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Formulation and evaluation of natural antitussive cough syrups

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Abstract: Among respiratory complications, cough is an important defensive pulmonary reflex that removes fluids, irritants or foreign substances from the respiratory tract. *Rosa damascene* Mill, petal extract is a traditional medicine and reported to use in the management of cough. In the present study rose petals extract samples were used to prepare natural antitussive cough syrups containing ivy leaf extract to observe synergistic effect of rose water in managing coughing in rats. Four cough formulations (A1, A2, A3 and A4) were prepared. The antitussive activity was observed at three dosage levels; 5ml, 10ml and 15ml. Cough was induced by the standard procedure using sulfur dioxide gas and ammonia. The antitussive activity was recorded by observing the coughing bouts. The result indicated that all formulations had a good effect on cough reduction at 90min but comparing the effect of formulations in all doses formulations, A2 followed by A3 and A4 at 30 minute time interval which is good in comparison with standard Diphenhydramine HCL and Dextromethorphan HBr 10ml in sulfur dioxide gas and ammonia induced cough. Hence, the results of the present study indicated the synergistic effect of rose water in the management of cough ailments.

Keywords: Antitussive, rose petals, management, cough, sulfur dioxide gas, ammonia.

INTRODUCTION

The phenomenon of coughing is an indicator of respiratory complaint which ultimately indicates tenderness of the chest and thorax (Gupta *et al*., 2009). Existing therapies currently available to manage cough are narrow due to lack of efficient and safe medications and also to exclude some common side effects.An alternative system of medicine, such as Greeco-Arabic, Ayurvedic and Traditional Chinese medicine (TCM), several plants have been reported to manage respiratory symptoms such as a cough (dry or unproductive) and irritation or inflammation of airways passages, expectoration (Gupta *et al*., 2009; Young & Smith, 2011, Jahan & Siddiqui, 2012) The numbers of plants having bioactive constituents are reported for managing the above symptoms. *Rosa damascena* (Rosaceae) is an erect shrub 1 to 2 meters in height.Flowers of this plant are big, showy and bright. *Rosa damascena* is mostly cultivated all over the world for visual beauty and scent (Ansari *et al*., 2017). In addition to perfuming effect flower petals and hips of *Rosa damascena* also used medicinally. Ancient medical books report several therapeutic effects of this plant, such as treating abdominal and chest pain, strengthening the heart, treating digestive problems, menstrual bleeding, and anti-inflammatory effects. A recent study also reported an anti-HIV, antioxidant and antitussive effects (Shehzad *et al*., 2020). *Rosa damascena* essential oil is reported to have an analgesic, hypnotic and antispasmodic effects. The plant is also used as a gentle laxative. North American Indian tribes used a decoction of the root as a cough remedy to ease children cough.

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In this study, effective herbal cough syrups were formulated and evaluated their antitussive activity. Formulations contained *Rosa damascena* petal extract (rose water) along with Ivy leaf extract, literature conforms that clinical trials on patients with different age groups suffering from respirotory discomfort feel ease after taking the formulations containing ivy leaf extract (Magdalena Olszanecka-Glinianowicz *et al*., 2020).

The present study shows the synergestic effect of both rose water and ivy leaf extract as an effective antitussive agent.

MATERIALS AND METHODS

Plant material

Rosa damascena flower collected from the local market and identified. The samples is kept in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy &Pharmaceutical Sciences, Karachi University.

Extraction of rose water

Rose water was prepared by the method described and reported earlier (Abidi *et al*., 2018) using the hydro distillation method in which 60g dried rose petal was distilled with 1L distilled water for 4hrs to yield 800ml of rose water that was kept in airtight jar at $4^{o}C$ in refrigerator.

Phytochemical analysis of rose water

The phytochemical analysis of rose water was performed by earlier published paper and also for the functional groups were determined through FT-IR was mentioned in the paper (Abidi *et al*., 2018).

Rosa damascena Mill.

