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ORIGINAL CONTRIBUTION

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Selective bronchodilatory effect of Rooibos tea (*Aspalathus linearis*) and its flavonoid, chrysoeriol

Summary *Background* Rooibos tea (*Aspalathus linearis*) is commonly used for hyperactive gastrointestinal, respiratory and cardiovascular disorders. *Aim of study* The aqueous extract of Rooibos tea (RT) was studied for the possible bronchodilator, antispasmodic and blood pressure lowering activities in an attempt to rationalize some of its medicinal uses. *Methods* Isolated tissue preparations, such as rabbit jejunum, aorta and guinea-pig trachea and atria were set up in appropriate physiological salt solutions and aerated with carbogen. For in vivo studies rats were anesthetized with pentothal sodium and blood pressure was measured through carotid artery cannulation. *Results* In jejunum, RT caused a concentration-dependent relaxation of low K^+ (25 mM)-induced contractions, with mild effect on the contractions induced by high K^+ (80 mM). In presence of glibenclamide, the relaxation of low K^+-induced contractions was prevented. Similarly, cromakalim caused glibenclamide-sensitive inhibition of low K^+, but not of high K^+, while verapamil did not differentiate in its inhibitory effect on contractions produced by the two concentrations of K^+. Like in jejunum, RT caused glibenclamide-sensitive relaxation of low K^+-induced contractions in trachea and aorta, but with a 20 times higher potency in trachea. In atria, RT was least potent with weak inhibitory effect on atrial force and rate of contractions. RT caused a dose-dependent fall in arterial blood pressure in rats under anesthesia. Among the tested pure compounds of Rooibos, chrysoeriol showed selective bronchodilator effect. Chrysoeriol (luteolin 3′-methyl ether) is a bioactive flavonoid known for antioxidant, antiinflammatory, antitumor, antimicrobial, antiviral, and free radical scavenging activities. *Conclusion* These results indicate that the bronchodilator, antispasmodic and blood pressure lowering effects of Rooibos tea are mediated predominantly through K_{ATP} channel activation with the selective bronchodilatory effect. This study provides a sound mechanistic basis for the wide medicinal use of Rooibos tea, with the therapeutic potential to be developed for congestive respiratory ailments.

Key words Rooibos tea – K_{ATP} channel activator – airway selectivity – hypotensive – chrysoeriol

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Introduction

Aspalathus linearis (Fabaceae) commonly known as Rooibos, is an indigenous South African plant. Local people of the Clanwilliam region, in north of Cape Town process its leaves to prepare herbal tea [1]. Rooibos tea is gaining popularity due to many health properties as evident by a rapidly growing number of its drinkers throughout the world, including China, Japan, Germany, England, Poland, Malaysia, South Korea, USA and of course South Africa. The total domestic and international sales of Rooibos in 1999 amounted to 6150 tons, of which 1800 tons (29%) were exported to 31 countries. In next year, the

quantity of Rooibos tea exported was two and a half times greater than the quantity exported in 1999, and the export of Rooibos tea continue to grow with five times increased sale in 2001 as compared to the previous years [2, 3]. Rooibos tea is commonly used for treating cardiac arrhythmias, colic, diarrhea [4], asthma [5] and hypertension [6]. It is known to contain aspalathin, chrysoeriol, orientin, isoorientin, vitexin, isovitexin, quercetin, isoquercetrin and rutin [4]. Rooibos tea has been known to possess antioxidant, antiaging [7], anticancer [8], antidiabetic [9] and antiinflammatroy [10] properties. In our earlier study, we observed that the Rooibos tea possesses antispasmodic activity predominantly through K^+ channel activation [11]. In this study, we attempted to explore its effect on airway and cardiovascular systems to provide scientific base for its medicinal use in asthma and hypertension. We observed that it elicits selective bronchodilator effect mediated through K_{ATP} channel activation. The airway selectivity of Rooibos tea may be due to the presence of chrysoeriol (Fig. 1) which also showed selective bronchodilator effect, like parent extract.

