

Comparative Cough Suppression of Chitosan Crab Extract of *Uca tangeri* and Dihydrocodeine

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Abstract

Cough is an innate primitive reflex and acts as a part of the body's immune system to protect against foreign materials from the respiratory tract. This study was done to investigate the cough suppression potential of *Uca tangeri*. A day before the test, guinea pigs were placed individually in a transparent chamber (60 × 36 × 60 cm) for 5 minutes before cough was induced by exposure to 15% citric acid, delivered using an Omron compressor nebulizer (rate of 0.4 ml/minutes and particle size 5µm) for 10 minutes. The animals were then monitored visually within this exposure time for cough; the latency and counts, of which, were taken as the basal values. The animals exhibiting 10 - 20 bouts of cough were selected for the study and fasted overnight but with access to water. The selected animals were randomly allotted to 5 groups (n=5 per group). The animals were treated orally thus: Group 1 was the control group and received 2 ml/kg of normal saline; group 2 received 25 mg/kg dihydrocodeine; Group 3 received 150 mg/kg extract; group 4 received 300 mg/kg extract and group 5 received 600 mg/kg of the extract. An hour after administration, they were re-exposed to citric acid aerosol (as earlier described) and the latency of cough and cough count were recorded. The procedure was repeated at hours 2 and 3 after treatment. Antitussive activity was then evaluated in each guinea-pig as the percentage reduction in the number of coughs also known as percentage suppression of cough and percentage increase in latency of cough. The results revealed that *Uca tangeri* exhibited a dose dependent percentage increase in cough latency period as well as percentage increase in suppression of cough which was inferior to dihydrocodeine, but significantly greater than normal saline and basal levels.

Keywords: Cough; Suppression; *Uca tangeri*; Chitosan Crab; Dihydrocodeine, Comparative.

INTRODUCTION

The body's immune system uses the involuntary primitive reflex of coughing to defend against foreign substances that invade the respiratory tract (Sharma et al., 2020). To eliminate debris, excessive mucus, irritants, microbes, or other substances from the respiratory tract, one may cough either voluntarily or involuntarily. Coughing is either voluntary or involuntary (Chung and Pavord, 2008). Coughing could be classified as acute, subacute, or chronic depending on the duration (Vally and Ihuma, 2016). Acute cough has the shortest duration. It is considered to be a cough with duration of less than two weeks. Sub-acute cough has a duration between three-eight weeks while chronic cough lasts for more than eight weeks (four weeks for children) (Vally and Ihuma, 2016).

Adults who have persistent coughing are increasingly being linked to pertussis (Irwin et al., 2006). The prevalence of chronic cough is 9.6% worldwide, with Oceania having the highest incidence (18.1%), and Africa having the lowest prevalence (2.3%), according to Song et al. (2015). Coughing is a symptom of several

diseases and health conditions, including the common cold, acute bronchitis, pneumonia, pertussis, flu, smoking, as well as asthma, tuberculosis, and lung cancer. Coughing can cause chest pain, congestion, and an irritated throat. Repeated coughing causes irritation and discomfort, which in turn leads to additional coughing (Irwin et al., 2006).

Cough is also brought on by a variety of microbes, including bacteria and viruses, which aids in the spread of the illness to new hosts. Regular coughing is typically brought on by a respiratory tract infection, but it can also be brought on by choking, smoking, air pollution, asthma, gastroesophageal reflux disease (GERD), post-nasal drip, chronic bronchitis, lung tumors, heart failure, and drugs like ACE inhibitors (Dicpinigaitis et al., 2009).

Crabs of various species have been employed in the treatment of various illnesses. *Cardisoma guanhumi* has been used to treat wounds, boils and bronchitis in Latin America, according to Alves and Alves (2011). *Goniopsis cruentata* is used to treat epilepsy and genital problems; *Plagusia depressa* serves as a complementary medicine for epilepsy; *Emerita portoricensis* on earaches' therapeutic applications; *Ocypode quadrata* in treating

asthma, hemorrhage in women, the flu, and to lessen the effects of Naquin poison intoxication (Pisces, Batrachoididae); *Ucides cordatus* in treating hemorrhage in women, urinary incontinence, osteoporosis, cough, asthma, tuberculosis, womb disorders, arthrosis, and bronchitis; and *Uca maracoani* in curing asthma, whooping cough.

