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Analgesic and gastrointestinal motility profile of essential oil from *Myrtus communis leaves*

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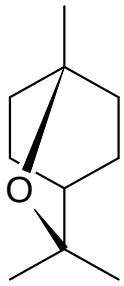
Abstract

In the present research work the essential oil of *Myrtus communis* Leaves were tested for their analgesic and gastrointestinal motility. The analgesic effect was evaluated in mice, using acetic acid induced writhing test and effect on GIT motility was also tested in mice using charcoal as marker in intestine. The essential oils showed dose dependent analgesic effect in compression with standard drug and significantly (P<0.05) inhibited the writhing at 100 and 150 mg/kg, while the analgesic effect was non-significant at the dose of 50 mg/kg. In case of GIT motility the oils were good laxative at low dose (50 mg/kg) while with increasing the dose the motility was not significant. It can be concluded that the oils should be use for constipation in low dose while for analgesic effect its high dose is required.

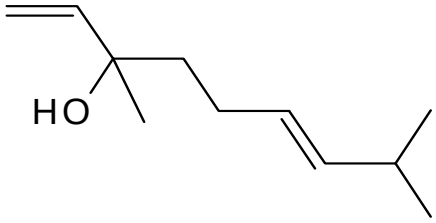
Keywords: *Myrtus communis, Analgesic; Gastrointestinal motility*

Introduction

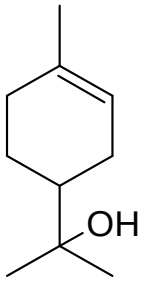
Myrtus communis L. (myrtle) is a common and widespread annual shrub, and the sole representative of the family, Myrtaceae in the Mediterranean Basin, that has been used since ancient times for medicinal, food and spices purposes. The leaves contain tannins, flavonoids such as quercetin, catechin and myricetin derivatives and volatile oils (Baytop, 1999). In essential oil of *M. communis* species, eucalyptol (**1**) was the predominant component (50.13 %). The other important components were linalool (**2**) (12.65%), terpineol (**3**) (7.57%) and limonene (**4**) (4.26%). According to another study essential oil of leaves of *M. communis* study containes pinene (**5**), limonene (**4**) and eucalyptol (**1**) of the (Flamini et al., 2004) . The decoction of leaves and fruits was useful for sore washing. The decoction of the leaves is still used for enemas and against respiratory diseases (Maccioni et al., 2007). The essential oil obtained from the leaves by steam distillation is also important in perfumery (Baytop, 1999). It is known to possess antioxidant, antibacterial, and antiseptic agent (Zanetti et al., 2010). The present study on the essential oil of *Myrtus communis* for analgesic and anti-constipating



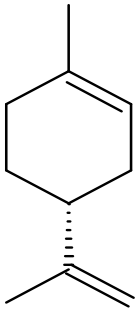
Eucalyptol **(1)**



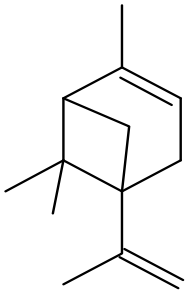
Linalool **(2)**



Terpineol **(3)**



Limonene **(4)**



Pinene **(5)**

actions is the part of our research work on Pakistani medicinal plants (Barkatullah et al., 2011; Ismail M et al., 2011; Muhammad N et al., 2012; Muhammad and Saeed, 2011; Muhammad et al., 2012; Rahman et al., 2011a; Rahman et al., 2011b; Raziq et al., 2011).

Material and methods

Plant material

The fresh leaves of *Myrtus communis* were collected from Peshawar, Pakistan in May 2011. After botanical authentication, the plant materials were washed under running tap water to remove adhering dust, Plant was air dried under shade and crushed into small pieces. The resulting material was subjected to hydro distillation (Muhammad and Saeed, 2011).

Extraction

The isolation of essential oils from *Myrtus communis* leaves is usually obtained by hydro distillation method with a Clevenger-type apparatus, according to the Italian Official Pharmacopoeia. These essential oils were screened for analgesic and anti-constipating effect.

Chemicals

Diclofenac sodium, castor oil, activated charcoal, Acetic acid, sterile normal saline was used in all experiments as control while extracts was prepared in normal saline.

Animals

Balb-C mice of either sex were used for experimental work. Animals were purchased from the Pharmacology Section of the Department of Pharmacy, University of Peshawar. The animals were maintained in standard laboratory conditions (25 ^{0}C and light/dark cycles i.e. 12/12 h) and were fed with standard food and water *ad libitum*.

