European Journal of Medicinal Plants
10(4): 1-7, 2015, Article no.EJMP.20483
ISSN: 2231-0894, NLM ID: 101583475

SCIENCEDOMAIN *international*
www.sciencedomain.org

Evaluation of *Ex-vivo* Anti-arthritic, Anti-inflammatory, Anti-cancerous and Thrombolytic Activities of *Mussaenda roxburghii* Leaf

Md. Irfan Amin Chowdury[1*], Mohammad Nazmul Alam[1], Sudipta Chowdhury[1], Md. Shahrear Biozid[1], Md. Faruk[1], Muhammad Moin Uddin Mazumdar[1] and Ahmad Ibtehaz Chowdhury[1]

[1]Department of Pharmacy, Faculty of Science and Engineering, International Islamic University Chittagong, 154/A, College Road, Chittagong-4203, Bangladesh.

Authors' contributions

This work was carried out in collaboration between all authors. Authors MIAC, MNA and SC designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MSB, MF, MMUM and AIC managed the analysis of the study and the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2015/20483
Editor(s):
(1) Marcello Iriti, Professor of Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
(1) James Adams, University of Southern California, USA.
(2) Abdullahi M. Nuhu, College of Science and Technology, Kaduna Polytechnic, Nigeria.
(3) Atef Mahmoud Mahmoud Attia, National Research Centre, Egypt.
Complete Peer review History: http://sciencedomain.org/review-history/11395

Original Research Article

Received 30th July 2015
Accepted 19th August 2015
Published 15th September 2015

ABSTRACT

Aim: The current study evaluates the anti-arthritic, anti-inflammatory, anti-cancerous and thrombolytic activities of methanolic extract of *Mussaenda roxburghii* leaf.
Place and Duration of study: This study was conducted in the Department of Pharmacy, Faculty of Science and Engineering, International Islamic University Chittagong, during the period between August 2013 and March 2014.
Methodology: Bovine serum albumin was used for the evaluation of anti-arthritic potential and methanolic plant extract was compared with diclofnac sodium. The anti-inflammatory activity was assessed by hypotonicity induced membrane lysis and methanolic plant extract was also compared with diclofenac sodium. Brine shrimp lethality bioassay was used to evaluate the anti-cancerous

**Corresponding author: E-mail: aminirfan504@gmail.com;*

activity of methanolic extract of *Mussaenda roxburghii*. Evaluation thrombolytic activity of methanolic extract of *Mussaenda roxburghii* was investigated by clot lysis method and methanolic extract was compared with streptokinase.

Results: In the case of anti-denaturation, methanolic extract of *Mussaenda roxburghii* exhibited 42.15% of inhibition at 31.25 µg/ml and 80.33% of inhibition at 1000 µg/ml concentration. For anti-inflammatory effect, methanolic extract showed 53.79% and 86.93% of membrane stabilization activity at 31.25 µg/ml and 1000 µg/ml concentration respectively. The extract showed minimal anticancerous activity, lethal concentration (LC_{50}) value of the extract was 282.03 µg/ml compared to vincristine sulphate (12.59 µg/ml). For the evaluation of the thrombolytic activity, methanolic leaf extract of *Mussaenda roxburghii* was treated with human blood. It showed promising activity which was about 49.09%.

Keywords: Mussaenda roxburghii; protein denaturation; anti-inflammatory; HRBC; brine shrimp; anti-cancer; cytotoxicity; clot lysis.

1. INTRODUCTION

Plant-based medicine contain significant amounts of bioactive compounds, which provide desirable health benefits. The World Health Organization reported that 80 % of the world populations rely chiefly on indigenous medicine and that the majority of traditional therapies involve the use of plant extracts or of their active constituents [1] and over 25% of modern medicines that are commonly used worldwide contains compounds extracted from medicinal plants [2]. The active principles differ from plants to plants due to their biodiversity and produce a definite physiological action on the human body that develops interest on their medicinal properties [3]. In recent years, there has been a revival in the use of traditional medicinal plants and therefore, pharmaceutical companies are investing a lot of money in developing natural products extracted from plants [4]. In Bangladesh thousands of plant species are known to have medicinal value [5] and ninety percent of the medicinal plants are wild sourced [6].

