medicinal properties (9).

Materials and Methods

Preparation of plant material

- Normally the plant materials are collected in fresh condition.
- Then these are cut into small pieces if necessary to make it suitable for grinding purpose.
- The leaves were dried for a period of 15 days under shade and ground.
- Finally dried in an oven at 40-45oC for 36 hrs.
- The materials are grinded into coarse powder with the help of a grinder and stored in an air tight container for further use.

Extraction

The ground leaves were soaked in sufficient amount of methanol for 15days at room temperature

- Occasional shaking stirring
- Filtered through a cotton plug followed by Whitman filter paper number.
- The solvent was evaporated with water bath to yield viscous mass.
- The viscous mass was kept at room temperature under a ceiling fan to get a dried extract.
- The prepared extract kept for further pharmacological screening.

Mice weighing about 28-32gm aged about 1.5 month were purchased from animal house of Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh. All the animals were acclimatized to new environment for a period of one week. During the experiment period the animals were kept in a well-ventilated animal house at 25. They were supplied with standard pellets and fresh drinking water. All the mice were kept in cage and maintained with natural 12h light and dark cycle.

Results and Discussion

The qualitative phytochemical screening was performed to ensure the presence or absence of secondary plant metabolites. In our investigation we have found positive results for Carbohydrate, alkaloids, phenol, glycoside and saponins. The result showed in (Table-1).

(++)= Present ,(+++)= Rapidly present

(-) = Absent.

S. no.	Test Name	Observation	
01	Carbohydrate Test	Molish Test	++
		Benedict Test	++
		Fehling A&B	+
02	Alkaloid Test	Mayer Test	_
		Wagners Test	+- confusion
03	Glycosides Test	+++	
04	Phytosterol Test	_	
05	Phenolic Test	++	
06	Flavoniods Test	_	
07	Protein Test	_	
08	Saponins Test	+ moderat	

Table-1: Phytochemical Screening of Micromelum pubescens

***In-vitro* Thrombolytic activity:**

Addition of 100 µl SK, a positive control (15,00,000 I.U.) to the clots along with 90 minutes of incubation at 37°C, showed 75% clot lysis. Clots when treated with 100 µl sterile distilled water (negative control) showed only negligible clot lysis 5.64%. The in-vitro thrombolytic activity study revealed that Micromelum pubescens showed 25.03%. The results are shown in (Table-2)

Number Of Volunteer	Weight Of Tube A (gm)	Weight Of Tube After Removing Lysis from Tube B (gm)	Weight Of Clot C=B-A (gm)	Weight of Tube with Clott after Removing Lysis with Extract Solution D(gm)	Final weight Of Lysis E=B-D (gm)	% of Clot Lysis	Average % of clot Lysis
01.	0.81	1.27	0.46	1.21	0.06	13.04	
02.	0.79	1.17	0.38	1.01	0.16	42.11	
03.	0.81	1.22	0.41	1.05	0.17	41.46	
04.	0.81	1.17	0.36	1.05	0.12	33.33	
05.	0.84	1.27	0.43	1.19	0.08	18.60	
06.	0.81	1.30	0.49	1.17	0.13	26.53	
07.	0.79	1.34	0.55	1.15	0.19	34.55	36.84
08.	0.81	1.01	0.02	0.97	0.04	20.00	
09.	0.81	1.06	0.25	0.94	0.12	48.00	±2.74
10	0.81	1.03	0.22	0.97	0.06	27.02	

Table-2: Thrombolytic assay of the methanolic extract of Micromelum pubescens

% clot lysis	
Water	2.74%
Micromelum pubescens	36.84%
Streptokinase	75%

Table-3: Clot lysis by water and Micromelum pubescens compared with streptokinase