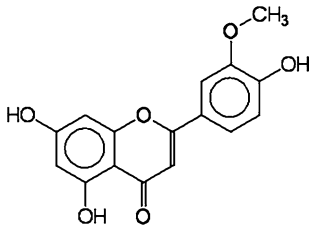

Fig. 1 Chemical structure of chrysoeriol

Materials and methods

Drugs and animals

Norepinephrine, potassium chloride, verapamil were obtained from Sigma Chemicals Co, St Louis, MO, USA, cromakalim and glibenclamide respectively from Tocris, Ellisviille, MO and RBI Chemicals Co, Natick, MA, USA. Pentothal sodium (thiopental sodium) and heparin injections were purchased from Abbot Laboratories, Karachi, Pakistan and Rotex Medica, Trittau, Germany respectively. Pure compounds: chrysoeriol, orientin and vitexin from ChromaDex, Santa Ana, CA, USA. Animals used in this study such as rabbits (1–1.2 kg), guinea-pigs (500–550 g) of local breed and Sprague-Dawley rats (200–220 g) of either sex, housed at the Animal House of the Aga Khan University, maintained at 23–25°C and were given standard diet and tap water *ad libitum*. Experiments performed complied with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council [12], approved by the Ethical Committee of the Aga Khan University.

Preparation of tea extract

Rooibos tea was bought from herbal market near Khayelitsha, Western Cape Province, South Africa. The aqueous extract of the tea was prepared by boiling 150 g dry tea in 1 liter distilled water for 10 min with subsequent standing for 20 min and cooling down to room temperature. The tea concentrate was filtered and then evaporated in a Buchi rotary evaporator yielding a thick, brown colored extract (RT) weighing 30.5 g (yield 20.4% w/w).

Preliminary phytochemical analysis

Phytochemical screening was carried out for the presence of flavonoids, tannins, saponins, alkaloids, anthraquinones, coumarins and sterols according to Evans [13] with some modifications. To measure the total amount of phenolic compounds, 1 ml of Folin-Ciocalteu Reagent was added to the extract solution adjusted to 46 ml by addition of distilled water [14]. After 3 min, 3 ml of Na_2CO_3 (2%) was added. Subsequently, the mixture was shaken on a shaker for 2 h at room temperature and then absorbance was noted at 760 nm. Phenolic content was expressed as mg of quercetin equivalent/g of the extract. Total flavonoid content was determined by the method previously described by Huang et al. [15]. Briefly, 1.5 ml of the extract solution was added to an equal volume of solution of 2% $AlCl_3 \cdot 6H_2O$ in methanol. The mixture was vigorously shaken and absorbance was read at 367 nm after 10 min of incubation. Flavonoid content was expressed as mg of quercetin equivalent/g of the extract. Absorption of the sample was measured by using a DU-70 Spectrophotometer.

In vitro experiments

Isolated tissue and *in-vivo* blood pressure experiments are performed according to Gilani et al. [16].

Rabbit jejunum

Rabbit, starved for 24 h was sacrificed by a blow on back of the head. Respective segments of 2-cm length were suspended individually in 10 ml tissue baths containing Tyrode's solution, maintained at 37°C and aerated with a mixture of 95% oxygen and 5% carbon dioxide (carbogen). Intestinal responses were

recorded isotonically using Bioscience transducers coupled to oscillograph.

Guinea-pig trachea

Trachea from guinea-pig killed by cervical dislocation was dissected out and kept in Kreb's solution. The preparation was then mounted in a 20 ml tissue bath containing Kreb's solution, maintained at 37°C and aerated with carbogen. A tension of 1 g was applied to each of the tracheal strip and was kept constant throughout the experiment. The changes in isometric tensions of the strips were measured via a force-displacement transducer (FT-03) using a Grass model 7 Polygraph (Grass Instrument Company, Quincy, MA, USA).

Rabbit aorta

Aortic rings of 2–3 mm width were individually mounted in 20 ml tissue baths containing Kreb's solution, at 37°C and aerated with carbogen. A resting tension of 2 g was applied to each tissue and was kept constant throughout the experiment. The changes in isometric tensions of the rings were measured using Polygraph.

Guinea-pig atria

Right atria isolated from guinea-pigs were mounted individually in 20 ml tissue baths containing Kreb's solution, at 32°C and aerated with carbogen. The tissues were allowed to beat spontaneously (due to the presence of pacemaker cells) under the resting tension of 1 g. Tension changes in the tissue were recorded using Polygraph.

