Pharmacological basis for the medicinal use of *Alcea rosea* in airways disorders and chemical characterization of its fixed oils through GC-MS

Muhammad Hanif[1,2], Malik Hassan Mehmood[1,3], Ghazala Ishrat[5], Safna Naozer Virji[1], Abdul Malik[4], Mansoor Ahmed[2]* and Anwarul-Hassan Gilani[1]

[1]Natural Product Research Division, Department of Biological and Biomedical Sciences, The Aga Khan University Medical College, Karachi, Sindh, Pakistan

[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi, Pakistan

[3]Department of Pharmacology, Faculty of Pharmaceutical Sciences, Government College University, Faisalabad, Pakistan

[4]Department of Pharmacology, College of Pharmacy, University of Sargodha, Pakistan

[5]Department of Pharmaceutics, Faculty of Pharmacy, Barrett Hodgson University, Karachi, Pakistan

Abstract: *Alcea rosea* L. also known as *Althea rosea* belongs to the Malvaceae family. This medicinal herb, traditionally used to treat several conditions including airway disorders like asthma and chronic bronchitis. This study evaluated the bronchodilatory effects and possible mechanism of *A. rosea* on guinea-pig tracheal tissues. Moreover lipophilic profiling of *A. rosea* has been carried out by using Gas-Chromatography-Mass-Spectrometry. A total of 19 compounds have been identified from the plant, *n*-hexane fraction. These compounds have been further confirmed from their Van den Dool and Kratz (*I*) Indices. Major class of metabolite identified from the plant includes fatty acid, saturated and unsaturated fatty acid esters. Hydrocarbons have also been detected from the *n*-hexane fraction. These fatty acid esters have not been reported previously by GC-MS and were identified first time from the flowers of *Alcea rosea. In-vitro* experiments were performed on guinea-pig tracheal tissues, mounted in Kreb's solution at 37°C and bubbled with carbogen. In isolated guinea-pig trachea, *A. rosea* inhibited carbamylcholine and K^+ (80 mM)-induced contractions, potentiated isoprenaline concentration-response curves (CRCs) and suppressed Ca^{2+} CRCs. These results suggest that *A. rosea* cause bronchodilation through dual inhibition of phosphodiesterase enzyme and Ca^{2+} influx, which substantiate its potential in airways disorders.

Keywords: *Alcea rosea*, bronchodilator, phosphodiesterase inhibitor, calcium channel blocker, GC-MS analysis.

INTRODUCTION

Airway disorders, including asthma and bronchitis, are highly prevalent in Pakistan and on the rise. The prevalence of asthma increased at a steady rate of about 10.1% per year from 1993 to 2003 (ISAAC, 2011). This increase may be attributed to the increase in air pollution and cigarette smoking (Braman, 2006). Asthma is a type I hypersensitivity reaction triggered by repeated exposure to certain allergens (Holgate, 2011). In this disease, bronchial smooth muscles are stimulated to contract leading to temporary and reversible airway obstruction (Wiggs *et al.,* 1992). A similar obstructive disease is chronic bronchitis, in which progressive inflammation of the bronchial mucous membrane results in constriction of the small airways (Landis *et al*., 2014). β-$_2$ agonists (like salbutamol) and anti-cholinergics (like ipratropium bromide) along with corticosteroids (like beclomethasone) are the conventional pharmacological agents used in the treatment of these disorders (Jadad *et al.,* 2000). In addition to the high cost of available therapeutic remedies to treat such chronic disorders, multiple side effects are also associated when used for a long duration. Therefore, there is a need for the development of newer, economical and safe alternative therapeutic options to treat such chronic disorders.

Alcea rosea also known as *Althaea rosea* commonly called 'Garden hollyhock' (local name: Gul-e-Khera) belongs to the mallow (Malvaceae) family (Munir *et al.,* 2012). It is a biennial plant, originally found in the Southwest provinces of China dating back to the 15th century. It is an ornamental plant that can grow up to the height of 8 feet in a wide variety of soils, preferably moist, well-drained (Shaheen *et al.,* 2010) and is distributed throughout temperate regions (Lim, 2014). The flowers are of multiple colours: red, purple, white, pink, yellow, or black-purple that grow as single flowers on an erect stalk (Lim, 2014). Flavonoids and anthocyanins have been isolated and extracted from *Alcea rosea* and are used in the manufacturing of natural drugs with anti-microbial and anti-inflammatory effects on the gastrointestinal tract (Munir *et al.,* 2012). Different parts of the plant are known to be effective against various disorders. For instance, flowers are edible or can be harvested after they bloom, dried and preserved for later

**Corresponding author:* e-mail: hanifbuzdar@gmail.com

use (Bown, 1995). They can be used as treatment for respiratory problems like cough and sore throat, constipation, dysmenorrhoea and haemorrhage (Duke, 2002; Duke & Ayensu, 1985).

