



EFFECT OF SOLVENTS ON EXTRACTION OF BIOACTIVE MASS PRESENT IN TITEYPATI (*ARTEMISIA VULGARIS* LINN) LEAVES RESPONSIBLE FOR BODY WEIGHT REDUCTION IN ALBINO RATS

Prasenjit Mitra ¹, Tanaya Ghosh ^{1,2} and Prasanta Kumar Mitra ^{3*}

¹Department of Biochemistry, North Bengal Medical College, Sushrutanagar 734012 Dist. Darjeeling, West Bengal, India.

¹Department of Biochemistry, All India Institute of Medical Sciences, Jodhpur, Rajasthan, India.

²Department of Medical Biotechnology, Sikkim Manipal Institute of Medical Sciences, Gangtok, Sikkim, India.

* Professor & Head, Department of Medical Biotechnology, Sikkim Manipal Institute of Medical Sciences, Gangtok, Sikkim, India.

ABSTRACT

Effect of solvents on extraction of bioactive mass present in Titeypati (*Artemisia vulgaris* Linn.) leaves responsible for body weight reduction in albino rats was studied. Five solvent systems were used. They were, 80 : 20 (v/v) ethanol – chloroform mixture, 80 : 20 (v/v) methanol – chloroform mixture, 80 : 20 (v/v) acetone – chloroform mixture, 80 : 20 (v/v) petroleum ether – chloroform mixture and 80 : 20 (v/v) acetone – petroleum ether mixture. In all cases extraction was carried out with same amount of solvent system at room temperature for a period of 30 minutes. Results showed that mass obtained after extraction of leaves of *A. precatarius* L. with 80 : 20 (v/v) ethanol – chloroform mixture had maximum body weight reduction activity in albino rats.

Key words: Extraction process, Body weight reduction activity, *Artemisia vulgaris* Linn.

INTRODUCTION

In analysis of medicinal plants, extraction is a very crucial step and necessary to obtain the targeted active compound(s) responsible for the desired effect (Fabricant DS, Farnsworth NR, 2001). Extraction is also needed for further separation and characterization of the active compound. Various methods such as heating under reflux, sonification, soxhlet extraction etc. are used for extraction of plant samples (Pharmacopoeia of the People's Republic of China, 2000; The Japanese Pharmacopoeia, 2001; United States Pharmacopoeia, 2002)

In extraction process solvents or mixture of solvents are used. Selection of solvent system largely depends on the specific nature of the bioactive compound being targeted. If the targeted compound is lipophilic in

nature, dichloromethane or a mixture of dichloro methane /methanol are used. In case of hydrophilic compounds polar solvents such as methanol, ethanol or ethyl-acetate are used (Cosa P *et al.*, 2006; Sasidharan S *et al.*, 2011). However, final selection of the solvent system depends on maximum extraction capacity of the bioactive compound by the solvent systems. Selection of solvent system is, therefore, through trial and error.

Recently we found that plant Titeypati (*Artemisia vulgaris* Linn.) could exert body weight loss in albino rats (Ghose Tanaya *et al.*, 2013). We intended to isolate the active compound responsible for the said activity. In connection with the isolation work we studied effects of different solvent systems to obtain active fraction. In this communication experimental details and results are being reported.

Corresponding Author:

Prasanta Kumar Mitra

Email: dr_pkmitra@rediffmail.com

MATERIALS AND METHODS

Plant material

Leaves of Titeypati (*Artemisia vulgaris* Linn.) were collected in morning hours (9 – 10 AM) from the medicinal plants garden of the University of North Bengal, Dist. Darjeeling, west Bengal, India during the periods July – August as we have noted that leaves of *Artemisia vulgaris* Linn. had maximum body weight losing property during this period. Results are under communication. Leaves were authenticated by the experts of the department of Botany of the said University. A voucher specimen was kept in the department of Medical Biotechnology, Sikkim Manipal Institute of Medical Sciences of Sikkim Manipal University, Gangtok, Sikkim, India for future references. Leaves were shade dried and powdered. The powder was used as the test drug.