Cough syrup formulation

4 sets of cough syrup formulations were prepared and marked as A1, A2, A3 and A4. The composition of all formulations are stated in table 1. In formulation A1, 100% hydrodistilled rose water was used, A2 contained 50% hydrodistilled rose water and 50% distilled water was used, A3 marketed rose water was used and in A4 formulation 50% marketed rose water and 50% distilled water were used.

Preparation of cough syrup

Methylparaben and propylparaben were added to the rose water and mixed well until a clear solution was formed. In a separate glass, the xanthan gum was soaked in a small amount of rose water. Sorbitol, glycerin and propylene glycol were mixed, then sucralose and honey flavor were added, after the completion of mixing, ivy leaf extract was added. Finally, mixed all of the above solutions in a separate beaker. Then added the potassium sorbate and citric acid. Heat until a clear solution is obtained. Add a volume of rose water to make 1l syrup.

Assessment of antitussive activity

An antitussive effect previously described by Miyagoshi *et al*. as well as those conducted by Pulok K. Mukherjee (Miyagoshi, Amagaya, & Ogihara, 1986; Mukherjee, 2002), were adopted to evaluate the antitussive effect of the cough syrup formulations A1, A2, A3 and A4.

Experimental animals used

Albino Wister rats of both sexes weighing 180 to 200g, obtained from the Department of Pharmacology, Faculty of Pharmacy & Pharmaceutical Sciences, University of Karachi, they were maintained with a standard diet, light cycle and darkness..

The international animal bioethical guidelines were strictly followed. This activity has been reviewed and approved by the IBC under guidelines provided by Helsinki declaration and guide for the care and use of laboratory animals, National Academy of Science, National Academy Press and Washington, DC. The institutional bioethics (IBC) certification number is IBCPH-22.

The animals were divided into 14 Groups for sulphur di oxide and ammonia induced antitussive activity respectively; Group I worked as the control group received only the normal saline, Group II received A1 formulation 5ml/70kg, GroupIII A1 10ml/70kg formulation, Group IV A1 15ml/70kg formulation, Group V A2 5ml/70kg formulation, GroupVI A2 10ml/70kg formulation, Group VII A2 15ml/70kg formulation, Group VIII received A3 formulation 5ml/70kg, GroupIX A3 10ml/70kg formulation, Group X A3 15ml/70kg formulation, Group XI received A4 formulation 5ml/70kg, GroupXII A410ml/70kg formulation, Group XIII A4 15ml/70kg formulation. Group XIV received the standard drug of Diphenhydramine HCL & Dextromethorphan HBr 10ml/70kg.

Sulfur dioxide (SO_2) induced cough

The procedure for the induction of cough with sulfur dioxide was carried out in a desicator. Sodium hydrogen sulfite was prepared by dissolving1g of sodium hydrogen sulfite in 2ml of refined water and 0.7ml of concentrated sulfuric acid, which is essential for the development of sulfur dioxide. The response continues as follows:

$2NaHSO_3 + H_2SO_3$-a $2SO_2$- + Na_2SO_4 +$2H_2O$

This protocol was followed for the formation of sulfur dioxide gas. The rats were exposed to the gas for 45 secs. Cough rates were observed in all animals in all groups 0 minutes before drug administration and 30, 60 and 90 minutes after drug administration (Marina *et al*., 2008).

Ammonium liquor induced cough activity

Independently all rodents of each group were put on a wire dressing stage in a desiccator and exposed to 0.3ml NH4OH (25%) by a nebulizer for 45 seconds. Following 45 seconds the rodents were eliminated from the desiccator and placed in a perception chamber for checking of recurrence of hack sessions for 5 minutes before the medication, 30, 60, a hour and half after drug administration.

Statistical analysis

Test results, communicated as mean ± standard deviation, SPSS adaptation 20.0, apply Tukey's single direction investigation of change (ANOVA) Post Hoc Test (Test results have been displayed as mean ± standard error mean by using SPSS 21. One way ANOVA followed by Post hoc Tukeys test was used for analysis.)