Despite its medicinal popular use in airway disorders like cough and bronchitis (Duke, 2002; Munir *et al.,* 2012) there has not been extensive research performed to support the effectiveness of *A. rosea*. Few literatures indicate that *Alcea rosea* root extract has the potential to reduce ethylene glycol-induced urolithiasis and supports the plants' anti-inflammatory and diuretic properties (Ahmadi *et al.,* 2012). Research done to detect the immunomodulatory activity of *Alcea rosea* indicates that the plant appears to be a B-lymphocyte polyclonal activator (El Ghaoui *et al.,* 2008). The antiviral activity of *Alcea rosea* against Human Immunodeficiency Virus types 1 and 2 (HIV 1 and HIV 2) has also been studied and it has been found that the methanol fraction of *Alcea rosea* does not possess potent cytotoxic activity (Asres *et al.,* 2001). Subjected to the degree of saturation and carbon chain length, there are about 3 dozen of fatty acids found abundantly in nature (Boullata, 2006). Human body is not capable to synthesize the so called essential fatty acids and these should only be taken in the diet (EI Alfy *et al.,* 2015).

Essential fatty acids are required in a number of processes starting from immunity building to normal cognitive function (Aluko, 2012; De Pablo & De Cienfuegos, 2000). However several reports indicated fatty acids intake lower asthma incidence, prevalence of asthma-related symptoms and improving lungs functions in human. (Kitz *et al.,* 2012). Moreover, they also play an important role in modulation of immune system by reducing proliferation of lymphocytes, decreasing cytokine production, activating phagocytosis and modifying the activity of defensive cells of human body (De Pablo & De Cienfuegos, 2000). Specifically, omega-3 and 6 fatty acids prevent normal cells from getting damaged due to effects of oxidative stress, by halting activation of NF-κB (Natural killer cell kappa-B) (Aluko, 2012). Currently, no scientific evidence is available to support its medicinal use in respiratory disorders. We hypothesized that *Alcea rosea* may contain certain bioactive phytochemical constituents that can cause bronchodilation. The current study is the first report elucidating the insight into mechanism in support of the bronchodilatory effect of *Alcea rosea* flowers.

MATERIALS AND METHODS

Plant material and preparation of crude extract of Alcea rosea and fractions

The crude extract was prepared from the flowers of *Alcea rosea*. A credentialed botanist from the Department of Botany, University of Karachi (Pakistan) authenticated the test material as flowers of *Alcea rosea*, which were purchased form Juria Bazar, Karachi, Sindh, Pakistan. As per protocol, a sample of the plant material was also submitted in the herbarium of the Department of Biological and Biomedical Sciences, Aga Khan University, Karachi. A sample voucher (G.H. No 91661) was submitted to the herbarium of the botany Department, Karachi University, Sindh, Pakistan. To prepare the crude extract, 775 g of *Alcea rosea* flowers (after the removal of adulterant material) were crushed and then soaked for three days in 3 litres of 70% aqueous-methanol with occasional shaking. At the end of three days, the solution was filtered through a muslin cloth, followed by filtration through a Whatman qualitative grade 1 filter paper (Williamson *et al*, 1998). After repeating this procedure of filtration thrice, the crude extract (Ar.Cr) was obtained by evaporating the collective deposit in a rotatory evaporator (Rota vapor BUCHI classical RE-111) connected to a recirculation chiller (model B-700) and a water bath (model 461) with hotness sustained in the range of 35-40^{o}C and pressure maintained below atmospheric pressure. The total yield of Ar.Cr. was 75.02g i.e. approximately 9.6%. Dried aqueous methanolic extract of *Alcea rosea* was then triturated with warm *n*-hexane to separate *n*-hexane soluble and insoluble portions.

Standard drugs and animals

Carbamylcholine, papaverine, isoprenaline hydrochloride and verapamil hydrochloride were acquired from Sigma Chemicals Co., St. Louis, MO, USA. Sodium chloride required for Kreb's (Ringer's) solution was purchased from BDH Laboratory Supplies, Pool, England; potassium chloride from Sigma Chemicals Co. and the remaining salts: calcium chloride, glucose, magnesium sulfate, sodium bicarbonate and potassium dihydrogen phosphate, were all obtained from Merck, Darmstadt, Germany. All the chemicals were solubilized in distilled water to produce varying concentrations.