Blood pressure (BP) in anaesthetized rats

Rats were anaesthetized with an intraperitoneal injection of thiopental sodium (70–90 mg/kg). The right carotid artery was cannulated by polyethylene tubing PE-50, which was connected to a pressure transducer (P23 XL) coupled with a Grass model 7 Polygraph. The left jugular vein was cannulated with similar tubing to facilitate the intravenous injection of the standard drugs and plant material. Arterial BP was allowed to return to the resting level between injections. Standard drugs and the tea extract (all prepared in saline) were then administered by i.v. injections and flushed in with 0.1 ml saline. Changes in BP were recognized as the difference between the steady state values before and the lowest readings after injection. Mean arterial blood pressure (MABP) was calculated as the diastolic BP plus one-third of pulse width.

Data analysis and statistics

All the data expressed are mean ± standard error of mean (SEM, n = number of experiments) and the median effective concentrations (EC_{50} values) with 95% confidence intervals (CI). The statistical parameter applied is the Student t-test. $P < 0.05$ noted as significantly different.

Results

Phytochemical screening

RT was found to contain flavonoids, tannins and saponins, while it tested negative for the presence of the rest of the classes of compounds. Total phenolic and flavonoid contents were respectively 120.0 ± 1.6 and 199.98 ± 1.93 mg of quercetin equivalent/g of the extract.

Effect on rabbit jejunum

RT caused mild inhibition of high K^+ (80 mM)-induced contractions, while completely relaxed the contractions induced by low K^+ (25 mM) with EC_{50} values of 2.3 mg/ml (1.8–2.9, 95% CI, $n = 4$). In presence of glibenclamide (3 μM), the inhibition of low K^+ (25 mM)-induced contractions was prevented (Fig. 2A). Similarly, cromakalim also caused glibenclamide-sensitive relaxation of the contractions induced by low K^+ (25 mM) with EC_{50} value of 0.04 μM (0.02–0.07, $n = 4$), without any effect on high K^+ (80 mM)-induced contractions (Fig. 2B), where as verapamil inhibited low K^+ (25 mM) and high K^+ (80 mM)-induced contractions at a similar concentration with EC_{50} values of 0.02 (0.02–0.03, $n = 5$) and 0.02 μM (0.01–0.04, $n = 5$) respectively (Fig. 2C).

Effect on guinea-pig trachea

RT was found devoid of any stimulant action when screened on the tracheal resting baseline. When tested against the high K^+ (80 mM)-induced contractions, RT exerted mild inhibitory effect, while produced complete relaxation of low K^+ (25 mM)-induced contractions with EC_{50} value of 0.08 mg/ml (0.05–0.11, $n = 5$). In presence of glibenclamide (3 μM), the relaxation of low K^+ (25 mM)-induced contractions was prevented (Fig. 3).

Effect on rabbit aorta

When tested on vascular preparations at resting tension, tea extract was found devoid of any vaso-