Mussaenda roxburghii (Family-Rubiaceae) is a large erect shrub, stem light brown in colour. *Mussaenda roxburghii* is a perennial shrub, which grows in the foothills and moist areas of valley. To treat boils disease, the paste obtained from leaf of this plant is applied in the infected areas [7,8]. For the treatment of jaundice, skin diseases, cuts, wounds and boils roots are used [9,10]. Ailments of bone fracture are alleviated by using the leaves [11]. Previous phytochemical investigation led to isolation of a new iridoid, shanzhiol [12].To know the potential of medicinal plants of [13,14], the current study has been initiated to determine the anti-inflammatory, anti-arthritic, anticancer and thrombolytic activities of *M. roxburghii*. It is also essential to reveal the pharmacological evidence for its folkloric uses as well as for the exploration of new drugs [8].

2. MATERIALS AND METHODS

2.1 Plant Material

Mussaenda roxburghii was collected from a local area (Bhatiary) of Chittagong district, Bangladesh and authenticated by the Botanist Dr. Shaikh Bokhtear Uddin, Assistant professor, Department of Botany, University of Chittagong, Bangladesh.

2.2 Preparation of Extract

The leaf was indirectly sun dried and ground. The ground (300 g) were soaked in sufficient amount of methanol for one week at room temperature with occasional shaking and stirring then filtered through a cotton plug followed by Whitman filter paper No. 1. The solvent was evaporated under vacuum at room temperature to yield semisolid. The extract was then preserved in a refrigerator till further use.

2.3 Reagents

The chemicals used were Bovine serum albumin (BSA), Diclofenac sodium, Sodium dihydrogen phosphate, Disodium hydrogen phosphate, Sodium Chloride, Dextrose, sodium citrate, citric acid, were purchased from Sigma-Aldrich. Commercially available lyophilized Streptokinase vial of 15 00000 I.U. was purchased from Durakinase, Dongkook Phama. Co. Ltd, South Korea. Absolute methanol (99.50%) and vincristine sulfate (VS) were purchased from Sigma-Aldrich, Munich, Germany. All chemicals in this investigation were of analytical reagent grade.

2.4 Inhibition of Protein Denaturation

Diclofenac sodium was used as standard for the inhibition of protein denaturation. The test solution (0.5 ml) contains 0.45 ml of bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of methanolic extract of *Mussaenda roxburghii*. The control solution (0.5 ml) contains 0.45 ml of bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of distilled water. Product control (0.5 ml) contains 0.45 ml of distilled water and 0.05 ml of methanol extract of *Mussaenda roxburghii*. Standard solution (0.5 ml) contains 0.45 ml of bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of diclofenac sodium. Six concentrations (31.25, 62.5, 125, 250, 500, and 1000 µg/ml) of methanol extract of *Mussaenda roxburghii* and diclofenac sodium (standard) were taken, respectively. All the solutions were adjusted to pH 6.3 using 1 N HCl. Samples were kept in the incubator at 37℃ for 20 min and the temperature was increased to keep the samples at 57℃ for 3 min. After cooling, 2.5 ml of phosphate buffer was added to the previous solutions. The absorbance was measured using UV-Visible spectrophotometer at 416 nm. The control clearly shows 100% protein denaturation. The results were compared with diclofenac sodium [15].

The percentage inhibition of protein denaturation of different concentrations is tabulated in Table 2. The percentage inhibition of protein denaturation can be calculated as:

> % inhibition = [100 - (OD of test solution - OD of product control)] ×100 Where OD = optical density.

The control represents 100% protein denaturation. The results were compared with diclofenac sodium.