The animals used for this study were Himalayan guinea-pigs, both male and female, weighing 350-550 g. The animals were housed at the Animal House of the Aga Khan University Hospital and the temperature maintained at 23-25°C. A standard diet and tap water ad libitum was provided to the animals, however, 24 hours prior to the experiment, food was withdrawn. The experiments performed on the animals were all approved by the Ethics Committee for Animal Care and Use (ECACU) at Aga Khan University, Karachi, Sindh, Pakistan in accordance with the rules of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (1996). ECACU Approval Number: 44-ECACU-BBS-18.

Isolated tissue preparation

After sacrificing guinea-pigs by cervical dislocation, their trachea was dissected out. The trachea was then cut into

2-3 mm wide tracheal rings, which were then opened by a longitudinal cut made on the ventral side, opposite the trachealis smooth muscle. This resulted in a tracheal ring with smooth muscle in the centre and hyaline cartilage at the periphery (Aqel, 1991). These rings were then placed in a tissue bath assembly, maintained at 37°C, in 10 ml of physiological Kreb's solution. Carbogen (95% oxygen and 5% carbon dioxide gases) was bubbled through the solution containing the tissue. The tracheal tissues were then allowed to acclimatize and equilibrate for approximately 30 minutes, after a fixed preload of 2 g was applied (Gilani *et al*, 2005). Next, the tissues were treated with either carbmylcholine or K^+ (80 mM) solution to check for smooth muscle response, followed by washing of the tissue bath and replacing the Kreb's solution. Dosing of carbmylcholine and K^+ (80 mM), followed by fresh Kreb's solution was repeated 2-3 times until the responses recorded were reproducible and ensured that the tissue had stabilized. To detect the bronchodilatory effects of *Alcea rosea* on tracheal smooth muscle, increasing doses of Ar.Cr (0.003-1.00 mg/ml) were administered. Papaverine was employed as the positive control to relax the carbmylcholine and K^+ (80 mM)-induced contractions, while the negative control was verapamil. Isoprenaline dose-response curves (DRCs) were produced by cumulative dosing of isoprenaline preceded by a carbamylcholine-induced sustained contraction (Abdel Haq *et al*., 2000). Increasing doses were added until the muscle had completely relaxed and the tracings returned to their baseline level. The tissues were then incubated with different doses of Ar.Cr, papaverine and verapamil for 30-45 minutes and isoprenaline DRCs were obtained. The logarithmic curves were then formed by plotting isoprenaline concentration on the X-axis against percentage of contraction on the Y-axis.

Furthermore, calcium DRCs were produced by initially placing the tissues in normal Kreb's solution for approximately 30 minutes. Next, in order to obtain a sustained contraction of the smooth muscle, K^+ (80 mM) solution was added. Once the steady contracted state was achieved, the tissues were washed with Ca^{2+}-free Kreb's solution, after which the tissues were washed with K^+-rich Ca^{2+}-free Kreb's solution multiple times. The repeated washing of the tissue with K^+-rich Ca^{2+}-free Kreb's solution was done to ensure that all the calcium stores of the tissues had been chelated. Subsequently, the addition of progressively increasing doses of $CaCl_2$ resulted in a stepwise contraction of the tissues. Once the $CaCl_2$ dosing was complete, the tissues were again washed with Ca^{2+}-free Kreb's solution followed by the repeated washing with K^+-rich Ca^{2+}-free Kreb's solution to confirm the chelation of calcium stores. The same tissues were then incubated for 45-60 minutes with a single dose of the plant extract. Increasing doses of $CaCl_2$ were once again added and the responses noted. The tissue was then washed in the same manner, first with Ca^{2+}-free Kreb's solution and then repeatedly with K^+-rich Ca^{2+}-free Kreb's solution. A second dose of Ar.Cr. now of a higher concentration, was administered to the same tissue in K^+-rich Ca^{2+}-free Kreb's solution and contractile responses were recorded after the dosing of increasing concentrations of $CaCl_2$. A logarithmic curve is then plotted with $CaCl_2$ concentration on the X-axis and the percentage of contraction on the Y-axis. Similarly, calcium DRCs were obtained, following the same procedure with papaverine and verapamil as positive and negative controls, respectively.

STATISTICAL ANALYSIS

All the data expressed are mean ± standard error of mean (S.E.M., *n*=number of experiments). Inhibitory effects are expressed as the median inhibitory concentrations (IC_{50}) with 95% confidence intervals (C.I.). DRCs were analyzed by non-linear regression using GraphPad4 program (GraphPAD, San Diego, CA, USA).

