Vol. 9/1 (2009) 79 - 84

JOURNAL OF NATURAL REMEDIES

Phytochemical and pharmacological investigation of extracts of *Merremia tridentata* Linn. (Convolvulaceae)

A. A. Bidkar*, A. P. Sherje, K. N. Gujar, U. S. Bagul, P. B. Miniyar, S. A. Aphale

Sinhgad Institute of Pharmacy, Narhe, Pune- 411 041 (M. S.)

Abstract

Merremia tridentata (Linn.) is a widely acclaimed Ayurvedic medicinal plant with wide ranging biological activities. It is used in the treatment of rheumatism, hemiplegia, piles swelling and urinary disorders. A literature survey revealed that the anti-inflammatory and wound healing properties of *M. tridentata* has not been evaluated so far. Hence objective of the present work was to evaluate these activities of the root extracts of the plant. The shade-dried roots of *M. tridentata* were defatted with petroleum ether and subjected to continuous hot extraction with ethyl alcohol. The resulting concentrated alcoholic extract was subjected to fractionation using petroleum ether (60-80°), solvent ether, ethyl acetate, butanone and butanol in succession. Preliminary phytochemical screening of extracts revealed the presence of flavonoids. All the fractions were subjected to screening for anti-inflammatory and wound healing properties. The anti-inflammatory activity was studied by carrageenan induced rat paw edema method. The wound healing activity was ascertained by resutured incision, excision, dead space wound (grass pith granuloma) model. The ethyl acetate, butanone and butanol extracts showed better wound healing activity followed by solvent ether and petroleum ether extracts. Hence from the results of phytochemical and pharmacological investigation it can be concluded that, among the various extracts of roots of *M. tridentata*; ethyl acetate, butanol, butanone, solvent ether extracts shows more significant anti-inflammatory and wound healing promotion activities compared to petroleum ether and solvent ether extracts. The above properties of roots of *M. tridentata* can be attributed to the presence of flavonoids.

Key words: *Merremia tridentata*, wound healing, anti-inflammatory, activities, *Merremia*, Convolvulaceae.

1. Introduction

Merremia tridentata Linn. (Convulvulaceae) is a widely distributed plant throughout India, Srilanka, Angola, Mauritius, Modagascar. It a perennial herb with a small woody root stock, stems with elongate, prostate, not twinning slender angular and glabrous structure [1, 2]. Phytochemically the plant has been reported to contain flavonoids quinone, phenolic acids, tannins, saponins, p-hydroxy benzoic acid, vanillic acid, and syrigic acid [3]. It is considered

* Corresponding author
Email: abhijeet_bidkar@rediffmail.com

bitter, astringent, calefacient and tonic [1]. It is used in the treatment of rheumatism, hemiplegia, piles swelling and urinary disorders. The decoction of root is also used for the same purpose [4]. The ethanolic extract of *M. tridentata* was used to study the effect on anti-inflammatory reactions [5]. The anti-inflammatory and wound healing activity of roots of *M. tridentata* has not been scientifically evaluated so far. Hence present work was undertaken to investigate these properties with various extracts of the roots of *M. tredentata*.

2. Materials and methods

2.1 Extraction of active constituent

The roots of *M. tridentata* were collected from Udupi, Manipal (India) in the month of December. It was authenticated at Department of Botany, Poornaprajna College, Udupi (Manipal) where sample specimen was deposited for future reference. The material was shade dried and powdered mechanically. All the reagents and chemicals were either of AR grade or spectroscopy grade. Spectral absorbance measurements were made with Shimadzu 1201 double beam spectrophotometer with 1 cm matched quartz cell. The IR study was carried out on FTIR NICOLET (USA) by applying KBr disc.

For preparation of extract about 1.5 Kg of the powdered drug was defatted with petroleum ether (60-80°) in six batches of 250 g in Soxhlet apparatus. The defatted marc was refluxed with 95% ethyl alcohol. The resulting extract was filtered, pooled, and the solvent removed under reduced pressure using a rotary flash evaporator. The residue obtained from ethanol extract was subjected to fractionation by using petroleum ether (60-80°), solvent ether, ethyl acetate, butanone, and butanol in succession.

