International Journal of Botany Studies
ISSN: 2455-541X; Impact Factor: RJIF 5.12
Received: 28-12-2018; Accepted: 02-02-2019
www.botanyjournals.com
Volume 4; Issue 2; March 2019; Page No. 07-10

In-vivo pharmacological investigation of root extract of *Myrtus communis* L. by using Animal model (*Mus musculus*)

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Abstract
The *Myrtus communis* is traditionally used for curing skin irritation and intestinal diseases. Therefore the present study was aimed to investigate the effect of *Myrtus communis* root extract (MCRE) for analgesic activity by acetic acid-induced writhing test, and tail immersion test, carrageenan induced model was used for evaluation of anti-inflammatory activity while the antispasmodic activity by intestinal transit of charcoal meal. Analgesic activity of (10 and 15mg/kg) showed normal results (51.5%, 59.3% respectively) but higher dose (20 mg/kg) showed good result (70.3%) compared to diclofenac sodium. Antispasmodic activity showed that the extract was dose-dependent higher dose showed longer distance covered by charcoal in the intestine. The results of anti-inflammatory activity showed that only MCRE at 20mg/kg revealed the effect to lessen the mice paw edema at one hour of administration compared to aspirin. Hence it is proved that MRCE contains analgesic, anti-inflammatory and antispasmodic agents which support its uses in traditional medicine. However; further studies are required for identification and isolation of bioactive constituents of *Myrtus communis*.

Keywords: Medicine, *Myrtus communis*, pharmacological activity, plants, root extract

1. Introduction
The complicated biochemical and biological process, which contain non-specific reaction and vascular tissues, the natural immune responses activate these for injury and infection [1]. Inflammation is the protective process in body against allergens, burns and infection but if inflammation become uncontrol it leads to etiologic factor and make a straight way for many other chronic diseases [2]. Plants have been used for different purposes like food, medicine, as resource of chemical products and for ornamental purpose. Medicine is the main field where plant is becoming an important subject of study growingly receptive to their use [3]. The plants are being used continually as medicines against different diseases. An essential role is being played by medicinal plants against inflammation [1]. Presently *Myrtus communis* is among the most broadly diffused vascular plant's species [4]. It is reported that most of the plants all over the world synthesize different chemicals that play a major role in human as well as animal health [2]. *Myrtus communis* an evergreen shrub of plant family Myrtaceae is distributed all over the world (typical of the Mediterranean maquis) recognized for its therapeutic (antiseptic and hypoglycaemic agent) food and cosmetic uses. The fruits are palatable mostly used as auxiliary to pepper in condiments, while essential oils provide fragrance and flavor [5]. *Myrtus communis* extract has been evaluated for its anti-inflammatory property by determining the carrageenan-induced paw edema suppression for one dose rats [6]. Leaves of *M. communis* described as hypoglycemic, anti-constipation, hemostatic, painkiller, antiseptic and stimulant [7]. Root is reported to have antibacterial properties [5]. Phyto-chemical examinations shown the existence of various eight bioactive chemical constituents i.e. anthocyanins, essential oil, fatty acids, flavonoids, tannins, terpenes, coumarins and phenols [8, 9]. Besides, the chemical synthesized medicines are either very expensive or have a side effect if used as an analgesic or for other inflammations. So, the aim and objective of our conducted study were to assess the pharmacogenetic features of root extract of *Myrtus communis* as an analgesic, tail-immersion, anti-inflammatory and antispasmodic activities.

2. Materials and Methods
2.1 Collection of plants
The fresh and healthy root *of Myrtus communis* was collected from Dir valley Pakistan, and authenticated by plant taxonomist. Root was washed with tap water to remove dust particles and impurities. Root dried at room temperature and grinded in a coffee grinder (Sunbeam EM0415).

2.2 Preparation of crude methanolic extract

The *Myrtus communis* root extract was taken following maceration technique [9]. Powder (250g) (weight by Fisher Scientific scale) was separately refluxed with 10 VOL (v/w) of analytical grade methanol kept in dark for three days; the filtered through Whatman's filter paper. After filtration, the filtrate was run in the rotary evaporator at a temperature of 45℃ under reduced pressure until all the solvent had been removed and the pure plant extract was obtained in the rotatory flask. The pure extract was collected in a china dish, which was used for further processing in the following activities.

