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BRONCHODILATOR, VASODILATOR AND SPASMOLYTIC ACTIVITIES OF METHANOLIC EXTRACT OF *MYRTUS COMMUNIS* L.

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The present study was undertaken to validate some of the folkloric claims about the effectiveness of the use of a *Myrtus communis* L. crude methanol extract (Mc.Cr) in gastrointestinal, respiratory and vascular diseases. Mc.Cr caused complete relaxation of spontaneous and K^+ (80 mM)-induced contractions in isolated rabbit jejunum. It caused right ward parallel shift of calcium concentration response curves. Mc.Cr exhibited relaxant effect on CCh- and K^+ (80 mM)-induced contractions in isolated rabbit tracheal preparations. Furthermore, Mc.Cr caused relaxation of phenylephrine (1 μM)- and K^+ (80 mM)-induced contractions in isolated rabbit aorta preparations. These effects were similar to verapamil, a standard calcium channel blocker. These findings could be the basis for explaining the spasmolytic, bronchodilator and vasodilator activities of the extract, through a possible calcium channel blocking activity.

K e y   w o r d s : *Myrtus communis, spasmolytic effect, bronchodilatory effect, vasorelaxant effect*

INTRODUCTION

Myrtus communis L. (Myrtaceae) is a well-known shrub diffused in all the world and known for its therapeutic, cosmetic and food uses. The myrtle fruits are edible and consumed as substitute for pepper in condiments (1), whereas its essential oils is source of flavor and fragrance (2). Fruits possess antiseptic, astringent, carminative, emmenagogue, dessicant, demulcent, analgesic, anti-inflammatory, haemostatic, antiemetic, lithotripsic, diuretic, stomachic, haemostatic, nephroprotective, antidote, antidiaphoretic and antidiabetic properties and are used as a cardiotonic, a hair tonic and a brain tonic (3-4). Leaves are claimed to be astringent, antiseptic, hypoglycaemic, laxative, analgesic, haemostatic and stimulant (5). The leaves are claimed to be useful in treatment of cerebral diseases, especially epilepsy, and in stomach diseases (6). The essential oil of the leaves has been esteemed in France as a disinfectant and an useful antiseptic and has also been used in certain respiratory and bladder diseases and recommended as a local application in rheumatic disease (7). The decoction of the leaves is still used for vaginal lavages, enemas and against respiratory diseases (8). Root is reported to have antibacterial properties (9). Fruits are eaten raw or cooked (10). The fruit has an aromatic flavour, it can be eaten fresh when ripe or can be dried and is then used as an aromatic food flavouring or used for an acid drink (1, 11). The leaves are used as flavouring in cooked savoury dishes (12). Dried fruits and flower buds are used to flavour sauces and syrups (1). An essential oil from the leaves and twigs is used as a condiment, especially when mixed with other spices (2, 13). The flowers have a sweet flavour and are used in salads (1). Leaves, flower as well as fruit contains essential oil which is used both in the flavor and fragrance industry (14).

Phytochemical investigations revealed the presence of essential oil (15), anthocyanins (16), fatty acids (17), coumarins (18), flavonoids (19), tannins (20), terpenes (21) and phenolics (22-25). The plant has traditionally been used to treat a number of ailments relevant to gastrointestinal respiratory systems and vessel related diseases. As part of our continuous studies to explore biological activities of medicinal plants (26-32), the present study was undertaken to validate some of folkloric uses of *M. communis*, in particular in the treatment of gastrointestinal, respiratory and vascular diseases.

MATERIALS AND METHODS

Plant material and preparation of extract

The aerial parts of *Myrtus communis* L. were collected from a rural area surrounding Multan, Pakistan in June, 2010, and were identified by the taxonomist, Saima Shehzadi, from the Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan and a voucher specimen (P.Fl. 742-3) was kept in the herbarium of this University. The plant material was shade dried and rendered free from possible adulterants through manual picking. The dried plant material was grinded to coarse powder with the help of special herbal grinder. The powdered material was soaked in 70% aqueous-methanol for 1 week in amber colored bottle with occasional shaking. The soaked material was passed through a double layered muslin cloth to remove vegetative debris. The fluid portion was filtered through Watman-1 filter paper. The procedure was repeated twice and the combined filtrate was evaporated on a rotary evaporator under reduced pressure at 40°C to a dark green coloring mass of thick

paste like consistency and approximate yield was 9.5%. The crude methanol extract of *Myrtus communis* (Mc.Cr) was solubilized in distilled water to be used in *in-vitro* experiments. All dilutions were made fresh on the day of experiment.