2.5 Anti-inflammatory Activity

2.5.1 The human red blood cell (HRBC) membrane stabilization method

In this method human red blood cell membrane was used for the hypotonicity induced membrane lysis. 2 ml blood was drawn from Healthy volunteer (human) who had not taken any NSAIDs for prior to the experiment. Then the blood was mixed with equal volume of Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) and centrifuged at 3,000 rpm. Isosaline was used for the washing of the packed cells. A 10% v/v suspension was made and kept at 4ºC undistributed before use. Six concentrations (31.25, 62.5, 125, 250, 500, 1000 µg/ml) of extracts were used and blood control (distilled water instead of hypo saline to produce 100% hemolysis) were separately mixed with 1 ml (0.15 M) of sodium phosphate buffer, 2 ml of hyposaline and 0.5 ml of 10% HRBC suspension was added to prepared. When drugs were omitted in blood control, erythrocyte suspension was absent in drug control. All the assay mixture were kept in incubator at 37ºC for 30 min and centrifuged at 3000 rpm for 20 min and hemoglobin content of supernant solution was estimated spectrophotometrically at 560 nm [16].

The percentage of HRBC membrane stabilization or protection was calculated by using the following formula:

$$\% \text{ of membrane stabilization value} = \frac{100 - [(\text{Drug test value} - \text{Drug control value}) \times 100]}{\text{Blood control value}}$$

Where, the blood control represented 100% lysis.

2.6 Anti-cancer Screening

Anticancer screening was performed by brine shrimp lethality bioassay which is used for screening bioactive compounds for the evaluation of cytotoxicity of the methanol extract [17,18]. In this experiment, *Artemia salina*, a simple zoological organism was used as a convenient monitor for the experiment. The eggs of the brine shrimp were collected from an aquarium shop (Dhaka, Bangladesh) and hatched in artificial seawater (3.8% NaCl solution) for 48 hrs. to develop into larval shrimp called nauplii.

Meyer Method was performed upon the brine shrimp for the evaluation of cytotoxic assay. The test samples (extract) were prepared by dissolving them in DMSO (not more than 50 µL in 5 mL solution) plus seawater (3.8% NaCl in water) to attain concentrations of 10, 50, 100, 150, 200 and 300 µg/ml.

A vial containing 50 µL DMSO diluted to 5 mL was used as a control. Standard vincristine sulfate was used as a positive control. Mature shrimps were placed into each of the experimental vials. After 24 h, the vials were observed using a magnifying glass, and the number of surviving nauplii in each vial was

counted. From these data, the percentage of lethality of the brine shrimp nauplii was calculated for each concentration using the following formula:

$$\% \text{ Mortality} = \frac{Nt}{No} \times 100\%$$

Where N_t = Number of dead nauplii after a 24-h incubation;

N_0 = Number of total nauplii transferred i.e., 10.

The LC_{50} (median lethal concentration) was determined from the log concentration versus % mortality curve.

2.7 Thrombolytic Activity

2.7.1 Blood specimen

Blood (2 ml) was collected from healthy human volunteers (n = 3) without a history of oral contraceptive or anticoagulant therapy. A 500 µl of blood was transferred to each of the three previously weighed microcentrifuge tubes to form clots.

2.7.2 Clot lysis

2 ml venous blood collected from the healthy volunteers and distributed in three different pre weighed sterile microcentrifuge tube (0.5 ml/tube) and kept in incubator at 37℃ for 45 min. After the formation of clotting, serum was completely removed without disturbing the clot and each tube having clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube – weight of tube alone). To each microcentrifuge tube containing pre-weighed clot, 100 µl of methanol extract of *Mussaenda roxburghii* added separately.100 µl of streptokinase used as positive control and 100 µl of distilled water used as a negative control which were separately added to the control tubes numbered. All the tubes were then incubated at 37℃ for 90 min and observed for clot lysis. After incubation, fluid released was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference in weight before and after clot lysis was expressed as percentage of clot lysis as shown below:

Percent (%) of clot lysis = (Weight of released clot /clot weight) ×100

The experiment was repeated with the blood samples of the 3 volunteers [19].

