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# Antimotility Effect of Hydroalcoholic Extract of Yarrow (*Achillea millefolium*) on the Guinea-Pig Ileum

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**Abstract:** The use of medicinal herbs among the general population gives rise to the possibility of therapeutic or toxic effects in patients that use these plants. The effects of *Achillea millefolium* hydroalcoholic extract on the contractile responses of the isolated guinea-pig ileum were investigated. The effect of the *Achillea millefolium* extracts at five concentrations ranging form 0.05 to 5 mg $mL^{-1}$ was tested. The terminal ileum was removed. Segments were fixed in an organ bath containing in Tyrode solution. Contraction changes in the tissues were monitored using force displacement transducer amplifier connected to physiograph. Each segment served as its own control. *Achillea millefolium* inhibited the contractile response in a dose-dependent manner. The 50% effective concentration values ($EC_{50}$) were calculated, which was 1.5 mg $mL^{-1}$. Regression analysis had shown that with increasing in extract concentration the effect of extract was increased. The coefficient of extract dose was 0.031 mg. Present results demonstrate that extract prepared from the plant of *Achillea millefolium* inhibited electrical induced contractions of the guinea-pig ileum when tested *in vitro*. This effect is dose dependent and reversible.

**Key words:** *Achillea millefolium*, guinea-pig ileum, antispasmodic, irritable bowel syndrome, anti diarrhea

## INTRODUCTION

The use of medicinal herbs among the general population gives rise to the possibility of therapeutic or toxic effects in patients seeking conventional medical assistance. Aerial parts from different species of the genus *Achillea* have been used in traditional and modern medicine as bitter aromatics, astringents, chemostyptics, choleretics and antiphlogistics (Brandley, 1992).

Yarrow (*Achillea millefolium*), extract supplied in polypropylene glycol, is reported to function as a biological additive in cosmetic products (Anonymous, 2001). Many compounds have been isolated from *Achillea* species, including flavonoids, sesquiterpene lactones and polyacetylenes. The extract contains alkanes, fatty acids, monoterpenes, guaiane, squiterpenes (rupicolin A and B, 1-deoxy-1alpha-peroxy-rupicolin A and B) and flavonoids (apigenin and centaureidin) (Stojanovic *et al*., 2005). Eucalyptol, camphor, alpha-terpineol, beta-pinene and borneol were the principal components comprising 60.7% of the oil. It also inhibited the nonenzymatic lipid peroxidation of rat liver homogenate. The extract showed antioxidant activity (Candan *et al*., 2003). The extract of this plant have shown other important effects such as antibacterial effects against *Streptococcus pneumoniae*, *Clostridium perfringens*, *Candida albicans*, *Mycobacterium smegmatis*, *Acinetobacter lwoffii* and *Candida krusei* (Candan *et al*., 2003; Stojanovic *et al*., 2005). Other reports have revealed the presence of compounds such as, 8-cineole, borneol and beta-caryophyllene in the extract (Agnihotri *et al*., 2005).

Among the other main components of this plants are artemetin and casticin (Falk *et al*., 1975). It has been reported that *Achillea nobilis* extract has an antispasmodic action on rat duodenum (Karamenderes *et al*., 2003). Swiss mice treated with this extracts had an increased number of metaphases in the germ epithelium that might be due to cytotoxic substances or substances stimulating cell proliferation (Montanari *et al*., 1998). On the other hand it has been revealed that the *Achillea nobilis* extract is active against a variety of anti tumor cell lines in mouse (Tozyo *et al*., 1994). In folk medicine anti diarrhea effect of this plant

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was reported. Therefore, the main aims of this study were to evaluate the *Achillea millefolium* hydroalcoholic extract on the motility properties of the terminal ileum of guinea pig.

## MATERIALS AND METHODS

**Plant material:** The leaves and flowers of plant of *Achillea millefolium* were collected in Semnan Province of Iran, in 2004 from the Semnan Botanicals Garden, The plant was identified by Dr. Etemadi in the School of Practical and Agricultural Sciences and the voucher specimen (No. 83-36) was preserved and deposited in, herbal library of School of Practical and Agricultural Sciences.