The individual fractions were subjected to various chemical tests for sterols, triterpenoids, glycosides, saponins, carbohydrates, alkaloids, flavonoids, tannins, and proteins for detection of chemical group. The results of qualitative chemical tests revealed the presence of flavonoids in solvent ether, ethyl acetate, butanol, and butanone fractions [6, 7]. The chemical identity of the compound was ascertained by using thin layer chromatography (TLC), UV-Visible spectroscopy and infra-red spectroscopic studies. The thin layer chromatographic study was performed using the solvents system of ethyl acetate: acetic acid: formic acid: water (50:11:11:28v/v). After TLC all fractions were subjected to column chromatography for separation and purification of compounds using silica Gel as adsorbent. The elution was carried out using chloroform alone and mixtures of chloroform and methanol in different proportions. Then all elutes form column chromatography for respective fractions were subjected to colour reactions and TLC for further confirmation of flavonoids. The identity of the flavoinoids was confirmed with UV-Visible spectroscopy and Infra-red spectroscopic studies.

2.2 Experimental animals

Albino mice of either sex weighing between 20 – 25 g of 90 days age were used for acute toxicity studies to determine LD_{50} of various fractions. Healthy adult albino rats of either sex weighing between 150-200 g were used for anti-inflammatory and wound healing study. They were maintained at standard housing condition with free access to food and water. All the animal experiments were carried out in accordance with National Institute of Health and Institutional Ethics Committee guidelines.

2.3 Acute toxicity study

The animals were fasted overnight prior to acute experimental procedure. The method of *Miller and Tainter* [8] was adopted for the

determination of LD_{50}. Various extracts were suspended in Tween 80 as a vehicle and were administered peritonially. LD_{50} was extrapolated by making use of graphical method to rats [9]. The one tenth of the lethal dose was taken as the effective (therapeutic) dose.

2.4 Anti-inflammatory study

The method of Winter [10] was adopted for this study. The animals were starved for 12 hrs prior to study. The animals were divided into seven groups (each of six animals) consisting of test, control and standard group. Inflammation was produced by 1% carrageenan in Tween 80 and was injected into the subplanter region of the left hind paw of the rat. The Group I animals were considered as control and were treated with 0.5 ml of Tween 80 solution given orally. The Group II animals served as reference standard and were given orally aspirin (200 mg/ Kg body weight) in a suspension of Tween 80. The Groups III to VII were treated with ethyl acetate, butanol, butanone, petroleum ether, and solvent ether extracts. The paw volume was measured before and 5 hrs after the carrageenan administration by volume displacement method. The difference between initial volume in control and test was used to calculate % inhibition. The data was statistically evaluated by One way ANOVA followed by Tuckey post test. The values of $P< 0.05$ were considered as statistically significant.

2.5 Wound healing study

For the assessment of the wound healing activity resutured incision method [11], dead space wound (granuloma studies) and excision wound model were used. For resutured incision wound method, under anesthesia two longitudinal paravertebral incisions of 6 cm length were made through the entire thickness of the skin at a distance of 1.5 cm from midline on each side of the depilated back. After complete haemostasis, the wound was closed by means of interrupted sutures placed at equidistance. The wounds were left undressed and the animals were caged individually. Group I served as control and was not given any treatment. Animals of Groups II to VIII were treated with different extracts of the drug in a suspension of Tween 80. Sutures were removed on eighth post wounding day. The skin breaking strength of the 10 days old wounds was measured by the method of Lee [12].

For excision study, the skin of the impressed area was excised to full thickness to obtain a wound area of about 500 mm^2. The extracts of the drug were administered orally to the animals. The percentage of wound closure was recorded on 4, 8, 12, 16 postwounding days.

Dead space wounds were created under light ether anesthesia, by subcutaneous implantation of sterilized cylindrical grass piths in the region of the axilla and groin, on both sides. The granulation tissue formed around the grass pith were harvested on the tenth postwounding day and subjected to breaking strength.