Animals

Male Wister strain rats, body weight between 35 and 40g, were used for this study. Animals were housed individually in polypropylene cages, maintained under standard conditions

like 12h light and 12h dark cycle, 20 - 30 degree centigrade, 35 - 60 % humidity. The animal experiment was approved by the ethics committee of the Institute. Rats were fed with standard rat pellet diet (Hindustan Lever Ltd., Mumbai, India) and provided water *ad libitum*.

Acute oral toxicity study

Acute toxicity studies were carried out on Swiss albino mice by the method of Ghosh MN (2005). Powdered leaves of *Artemisia vulgaris* Linn. was given at doses of 1, 2, 5, 10 and 30 mg/kg to different groups of mice each group containing six animals. Watery suspension of the test drug was given to the animals orally through a feeding tube. After administering the test drug, the animals were observed for the first three hours for any toxic symptoms followed by observation at regular intervals for 24 hours up to seven days. At the end of the study, the animals were also observed for general organ toxicity, morphological behavior and mortality.

Chemicals

All chemicals used in this study were purchased from Sigma Chemical Company, Mumbai. Chemicals were of analytical grade with high purity.

Experimental design

Leaves of *Artemisia vulgaris* Linn. were properly washed, shade dried and powdered. 100g of this powder were separately extracted in five sets of experiments with

a) 1000 ml of 80 : 20 (v/v) ethanol – chloroform mixture.

b) 1000 ml of 80 : 20 (v/v) methanol – chloroform mixture.

c) 1000 ml of 80 : 20 (v/v) acetone – chloroform mixture.

d) 1000 ml of 80 : 20 (v/v) petroleum ether – chloroform mixture.

e) 1000 ml of 80 : 20 (v/v) acetone – petroleum ether mixture.

Extraction in each case was for ½ h in a soxhlet machine at room temperature (35-37 °C) . It was then centrifuged. Supernatant was collected and evaporated to dryness by applying vacuum pressure. Dry brown mass was obtained.

Rats were divided into two groups of 8 each. First group of animals took normal diet while animals of the second group, in addition to normal diet, took isolated dry brown mass obtained after solvent extraction in the dose of 1.0g/kg body weight daily through oral route. Dose selection was as per our earlier study (Ghose Tanaya *et al.*, 2013). Experiment was continued for 40 days. Separate rats were used for different solvent extraction groups.

Growth of rats

Growth of rats was measured on 10th, 20th, 30th and 40th day. Overall behavior of the animals was noted.

Statistical analysis

The values were expressed as mean ± SEM and were analyzed using one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS). Differences between means were tested employing Duncan's multiple comparison test and significance was set at $p < 0.05$.

RESULTS

Acute toxicity studies

Acute toxicity studies revealed that leaves of *Artemisia vulgaris* Linn. did not produce any toxic symptoms when administered orally to mice in doses of 1, 2, 5, 10 and 30 mg/kg. Animals were healthy, cheerful and behaved normal throughout the experimental period. No death of animal was recorded during seven days of experiment.

Table – 1 shows effect of isolated brown mass after extraction of *Artemisia vulgaris* Linn. leaves powder with 1000 ml of 80 : 20 (v/v) ethanol – chloroform mixture on body weight of rats. It appears from the table that the isolated brown mass from *Artemisia vulgaris* Linn. could decrease body weight of rats from 20th day up to 40 days of experiment and the results were statistically significant at the level of $p < 0.01$ on day 20 and day 30 and at the level of $p < 0.001$ when compared with the control group.

Table – 2 shows effect of isolated brown mass after extraction of *Artemisia vulgaris* Linn. leaves powder with 1000 ml of 80 : 20 (v/v) methanol – chloroform mixture on body weight of rats. It appears from the table that the isolated brown mass from *Artemisia vulgaris* Linn. could not decrease body weight of rats even on 40th day of experiment.