GC-MS for identification of constituents

The hexane soluble fraction of Alcea rosea was screening to gas chromatography and mass spectroscopy to identify non-polar phytochemical constituents.

GC-MS analysis

GC-MS analysis was carried out on Agilent 7890A gas chromatograph system attached with Agilent 7000 GC-mass spectrometer on EI positive mode at an electron energy of 70 eV preset at 250°C. HP-5MS capillary column (30m, 0.25mm ID and 0.25µm film thickness) was used for analyses, the column temperature was programmed as follows: 50°C for 10 minutes and then rise at a rate of 6°C /min to 180°C and hold for 20 min, again rise from 180°C to 290°C at 15°C /min and finally hold for 23 min at 290°C. The carrier gas used was helium at a flow rate of 1.2ml/min and injection port temperature was kept at 250°C.

Identification of compounds

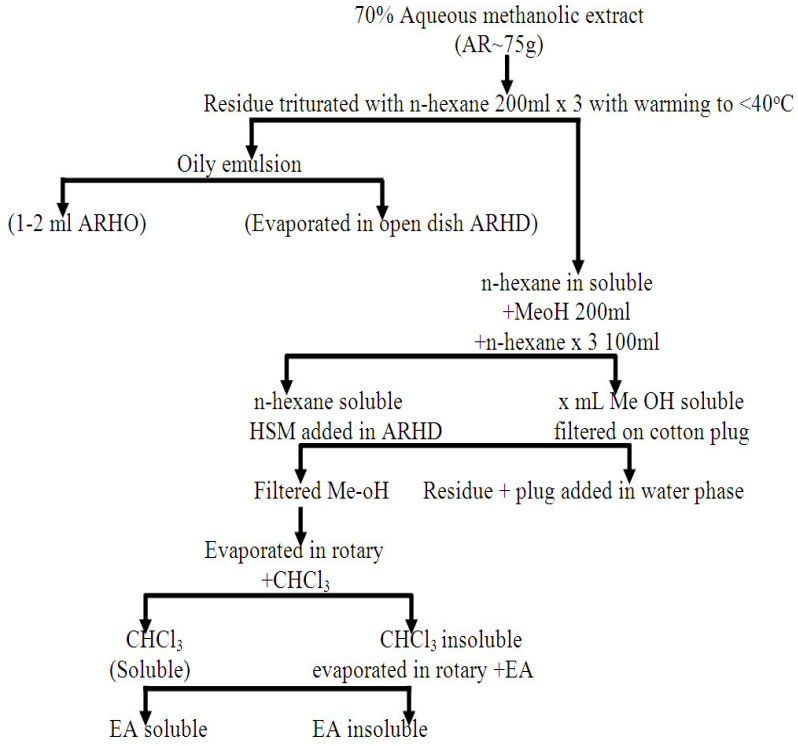
Lipophilic components including fatty acids methyl esters (FAMEs) were identified through comparing their spectra from NIST library, 2005. These compounds were further confirmed by matching their retention indices with the reported data. The retention indices of compounds were calculated by Van den Dool and Kratz *(I)* method (Van den Dool & Kratz, 1963).

RESULTS

Effects of A. rosea on guinea-pig tracheal tissue

Fig. 1 demonstrates the bronchodilatory effects of Ar.Cr., papaverine and verapamil on carbamylcholine and K+ (80 mM)-induced contraction of isolated guinea-pig tracheal tissues. The respective IC50 values for Ar. Cr., papaverine

Flowchart explaining extraction method of Alcea rosea and its various fractions



AR: *Alcea rosea* extract in 70 % Methanol (Me-OH)
ARHO: Extract with n-Hexane trituration (Oily)
ARHD: Extract with n-Hexane trituration (Dry)
HSM: Hexane solution from Methanol solution.
EA: Ethyl acetate