RESULTS

The antitussive activity of cough syrup formulations A1, A2, A3and A4 at 3 different doses 5ml/70kg, 10 ml/70kg & 15 ml/70kg, control and standard were mentioned in

Graph of antitussive cough syrup formulations A1, A2, A3, A4 and standard drug on ammonia and sulphur-di-oxide induced cough

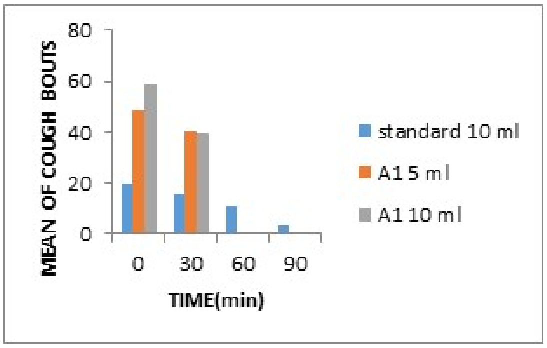

Fig. 1: A1 formulation ammonia induced cough method

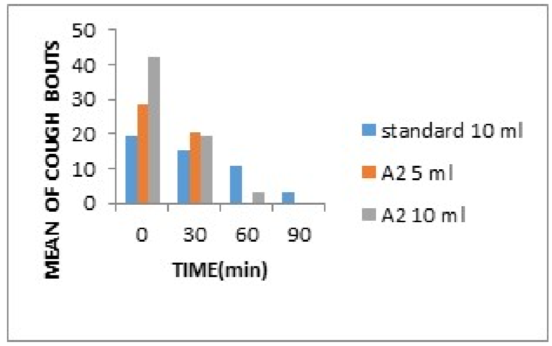

Fig. 2: A2 formulation ammonia induced cough method

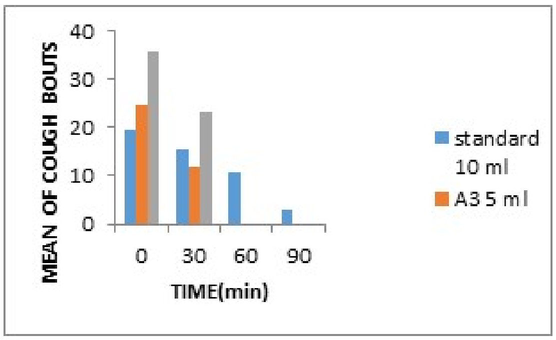

Fig. 3: A3 formulation ammonia induced cough method

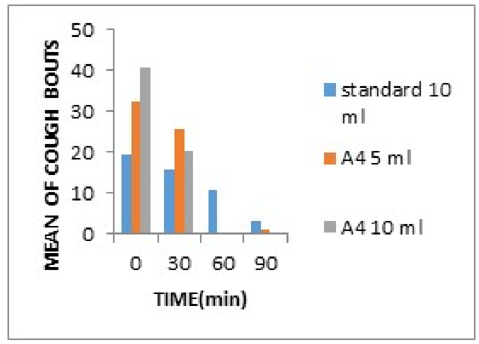

Fig. 4: A4 formulation ammonia induced cough method

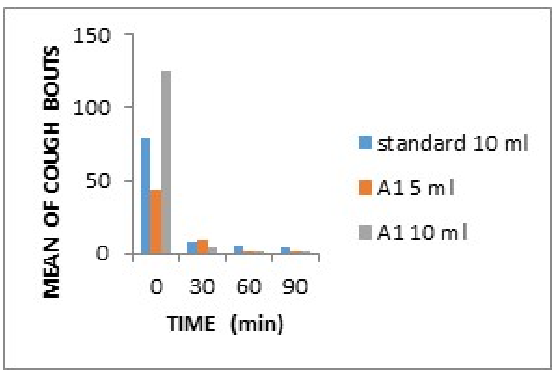

Fig. 5: A1 formulation SO_2 induced cough method

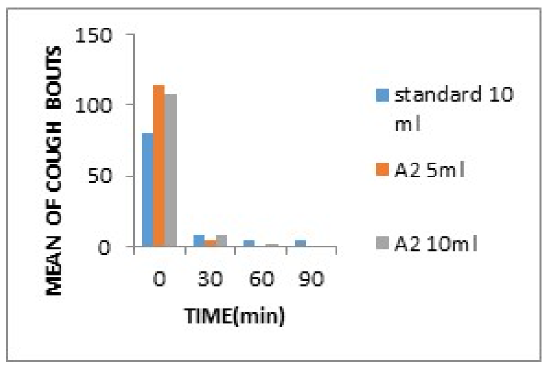

Fig. 6: A2 formulation SO_2 induced cough method

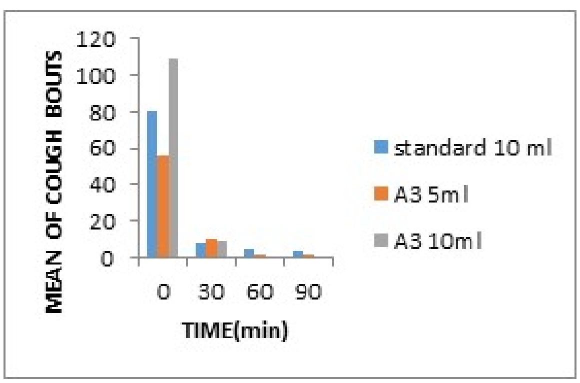

Fig. 7: A3 formulation SO_2 induced cough method

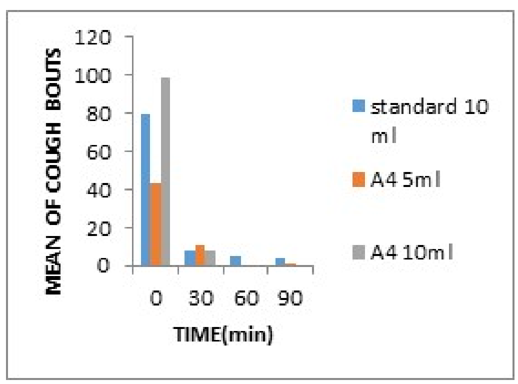

Fig. 8: A4 formulation SO_2 induced cough method

Table 1: Composition of cough syrups A1, A2, A3 and A4

S. No.	Ingredient	Used as	Amount (Gram)
1.	Methylparaben	Preservative	1.20
2.	Propylparaben	Preservative	0.20
3.	Potassium sorbate	Pharmaceutical preservative	1.50
4.	Xanthan gum	Thickening agent	2.50
5.	Citric acid	Preservative	0.70
6.	Ivy leaf extract	Supporting material	7.14