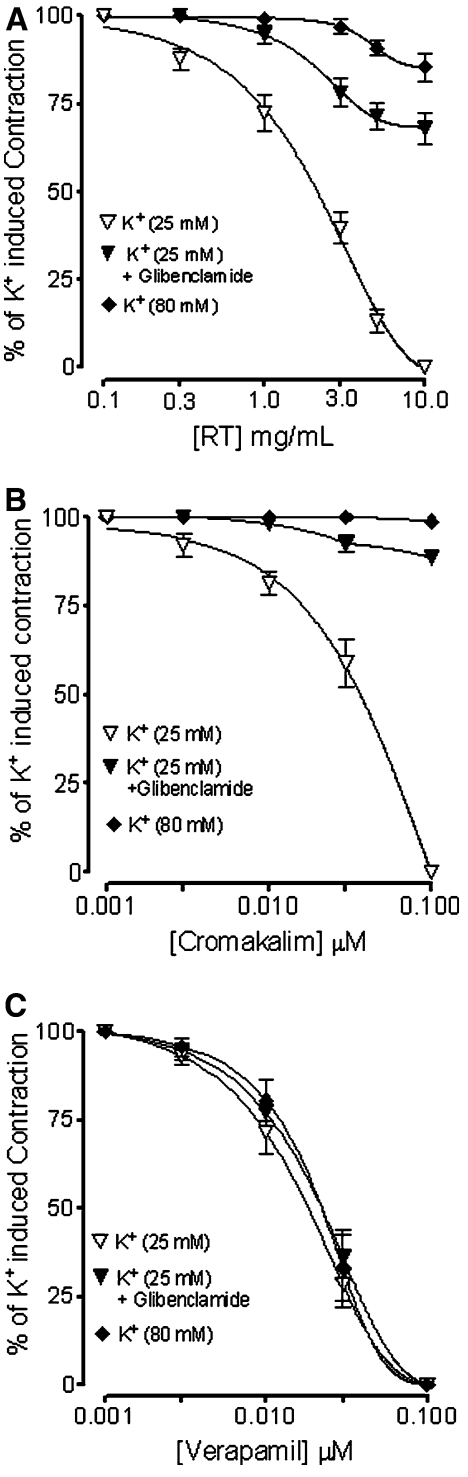

Fig. 2 Concentration-response curves showing the comparison of (**A**) Rooibos tea extract (RT), (**B**) cromakalim and (**C**) verapamil for the inhibitory effect against low K^+ (25 mM), in the absence (▽) and presence (▼) of glibenclamide (3 µM) and high K^+ (80 mM)-induced contractions (♦) in isolated rabbit jejunum preparations. Symbols represent mean ± SEM, $n = 4–5$

constrictor effect. When tested against high K^+ (80 mM)-induced contractions, RT caused slight inhibition, while completely relaxed the contractions induced by low K^+ (25 mM) with EC_{50} value of 1.5 mg/ml (0.87–2.6, $n = 4$). In presence of glibenclamide (3 µM), the relaxation of low K^+ (25 mM)-induced contractions was prevented (Fig. 3).

Effect on guinea-pig atria

RT exhibited mild inhibitory effect on atrial force (35%) and rate (29%) of spontaneous contractions ($n = 4$), when tested at higher concentration of 10 mg/ml (Fig. 4). Due to weak cardiac-depressant profile of the extract with low potency, it was not possible to study the interaction with glibenclamide.

Effect on BP in anaesthetized rats

Intravenous administration of RT produced a dose-dependent fall in MABP (mmHg) of normotensive anaesthetized rats. The doses of 10, 30 and 100 mg/kg induced a fall in MABP (mean ± SEM) of 8.8 ± 1.7%, 15.2 ± 2.04% and 35.25 ± 2.9% respectively. Pretreatment of animals with glibenclamide (3 mg/kg) partially blocked the hypotensive effects, reducing the respective original responses of the MABP to 4.6 ± 0.52%, 6.8 ± 0.59% ($P < 0.05$) and 10.11 ± 2.1% ($P < 0.001$). Cromakalim (10 µg/kg) also produced hypotensive effect, abolished by glibenclamide (Fig. 5).

Effect of pure compounds

In isolated jejunum, trachea and aortic preparations, chrysoeriol caused concentration-dependent, glibenclamide-sensitive relaxation of low K^+ (25 mM)-induced contractions with respective EC_{50} values of 41.3 ($n = 3$), 1.43 ($n = 4$) and 61 µg/ml ($n = 2$), without any effect on high K^+ (80 mM)-induced contractions. Vitexin inhibited low K^+ (25 mM)-induced contractions in jejunum and trachea with respective EC_{50} values of 170.6 ($n = 1$) and 214 µg/ml ($n = 1$) while orientin relaxed it with EC_{50} value of 12.3 ($n = 2$) in jejunum, but did not cause any effect upto 3.0 mg/ml in trachea.

Discussion

The results of our preliminary phytochemical analysis reveal that the aqueous extract of Rooibos tea contains flavonoids, tannins, and saponins, which is in accordance with the previous findings [4], except that the presence of saponins is reported for the first time.