Table 1: Fixed oil composition of *Alcea rosea* flowers by GC-MS

Constituent (given number)* IUPAC/trivial name	KI_{Cal}	KI_{Lit}	References
2-Methyl heptanoic acid (01)	1127	1141	Yan *et al.*, 2015
n-Dodecane (02)	1211	1200	Vibha *et al.*, 2009, Xu *et al.*, 2003
n-Tridecane (03)	1309	1300	Jerkovic *et al.*, 2012
n-Tetradecane (04)	1407	1396	Mehtab *et al.*, 2018
n-Pentadecane (05)	1505	1499	Javidnia *et al.,* 2004
n-Hexadecane (06)	1604	1596	Mehtab *et al.*, 2018
n-Heptadecane (07)	1693	1707	Moein *et al.*, 2010
Tetradecanoic acid , methyl ester (08)	1717	1722	Bouzidi *et al.*, 2016
n-Octadecane (09)	1784	1800	Rouis-Soussi *et al.*, 2014
n-Nonadecane (10)	1882	1900	Moein *et al.*, 2010
9-Hexadecenoic acid, methyl ester, *(Z)*- (11)	1889	1880	Bouzidi *et al.*, 2016
n-Hexadecanoic acid methyl ester (12)	1929	1918	Mehtab *et al.*, 2018
n-Hexadecanoic acid (13)	1979	1970	Kowalski., 2005
Heptadecanoic acid, methyl ester (14)	2009	1994	Zouari., 2011
9,12-Octadecadienoic acid *(Z,Z)-,* methyl ester(15)	2088	2090	Bouzidi *et al.*, 2016
9-Octadecenoic acid, methyl ester (16)	2096	2086	Bouzidi *et al.*, 2016
n-Octadecanoic acid, methyl ester (17)	2124	2131	Mehtab *et al.*, 2018
n-Eicosanoic acid, methyl ester (18)	2330	2330	Leffingwell., 2013
n-Docosanoic acid, methyl ester (19)	2538	2531	Kowalski., 2005

KI cal. = Linear retention indices of samples; KI lit. = Linear retention indices cited in literature. No. in bold shows the elution order of compounds

and verapamil against carbamylcholine-induced contractions were 0.22mg/mL (0.15-0.32, 95% C.I., n=4), 0.73µM (0.46-1.16, 95% C.I., n=3) and 0.32µM (0.19–0.54, 95% C.I., n=1). When tested against K+ (80mM)-induced contractions, Ar.Cr. (fig. 1a) was found to inhibit K+ (80mM)-induced contractions at a similar concentration as that of carbamylcholine-induced contraction. The IC50 value for Ar.Cr. against K+ (80 mM)-induced contractions was 0.07mg/mL (0.14–0.27, 95% C.I., n=4), while IC50 value against carbamylcholine-induced contractions was 0.22mg/mL (0.15–0.31, 95% C.I., n=4).

Papaverine also showed a similar pattern on carbamylcholine-induced and K+ (80 mM)-induced contractions (fig. 1b) with respective IC50 values of 0.73µM (0.46–1.16, 95% C.I., n=3) and 1.218 µM (0.65-2.30, 95% C.I., n=3). In contrast with papaverine, verapamil was more potent against K+ (80 mM)-induced contractions ($p<0.001$) with the IC50 value of 0.14µM (0.07-0.25, 95% C.I., n=4) as compared to carbamylcholine-induced contraction i.e. IC50 of 0.32µM (0.19-0.55, 95% C.I., n=4) as shown in fig. 1c.

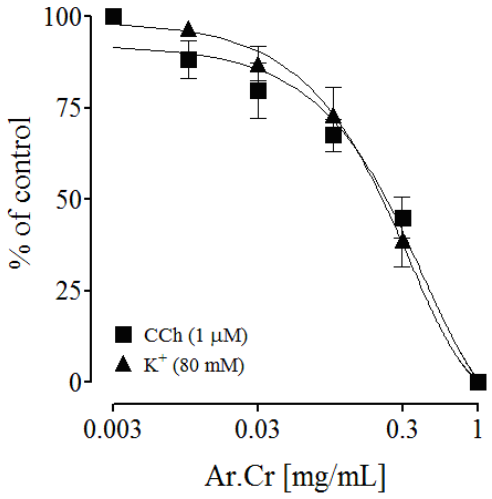


Fig. 1 a): Effects of crude extract of *Alcea rosea* L. (Ar.Cr.) on carbamylcholine and K^+ (80 mM)-induced contractions in isolated guinea-pig trachea. (Values are expressed as mean ± S.E.M.; number of observations =1-4)

Isoprenaline dose-response curves

To further elucidate the bronchodilatory mechanism in Ar.Cr. isoprenaline DRCs were plotted in the absence and presence of progressively higher doses of Ar.Cr. (fig. 2). Ar.Cr. produced a statistically significant leftward shift in the isoprenaline DRCs at doses of 0.01mg/ml and 0.03mg/ml. IC50 values of isoprenaline in the absence of Ar.Cr, dose of 0.01mg/ml of Ar.Cr and dose of 0.03 mg/ml of Ar.Cr were 1.2660µM (0.58-2.79, 95% C.I., n=3), 0.39µM (0.22–0.68, 95% C.I., n=3) and 0.10µM (0.06–0.17, 95% C.I., n=3) respectively.