3. Result and discussion

The preliminary qualitative chemical test carried out revealed the presence of flavonoids in solvent ether, ethyl acetate, butanol, and butanone fractions. The TLC study showed the Rf values for ethyl acetate, butanol, and butanone fractions as 0.822, 0.814, and 0.848 respectively. Of the several elutes from column chromatography for ethyl acetate fraction chloroform: methanol (70:30v/v) gave a positive colour reaction for flavonoids. The identity of the same elute confirmed by TLC study using same solvent system of ethyl acetate: acetic acid: formic acid: water (50:11:11:28v/v). The Rf value was found to be 0.822.

Similarly, of the several elutes form the column chromatography for butanol and butanone

fractions, the chloroform: methanol (60:40v/v) elute and chloroform: methanol (50:50v/v) elute gave positive colour reactions for flavonoids. Further, these elute showed same Rf as obtained for flavonoids during TLC studies of butanol (0.848) and butanone (0.814) fractions. The ethyl acetate extract had shown the absorption at 223 nm and 246 nm whereas butanol fraction at 233 nm and butanone at 238 nm. The peaks obtained from the IR studies indicate the presence of flavone, isoflavone, and flavonol derivatives which were further confirmed from the absorption values obtained by the UV-Visible spectrophotometric study [3]. The results of IR spectroscopic studies are given in Table 3.

In order to find suitable solvent for extraction and fractionation five different fractions of the ethanol extract were studied for pharmacological study and their comparison was made for both the activities. All the fractions were then subjected to acute toxicity study, anti-inflammatory and wound healing study. One tenth of the lethal dose was selected for evaluation of anti-inflammatory and wound healing properties. The lethal dose for petroleum was found to be 2500 mg/Kg whereas 3000 mg/ Kg for the remaining extracts. Table 1 shows the effects of Aspirin (standard) and all extracts of roots of *M. tredentata* on carrageenan induced rat paw edema. In the present study ethyl acetate, butanone, butanol, and solvent ether fractions showed significant inhibition of rat paw edema i.e. 87%, 68%, 72%, and 84% respectively and insignificant for petroleum ether (23%) when compared to control.

Table 2 shows the tensile strength values (g) of resutured incision, excision and grass pith granuloma model on the tenth post-wounding day. All the five fractions showed considerable difference in response in all the above said wound model showed tensile strength values with ethyl acetate values as (244.7±5.57), butanone fraction (232.89±12.47), butanol fraction (236.1±17.63), petroleum ether fraction (213.0±12.26), and solvent ether fraction (215.4±11.58) which is little higher than control. In grass pith granuloma model, butanol (32.76±24.87), butanone (335.7±18.09), and ethyl acetate (264.1±4.52) fractions showed relatively more wound healing activity when compared to petroleum ether (210.0±1.76) and solvent ether (221.7±11.85) extracts.

Table 1. Effects of Aspirin and Extracts on carrageenan induced rat paw edema.

Sr. No.	Group	Dose	Mean paw volume in ml at 5^{th} hr (Mean± Standard Error)	% Inhibition of paw edema
1	Control	0.5 ml	0.8167 ± 0.060	-
2	Standard	200 mg/Kg	0.3333 ± 0.333*	59.75
3	Petroleum ether	250 mg/Kg	0.4833 ± 0.60*	23.00
4	Solvent ether	300 mg/Kg	0.1500 ± 0.022*	84.14
5	Ethyl acetate	300 mg/Kg	0.1333 ± 0.210*	86.58
6	Butanol	300 mg/Kg	0.2333 ± 0.66*	71.95
7	Butanone	300 mg/Kg	0.2667 ± 0.210*	68.29

Values are mean ± SEM (standard error mean), n=6 in each group; df=6,35; *P<0.05 as compared to control.

4. Conclusion

From the results obtained by pharmacological study it can be concluded that solvent ether, ethyl acetate, butanol, butanone fractions showed more significant anti-inflammatory activity compared to control whereas it was insignificant for petroleum ether. The wound healing activity was more significant for ethyl acetate, butanone, and butanol than petroleum ether and solvent ether when compared to control. The phytochemical investigation carried out on the root extract of *M. tridentata* revealed the presence of flavonoids. Flavonoids are also known to promote the wound-healing process

Table 2. Mean tensile strength values (g) of wound models on tenth post wounding day.