Chemicals

Acetylcholine chloride, carbachol (CCh), potassium chloride, verapamil hydrochloride, phenylephrine (PE) and magnesium chloride were purchased from Sigma Chemicals Co. St Louis, MO, USA. Calcium chloride, glucose, magnesium sulphate, potassium dihydrogen phosphate, sodium bicarbonate, sodium dihydrogen phosphate, and methanol were obtained from Merck, Darmstadt, Germany. Sodium chloride and sodium hydroxide were purchased from BDH Laboratory supplies, Poole, England. The chemicals used in the experiments were of highest purity and reagent analytical research grade. Stock solutions and subsequent dilutions were made fresh in distilled water on the day of experiment. The drugs were rendered soluble in vehicles which were without any effect on tissue contractility in control experiments.

Animals and housing conditions

Animals used were local strain rabbits (male/female; 1.0–1.8 kg), housed under controlled environmental condition (23–25°C) at the animal house of Faculty of Pharmacy, Bahauddin Zakariya University, Multan. The animals were provided with fresh green fodder and tap water *ad libitum,* deprived of food 24 hours prior to the experiments but were given free access to water. Rabbits used for *in vitro* studies were sacrificed following a blow on back of head.

All the experiments on animals were performed in compliance with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences (NRC, 1996), approved by the Ethical Committee of Bahauddin Zakariya University, Multan, Pakistan.

In vitro experiments

The experiments on isolated tissues were performed by adoption of procedures as described previously (33-34). Briefly, we used freshly prepared jejunum, tracheal and aortic tissue segments from the rabbit and maintained adequately well in the respective buffer solutions. The detailed elaboration of each tissue extraction procedure is described below under the respective heading.

1. Isolated rabbit jejunum preparations

The plant extract was tested on isolated rabbit jejunum preparations for possible presence of spasmolytic activity. Rabbit was dissected to remove jejunum and placed in Tyrode physiological salt solution maintained at 37°C and aerated with carbogen (95% O_2 and 5% CO_2). The tissue were cut into segments about 2 cm in length, rendered free of adhering mesenteries and were subsequently suspended in isolated tissue baths containing Tyrode's solution, at 37°C and aerated with carbogen. The composition of the Tyrode's solution (mM) was: KCl (2.68), NaCl (136.9), $MgCl_2$ (1.05), $NaHCO_3$ (11.90), NaH_2PO_4 (0.42), $CaCl_2$ (1.8) and glucose (5.55). A preload of 1 g was applied and intestinal contractions were recorded isotonically through Powerlab Data Acquisition System (AD Instruments, Sydney, Australia). The tissues were allowed to equilibrate for about 30 min prior to exposure to any drug or test material. The isolated rabbit jejunum preparations exhibit spontaneous rhythmic contractions and allow testing of the relaxant (spasmolytic) effect without application of an agonist (34). The response observed on addition of test material to isolated tissue bath was quantified by dose addition in cumulative manner. The observed relaxant effects on the part of test substance was quantified as the percent decrease in spontaneous contractions of the preparation recorded immediately prior to the addition of test substances.

The possible mechanism of the relaxant activity of the test material was investigated through the relaxation of K^+ (80 mM)-induced sustained spasmodic contractions (35). The test material was added to isolated tissue bath in a cumulative manner to relax sustained contractions in concentration-dependent manner (36). The observed relaxant effect of the test material on K^+ (80 mM)-induced contraction was expressed as percent of the control contractile response.

Calcium channel blocking effect of the test substance was confirmed by the method described previously (37). The isolated rabbit jejunum preparation was allowed to stabilize in normal Tyrode's solution, which was subsequently replaced for 30 min with Ca^{2+}-free Tyrode's solution to which EDTA (0.1 mM) was added in order to remove calcium from the tissue. The isolated tissue bath solution was further replaced with K^+-rich and Ca^{2+}-free Tyrode'ssolution, having the following composition (mM): KCl (50), NaCl (91.04), $MgCl_2$ (1.05), $NaHCO_3$ (11.90), NaH_2PO_4 (0.42), glucose (5.55) and EDTA (0.1). Subsequent to incubation period of 30 min., Ca^{2+} was added to the tissue bath in cumulative manner to obtain control calcium concentration-response curves (CRCs). The control calcium concentration-response curves are prepared in duplicate and tissue was then washed and allowed to be equilibrated in the presence of plant extract for 1 hour prior to recording of the concentration response curves of Ca^{2+} for comparison to the control concentration response curves. The CRCs of Ca^{2+} were recorded in the presence of different concentrations of the plant extract in tissue bath.