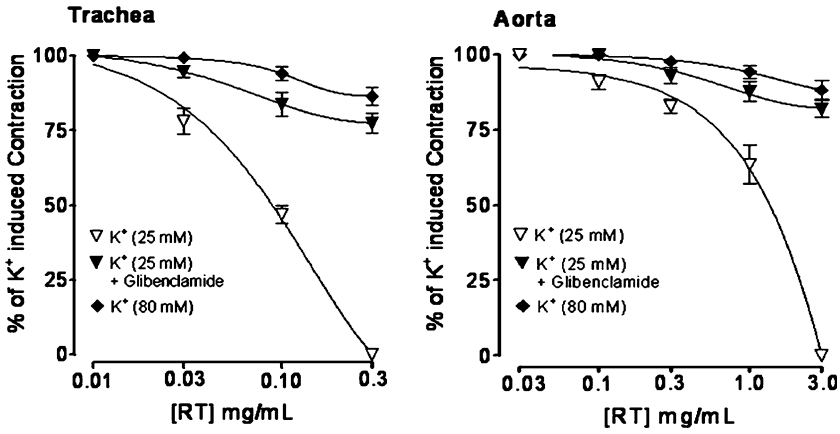

Fig. 3 Concentration-response curves showing the effect of Rooibos tea extract (RT) on low K^+ (25 mM), in the absence (▽) and presence (▼) of glibenclamide (3 μM) and high K^+ (80 mM)-induced contractions (♦) in isolated guinea-pig trachea and rabbit aorta preparations. Symbols represent mean ± SEM, $n = 4–5$

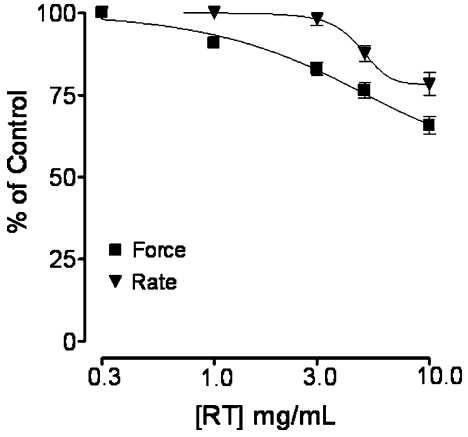

Fig. 4 Effect of Rooibos tea extract (RT) on force and rate of spontaneously beating isolated guinea-pig right atrial preparations. Symbols represent mean ± SEM, $n = 4$

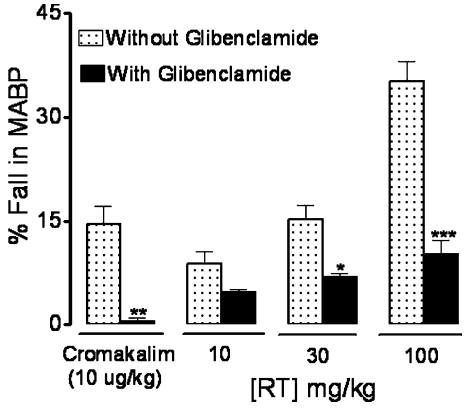

Fig. 5 Histogram showing effects of cromakalim and Rooibos tea extract (RT) on mean arterial blood pressure (MABP) in the absence and presence of glibenclamide (3 mg/kg) in anaesthetized rats. Values shown represents mean ± SEM, $n = 4$. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ vs. without glibenclamide

In our earlier study, we reported that the antispasmodic effect of Rooibos tea is mediated predominantly through K_{ATP} channel activation [11] due to its selective inhibitory effect against low K^+-induced contractions, in comparison to high K^+-induced contractions, blocked by glibenclamide, a specific blocker of the ATP-dependent K^+ channels [17].

Potassium channel openers are relatively a new class of drugs with a wide range of potential therapeutic uses like asthma, hypertension, gastrointestinal spasms and urinary incontinence [18]. These compounds open K^+ channels, cause membrane hyperpolarization through the increase in K^+ efflux, thus causing decrease in the intracellular free Ca^{++} and smooth muscle relaxation. Based on the medicinal use of Rooibos tea in asthma [5] and potential therapeutic use of K^+ channel openers in this disorder [19], the extract was studied in trachea for the possible bronchodilator effect. Like in gut and vascular smooth muscle preparations, the extract produced glibenclamide-sensitive relaxation of low K^+-induced contractions with mild effect on the contractions induced by high K^+, similar to a standard K_{ATP} channel opener cromakalim [20], while verapamil, a Ca^{++} antagonist [20] inhibited low and high K^+-induced contractions at similar concentrations, indicating the presence of K_{ATP} channel dependent bronchodilatory substance(s) in Rooibos tea. Interestingly, it showed selectivity in its inhibitory effect on trachea, as it exhibited its effect at a concentration about 20 times less than those produced K^+ channel dependent inhibitory effect in other smooth muscle tissues. K_{ATP} channels are known to be heterogeneous [21] and some K_{ATP} channel openers are found to exhibit selectivity for some organ systems. For example, a second generation K_{ATP} channel activator, HOE 234 (rilmakalim; (3S, 4R)-3-hydroxyl-2,2-dimethyl-4-(2-oxo-1-pyrrolidinyl)-6-phenylsulfonylchroman hemihydrate) is bronchial smooth muscle selective, while

