

Antimotility Effect of *Machiluss odoratissima* & *Sonchus wightianus* from Nepal

Amit Subedi^{1,2,3*}, Dipak Khakural¹, Sadhana Amatya¹, Tirtha Maiya Shrestha⁴
and Mohan Prasad Amatya⁵

¹Department of Pharmacy, Institute of Medicine, Tribhuvan University, Kathmandu, Nepal

²Department Antibiotics laboratory, Advanced Science Institute –RIKEN, Saitama, Japan

³College of Pharmacy, Yeungnam University, Gyeongbuk, South Korea

⁴Department of Pharmacy, Kathmandu University, Kathmandu, Nepal

⁵Department of Plant Resources, Kathmandu University, Kathmandu, Nepal

ABSTRACT

The extracts of *Machiluss odoratissima* & *Sonchus wightianus* were subjected to investigate phytochemical, cytotoxic and antimotility properties. The phytochemical screening showed the presence of different compounds. The cytotoxic screening showed that both the extracts are non-toxic. The in vivo study showed the presence of potent antimotility compounds in both plants.

Keywords: *Machiluss odoratissima*, *Sonchus wightianus*, antimotility, Nepal

1. INTRODUCTION

Diarrheal disease is responsible for deaths of millions of children especially in developing countries [1]. The commonly used antimotility drugs, loperamide and diphenoxylate, are not free from associated risk and have limited use in children. Thus, for the search of new, safe and efficacious antimotility drugs, present study was carried out.

Plants have been the source of number of chemical identity with pharmacological values. Though many plants in developing countries are used as traditional medicine, the science behind their use is still unknown. Besides traditionally used medicinal plants, there are still many other plants with their medicinal values unknown and unexplored.

S. wightianus, locally called Dudhe, of asteraceae family is found in 700-4500 m in Nepal while *M. odoratissima*, locally called Kaulo, of lauraceae family is found in 2100-2300 m on lower part of Himalaya [2]. *S. wightianus* is traditionally used during diarrhea [3] but not yet evaluated scientifically. *M. odoratissima* is not used traditionally for medicinal purpose but reported to have antioxidant and antimicrobial property [2]. Thus, in our present study we chose one traditionally used medicinal plant and

one with no traditional use to evaluate their antimotility effect scientifically.

2. MATERIALS AND METHODS

Barks of *M. odoratissima* and leaves of *S. wightianus* were collected from Phulchowki and Sanepa respectively of Kathmandu Valley during the month of November. Brine shrimp from San Francisco Bay (USA) was used and Swiss albino mouse were purchased from Department of plant resource, Kathmandu, Nepal.

2.1 Extract Preparation

M. odoratissima extract – Briefly, cleaned, air dried, and powdered barks were macerated by methanol. The methanolic fraction was defatted by using petroleum ether. Finally, the methanolic portion was dried by vacuum evaporator at temperature below 40°C to obtain dry powder.

S. wightianus extract – Briefly, the cleaned, air dried and powdered leaves were successively extracted in Soxhlet apparatus by petroleum ether, diethyl ether, methanol and water. The extracts were dried by keeping in water bath below 70°C. Methanolic portion was used during all the experiments.

2.2 Phytochemical screening

Phytochemical screening of the extracts was done to identify the main groups of chemical constituents by their color reactions as described elsewhere [2, 4].

2.3 Brine shrimp Bioassay

Brine shrimp bioassay was done according to Carballo [5]. Briefly, artificial seawater was prepared and brine shrimps were hatched in beaker by sparkling brine shrimp eggs at 1gm/ltr. It was continuously illuminated with table lamp and temperature was maintained at 25-30°C. After 48 hr, ten highly active phototropic nauplii were transferred to test tube. Stock solutions of the extracts were prepared and required volume was transferred to test

* Corresponding author: Email: amitsubedi@gmail.com

tube and final volume was adjusted to 5 ml by adding sea water. Dry yeast suspension was added in each test tube as a source of food. After 24 hrs, the number of dead nauplii in each test tube was counted against bright background. The percentage mortality of brine shrimps was calculated at each concentration and control. LC₅₀ (lethal concentration for 50% population) of the extracts were determined by regression equation obtained by plotting mean % mortality against logarithm of concentration. Results shown are average of five experiments for each dose.