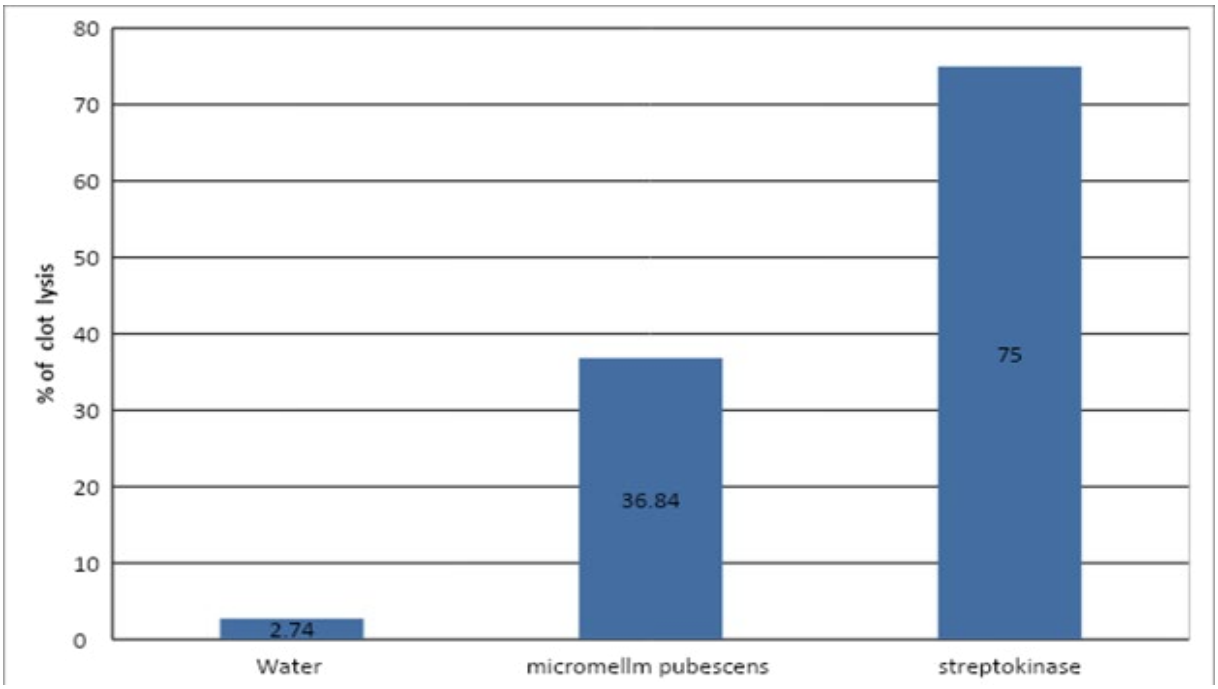

Figure-1: Clot lysis by water and Micromelum pubescens compared with streptokinase

Egg albumin denaturation:

The plant extract inhibited the protein denaturation by from a lowest 12.96% when conc. was 62.5µg/ml to the highest % of inhibition was 313.48% when concentration was 1000 µg/ml as compared to standard drug Diclofenac-Na inhibited by 325% at 800 µg/ml which is shown in (Table 4 & Figure 2).

No	Concentration µg/ml	Test Solution Abs.	% of Protein inhibition By Extract	% of Protein Inhibition By Diclofenac-Na
1	62.5	0.436	12.96%	19.66%
2	125	0.533	37.31%	40.76%
3	250	0.626	62.18%	83.7%
4	500	0.965	150.26%	165.5%
5	1000	1.596	313.48%	325%