most of the first generation molecules, such as lemakalim and bimakalim seem to exhibit a greater potency in the vasculature, while S 0121 ((-)-3R,4S,5′R-6-cyano-3,4-dihydro-2,2-dimethyl-*trans*-4-(2′-oxox-5′-methyl-pyrrolidin-1′-yl)-2*H*-1-benzopyran-3-01), another K channel opener, was reported to exhibit selective inhibitory effects on uterine smooth muscles [22]. It is possible that Rooibos tea contains K_{ATP} activating constituent(s) that exhibit tracheal selectivity, hence strengthening the evidence of K_{ATP} channel heterogeneity and can be considered a better candidate to meet the desire of the organ-selective therapeutic agent development.

We have previously tested some of the known commercially available compounds of Rooibos tea, such as chrysoeriol, orientin and vitexin in jejunum and were found to exhibit K_{ATP} channel opening effect [11]. In the present study, these compounds were studied further in trachea and aorta to see if any of them show selectivity in the airway like the parent extract. Among the three, chrysoeriol was found to be 25 times more potent in trachea than jejunum and 40 times than aorta, while the potency of vitexin in jejunum and trachea was comparable. Orientin was inactive in trachea, while showed a potent antispasmodic effect in jejunum. Due to limited supply of the pure compounds they could not be studied further.

The extract exerted mild inhibitory effect against high K^+-induced contractions, showing weak Ca^{++} antagonist effect, which may be due to the presence of quercetin, luteolin and rutin (known Rooibos constituents), previously reported as Ca^{++} influx inhibitors [11, 23, 24].

When injected intravenously in rats under anesthesia, Rooibos tea extract evoked a dose-dependent fall in BP, which is in line with its medicinal use in hypertension. The BP lowering effect was partially blocked by glibenclamide, indicating a K_{ATP} channel opening effect, along with some additional mechanism, possibly due to the presence of Ca^{++} antagonists. In aorta, the tea extract also produced glibenclamide sensitive relaxation of low K^+-induced contractions with weak effect on high K^+-induced contractions, confirming the involvement of K_{ATP} channel activating mechanism in the vasodilator effect of the plant extract, and this study may explain the use of Rooibos tea in hypertension.

When tested on spontaneously contracting right atria, tea extract produced a weak inhibitory effect on atrial force and rate of contractions. It has been argued that sensitivity of the cardiac cells towards K_{ATP} channel openers is much less, compared with smooth muscle cells [25]. Previous studies have shown that the cardiac inhibitory effects of cromakalim occur at concentration, 30–100-folds greater than that causing smooth muscle relaxation [26]. In an earlier study it was found inactive up to the concentration of 30 μM, while at 100 μM, a small negative inotropic effect (20%) was observed in half of the preparations studied [27]. Role of K_{ATP} channels under physiological condition of heart is not well marked [28], moreover, there is an existence of different structural isoforms of K_{ATP} channels in cardiac and smooth muscles which may explain the relatively weak inhibitory effect of Rooibos on cardiac muscles [29].

This study shows that the aqueous extract of Roobios tea possesses smooth muscle relaxing effect mediated possibly through dominant K^+ channel activation along with weak Ca^{++} antagonist mechanisms. The selective bronchodilatory effect of the tea extract was shared by one of its known flavonoid compound namely, chrysoeriol, while orientin was found selective for its inhibitory effect on gut, though further studies are required to draw a firm conclusion. This study may explain the medicinal use of Rooibos tea in hyperactive gastrointestinal, respiratory and cardiovascular diseases with the potential to be developed as a remedy for the congestive airway disorders.

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