2.4 Antimotility Effect

Charcoal meal test was used to evaluate the efficacy of a compound to inhibit gastrointestinal motility in small intestine as described by Rouf [6]. Ethical approval for animal experiments was taken from Institutional Review Board of Institute of Medicine, Kathmandu, Nepal. Briefly, mouse of 20-25 gm were starved for 20 hrs prior to experiments and were divided into 4 groups of 4 mice each. Control group received distilled water at 10 ml/kg, positive control group received atropine at 5 mg/kg and test group received extract intraperitoneally at specified dose prepared in volume of 10ml/kg. After 30 minutes, each group received 1 ml charcoal meal per oral (charcoal meal = 12 gm activated charcoal, 2 gm gum tragacanth diluted to 130 ml by distilled water). After 30 minutes of charcoal meal, each mouse was killed. The abdomen was opened and intestine from pylorus to ileocaecal junction was cut and its length measured. The distance which charcoal meal travelled from pylorus was measured and expressed as percentage of total length.

Intestinal Motility =

$$\frac{\text{Distance travelled by charcoal meal}}{\text{Total length of intestine}} \times 100\% \quad (1)$$

3. RESULTS AND DISCUSSION

3.1 Extractive values and Phytochemical Screening

The extracts were prepared as described and the extractive values are shown in Table I. The phytochemical screening showed that *M. odoratissima* contains terpenoids, tannins, deoxy sugar, saponin and phenolic compounds while *S. wightianus* contains glycoside, steroid, tannin, flavonoids, deoxy sugar and reducing sugar as shown in Table II.

TABLE I. EXTRACTIVE VALUES OF *M.ODORATISSIMA* AND *S.WIGHTIANUS* IN DIFFERENT SOLVENT SYSTEM

Plant	Solvent	Extractive Value (%)
<i>M. odoratissima</i>	Methanol	14.52
<i>S. wightianus</i>	Petroleum Ether	3.773
	Diethyl Ether	1.831
	Methanol	20.35
	Water	0.943

TABLE II. PHYTOCHEMICAL SCREENING OF DIFFERENT EXTRACTS OF *M.ODORATISSIMA* AND *S.WIGHTIANUS*. PE = PETROLEUM ETHER, DE = DIETHYL ETHER, MET = METHANOL

Test compound	<i>M. odoratissima</i>	<i>S. wightianus</i>			
		PE	DE	Met	Water
Alkaloids	-	-	-	-	-
Glycoside	-	-	-	+	+
Terpenoids	+	-	-	-	-
Steroid	-	+	+	-	-
Tannin	+	-	-	+	+
Flavone aglycone	-	-	-	-	-
Flavonoids	-	-	-	+	+
Deoxy sugar	+	+	+	+	+
Saponin	+	-	-	-	-
Coumarin	-	-	-	-	-
Phenolic compounds	+	-	-	-	-
Reducing Sugar	-	-	-	+	+

3.2 Brine shrimp Bioassay

The brine shrimp bioassay showed that the LC₅₀ for methanolic extract of *M. odoratissima* is 48046 µg/ml while that of *S. wightianus* is 2248.72 µg/ml as shown in Table III. The LC₅₀ values of both the extracts are very higher than nontoxic levels [7]. So it is likely that the extract or the lead compound responsible for the pharmacological effect has very low or almost no toxic effect.

TABLE III. BRINE SHRIMP BIOASSAY OF DIFFERENT EXTRACTS OF *M.ODORATISSIMA* AND *S.WIGHTIANUS*.

Extract	Dose (µg/ml)	Mean Mortality (%)	LC50 (µg/ml)
Control	0	0±0	-
<i>M. odoratissima</i>	100	1 1.67±1.67	48046.00
	500	20.00±3.65	
	1000	26.67±3.07	
<i>S. wightianus</i>	100	10±1.67	2248.72
	500	20.00±0.00	
	1000	43.33±3.33	

3.3 Antimotility Effect

The extract of *S.wightianus* and *M. odoratissima* showed potent antimotility effect in Swiss albino mice as shown (Fig.1). The extract of *S. wightianus* was more potent than that of *M. odoratissima*. The present in vivo study showed that extracts of both the plant possess promising antimotility effect. The crude extract showed more potent action than atropine. Thus we can conclude that both the plants possess potent and safe antimotility compound.