**Extraction and isolation:** Forty five grams of air-dried material were exhaustively extracted with 350 mL methanol by soxhlet extraction. The methanol extract was filtered and concentrated using a rotary evaporator. After filtration, the extract was concentrated to yield 100 mL of a brown residue.

**Animals and assay arrangement:** After approval from the Animal Care and Use Committee at the University was achieved, male guinea pigs (250-400 g) were obtained from the Pasture Institute (Tehran, Iran). Animals were kept at the university animal house at 12 h dark-light cycle at 22-24°C for a week for adaptation. All procedures for care were executed in accordance with national and international guidelines for the care and use of laboratory animals. One day before the test the guinea pigs were fasted overnight with free access to water. The animals were sacrificed by cervical dislocation. The abdomen was opened and the terminal ileum was carefully dissected. The ileum was excised, flushed of luminal contents, placed in Tyrode's solution at room temperature. The composition of the Tyrode's solution was as follows (mM): NaCl 136.9, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.0, $NaHCO_3$ 11.9, $NaH_2PO_4$ 0.4 and Glucose 5.0. The temperature of the tissue bath was kept at 37°C, Whole segments (2 cm long) were set up for recording of isometric contractions along their longitudinal axis in a jacketed organ bath containing 50 mL of gassed (bubbled with 5% $CO_2$ and 95% $O_2$) Tyrode solution. One end of each strip was bound to a glass holder and the other end was connected by a silk thread to a strain-gauge transducer (Harvard Apparatus Limited, MA, USA) to monitor the mechanical activity. Muscle strip was loaded with a resting tension of 0.5 g and after at least 60 min of equilibration and bubbling with 95% $O_2$ and 5% $CO_2$, preparations were submitted to field stimulation with rectangular 1-ms pulses of supramaximal voltage (ca. 45 V), delivered at 0.1 Hz (Narco-Bio-System. Houston, Texas, USA), via platinum electrodes 10 mm in length and 10 mm apart. Electrical field stimulation of the guinea pig ileal segments induced twitch contractions. Muscle tension was recorded isometrically. Contraction changes in the tissues were monitored using force displacement transducer amplifier connected to a physiograph (Harvard Apparatus Limited, MA, USA). At first a 5 minute control period was recorded then the test substance (extract) was added to the bath in a volume of 0.5 mL at different concentrations (0.05, 0.15, 0.5, 1.5 and 5.0 mg $mL^{-1}$) therefore each segment served as its own control. After each experiment the tissue was washed twice. All the responses were recorded for a 5 min period. Each test substance was first examined at a concentration of 0.05 mg $mL^{-1}$ and followed with increased concentrations to evaluate the effect of different *Achillea millefolium* concentrations on intestinal motility. The extract concentrations were administered into the bath to the serosal surface of the ileum.

**Statistical analysis:** The effects of the plant crude extract or controls were determined by comparing the frequency and the amplitude of the control and test materials. Amplitudes were calculated from the physiograph tracings, using an analog-digital tablet and specially designed software. All the results are expressed as the mean±SD (N = 5). Each segment served as its own control. Paired Student's t-test was used for statistical analysis. Quantitative data are presented as medians and 95% confidence intervals. The 50% maximum effective concentration value ($EC_{50}$ value) was calculated. $EC_{50}$ value for contraction inhibition was derived from a nonlinear regression model based on sigmoidal dose response curve (variable) and computed using (Curve expert, 1.3). Also for the evaluation of correlation between doses and effects of extract regression analysis (linear model) was used. A p-value of $<0.05$ was considered to be significant for all tests analysis. The software package SPSS for Windows, version 11.5, (SPSS, Chicago, IL).

## RESULTS

**Antispasmodic activity of *Achillea millefolium*:** Analysis of the dose-response curves for contractions showed significant quantitative differences in the action of the *Achillea millefolium* on peristalsis (Fig. 1) which presents an Antispasmodic activity for *Achillea millefolium* extract.