2. Isolated rabbit tracheal preparations

Rabbit trachea was dissected out as described previously (38-40) and kept in Krebs solution having the following composition (mM): NaCl (118.2), $NaHCO_3$ (25.0), $CaCl_2$ (2.5), KCl (4.7), KH_2PO_4 (1.3), $MgSO_4$ (1.2) and glucose (11.7). The trachea was cleaned free from the surrounding fatty tissues and rings of 2–3 mm width containing 2–3 cartilages were prepared. Each ring was opened by a longitudinal incision on the ventral side opposite to the smooth muscles layer to form a strip with smooth muscles layer in middle and cartilages on both sides. These tracheal tissues were mounted in 20 ml organ bath containing Krebs solution being maintained at 37°C and aerated with carbogen. A preload tension of 1 g was applied and tissue preparations were allowed to be equilibrated for 1 hour prior to any challenge by the drug. The Mc.Cr was added to the isolated tissue bath in different concentrations in cumulative manner to assess possible relaxant effect on carbachol (1 μM)- and high K^+ (80 mM)-induced sustained contractions. The isometric contractile responses were recorded through a Powerlab Data Acquisition System (AD Instruments, Sydney, Australia) linked to a computer installed with a Lab Chart software (Version 7). The standard drug, Verapamil, with Ca^{2+} channel blocking effect was tested on carbachol (1 μM)- and K^+ (80 mM)- induced spastic contractions for confirmation of the possible mode of action.

3. Isolated rabbit aorta preparation

The descending thoracic aorta was dissected out and kept in Krebs solution at 37°C and aerated with carbogen. It was cut into

rings of about 2–3mm in width and each ring and was mounted in a tissue bath containing Krebs solution at 37°C and aerated with carbogen. A pre-load of 2 g was applied to each preparation and allowed to equilibrate for a period of 1 hour. Vasorelaxant effects were assessed on phenylephrine (1 μM)- and K^+ (80 mM)-induced spastic contractions in isolated tissue preparations. Changes in isometric tension of aortic rings were recorded *via* a force-displacement transducer coupled to Powerlab Data Acquisition System (AD Instruments, Sydney, Australia) linked to computer installed with Lab Chart software (version 7).

RESULTS

Effect on isolated rabbit jejunum preparations

Mc.Cr exhibited inhibition of the spontaneous contractions of the isolated rabbit jejunum in a dose dependent manner, at the dose range of 0.1–5.0 mg/ml, with an half maximal effective concentration (EC_{50}) value of 1.70 mg/ml (95% CI: 1.65–1.75; n=5). It also caused a dose dependent relaxation of K^+ (80 mM)-induced contractions at a concentration range of 0.1–5.0 mg/ml with an EC_{50} value of 2.90 mg/ml (95% CI: 2.85–2.95; n=5). These findings are comparable to the verapamil, which caused relaxation of the spontaneous as well as K^+ (80 mM)-induced contraction in rabbit jejunum with

respective EC_{50} value of 0.32 μM (95% CI: 0.29–0.39; n=5) and 0.105 μM (95% CI: 0.102–0.109; n=5) (*Fig. 1*).

Effect on Ca²⁺concentration-response curves

The Mc.Cr was also tested on the control CRCs of calcium. The medium was rendered Ca^{2+} free during which the spontaneous contractions of the isolated rabbit jejunum preparations were abolished completely but the cumulative addition of Ca^{2+} (0.1–6.4 mM) caused a stepwise revival of the contractile activity in tissue and maximal contraction was attained at tissue bath concentration of 6.4 mM of Ca^{2+}, which was considered to be 100%. The tissue incubated with Mc.Cr at concentration range of 1.0–3.0 mg/ml for 35 min shifted the concentration response curve of Ca^{2+} towards right. These effects were found to be comparable to those produced by verapamil (0.1–0.3 μM), (*Fig. 2*, n=5).