7.	Sorbitol	Sweetener (Diet sugar)	200.0
8.	Glycerine	Soothing agent	25.0
9.	Propylene glycol	Non-active enabling agent or excipient	50.0
10.	Sucralose	Artificial sweetener	1.50
11.	Honey flavor	Flavoring agent	2.5
12.	• Rosewater (lab distilled) for A1 • 50%lab distilled rose water + 50% distilled water) for A2 • Rosewater (marketed) for A3 • 50% marketed rose water + 50% distilled water for A4	Active ingredients	Q.S for 1 l

Table 2: Antitussive activity of cough syrup formulations A1, A2, A3, A4, and standard drugs on sulphur di oxide induced cough

Treatment	Dose	Frequency of cough (mean ± SEM)			
		0 min	30min	60 min	90min
Control		119.66±29.33	6.66±0.33	16.66±3.33	20.00±5.00
Standard **	10ml	80.00±29.67	8.00±5.13	4.66±2.02*	4.33±2.60*
A1	5ml	44.00±26.68	9.00±2.08	0.66±0.33*	0.33±0.33*
	10ml	125.00±2.51	4.33±1.76	0.66±0.66*	0.66±0.66*
	15ml	83.33±2.84	3.00±0.57	0.00±0.00*	0.00±0.00*
A2	5ml	114.66±3.84	4.33±0.66	0.00±0.00*	0.00±0.00*
	10ml	107.66±7.85	8.33±2.18	1.66±1.66*	0.00±0.00*
	15ml	86.00±9.60	19.33±9.33	2.33±2.33*	0.66±0.66*
A3	5ml	55.66±12.99	10.33±1.20	1.66±0.88*	1.33±1.33*
	10ml	109.00±7.00	9.66±1.85	0.00±0.00*	0.00±0.00*
	15ml	86.00±9.60	6.00±2.08	0.00±0.00*	0.00±0.00*
A4	5ml	43.66±13.24	10.33±0.88	0.00±0.00*	1.00±0.57*
	10ml	98.66±3.48	8.33±1.76	0.00±0.00*	0.00±0.00*
	15ml	60.00±13.65	6.00±0.57	0.33±0.33*	0.00±0.00*