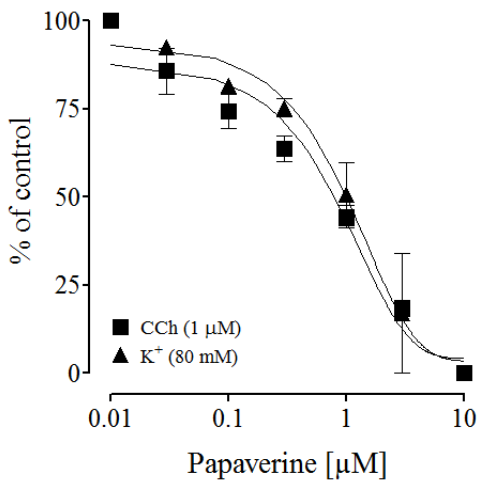


Fig. 1 b): Effects of Papaverine on carbamylcholine and K^+ (80 mM)-induced contractions in isolated guinea-pig trachea. (Values are expressed as mean ± S.E.M.; number of observations =1-4)

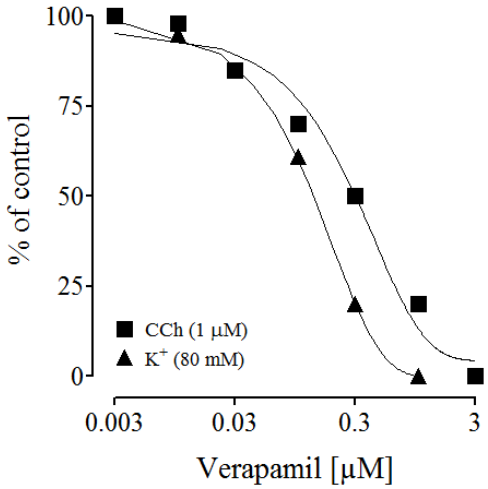


Fig. 1 c): Effects of Verapamil on carbamylcholine and K^+ (80 mM)-induced contractions in isolated guinea-pig trachea. (Values are expressed as mean ± S.E.M.; number of observations =1-4)

Calcium dose-response curves

Pre-treatment of tissues with the plant extract caused a rightward shift in calcium DRCs with a significant suppression ($p<0.001$, one-way analysis of variance (ANOVA) followed by Dunnett's test) of the maximum response (taken as 100%) to a level of 69.0±1.58% (n=4) and 49.75±1.38% (n=4), at 0.1mg/mL and 0.3mg/mL respectively (fig. 3a). Papaverine also produced a rightward shift in the DRCs of Ca2+ and attenuated its maximum response to 72.1% (n=1) and 49.4% (n=1), at tested concentrations of 3 and 10µM respectively (fig. 3b). Similarly, verapamil shifted the calcium DRCs to the right with a significant suppression of the maximum effect to a level of 52.5%±1.5% (n=2) and 30.5%±1.25% (n=2) at 0.01 and 0.03 µM respectively (fig. 3c).

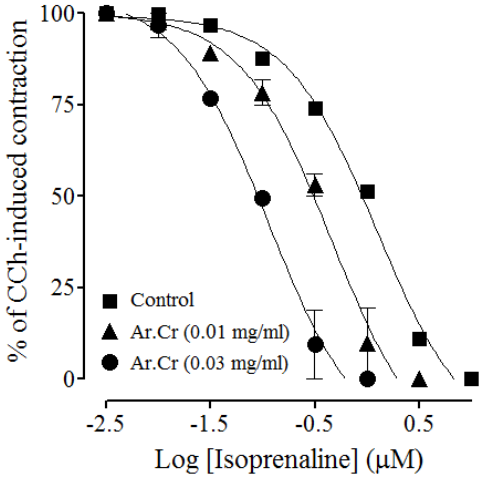


Fig. 2: Effect of Ar.Cr. on Isoprenaline dose-response curves in isolated guinea-pig trachea. (Values are expressed as mean ± S.E.M.; number of observations=1-3)