2.3 Experimental Animals

The National Institute of Health (NIH) in Islamabad supplied Albino mice of both genders of Swiss origin. The animals were alternatively kept in 12 hours of daylight and 12 hours of darkness. The averages temperature was (25±3℃) with 50-55% humidity. Before the commencement of the experiment, the animals were fed with standard animals feed and acclimatized to their surroundings.

2.4 Analgesic activity

Albino Swiss mice of both sexes lived on fast for almost twenty-fourhours earlier of the experiment. Both sexes were randomly organized into six groups. Group one was treated with normal saline(intraperitoneally 10 ml / kg) for negative control, while group 2 was treated with diclofenac sodium for positive control(50 ml / kg). Group 3, 4, 5 were injected with a methanol extract of 10, 15, 20 mg / kg, and the remaining group No 6 was only injected with acetic acid. After 30 minutes of the pretreatment, injected of 1% acetic acid into the peritoneal cavity of mice induced pain. The writhing that occurred within the next 10 minute following acetic acid administration were recorded for 10 minutes [10].

The percent inhibition was calculated by

% Inhibition = X 100Where, A= Average number of writhing of the control group. B= Average number of writhing of the test group.

2.5 Tail Immersion Test

The control analgesic activity was evaluated by tail immersion test. Mice were divided into groups of two animals each. The lower portion 5 cm of the tail was immersed in a beaker of water maintained at 55± 0.5℃. The time in second for tail withdrawal from the water was taken as the reaction time with a cut off time of immersion at 10sec. The reaction time was measured 1 hour before and after oral administration of extracts (10, 15 and 20mg/kg, per oral) or distilled water (10ml/kg), Ibuprofen (100mg/kg) was administrated subcutaneously 30 min before the test.

2.6 Antispasmodic activity

2.6.1 Charcoal movement activity

The animals were divided into groups of 6 mice in control, positive and experimental groups. Each mouse was given orally 1 ml of the charcoal meal (3 percent deactivated charcoal in 2 percent aqueous tween 80 orally). Methanolic root extract of *M. communis* at doses of 10, 15 and 20mg/kg given to test group. Castor oil (10ml/kg) given to the positive control group and distilled water (10ml/kg) to control group. Each mouse was slaughtered after 50 minutes of charcoal treatment and distance covered by the charcoal meal from the pylorus to caecum was measured [11].

2.7 Anti-inflammatory activity

The initial right hind paw volume of the rats was measured using a pale thermometer. Male mice weighting 20-25g were divided into 4 groups of 6 mice each. The control group received solvent (10ml/kg) group. The group two received a reference drug aspirin at the dose of 200mg/kg, 3 and 4 received methanolic root extract of *M. commuis*at the dose of 10, 15, and 20mg/kg. After 30min each rat in all groups was injected with 0.01ml of 1%carrageenan in normal saline in a subplantar region of the right hind paw. The difference in footpad thickness between the right and left foot was measured with a pair of dial thickness gauge calipers (Ozaki Co., Tokyo, Japan). Mean values of treated groups were compared with mean values of a control group [12].

3. Results and Discussion

3.1 Analgesic Activity

3.1.1 Analgesic activity of *Myrtus communis* on acetic acid induced writhing in mice.

The analgesic effects induced by different doses of extract showed in (Table 1) that two different doses (10,15mg/kg) of Methanolic extract of the plant showed normal result (51.5% & 59.3%) but higher dose (20mg/kg) showed significant analgesic action (70.3%) compared to the standard drug diclofenac sodium (78.1%). Our results are strengthening by [10] carried out analgesic activity of *S. calva*, [13]on *Cnestis ferruginea* Similar work was reported by [14],on *C. campestris* and [15] on *Cassia uniflora*o brained significant analgesic effect at higher dose, which significantly reduce the number of writhing. These evidences strengthen the validity of present study.

Table 1: Analgesic activity of *Myrtus communis* on acetic acid induced writhing in mice.

S.No.	Treatment	Dose	No of writhing	% of Writhing
1	Normal saline	10mg/kg	64± 3.21	
2	Diclofenic sodium	10mg/kg	14± 1.45	78.1
3	MCRE	10mg/kg	31±1.12	51.5
4		15mg/kg	26 ±3.10	59.3
5		20mg/kg	19±1.98	70.3

MCRE= Myrtus communis Root Extract

3.1.2 Tail immersion activity

In tail immersion test MCRE exhibit significant with analgesic effect at a higher dose when drawing his tail after 60min administration of dose with the average 8.95±0.05, 8.95±0.05 and 9.95±0.09 at 10mg/kg, 15mg/kg and 20mg/kg.)