*P <0.05 significant as compared to control, ** Diphenhydramine HCL and Dextromethorphan HBr
Values are recorded as MEAN ± SEM
Mean difference is significant at the 0.05 level (P<0.05, Tukey's test) as analyzed by one –way analysis of variance (ANOVA)

table 2 when the cough is induced by sulphur dioxide.While table 3 shows the results of ammonia-induced cough at two different dose levels 10ml/70kg and 5ml/70kg.

STATISTICAL ANALYSIS

Test results, communicated as mean ± standard deviation, SPSS adaptation 20.0, apply Tukey's single direction investigation of change (ANOVA) Post Hoc Test. The results show that after 60 & 90 minutes, all models showed significant suppression of cough P <0.05.

DISCUSSION

Nature participate a significant role in invention of new medicinal agents which play a vital role as a chief therapeutic components. According to world health

Table 3: Antitussive activity of cough syrup formulations A1, A2, A3, A4, and standard drugs on ammonia induced cough

Treatment	Dose	Frequency of cough (mean±SEM)			
		0 min	30min	60 min	90min
Control		58.30 ± 2.20	50.64±4.27	32.46±2.03	22.00±4.00
Standard **	10ml	19.50 ± 1.73	15.54±2.76	10.66±0.02*	3.12±2.60
A1	5ml	48.40 ± 5.51	40.22±2.26	0.76±0.83*	0.63±0.33
	10ml	58.60 ± 3.11	39.32±2.43	0.00±0.00*	0.00±0.00
A2	5ml	28.60 ± 0.74	20.21±0.67	0.00±0.00	0.00±0.00
	10ml	42.20 ± 1.67	19.64±3.32	3.27±2.33	0.00±0.00
A3	5ml	24.88 ± 1.50	11.92±4.32	0.00±0.00	0.00±0.00
	10ml	35.90 ± 5.80	23.43±1.27	0.00±0.00	0.00±0.00
A4	5ml	32.62±1.27	25.87±0.98	0.00±0.00	1.00±0.57
	10ml	40.65±3.27	20.27±1.19	0.00±0.00	0.00±0.00

*P <0.05 significant as compared to control, ** Diphenhydramine HCL and Dextromethorphan HBr
Values are recorded as MEAN ± SEM
Mean difference is significant at the 0.05 level (P<0.05, Tukey's test) as analyzed by one –way analysis of variance (ANOVA)

organization about eighty percent of the world populations are relay on nature specially on plants as a initial source of treatment (Committee on Herbal Medicinal Products (HMPC) 2005, Rev. 2). The current research was to determine the biological activity of rose water obtained from damask rose petals, the most important focus of this work was to assess the therapeutic value of rose water and ivy leaf extracts to establish a relationship between traditional use and the results obtained through drug screening test.

Cough is one of the most common symptoms accompanying a patient on the way to the doctor for medical help. Under normal conditions, the most important task of coughing is to protect the airways from inhalation of irritants and stimulation of the afferent vagus nerves activates the cough reflex in the extra pulmonary airways and is mediated by the brainstem (Young *et al*., 2011).