2.8 Statistical Analysis

The results were expressed as mean of the three repetitions and standard deviations were calculated. Statistical comparisons were made using the Independent t-test and $P<0.01$, $P<0.05$ and $P< 0.001$ was considered as significant. Statistical significance between % of clot lysis by streptokinase and plant extracts was performed by paired *t*-test analysis. All of the tests were performed by using the software SPSS version 20.0 (SPSS for Windows, IBM Corporation, New York, USA). Expression of data was expressed as mean ± SD. The mean difference between positive and negative controls was considered significant at $P< 0.05$ and $P< 0.001$.

3. RESULTS AND DISCUSSION

3.1 Anti- arthritic Study

Six different concentrations of methanolic extract of *Mussaenda roxburghii* and diclofenac sodium were experimented for anti-denaturation activity [15]. It exhibited significant percentage of inhibition in anti-denaturation study (Table 1). In case of lower concentration, methanolic extract of *Mussaenda roxburghii* showed 42.15% where the standard drug diclofenac sodium showed 51.65% of inhibition and in higher concentration, the extract of *Mussaenda roxburghii* exhibited the 80.33% of inhibition whereas the diclofenac sodium exhibited 85.49% of inhibition of protein denaturation.

3.2 Anti-inflammatory Study

Methanolic extract of *Mussaenda roxburghii* was studied for *ex vivo* anti-inflammatory activity by human red blood cell membrane stabilization method [20] which is reported in Table 2. The *ex vivo* anti-inflammatory activity of the extracts were concentration dependent, with the increasing concentration, the activity is also increased. Here, methanol extract of *Mussaenda roxburghii* showed 86.93% of membrane stabilization at 1000 µg/ml concentration and 53.79% at 31.25 µg/ml. All the results were compared with standard Diclofenac which showed 94.44 % and 65.56% at 1000 µg/ml and 31.25 µg/ml respectively.

3.3 Anti-cancer Screening

For the anti-cancer screening, Meyer method was performed to determine the lethality of methanolic crude extract of *Mussaenda roxburghii*. The lethality of leaf was observed on

Artemia salina after sample exposure for 24 h. Vincristine sulfate and vehicle was used as positive control and negative control respectively which were also used to compare the toxic effects of the methanolic extract [18]. At six different concentrations (10 to 500 µg/mL), percent mortality of brine shrimp of the plant extract has been presented in Table 3. From the table, it is clear that methanolic extract concentrations are directly proportional to the percentage of mortality. The LC_{50} value for the standard drug vincristine sulfate showed 12.59 µg/ml whereas LC_{50} values of methanolic extract of *Mussaenda roxburghii* exhibited 282.03 µg/ml. In case of negative control group, no mortality was observed.

3.4 Thrombolytic Activity

Streptokinase and distilled water was used as positive control and negative control respectively. Here, streptokinase showed 62.59±2.28% clot lysis and negative control exhibited 4.92 ± 0.98% which is negligible. The mean difference in clot lysis percentage between positive and negative control was very significant (p value < 0.05). Treatment of clots with *Mussaenda roxburghii* extract provided the clot lysis [21] 49.09±1.73%. The mean percentage of clot lysis by *Mussaenda roxburghii* was statistically significant (p value < 0.001). *Mussaenda roxburghii* exihibited the values which were significant (p value < 0.001) compared to both positive control (streptokinase) and negative control (water). Percent clot lysis obtained after treating the clots with methanolic extract and appropriate controls is shown in Table 4.