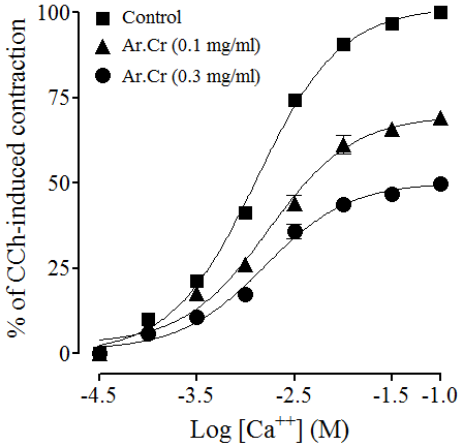


Fig. 3a): Effects of crude extract of *Alcea rosea* L. (Ar.Cr.) on calcium dose-response curves in isolated guinea-pig trachea. (Values are expressed as mean ± S.E.M.; number of observations =1-4)

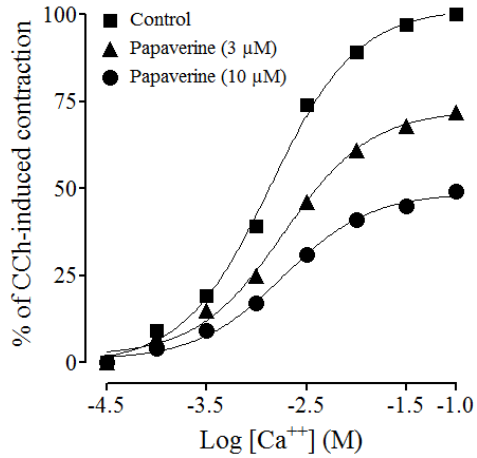


Fig. 3b): Effects of Papaverine on calcium dose-response curves in isolated guinea-pig trachea. (Values are expressed as mean ± S.E.M.; number of observations=1-4).

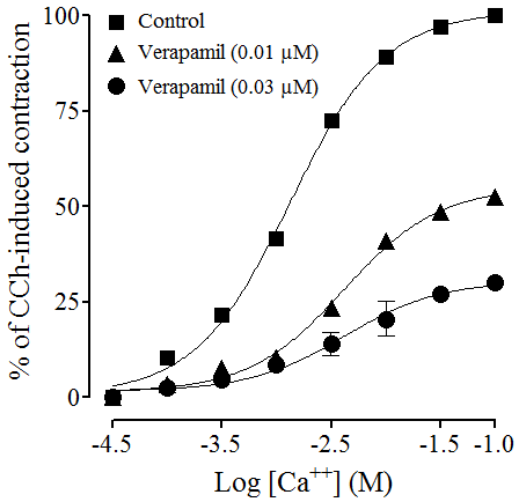


Fig. 3c): Effects of Verapamil on calcium dose-response curves in isolated guinea-pig trachea. (Values are expressed as mean ± S.E.M.; number of observations=1-4).

DISCUSSION

On account of its reputed use in bronchitis and asthma (Duke, 2002; Munir *et al*., 2012), the extract was tested for its possible bronchodilator effect in isolated guinea-pig tracheal strips, where it relaxed both CCh- and K^+ (80 mM)-induced contractions with similar potency. Papaverine, a known phosphodiesterase (PDE) and Ca^{2+}-channel blocker (Benyahia *et al,* 2012), showed similar effect as observed with *A. rosea*. *Alcea rosea* extract relaxed carbmylcholine and K^+ (80mM)-induced contractions with similar potency as seen with papaverine. Verapamil, a Ca^{2+}-channel antagonist (Katzung *et al.,* 2009), however, was more potent against K^+-induced contractions as compared to its effect on carbmylcholine induced contractions.

Bronchodilation can be mediated by several mechanisms, like PDE inhibition and Ca^{2+}-channel antagonism. Isoprenaline dose-response curves (DRCs) were plotted in tracheal tissues pre-treated with Ar.Cr.; this showed a left shift with increasing concentrations similar to that of papaverine. The Ca^{2+} DRCs produced with Ar.Cr. also showed a potentiating effect of the plant extract. This effect was similar to that of papaverine. Ca^{2+} DRCs with verapamil showed a more potent response compared to Ar.Cr. and papaverine. This confirms the presence of both Ca^{2+}-channel antagonist and PDE inhibitory activities in *Alcea rosea* L. PDE inhibitors like papaverine are known to cause bronchodilation by increasing intracellular levels of cyclic 3',5'-adenosine monophosphate (cAMP) (Hirota *et al.,* 2001). Under physiological conditions, the enzyme cAMP PDE is responsible for the degradation of cAMP. PDE inhibitors directly act on and inhibit the action of PDE resulting in increased levels of intracellular cAMP (Fan Chung, 2006). Increase in cAMP is known to cause

smooth muscle relaxation resulting in bronchodilatory effects, which may be the probable mechanism of action of Ar.Cr. β-adrenergic receptor agonists like isoprenaline activate adenylyl cyclase via the α-subunit of G_s-protein coupled receptors. This results in an increase in cAMP due to increased degradation of ATP to cAMP (Spina, 2014). In tissues pre-treated with *A. rosea* inhibition of PDE resulted in accumulation of cAMP and thereby, potentiating the bronchodilatory effects of isoprenaline.