Table – 3 shows effect of isolated brown mass after extraction of *Artemisia vulgaris* Linn. leaves powder with 1000 ml of 10 : 1 (v/v) acetone – chloroform mixture on body weight of rats. It appears from the table that the isolated brown mass from *Artemisia vulgaris* Linn. could decrease body weight of rats even from 20th day up to 40th day of experiment but the result was not statistically significant when compared with control group.

Table – 4 shows effect of isolated brown mass after extraction of *Artemisia vulgaris* Linn. leaves powder with 1000 ml of 80 : 20 (v/v) petroleum ether – chloroform mixture on body weight of rats. It appears from the table that the isolated brown mass from *Artemisia vulgaris* Linn. could not decrease body weight of rats even on 40th days of experiment.

Table – 5 shows effect of isolated brown mass after extraction of *Artemisia vulgaris* Linn. leaves powder with 1000 ml of 80 : 20 (v/v) acetone – petroleum ether mixture on body weight of rats. It appears from the table that the isolated brown mass from *Artemisia vulgaris* Linn. could decrease body weight of rats from 20th day up to 40th days of experiment but the results were not statistically significant when compared with the control group.

Table 1. Showing effect of isolated brown mass after extraction of *Artemisia vulgaris* Linn. leaves powder with 1000 ml of 80 : 20 (v/v) ethanol – chloroform mixture on body weight of rats. (Changes of body weight in gram)

Group	Treatment	10 th day	20 th day	30 th day	40 th day
1	Normal	41.9 ± 2.3	58.2 ± 2.1	62.9 ± 2.3	70.2 ± 2.2
2	Isolated brown mass from <i>Artemisia vulgaris</i> Linn. leaves	39.5 ± 1.3	50.0 ± 1.4*	52.1 ± 1.2*	55.3 ± 1.5**

*p<0.01, ** p< 0.001.

Table 2. Showing effect of isolated brown mass after extraction of *Artemisia vulgaris* Linn. leaves powder with 1000 ml of 80 : 20 (v/v) methanol – chloroform mixture on body weight of rats. (Changes of body weight in gram)

Group	Treatment	10 th day	20 th day	30 th day	40 th day
1	Normal	38.9 ± 2.1	59.2 ± 2.3	60.9 ± 2.3	68.5 ± 2.9
2	Isolated brown mass from <i>Artemisia vulgaris</i> Linn. leaves	36.2 ± 1.1	55.5 ± 1.9	58.1 ± 1.7	66.3 ± 1.8

Table 3. Showing effect of isolated brown mass after extraction of *Artemisia vulgaris* Linn. leaves powder with 1000 ml of 80 : 20 (v/v) acetone – chloroform mixture on body weight of rats. (Changes of body weight in gram)

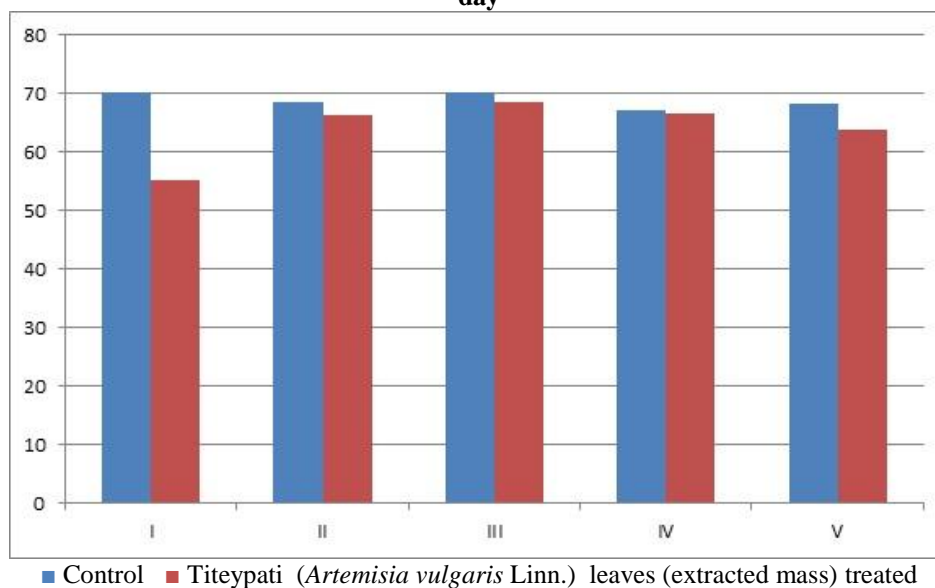
Group	Treatment	10 th day	20 th day	30 th day	40 th day
1	Normal	41.7 ± 1.7	58.9 ± 1.8	61.8 ± 1.7	70.1 ± 2.2
2	Isolated brown mass from <i>Artemisia vulgaris</i> Linn. leaves	39.7 ± 1.2	55.7 ± 1.7	58.3 ± 1.5	68.5 ± 1.7