Analgesic activity

BALB/c mice (18 -22 g) of either sex were fasted for 2 h before starting experiment. Animals were divided in five groups (n=6). Group I was treated with normal saline (10 ml/kg i.p), which served as negative control, while group II was treated with diclofenac sodium (10 mg/ kg i.p) and the remaining three groups were treated with essential oils at the doses of 50, 100 and 150 mg/kg i.p. After 30 min of saline, diclofenac sodium and oils administration, the animals were treated i.p. with 1% acetic acid. The writhing was counted after 5 min of acetic acid injection and the number of abdominal constrictions (writhing) was counted for 10 min (Butterweck et al., 2004). The percent analgesia was calculated using formula: Percent analgesia = 100- no, of writhing in tested animals/ no, of writhing in control animals ×100

GIT Motility test

For this purpose the animals were fasted 18 – 24 hr before the start of experiment. Animals were divided in five groups each of six animals. First group were given normal saline i.p, groups II was treated with castor oil (10 ml/kg) as stander drug, remaining three groups were treated with plant essential oil (50,100 and 200 mg/kg i.p), after 30 min of injecting saline, castor oil and essential oils 0.5 ml a 10 % charcoal suspension in 5 % gum acacia was administer P.O. After 15 min of administering charcoal the animal was killed by cervical dislocation and dissected out. The dissected animal was place on clean surface and measures the distance cover by charcoal (Marona and Lucchesi, 2004). The percent motility was calculated using formula: Percent Motility = 100- Distance covered / total length of intestine ×100

Statistical analysis

The results were articulated as mean ± SEM of six animals. For statistical analysis, ANOVA was followed by post hoc Dunnett's test for multiple comparisons. Effects were considered to be significant at the $P < 0.05$ level.

Results and Discussion

The analgesic effects of essential oils were tested in mice of either sex at the dose of 50, 100 and 150 mg/kg body weight. The numbers of writhing were significantly decreases by the essential oils of our selected plant. The dose dependant analgesic effect of the essential oils is presented in Table 1 and percent analgesia was 43.54, 70.20 and 72.67 at the tested doses of 50, 100, 150 mg/kg of essential oils respectively as shown in Figure 1. The essential

Table 1. Analgesic activities of essential oil from the *leaves Myrtus communis*

Treatment	Dose	No, of writhing (mean)
Normal saline	10ml/kg	65±3.46
Diclofenac sodium	10mg/kg	17.6**±1.14
Essential oil	50 mg/kg	35.00*±2.00
	100 mg/kg	19.37**±1.53
	150 mg/kg	17.70**±2.7

Values (mean ± SEM) present the writing after treatment with diclofenac sodium (10mg/kg), saline (10 ml/kg) and oils (50, 100 and 150 mg/kg). The data was analyzed by ANOVA followed by Dunnett's test. Asterisks indicated statistically significant values from control. *P < 0.05, **P<0.01

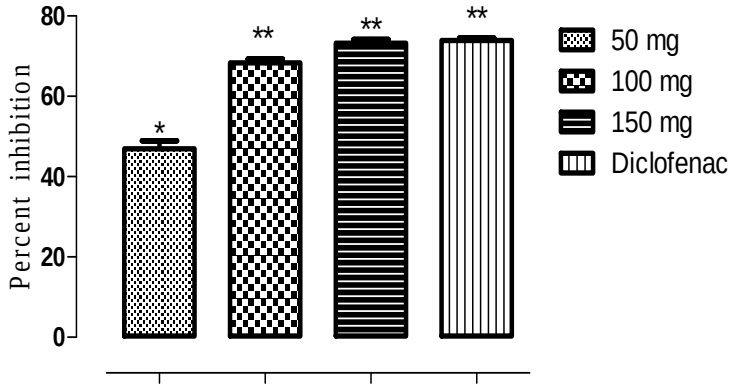


Figure 1. Analgesic effect essential oil of *Myrtus communis* in mice. Bars present the percent inhibition of writing after treatment with diclofenac sodium (10mg/kg), normal saline (10 ml/kg) and oils (50, 100 and 150 mg/kg). The data was analyzed by ANOVA followed by Dunnett's test. Asterisks indicated statistically signifycant values from control. *P < 0.05, **P<0.01.

than the standard drug (castor oil) as shown in Table 2. The maximum GIT activity was observed at 50 mg/kg but with increasing dose the motility was decreased. The percent increased in GIT motility is presented in Figure 2 **.**The analgesic effects essential oils of the leaves of *Myrtus communis* were investigated in acetic acid induced writhing pain model. This model was used for the analgesic affect, because of its sensitivity that could give different grades of injurious stimuli in chemically induced tissue damage (Victor et al., 2009). Similarly, the acetic acid induced writhing has been used to evaluate analgesic effects of drugs and the response is thought to be mediated by peritoneal mast cells, acid sensing ion channels and the prostaglandin pathways (Ranjit et al., 2006).