Table-4: Anti-inflammatory activity of Micromelum pubescens

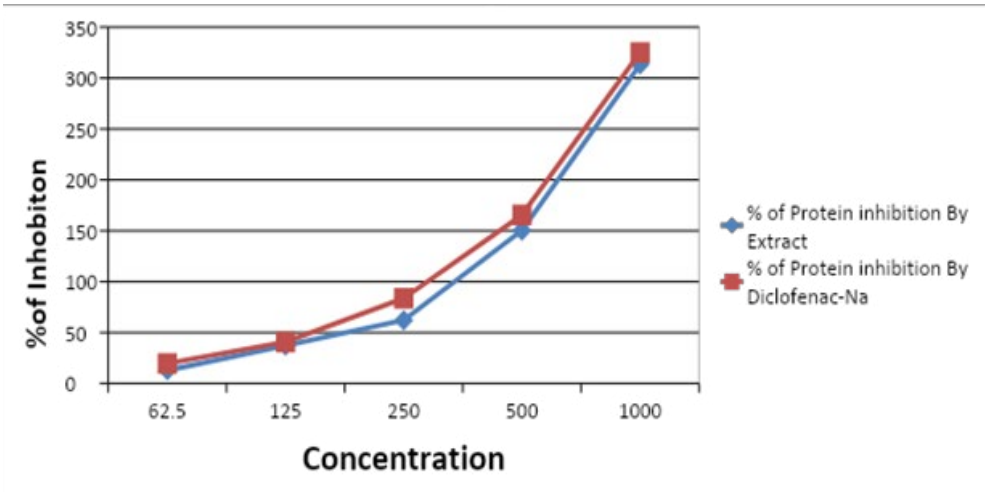

Figure 2: %of protein inhibition of Micromelum pubescens with compare to standard Diclofenac-Na.

***In-vivo* Antidepressant activity: Force Swim Test (FST):**

The possible antidepressant effect of MEMP after oral administration was studied in the forced swimming test. In this test animals treated with two doses of MEMP 200 and 400mg/kg, showed decreases in their immobility times, which was significant 157.33 ± 3.20 second and 134 ± 2.25second and respectively; when compared with control 1187.67 ± 2.52 second. Similarly, animals treated with Imipramine 10 mg/kg, as expected, showed a significant decrease in the immobility time 177.67 ± 2.52 Each value represents Mean ± S.D, n=3

Immobile time(second)

MICE NO	Control	Imipramine 10 mg/kg	MEMP-200	MEMP-400
1	185	78	160	132
2	188	75	155	155
3	190	80	157	157
Mean	187.67	177.67	157.33	134.63
SD	2.52	2.52	3.20	2.25
SEM	1.03	1.03	1.03	3.63

MEMP=Methanol extract of Micromelum pubescens

Table 5: Effect of the methanol extract of Micromelum pubescens anti-depression test on force swim test in mice

MEMP=Methanol extract of Micromelum pubescens

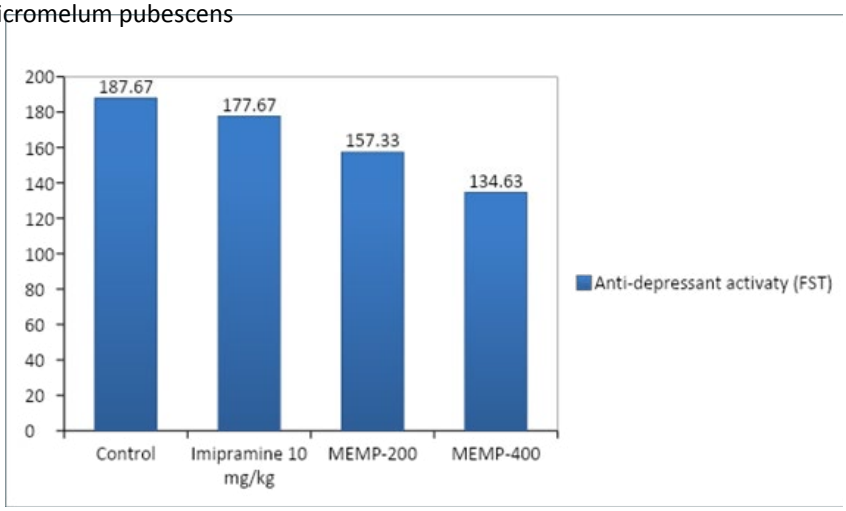

Figure 3: Effect of the methanol extract of Micromelum pubescens anti- depression test on force swim test in mice

Tail suspension test (TST):

In this test animals treated with two doses of MEMP 200 and 400 mg/kg, Showed decreases in their immobility times, which was significant 181.67± 3.60 s and 123 ± 4.40 second respectively when compared with control (206.63 ± 1.50).Similarly, animals treated with Imipramine (10 mg/kg), as expected, showed a significant decrease in the immobility time (81.30± 4.71).