Table 4. Showing effect of isolated brown mass after extraction of *Artemisia vulgaris* Linn. leaves powder with 1000 ml of 80 : 20 (v/v) petroleum ether – chloroform mixture on body weight of rats. (Changes of body weight in gram)

Group	Treatment	10 th day	20 th day	30 th day	40 th day
1	Normal	38.8 ± 1.2	58.9 ± 1.7	61.9 ± 1.8	67.2 ± 2.1
2	Isolated brown mass from <i>Artemisia vulgaris</i> Linn. leaves	37.2 ± 1.0	56.0 ± 1.6	58.8 ± 1.6	66.5 ± 1.6

Table 5. Showing effect of isolated brown mass after extraction of *Artemisia vulgaris* Linn. leaves powder with 1000 ml of 80 : 20 (v/v) acetone -- petroleum ether mixture on body weight of rats. (Changes of body weight in gram)

Group	Treatment	10 th day	20 th day	30 th day	40 th day
1	Normal	38.1 ± 1.5	59.8 ± 2.6	61.5 ± 2.6	68.2 ± 2.9
2	Isolated brown mass from <i>Artemisia vulgaris</i> Linn. leaves	35.9 ± 1.7	54.1 ± 1.6	57.1 ± 1.7	63.9 ± 1.9

Fig. 1. Leaves of Titeypati (*Artemisia vulgaris* L.)**Fig 2. Effect of extracted mass from leaves of Titeypati (*Artemisia vulgaris* Linn.) on body weight (g) of rats on 40th day**

■ Control ■ Titeypati (*Artemisia vulgaris* Linn.) leaves (extracted mass) treated
 1. 80 : 20 (v/v) ethanol – chloroform mixture II. 80 : 20 (v/v) methanol – chloroform mixture III. 80 : 20 (v/v) acetone – chloroform mixture IV. 80 : 20 (v/v) petroleum ether – chloroform mixture V. 80 : 20 (v/v) acetone – petroleum ether mixture

DISCUSSION

Titeypati (*Artemisia vulgaris* Linn.) is a perennial shrubby aromatic plant throughout the hills of India. It belongs to the family Asteraceae and is abundant in Sikkim and Darjeeling Himalayas in the middle and upper hill forest up to the height of 2000- 5000 ft. The plant has different names : Titeypati in Nepali, Tuk – gnyel in Lepcha, Dhama naga in Tibetan, Dona in Hindi, Nagdamini in Bengali, Barha in Sanskrit and Indian

worm wood in English. The whole plant has medicinal values. Medical uses of the plant as recorded in Ayurvedic literature are: used as appetizer, cures “kapha”, asthma and itching, prevents convulsion. Water extract of the plant is good larvicide like kerosene. It has also feeble insecticidal property. It is antibacterial and antifungal too (Chopra Col Sir RN, Chopra IC.1958; Gurung Bejoy , 2002). Modern researchers established anti microbial property, hepatoprotective activity of

Titeypati (Ghosh Tanaya, *et al.*, 2013; Mitra Prasanta Kumar, 2014 a,b,c; Mitra Prasenjit *et al.*, 2016).