Maximum clot lysis (62.59±2.28)% was observed in clot treated with streptokinase (streptokinase). The MR plant extract showed clot lysis (49.09±1.73)% and water (as a negative control) showed (4.92±0.98)% clot lysis. Here, MR= *Mussaenda roxburghii.*

Table 1. Percent inhibition of protein denaturation of *Mussaenda roxburghii*

Concentration (µg/ml)	Percent of inhibition in protein denaturation	
	MR	Diclofenac sodium
31.25	42.15%±1.03*	51.65%±1.50
62.5	54.27%±2.09**	61.29%±1.17
125	57.21%±2.06*	64.52%±1.24
250	65.61%±2.83**	74.19%±2.06
500	71.75%±1.56*	80.65%±1.57
1000	80.33%±1.19**	85.49%±1.49

*Values are expressed as mean±SEM of three replicate (n=3). **P<0.01, *P<0.05*

Table 2. Percent stabilization of membrane of *Mussaenda roxburghii*

Concentration (µg/ml)	Percent of membrane stabilization	
	MR	Diclofenac sodium
31.25	53.79%±1.54**	65.56%±1.47
62.5	63.32%±1.93*	73.33%±2.08
125	68.42%±2.20*	81.10%±0.91
250	75.43%±1.22*	86.67%±1.33
500	80.60%±1.35*	90.56%± 2.30
1000	86.93%±2.38*	94.44%±1.96

*Values are expressed as mean±SEM of three replicate (n=3). **P<0.01, *P<0.05*

Table 3. % of mortality of the extract at six concentrations

Concentration (ug/ml)	LogC	% of mortality	
		MR	Vincristine sulphate
10	1	10	40
50	1.699	10	80
100	2	30	100
200	2.301	50	100
300	2.477	60	100
500	2.699	70	100
LC_{50}		282.03	12.59

Table 4. Effect of both extracts (10 mg/ml) on *in-vitro* clot lysis

Treatment	% of clot lysis (Mean±S. D.)
Positive control	62.59±2.28**
Negative control	4.92±0.98
MR	49.09±1.73*

**MR= Mussaenda roxburghii, Positive control= Streptokinase, Negative control= Distilled Water Statistical representation was performed by using paired t-test. Percentage of clot lysis is represented as mean ± S.D. *P<0.001, **P<0.05 compared to control*

4. CONCLUSION

In conclusion, methnolic extract of *Mussaenda roxburghii* leaf possess significant anti-arthritic, anti-inflammatory, thrombolytic activities and minimal anti-cancerous activity ex vivo. It would be fascinating to investigate the mechanism underlying percentage of inhibition of protein denaturation, percentage of inhibition of hemolysis, anti-cancerous activity and clot lytic effects demonstrated by *Mussaenda roxburghii* extract. However, these activities might be due to the presence of bioactive or inhibitory compounds or synergism by the existence of some compounds. A lot of constituents might be present in this extract, such as tannin, polyphenols flavonoids, alkaloids, saponin and so on [18]. In addition, comprehensive research are required to figure out the specific active anti-arthritic, anti-inflammatory, anti-cancerous and thrombolytic compounds present in this methanolic leaf extract.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Only few ml blood was drawn from healthy volunteers by the permission of the Head of the department. Volunteers were the student of our department, which is Department of Pharmacy, International Islamic University Chittagong.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. World Health Organization. Summary of WHO guidelines for the assessment of herbal medicines. Herbal Gram. 1993;28: 13-14.
2. Chauhan AK, Varma A. A textbook of Molecular Biotechnology. First edition, I.K. International (Pvt.) Ltd. 2009;818.
3. Shammi SJ, Islam R, Zaman AU, Majumder R, Alam B. Comparative Pharmacological Studies of *Abelmoschuse sculentus* Linn. Fruits and Seeds Global J. Pharmacol. 2014;8(1):98-106.
4. Shohayeb M, Hameed ESSA, Bazaid SA, Maghrabi I. Antibacterial and Antifungal Activity of *Rosa damascena* MILL. Essential Oil, Different Extracts of Rose Petals. Global J. Pharmacol. 2014;8(1): 01-07.
5. Mollika S, Islam N, Parvin N, Kabir A, Sayem MW, Luthfunnesa, Saha R. Evaluation of Analgesic, Anti-Inflammatory and CNS Activities of the Methanolic Extract of *Syzygium samarangense* Leave. Global J Pharmacol. 2014;8(1):39-s46.
6. Ghani A. Medicinal plants of Bangladesh: Chemical constituents and uses. Asiatic Society of Bangladesh, Dhaka; 1998.
7. Rahman MA. Indigenous knowledge of herbal medicines in Bangladesh and treatment of skin diseases by tribal communities of the hill tracts districts, Bangladesh J Bot. 2010;39:169-177.
8. Islam F, Kuddus MR, Latif F, Hossain MK. Preliminary antimicrobial activity and cytotoxicity of leaf extracts of *Mussaenda roxburghii* Hook. f. Bol Latinoam Caribe Plant Med Aromat. 2013;12(6):612–617.
9. Saha J, Sarkar PK, Chattopadhyay S. A survey of ethno medicinal plants of Darjeeling hills for their antimicrobial and antioxidant activities. Indian Journal of Natural Products and Resources. 2011;2: 479-492.
10. Patil SA, Joshi VG. Evaluation of antibacterial and wound healing activity of leaves of *Mussaenda frondosa* Linn. International Journal of Research in Pharmaceutical and Biomedical Sciences. 2011;2:147-154.
11. Das HB, Majumdar K, Datta BK, Ray D. Ethnobotanical uses of some medicinal plants by Tripuri and Reang tribes of Tripura. Natural Product Radiance. 2009;8: 172-180.
12. Chandra DU, Ghosh R, Chowdhury S, Dinda B. New iridoid from aerial parts of *Mussaenda roxburghii*. Nat Prod Commun. 2012;7:1- 2.
13. Kuddus MR, Aktar F, Miah MK, Baki MA, Rashid MA. Polyphenols content, cytotoxic, membrane stabilizing and