In painful conditions, the cough becomes violent and patients often self-medicate for an acute cough associated with temporary upper respiratory infections ("colds"). Nonetheless, some genuine respiratory infections, like asthma, ongoing chronic obstructive pneumonic disease (COPD) and cellular breakdown in the lungs, regularly have a persistent hack (Mackenzie *et al*., 2004). So a chronic cough is another serious health problem than an acute or subacute cough. Existing antitussive drugs are generally divided into two categories, that is, central and peripheral, according to their site of action. Central cough suppressants are the majority of cough suppressants currently in use (Reynolds *et al*., 2004). In spite of the fact that narcotics, including codeine phosphate, are the best antitussives, their utilization is restricted by undesirable incidental effects like reliance (Kamei *et al*., 2002). Peripheral antitussives, such as levodopropizine and guaifenesin, usually inhibit the airway subtype that causes cough, but these drugs are not widely used. (Dicpinigaitis *et al*., 2006) As a result, an effective and well-tolerated cough medicine has yet to be found for clinical needs. Cough syrup formulation was an easily administered route of drug in patient suffering from engulfing problem (Chang *et al*., 2008).

This study shows the effect of rosewater cough syrup on sulfur dioxide-induced cough in experimental animals at 3 different doses levels:5ml / 70kg, 10ml / 70kg, &15ml / 70kg, as well as the use of four different compositions A1 (laboratory distillation, rose water), A2 (50% laboratory distilled rose water + 50% distilled water), A3 (commercial rose water) and A4 (50% commercial rose water + 50% distilled water) versus diphenhydramine Standard HCL and dextromethorphan HBr 10 ml/70kg. At 30 minutes after administration of sulfur dioxide and cough syrup, number of coughing bouts in A1 at three different dosage levels are as follows: mean ± SEM (9.00±2.08, 4.33±1.76 and 3.00±0.57) A2 (4.33±0.66, 8.33±2.18, 19.33±9.33) and then the A3, which looks like this (10.33±1.20, 9, 66±1.85 and 6.00±2.08) A4 (10.33±0.88, 8.33±1.76, 6.00±0.57) by comparing the result with the standard, it was observed that the standard diphenhydramine HCL and dextromethorphan HBr10ml/ 70kg shows 8.00±5.13 coughing bouts which is more than formula A1. The results show that after 60 and 90 minutes, all samples show significant suppression of cough P<0.05. Cough reduction is mentioned in figs. 5-8.While the antitussive effect of cough syrup formulation (A1, A2, A3, A4) at two different dose level that is at 10ml/70kg and 5ml/70 kg, when Cough induced by ammonia shows that by comparing the results with the standard at 10ml/70kg dose the frequency of cough bouts is (3.12±2.60) which is again more than A1 formulation. Comparing the results, all cough syrup formulations showed better result against standard drug which are Diphenhydramine HCL and Dextromethorphan HBr.

The study explains the potent antitussive effect of all four formulation designs with the help of *Rosa damascene* petal extract. The cough suppressing effect of rose water is concentration dependant. The higher the concentration of rose extract shows significant inhibition in cough bouts. in the present study standard antitussive, methods were used for the evaluation of antitussive effects of rose water described by the several research investigators (Forsberg *et al*., 1988) as it was previously reported that rose water has the bronchodilatory effect which is responsible to show antitussive effect (Karlsson *et al*., 1990). The another possible antitussive effect of *Rosa damascena* extract was the inhibition of nonadrenergic noncholinergic (NANC) nervous system stimulation (Linden *et al*., 1993). While some research shows that the antitussive effect of this plant is due to its tachykinin inhibitory substance (s) content which reduces the inflammation, as the rose plant is the potent anti-inflammatory agent (Zhao, Wang, & Green, 1999). By comparing the result obtained by two different methods that is sulphur di oxide induced cough and ammonia induced cough, A1 formulation shows the significant inhibitory effect in comparison of all (A2, A3, A4) formulations because of containing the highest concentration of lab-based extracted rose water, shown in fig 1 and 5, at 60 min in both methods total suppression in cough bouts observe as compare to the other (A2, A3, A4) formulation. Graphical representation cough syrup formulations shown in figs. 1-8.

CONCLUSION

Rosa damascena Mill. petal extract (rose water) is traditionally used to manage the cough. In the present study flower, petals achieved therapeutic effects to relieve cough and reduce airway inflammation. The pharmacological result also demonstrates that rose water from *Rosa damascena* was more effective in relieving cough due to the presence of polyphenolic compounds (Abidi *et al*., 2018) especially flavonoids.

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