Membrane depolarization caused by K^+ (80 mM) solution (Ratz *et al.,* 2005) administration leads to the opening of voltage-gated Ca^{2+}-channels followed by Ca^{2+} influx and release of Ca^{2+} stores from the sarcoplasmic reticulum (Miura *et al,* 1992). Increased intracellular Ca^{2+} causes the activation of calmodulin, which in turn activates myosin light-chain kinase leading to smooth muscle contraction (Barnes, 1983). Placing the tissue in Ca^{2+}-free Kreb's solution and repeated washing with high K^+-rich, Ca^{2+}-free Kreb's solution ensured the removal of all intracellular Ca^{2+} and the depletion of Ca^{2+} stores. Exogenous administration of $CaCl_2$ to the tissue incubated with Ar.Cr. showed suppression of overall contractility, thus confirming the Ca^{2+}-channel blocking activity of *Alcea rosea* L.

Plant extracts contain a variety of constituents and therefore, different effects predominate at different doses of plant extracts. In our experiments, we noted that low doses (0.01-0.03 mg/ml) of Ar.Cr. resulted in a leftward shift of isoprenaline DRCs. On the other hand, high doses (0.1-0.3 mg/ml) of Ar.Cr. caused rightward shift and suppression of the maximal response in Ca^{2+} DRCs. This suggests that the PDE inhibitory activity of Ar.Cr. prevails at lower concentrations, while at higher concentrations, Ca^{2+}-channel blockade predominates. Beside antioxidant capacities of fatty acid esters, bronchodilation action is usually attributed as being a property of long-chain unsaturated fatty acid esters more specifically 9-octadecenoic acid, methyl ester and 9,12-octadecadienoic acid (*Z, Z*)-, methyl ester (Choi *et al*., 2013). 9-octadecenoic acid methyl ester which is reported in *C. tenuifolia* seed oil. The oil showed outstanding *in-vivo* anti-oxidative activity as well as bronchodilator activity (Wei *et al*., 2016 & Li *et al.,* 2013). The *n-hexane* fraction of *Alcea rosea* flower is screened for lipid profiling using GC-MS and subjected to bronchodilation activity. Fixed oil composition showed that it contains fatty acid esters as its major components. Fatty acids are well known for bronchodilation, asthma, synergistic for anticancer agents and neuroprotective (Aluko, 2012; De Pablo & De Cienfuegos, 2000, Barros *et al.,* 2011). In this study, fatty acid and their esters have been reported for the first time from *A. rosea.* The study revealed that hexane fraction of *A. rosea* is equally enriched with virtuous fatty acid esters such as 9-octadecenoic acid methyl ester, 9,12-octadecadienoic acid (*Z, Z)* methyl ester and 9-hexadecanoic acid methyl ester, (*Z)* showing its possible utility for medicinal purposes.

Contemporary, well-established pharmacological agents like β-2 agonists and PDE inhibitors have been considered the drugs of choice in asthma and bronchitis (Spina, 2014). The Ca^{2+}-channel blockers have also been useful in the treatment of these conditions (Barnes, 1983). *Alcea rosea* L., with its assessed Ca^{2+}-channel antagonist and PDE -inhibitory activities, would be a suitable alternative phytomedicine for the treatment of airway disorders. Furthermore, *Alcea rosea* L. has been traditionally known to have several effects on multiple systems like cardiovascular system and gastrointestinal system (Duke, 2002), which would be expected with its given and suggested mechanisms of action.

CONCLUSION

In conclusion, 19 compounds from *A. rosea* flowers have been identified. These compounds have not been reported previously by GC-MS. The major class of compounds among all identified constituents are fatty acid and their esters. The *invitro* results of this study show that the flowers of *Alcea rosea* L. possess bronchodilatory activity possibly mediated through dual mechanism involving Ca^{2+}-channel blockade and PDE inhibitory pathways. Further *in-vivo* studies are required to confirm these findings and phytochemical studies are needed to identify and isolate the chemical constituents in *Alcea rosea* responsible for these pharmacological actions.

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