Additions of *Achillea millefolium* (0.015 mg to 5 mg $mL^{-1}$) induced concentration-dependent depression of contraction evoked by field stimulation in ileal

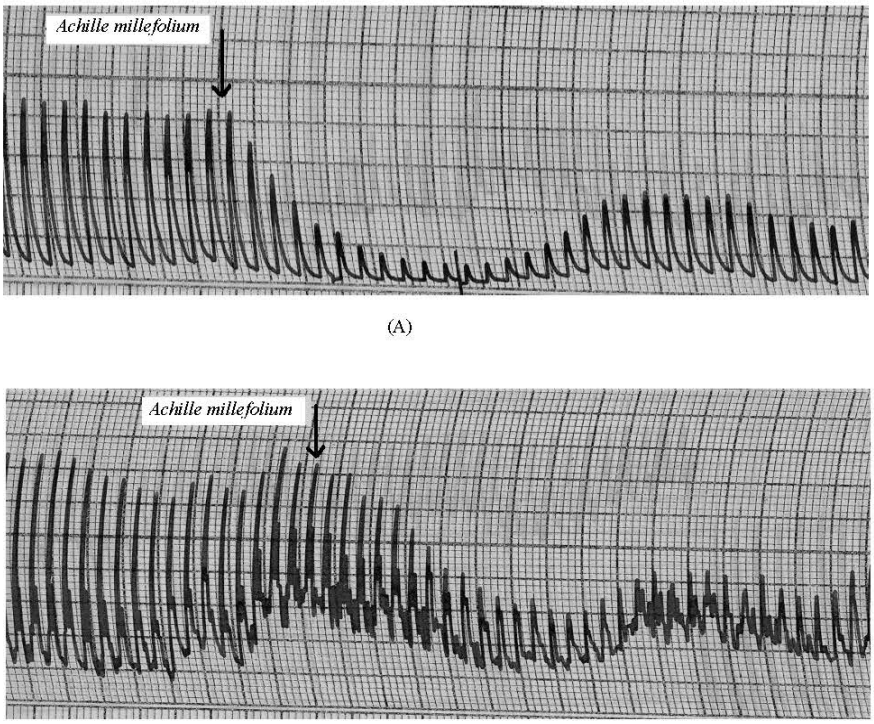


Fig. 1: The effect of *Achillea millefolium* extract in 5 mg $mL^{-1}$ (A) and 1.5 mg $mL^{-1}$ (B) on the small intestine in guinea pig

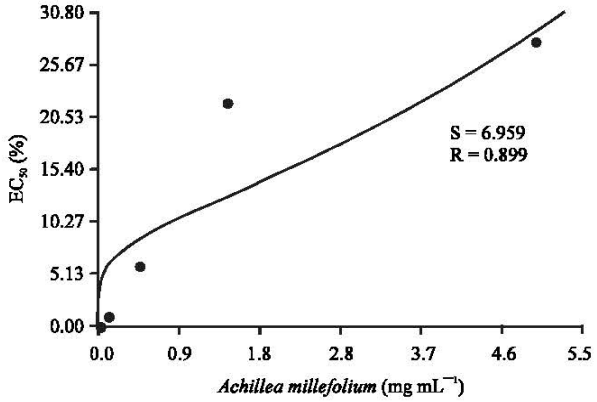


Fig. 2: The $EC_{50}$ value of *Achillea millefolium* extract on the small intestine in guinea pig

segments (n = 5 per group), with a mean $EC_{50}$ value of 1.5 mg $mL^{-1}$ and maximal inhibition of 32±2.9%. An additional dose of 15 mg $mL^{-1}$ was used to test the possible effect of higher concentrations of the extract on ileum contractions which did not increase the observed antispasmodic effect. *Achillea millefolium* inhibited the contractile response in a dose-dependent manner (0.05 mg $mL^{-1}$ = 0%; 0.15 mg $mL^{-1}$ = 1%; 0.5 mg $mL^{-1}$ = 7%; 1.5 mg $mL^{-1}$ = 22%; 5 mg $mL^{-1}$ = 28% of inhibition). The data presented in Table 1 show's inhibitory effects of *Achillea millefolium* on electrical induced contractions of the guinea-pig ileum. The $EC_{50}$ value induced from the graph for the methanol extract of *Achillea millefolium* on guinea-pig ileum was calculated as 1.5 mg $mL^{-1}$ (Fig. 2).