thrombolytic activities of *Sarcolobus globosus*. A medicinal plant from Sundarban forest, Bol Latinoam Caribe Plant Med Aromat. 2011;10:363-368.

14. Kuddus MR, Rumi F, Kaisar MA, Hasan CM, Rashid MA. Trans-isoferulic acid from Curcuma longa. Bol Latinoam Caribe Plant Med Aromat. 2010;9:319–321.
15. Alam MN, Biozid MS, Chowdhury AI, Mazumdar MMU, Chowdhury S, Chowdury MIA. Anti-arthritic and cytotoxic effects of methanolic extract of *Ixora nigricans* leaf. Journal of Medicinal Plants Research. 2015;9(25):719-723.
16. Kumar V, Bhat ZA, Kumar D, Bohra P, Sheela S. *In vitro* anti-inflammatory activity of leaf extracts of *Basella alba* Linn. Var. alba. Int J Drug Dev Res. 2011;3(2): 176-179.
17. Zhao GY, Hui JK, Rupprecht JL, McLaughli KV: Additional bioactive compounds and trilobacin, a novel highly cytotoxic acetogenin, from the bark of *Asimina triloba*. J Nat Prod. 1992;55: 347–356.
18. Billah MM, Islam R, Khatun H, Parvin S, Islam E, Islam SMA, Mia AA. Antibacterial, antidiarrhoeal, and cytotoxic activities of methanol extract and its fractions of *Caesalpinia bonducella* (L.) Roxb leaves. BMC Complementary and Alternative Medicine. 2013;13:101.
19. Prasad S, Kashyap RS, Deopujari JY, Purohit HJ, Taori GM, Daginawala HF. Development of an *in vitro* model to study clot lysis activity of thrombolytic drugs. Thromb J. 2006;4:14.
20. Biozid MS, Rahman MM, Alam MN, Sayeed MA, Chowdhury AI, Faruk M, Abedin MJ. *In-vitro* comparative study of anti-inflammatory and anti-arthritic effects of *Flemingia stricta* Roxb and *Nymphaea nouchali* leaf. Int J Pharm Pharm Sci. 2015;7(8):49-52.
21. Islam MN, Fahad MAB, Hossain MR, Rashid MM, Ferdous MR, Mukti M. *In vitro* Cytotoxic and Thrombolytic activity of methanolic extract of Bauhinia acuminate leaves. UK Journal of Pharmaceutical and Biosciences. 2014;2(2):05-07.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/11395