Table 2. GIT mortality activities of essential oil from the *leaves Myrtus communis*.

Treatment	Dose	Total length of intestine	Distance covered by charcoal
Control	10ml/kg	59.33±0.07	15.17±0.27
Castor oils	10ml/kg	55.75±0.10	33.25**±0.17
Essential oils	50 mg/kg	45.57±0.58	10.67**±0.58
	100mg/kg	47±2.65	18.00**±1.00
	150 mg/kg	47±2.65	28.00*±1.00

Values (mean ± SEM) present the writing after treatment with diclofenac sodium (10mg/kg), saline (10 ml/kg) and oils (50, 100 and 150 mg/kg). The data was analyzed by ANOVA followed by Dunnett's test. Asterisks indicated statistically significant values from control. *P < 0.05, **P<0.01.

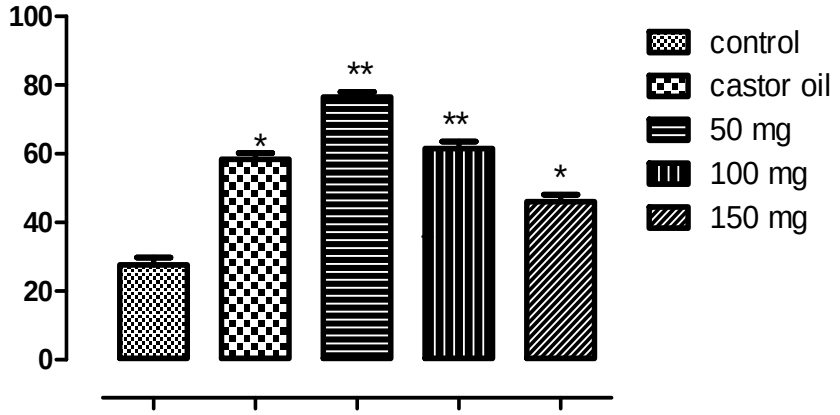


Figure 2. GIT motility effect of essential oil from *Myrtus communis* in mice. Bar presents the percent movement of charcoal in GIT after treatment with castor oils (10ml/kg) and oils (50, 100 and 150 mg/kg). The data was analyzed by ANOVA followed by Dunnett's test. Asterisks indicated statistically significant values from control. **P* < 0.05, ***P*<0.01.

The essential oils of *Myrtus communis* has a significant inhibition in number of the writhing in each mouse (Nuhu et al., 2010). The intraperitoneal injection of acetic acid produces an abdominal writhing response due to sensitization of chemo-sensitive nociceptors by prostaglandins. Increase level of protanoids as well as lipoxygenase products have been found in the peritoneal fluid after the injection of the acetic acid. The analgesic effect of any plant extract may therefore be due to either its action on visceral receptors sensitive to acetic acid, to the inhibition of the production of algogenic substances or the inhibition at the central level of the transmission of painful message (Ranjit et al., 2006).

Constipation is one of GIT disorder and its major complication is pills/hemorrhoid, which is chronic disorder and need surgery. Although there is number of constipation reliving compounds in market but having a lot of side effects, therefore the search for natural anti-constipating agents is carried out in various research institutes. In this research work *Myrtus communis* is tested for their anti-constipating effects on mice, using activated charcoal as marker. This method is simple and non expensive for testing the chemicals or plant extracts for GIT motility study. It is shown that the traveling of charcoal in intestine was mostly upto 20 cm while the movement of charcoal was significant with essential oil of *Myrtus comm.-unis.* It is still very interesting to say that these essential oils are purgative at low dose and good analgesic at high dose. So if these oils are used for the analgesic purpose there will be no chance of loss motion as these oils are not laxative at high dose. In conclusion these essential oils can be used as analgesic and laxative in traditional medicine.

Conflict of interest

There is no conflict of interest associated with the authors of this paper.

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