The extract of *S. wightianus* showed dose dependent activity. Surprisingly, the effect of extract of *M. odoratissima* was not dose dependent (Fig. 1). From this study we could not find out the reason but it is possible that the *M. odoratissima* extract contains promotility and antimotility compounds and the effect of antimotility compound got saturated at

50 mg/kg while the promotility compound still showing dose dependent effect at 50 mg/kg.

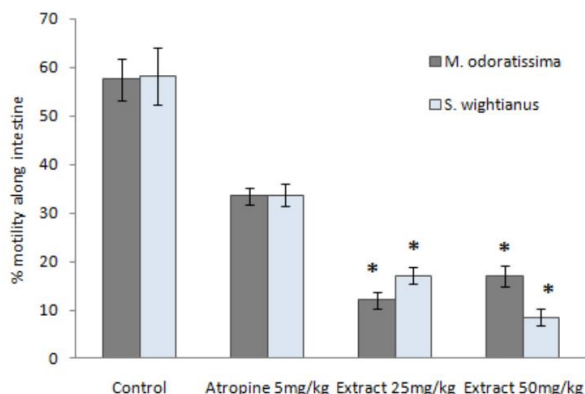


Figure 1. Antimotility effect of extracts of *M.odoratissima* and *S.wightianus* in Swiss albino mice. Data are mean \pm S.E of 4 mice. *, statistically significant ($p < 0.05$) and indicates significance against both the control and atropine group..

4. CONCLUSIONS

The present study showed that both plants contain compounds with significant antimotility effect. Extracts from both plants can be used to develop new, potent and safe antimotility drug. This study shows that scientific study also needs to be focused on plants without traditional medicinal values in the search of novel drug. Though we have not yet characterized the compound responsible for antimotility effect, detailed studies of both plants involving isolation, purification and characterization of responsible compound(s) with their pharmacological effects are going on and will be reported in future.

ACKNOWLEDGMENT

We would like to give special thanks Purna Bahadur Rana and Bishal Raj Adhikari for their assistance and contribution during the study.

REFERENCES

- [1] Ojewole JAO, Awe EO, Chiwororo WDH, 2008. Antidiarrhoeal activity of *Psidium guajava* Linn. (Myrtaceae) leaf aqueous extracts in rodents. *J. Smooth Muscle Res.*, 44(6), 195-207
- [2] Subedi A, Amatya MP, Shrestha TM, Mishra SK, Pokhrel BM, 2012. Antioxidant and antibacterial activity of methanolic extracts of *Machilus odoratissima*. *KUSET*, 8(1), 73-80.
- [3] Namsa ND, Tangjang S, 2011. Natural pharmacopoeia used in traditional system of medicine for the treatment of diarrhoea in Arunachal Pradesh. *Medicinal Plants - International Journal of Phytomedicines and Related Industries*, 3(3), 191-201.
- [4] Paudel A, Panthee S, Shakya S, Amatya S, Shrestha TM, Amatya MP, 2011. Phytochemical and antibacterial properties of *Rhododendron campanulatum* from Nepal. *Journal of Traditional Medicines*, 6(6), 252-258.
- [5] Carballo JL, Hernandez-Inda ZL, Perez P, Garcia-Gravalos M, 2002. A comparison between two brine shrimp assays to detect in vitro cytotoxicity in marine natural products. *BMC Biotechnology*, 2(17).
- [6] Rouf R, Uddin SJ, Shilpi JA, Alamgir M, 2007. Assessment of anti diarrhoeal activity of the methanol extract of *Xylocarpus granatum* bark in mice model. *Journal of Ethnopharmacology*, 109(3), 539-542.
- [7] Moshi MJ, Innocent E, Otieno JN, Magadula JJ, Nondo RSO, Otieno DF, Wensheit A, Mbabazi P, 2010. Antimicrobial and brine shrimp activity of *Acanthus pubescens* root extracts. *TJHR*, 12(2).