Recently, we noted that Titeypati could exert body weight loss in albino rats study (Ghose Tanaya *et al.*, 2013). We intended to isolate the active mass responsible for the said activity. In connection with the isolation work we studied effects of different solvent systems to obtain active fraction. Five solvent systems were used. They were, 80 : 20 (v/v) ethanol – chloroform mixture, 80 : 20 (v/v) methanol – chloroform mixture, 80 : 20 (v/v) acetone – chloroform mixture, 80 : 20 (v/v) petroleum ether – chloroform mixture and 80 : 20 (v/v) acetone – petroleum ether mixture. In all cases extraction was carried out with same amount of solvent system at

room temperature for a period of 30 minutes. Results showed that mass obtained after extraction of leaves of *Artemisia vulgaris* Linn. with 80 : 20 (v/v) ethanol – chloroform mixture had maximum body weight reduction activity in albino rats (Figure – 2).

CONCLUSION

Effect of solvent on extracted mass from leaves of Titeypati (*Artemisia vulgaris* Linn.) on body weight of rats was studied. Results showed that mass obtained after extraction with 80 : 20 (v/v) ethanol – chloroform mixture could exert maximum body weight loss in rats.

REFERENCES

- Chopra, Col Sir RN and Chopra IC. Indigenous drops of India , U. N. Dhar and sons Private Limited, Kolkata, 1958, P. 562.
- Cosa P, Vlietinck AJ, Berghe DV, Mae, L. Anti-infective potential of natural products: How to develop a stronger in vitro 'proof-of-concept'. *J. Ethnopharmacol.* 106, 2006, 290–302.
- Fabricant DS, Farnsworth NR. The value of plants used in traditional medicine for drug discovery. *Environ. Health Perspect.* 109, 2001, 69–75.
- Ghose Tanaya, Mitra Prasenjit, Mitra Prasanta Kumar, Effect of Leaves of *Artemisia vulgaris* L. on Growth of Rats. *Int. J. Herb Med.*, 1(2), 2013,30-34.
- Ghosh MN. Toxicity studies in fundamentals of experimental pharmacology. Hilton and Company, Kolkata. 2005, 190-7.
- Ghosh Tanaya, Mitra Prasenjit, Mitra Prasanta Kumar, Antibacterial activity of leaves of Titeypati (*Artemisia vulgaris* Linn.). *Bioscience Guardian*, 3(1), 2013,107-112.
- Gurung Bejoy. The medicinal plants of Sikkim Himalaya, J B Gurung (Pub), Gangtok, Sikkim, 2002, p. 35.
- Mitra Prasanta Kumar. Antibacterial activity of an isolated compound (AV – 2) from the leaves of Titeypati (*Artemisia vulgaris* Linn.). *Mintage Journal of Pharmaceutical & Medical Sciences*, 3(3), 2014, 12-14.
- Mitra Prasanta Kumar. Antibacterial activity of an isolated compound (AV-1) from the leaves of Titeypati (*Artemisia vulgaris* Linn.). *World Journal of Pharmaceutical Research*, 3(3), 2014, 4925 – 33.
- Mitra Prasanta Kumar. Seasonal variation in antibacterial activity of leaves of titeypati (*Artemisia vulgaris* L.). *J Pharma Biol.*4(4), 2014, 173-176.
- Mitra Prasenjit, Tanaya Ghosh and Prasanta Kumar Mitra. Seasonal Variation in Hepatoprotective Activity of Titeypati (*Artemisia vulgaris* L.) Leaves on Antitubercular Drugs Induced Hepatotoxicity in Rats. *SMU Medical Journal*, 3(1), 2016,763-774.
- Pharmacopoeia of the People's Republic of China, English ed., The Pharmacopoeia Commission of PRC, Beijing., 2000.
- Sasidharan S, Chen Y , Saravanan D, KM, Sundram KM, Yoga Latha L Extraction, isolation and characterization of bioactive compounds from plants' extracts. *Afr J Tradit Complement Altern Med.* 8(1), 2011,1-10
- The Japanese Pharmacopoeia, Fourteenth ed., JP XIII, The Society of Japanese Pharmacopoeia, Japan.2001.
- United States Pharmacopoeia and National Formulary, USP 25, NF 19, United States Pharmacopoeial Convention Inc., Rockville.2002.