Immobile time(second)

MICE NO	Control	Imipramine 10 mg/kg	MEMP-400	MEMP-200
1	202	75	120	180
2	205	82	125	175
3	208	85	127	190
Mean	206.63	81.30	123	181.67
SD	1.50	4.71	4.40	3.60
SEM	0.61	1.92	1.49	3.12

MEMP=Methanol extract of Micromelum pubescens

Table 6: Effect of the methanol extract of Micromelum pubescens anti- depression test on Tail Suspension test in mice

MEMP=Methanol extract of Micromelum pubescens

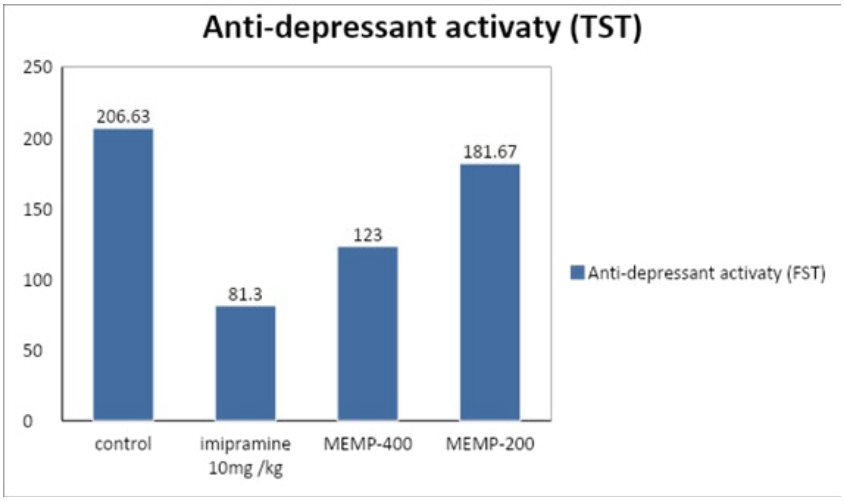

Figure 4: Effect of the methanol extract of Micromelum pubescens anti- depression test on Tail Suspension test in mice

Conclusion

According to study of the results of this plant extract it is proved that the plant may be a source of effective herbal drug. The outcome indicates that Micromelum pubescens have possess moderate thrombolytic and antidepressant activity. History says that, natural products are nothing new as the agents for treating various diseases. Naturally plants not only provide us foodstuff, shelter but also they provide remedies for many years. Different chemical constituents contained in plant exhibit different activities in alleviation of abnormal health condition of human beings or animals. In case of traditional medicine, the practitioners are appreciated to use different parts of plant because of having several chemical constituents in them which fulfill their wants.

References

1.KARTHISHWARAN,K.,MIRUNALINI,S.,DHAMODHARAN, G.,KRISHNAVENI, M. & ARULMOZHI, V. 2010. Phytochemical investigation of methanolic extract of the leaves of Pergularia daemia. J Biol Sci, 10, 242-246.

2. AZWANIDA, N. 2015. A review on the extraction methods use in medicinal plants, principle, strength and limitation. Med Aromat Plants, 4, 2167-0412.1000196.

3. PORSOLT, R., BERTIN, A. & JALFRE, M. 1977a. Behavioral despair in mice: a primary screening test for antidepressants. Archives internationales de pharmacodynamie et de therapie, 229, 327-336

4. CASTILLO, R., CARLAT, D., MILLON, T., MILLON, C., MEAGHER, S., GROSSMAN, S., ROWENA, R., MORRISON, J. & ASSOCIATION, A. P. 2007. Diagnostic and statistical manual of mental disorders, Washington, DC: American Psychiatric Association Press.

5. KESSLER, R. C., MCGONAGLE, K. A., ZHAO, S., NELSON, C. B., HUGHES, M., ESHLEMAN, S., WITTCHEN, H.-U. & KENDLER, K. S. 1994. Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States: results from the National Comorbidity Survey. Archives of general psychiatry, 51, 8-19.

6. HIGAZI, A. A.-R., KATZ, I., MAYER, M. & WEISS, A. T. 1993. The effect of ultrasonic irradiation and temperature on fibrinolytic activity in vitro. Thrombosis research, 69, 251-253.

7.PRASAD, S., KASHYAP, R. S., DEOPUJARI, J. Y., PUROHIT, H. J., TAORI, G. M. & DAGINAWALA, H. F. 2006. Development of an in vitro model to study clot lysis activity of thrombolytic drugs. Thrombosis Journal, 4, 14.