Effect on isolated rabbit tracheal preparations

The Mc.Cr caused complete relaxation of K^+ (80 mM)-induced and carbachol (1 μM)-induced contractions in isolated rabbit tracheal preparation at 3.0 mg/ml and 5.0 mg/ml with respective EC_{50} values of 0.788 mg/ml (95% CI: 0.70–0.89; n=5) and 1.07 mg/ml (95% CI: 0.99–1.94; n=5). Similarly, verapamil also caused the relaxation of high K^+ (80 mM) and carbachol
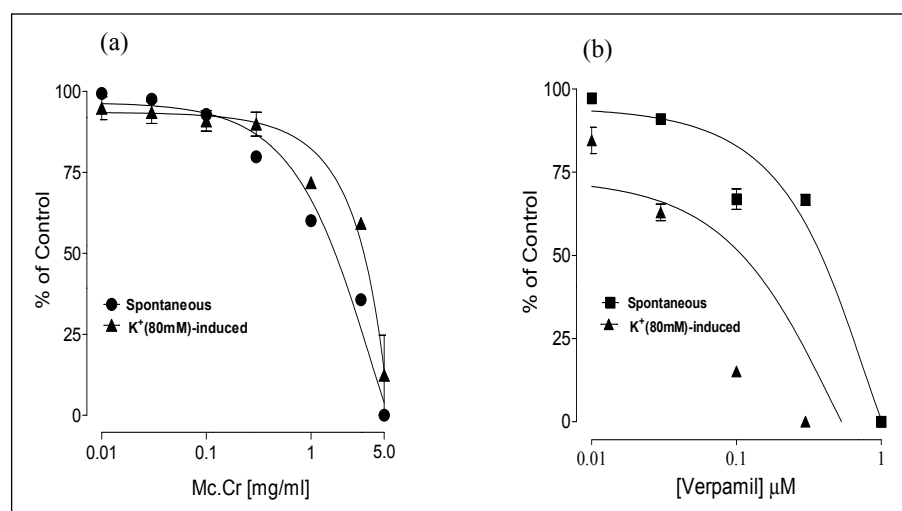

Fig. 1. Concentration dependent inhibitory effects of crude extract of (a) *Myrtus communis* (Mc.Cr) and (b) verapamil on spontaneous and high K⁺- (80 mM) induced contractions on rabbit jejunum preparation. Values are expressed as the mean ±S.E.M., n=5.
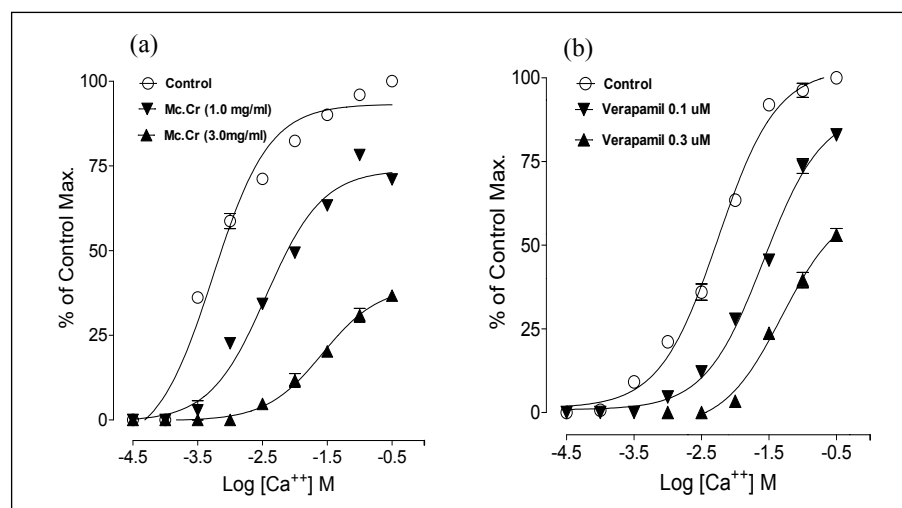