Various researchers performed similar work like [16] conducted analgesic activity of ketamine, Phencylidine,and piperidine[17]. Reported the analgesic effect of the root of *Plambago zeylanica*[18]. Conducted tail immersion test of methanolic and chloroform extract of *Centella axiatica*. These all workers carried out the similar studies that strengthen the present finding.

Table 2: Result of Tail Immersion Test of methanolic Root extracts of *Myrtus communis.*

S. No.	Treatment	Dose	0 min	30min	60min
1	Control			7.01±0.15	7.10±0.21
2	Normal Saline	100mg/kg	0.82±0.05	0.82±0.05	0.82±0.05
3	Ibuprofen	100mg/kg	7.01±0.15	3.92± 0.07	3.99± 0.09
4	MCRE	10mg/kg	0.85 0.03	8.31±0.05	8.95±0.05
5		15mg/kg	8.65±0.12	8.51±0.12	8.83±0.15
6		20mg/kg	8.93±0.10	9.23±0.21	9.95±0.09

MCRE= *Myrtus communis* Root Extract

3.2 Antispasmodic activity

The crude methanolic extract result showed in (Table 3, Fig. 3) a dose determined increase in intestinal motility. The percent increase in intestinal motility of the MCRE percent increase in intestinal motility was 28.4%, 25.5%, and 36.9%. The best result was showed by MCRE at a high dose of 20mg. comparably antispasmodic activity of other plants was investigated by different workers e.g. *Mascarenhasiaar borescens* [19]; *Myrsine africana* [20]; *Mirabilis jalapa* [21] and *Pimpinella anisum* [22].

Table 3: Result of Antispasmodic activity of methanolic Root extracts of *Myrtus communis*

S.No.	Treatment	Dose	Total length-of intestine	Distance covered-by charcoal	%Distance covered by charcoal
1	Control	10mg/kg	52.21±0.05	16.15±0.31	30.9
2	Castor oil	10mg/kg	58.62±0.17	38.35±0.25	64.4
3	MCRE	10mg/kg	45.5±1.22	12.95± 1.32	28.4
4		15mg/kg	55.2±3.11	14.1± 1.89	25.5
		20mg/kg	49.2±2.01	18.2 ±1.67	36.9

MCRE= *Myrtus communis* Root Extract

3.3 Anti-inflammatory Activity

The carrageenan injection (phlogistic agents) caused localized edema. The swelling amplified more and more to an extreme volume afterward the carrageenan injection. In pretreated with aspirin (200 mg/kg) had a majordecrease of paw edema at 30min after the aspirin administration and continued up to 60min with 5.7% respectively compared to the control group.

Only MCRE at a dose of 20mg/kg exhibited the anti-inflammatory effect to reduce the mice paw edema at I hour after administration respectively compared to the control group. Similarly, inflammation property of some other plants was carried out by different researchers e.g. *Scaphiumcnophorum* [23], Turkish Myrtus species [24], *Tridax procumbens* Linn. [25]. The current conclusions agree with these researchers submitting the fact that *M. communis* has noteworthy anti-inflammatory influence.

Table 4: Result of Anti Inflammatory Activity of methanolic Root extracts of *Myrtus communis.*

S.No.	Treatment	Dose	0 min	30min	60min
1	Control		4.6±0.7	4.94±0.14	5.7±0.07
2	Asprin	200mg/kg		4.51±0.12	4.72±0.11
3	MCRE	10mg/kg	1.64±0.3	1.86±0.11	1.93±0.11
4		15mg/kg	1.63±0.15	1.86±0.05	2.06±0.11
5		20mg/kg	1.76±0.15	2.2±0.2	3.03±0.32

MCRE= *Myrtus communis* Root Extract

The conducted study indicated that the root extract of plant "*M. communis"* having an excellent biochemical and biological activities. The root extract shows an excellent anti-inflammatory when it was compared with the control group.

Conclusion

Based on the result obtained in the present study, it is concluded that methanolic root extract of *M. communis* has significant potential for analgesic, anti-spasmodic, anti-inflammatory activities. Moreover, the further investigations are required to understand the mechanisms of action and isolation of chemical compounds responsible for these pharmacological activities.

Acknowledgment

The authors would like to extend their sincere appreciation to the Prof. Abdul Qadeer, Chairman Department of Botany Government Superior Science College Peshawar, Pakistan for providing all research facilities.

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