Sr. No	Group	Tensile strength on tenth day (g ± SEM)	
		Resutured Incision model	Grass pith granuloma model
1	Control	144.3± 9.937	155.4 ± 14.15
2	Petroleum ether	213.0 ± 12.26*	210.0 ± 11.76**
3	Solvent ether	215.41 ± 11.58*	221.65 ± 11.85**
4	Butanol	236.1 ± 17.63*	332.76 ± 24.87*
5.	Butanone	232.89 ± 12.47*	335.7 ± 18.09*
6.	Ethyl acetate	243.7 ± 5.57*	264.1 ± 4.52*

Values are mean ± SEM (standard error mean), n=6 in each group; df=6,35; *P<0.05, **P>0.05 as compared to control.

Table 3. Results of Infrared Spectroscopic Studies.

Sr.No	Fractions Used	Peak at cm^{-1}	Inference Stretching / Deformation
01	Ethyl acetate	3425.30	Broad O - H Stretching
		2918.89	C - H Stretching of alkane
		1696.09	C = O Stretching
		1442.89	C – H Deformation of alkane
		806.79	C – H Deformation of aromatic nucleus
02	Butanone	3407.20	Broad O - H Stretching
		2925.56	C - H Stretching of alkane
		1702.92	C = O Stretching
		1448.57	C – H Deformation of alkane
		616.12	C – H Deformation of aromatic nucleus
03	Butanol	3412.95	Broad O - H Stretching
		2925.06	C - H Stretching of alkane
		1708.45	C = O Stretching
		1449.07	C – H Deformation of alkane
		670.69	C – H Deformation of aromatic nucleus

mainly due to their astringent and antimicrobial property, which seems to be responsible for wound contraction and increased rate of epithelialisation [13]. The flavonoids also exhibit anti-inflammatory activity [14]. Thus, various root extracts of the plant are capable of producing anti-inflammatory and wound healing activity and these properties can be attributed to the presence of flavonoids.

5. Acknowledgement

The authors are thankful to Dr. S. Gopalkrishnan Bhat, Professor, Department of Botany, Poornaprajna College, Udupi (Manipal) for authentication of the drug. We also extend our sincere thanks to Prof. M. N. Navale, *Hon*. President, Sinhgad Technical Education Society, Pune for providing the necessary facilities.

References

1. *The Wealth of India*: A Dictionary of Raw Materials and Industrial Products. (1962) New Delhi; 342-349.
2. Kirtikar KR, Basu BD. (1935) *Indian Medicinal Plants*, Lakit Mohan Basu, Allahabad; 1964-1966.
3. Nair GG. (1986) *Current Sci*. 55 (19): 961- 968.
4. Nadkarni AK. (1954) *Indian Materia Medica, Popular Book depot*, Mumbai; 126-128.
5. Vedavathy S, Narayanrao K. (1995) *Indian Drugs*. 32(9): 427-432.
6. Markham KR. (1975) In: Harbone JB, Mabry TJ, Mabry H. (Eds.) *The Flavonoids*, Academic Press, New York; 51.
7. Markham KR. (1975) In: Harbone JB, Mabry TJ, Mabry H. (Eds.) *The Flavonoids*, Academic Press, New York; 647-648.
8. Miller, Tainter. (1944) In: Turner RA. (Eds.) *Screening Methods in Pharmacology*, Academic Press, New York.
9. Paget GE, Barnes JM. (1983), In: Laurence DR, Bacharach AC. (Eds.) *Evaluation of Drug Activities*, Academic Press New York; 115.
10. Winter CA, Risely EA, Nuss GW. (1962) *Proc. Soc. Exp. Bio. Med*. 3: 544-547.
11. Ehrlich HP, Hunt TK. (1969) *Ann. Surg*. 170(2): 203-206.
12. Lee KH.(1968) *J. Pharm. Sci*. 57(6): 1042-1043.
13. Tsuchiya H, Sato M, Miyazaki T, Fujiwara S, Tanigaki S, Ohyama M, *et al*. (1996) *J. Ethnopharmaco*l. 50: 27-34.
14. Onwukaeme ND. (1995) *J. Ethnopharmacol*. 46: 121-124.