Table 1: Effect of *Achillea millefolium* extract on the small intestine in guinea pig

| Dose of drug | Paired difference | | 95% CI | | |
|---|---|---|---|---|---|
| (mg) | Mean | SD | Upper | Lower | Significant (2-tailed) |
| 0.15 | -1.166 | 2.551 | -7.5054 | 5.1721 | 0.511 |
| 0.5 | -1.470 | 1.616 | -2.9650 | 0.0251 | 0.052 |
| 1.5 | -4.576 | 3.242 | -7.9790 | -1.174 | 0.018 |
| 5 | -6.363 | 5.578 | 11.0270 | -1.700 | 0.014 |

CI = Confidence International; SD = Standard Deviation

Regression analysis showed that with increase in extract concentration the effect of extract was increased (Coefficient = 0.031, constant = 0.094, $p<0.015$). For one percent increase in extract effect coefficient of drug dose was 0.031 mg.

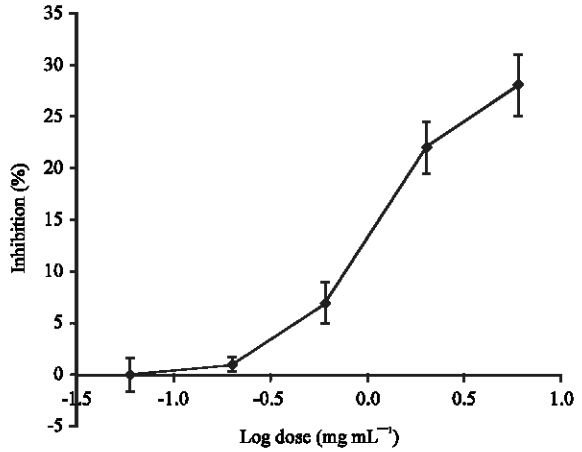


Fig. 3: Log dose-response curve for the *Achillea millefolium* extract on the small intestine in guinea pig

## DISCUSSION

Ethnopharmacological results have always been among the common useful ways for the discovery of biological active compounds from plants (Cordell *et al*., 1991). Present results demonstrated that extract prepared from the plant of *Achillea millefolium* inhibited electrical induced contractions of the guinea-pig ileum when tested *in vitro* (Fig. 3). This effect was dose dependent and reversible. The data presented in this study shows that *Achillea millefolium* extract is an inhibitor of the contractions of the guinea-pig ileum, with $EC_{50}$ calculated as 1.5 mg $mL^{-1}$.

The gut is innervated by the intrinsic primary afferent neurons located in the submucosal and myenteric plexuses. Also it is innervated by the sympathetic and parasympathetic neurons of the autonomic nervous system, which serve as the neuronal connections between the gut and the brain (Gershon, 2003). Cholinergic nerves play an important role in the excitation of the intestine musculature. It has been shown that 0.1 HZ stimulation of the Guinea pig ileum can cause the acetylcholine release from the nerve fibers remained in the tissue and the resulted contractions are reversible using atropine which is a proof for the involvement of acetylcholine in this effect (Puig *et al*., 1977). Therefore it is probable that the observed antispasmodic effect of the extract is due to an interaction with acetylcholine activity. There are several reports which have studied the actions of *Achillea millefolium* plant such as; anticonflict-like effects of aqueous extract of its flowers in female Wistar rat (Molina-Hernandez *et al*., 2004) and also its antioxidant, antibacterial and antifungal activities.

The presence of flavonoids have been reported in the extract (Stojanovic *et al*., 2005) which may take part in antispasmodic activity of the *Achillea millefolium* extract as the spasmolytic activity of flavonoids has been shown in rabbit jejunum (Amor *et al*., 2005). The clarification of definite mechanism for the antispasmodic effects of *Achillea millefolium* needs to be investigated further by methods such as immunohistochemistry and binding study. The decreased small intestinal contractions suggest that *Achillea millefolium* may be useful in the treatment of diarrhea predominant irritable bowel syndrome.

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