Fig. 2. Concentration response curves of Ca⁺⁺ in the absence and presence of increasing concentrations of crude extract of (a) *Myrtus communis* (Mc.Cr) and (b) verapamil in isolated rabbit jejunum preparations. Values are expressed as the mean ±S.E.M., n=5.
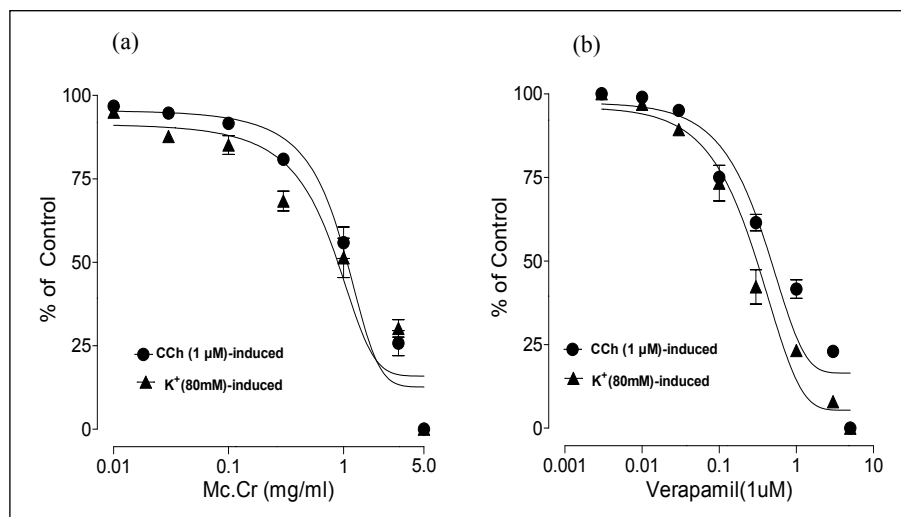
Fig. 3. Concentration dependent inhibitory effect of crude extract of (a) *Myrtus communis* (Mc.Cr) and (b) verapamil on CCh and K+ (80 mM)-induced contractions on isolated rabbit tracheal preparations. Values are expressed as the mean ±S.E.M., n=5.
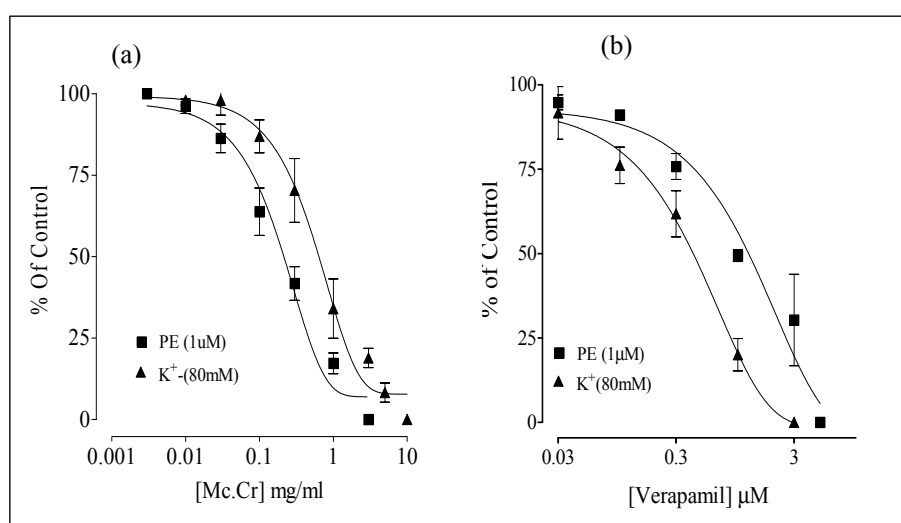

Fig. 4. Concentration dependent inhibitory effect of crude extract of (a) *Myrtus communis* (Mc.Cr) and (b) verapamil on PE (1 μM) and K+ (80 mM)-induced contractions on isolated rabbit aorta preparations. Values are expressed as the mean ±S.E.M., n=5.

(1 μM)-induced contractions with respective EC_{50} values of 0.298 μM (95% CI: 0.260–0.36; n=5) and 0.461 μM (95% CI: 0.39–0.490; n=5) (*Fig. 3*).

Effect on rabbit aorta rings preparations

The isolated rabbit aorta rings on exposure to phenylephrine (1 μM) as well as K+ (80 mM), demonstrated a consistent contractile activity. The rings were relaxed following the addition of Mc.Cr to isolated tissues bath in a concentration dependent manner, at 5.0 mg/ml and 10.0 mg/ml, with respective EC_{50} values of 0.21 mg/ml (95% CI: 0.18–0.28; n=5) and 0.65 mg/ml (95% CI: 0.46–0.94, n=5).Verapamil, being a standard Ca^{2+} channel blocker, also relaxed the phenylephrine (1 μM)- and K+ (80 mM)-induced contractions with EC_{50} values of 0.92 μM (95% CI: 0.81–0.98; n=5) and 0.39 μM (95% CI: 0.34–0.43; n=5), respectively (*Fig. 4*).

DISCUSSION

The herbal medicines are continuously used throughout world for prevention and treatment of different ailments and the natural products have significant contribution toward pharmaceutical industry as a source of potent medicinal agent (41). Plants have shown potential to facilitate management of gastrointestinal, respiratory and cardiovascular ailments (42).

Myrtus communis has a folkloric repute to provide relief in diarrhea and dysentery, hence its methanol extract, Mc.Cr, was applied on spontaneous contractions of isolated rabbit jejunum preparations for the evaluation of its possible spasmolytic effect. The plant extract demonstrated a spasmolytic potential through the suppression of the spontaneous contractions. The contractile activity in smooth muscle preparations is known to be dependent upon increased cytoplasmic concentration of free Ca^{2+} prior to activation of contractile elements (43). The increased intracellular Ca^{2+} is reported to be either influenced through L-type voltage dependent Ca^{2+} channels (VDCs) or to be released from intracellular stores in sarcoplasmic reticulum. Recent findings (44) suggested the involvement of T-type Ca^{2+} channels ($Ca_v3.2$: low voltage activated) in various functions of colonic regulation particularly mucosal cytoprotection and therefore contribution of this type of Ca^{2+} channels cannot be ruled out as a contractile element. The spontaneous contractions of the jejunum are known to be regulated by action potential which appears through rapid influx of Ca^{2+} *via* VDCs at the climax of periodic depolarization (45). The observed suppression of the spontaneous movements in isolated rabbit jejunum preparations may be due to interference either with influx of Ca^{2+} through VDCs or Ca^{2+} release from intracellular storage sites. In an attempt to explore the mechanism of the above-mentioned Mc.Cr-induced suppressant activity on spontaneous contractions, it was tested on K+ (80 mM)-induced contraction in isolated

rabbit jejunum preparations and found to exert comprehensive relaxant effect. The exposure to K^+ (>30 mM) causes contractions in smooth muscle preparations through the influx of extracellular Ca^{2+} subsequent to opening of VDCs (46). Substances capable to inhibit of K^+ (80 mM)-induced contraction are supposed to be inhibitors of Ca^{2+} influx (47). Similarly, verapamil, an established Ca^{2+} channel blocker (48), was also found to exert suppression of spontaneous as well as K^+ (80 mM)-induced contractions in isolated tissue preparations. The Ca^{2+} antagonist activity on the part of constituent(s) of *Myrtus communis* was further confirmed as Mc.Cr caused right ward shifting of the Ca^{2+} CRCs constructed in K^+-rich and Ca^{2+}-free medium. The Ca^{2+} channel blockers are found to be an important therapeutic class, the prevalent features of which is concentration-dependent inhibition of the slow entry of Ca^{2+} as well as reversal of the response to Ca^{2+} (49).

Myrtus communis has folkloric uses also in respiratory ailments (1). Its methanol extract was found to exhibit relaxant effect on carbachol (1 µM)- and K^+ (80 mM)-induced contractions in isolated rabbit tracheal preparations. The extract exerted relaxant effect on carbachol- as well as K^+ (80 mM)-induced contractions in a manner comparable to verapamil. The observed nonspecific relaxant effect is likely to be mediated through a Ca^{2+} channel blocking mechanism. The Ca^{2+} channel blockers are reported to provide relief in respiratory congestions (50) and availability of such activity of Mc.Cr may provide scientific basis for the traditional use of the plant in respiratory stress.

The respiratory disorders have an etiological association with cardiovascular diseases (51), hence the plant extract was further investigated for its possible vasodilator effects. Mc.Cr exerted relaxant effect on phenylephrine (1 µM)- and K^+ (80 mM)-induced contractions in isolated rabbit aortic preparations similar to verapamil. The phenylephrine-induced contraction of vascular smooth muscles is mediated through the increased cytosolic Ca^{2+} *via* both Ca^{2+} influx through receptor operated channels as well as the Ca^{2+} released from intracellular stores (51). Previously (52) it was also shown that high KCl induced contraction in aortic tissue is due to membrane depolarization, resulting to the increased of calcium influx possibly through L or T-type of Ca^{2+} channels. Therefore, we can speculate the relaxation of both phenylephrine as well as K^+-induced contractions by Mc.Cr was possibly mediated through a Ca^{2+} channel blocking effect.

The present study was undertaken to validate the folkloric use of *Myrtus communis* in the management of multiple ailments. *In vitro* studies on isolated tissue preparations demonstrated that crude methanol extract of *Myrtus communis* possess spasmolytic, bronchodilator and vasodilator activities possibly due to blockade of voltage dependent calcium channels.

Conflict of interests: None declared.

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