Dev Roy (2014) gathered 22 species of brachyuran crabs from various regions of the world, including India, Nepal, and Brazil. These crabs are primarily used to treat conditions like whooping cough, bronchitis, pneumonia, asthma, osteoporosis, wounds, boils, womb disorders, tuberculosis, earache, burns, and epilepsy, while hermit crabs are used to treat earache, urethritis; malaria. This research examines *Uca tangeri* and dihydrocodeine's capability to suppress coughing.

MATERIALS AND METHODS

Animals

25 adult guinea pigs of either sex weighing 460-600g were obtained from an animal facility in Ogoni, Rivers State, and brought to the animal house of Department of Pharmacology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Nigeria. All animals were allowed two weeks acclimatization in the animal facility of the Department of Pharmacology, University of Port Harcourt. They were all allowed free access food and tap water and were exposed to natural light-dark cycle and room temperature. All animals were handled according to standard protocols for the use of laboratory animals (National Institute of Health, 2002).

Site of Collection of crab samples

The samples were collected at Sivivilagbara along the Dor Nwezor channel of Bodo creek and Buguma creek. The Buguma Creek is a tributary of the Bonny River which is located southeast of the Niger Delta between longitude 6°51'E and 49.8'E and latitude 4°43'N and 47.8'N in Asari-Toru Local Government Area of Rivers State. The creek system consists of the main creek channel with other associated interconnecting creeks which are interconnected and surround Buguma and Ido communities. The creek serves as a source of tidal water for Nigerian Institute for Oceanography and Marine Research/Buguma Brackish Water Experimental Fish Farm, which was constructed between 1963 and 1966. The New Calabar River brings the salty ocean water as tidal flows diurnally to the fish ponds (Dublin-Green and Ojanuga, 1999).

Sample Collection and Identification

Samples were collected from the creeks. *Uca tangeri* were collected at low tide in the mangrove shores by hand picking. The samples collected were transferred into perforated plastic containers to allow for air during

transportation and was transported to the Pharmacognosy Research Laboratory, Department of Pharmacognosy, University of Port Harcourt. The samples were identified using Food and Agriculture Organization species identification sheets for fresh water and marine crab species.



Figure 1. *Uca tangeri*.

Method of Extraction

According to Shahidi and Synowiecki (1991), 60 of the freshly collected crabs (*U. tangeri*) were sacrificed and the shell separated from the meat and washed with tap water to remove all impurities. The crab shells and meat were then transferred to the oven and dried at 70°C until they were completely dry. Using a laboratory mortar and pestle, the dried crab shells and meat were ground and sieved into the size of 500µm.

Carotenoids Extraction

Four gram of the sieved crab shell was measured using WANT precision electric weighing balance into a beaker and 200ml of cod liver oil was added and stirred with magnetic stirrer until it was completely mixed for 20minutes. The beaker was then transferred into a water bath at a temperature of 60°C and allowed for 30 minutes. The mixture was then filtered with a white handkerchief to drain off the oil and the residue transferred into a beaker.

Deproteinization

The residue from the carotenoids extract was treated with 2% potassium hydroxide (KOH) at a ratio of 1:20 w/v and was stirred continuously for 2 hours at a temperature of 90°C to remove protein from the crab. The sample was filtered and the residues were continuously washed with distilled water until the pH became neutral i.e., pH=7. This was done to ensure that all the salt had been removed after removing the protein. The deproteinized crab was transferred into an oven and dried at 60°C until it was completely dry (Shahidi and Synowiecki, 1991).

Demineralization

2.5% w/v of hydrochloric acid was used at room temperature (23°C) for 6 hours to remove the mineral content of the deproteinized crab materials at a ratio of 1:20 w/v. The samples were filtered and washed with tap water until the pH was neutral. The demineralized crab material was then transferred to the oven and dried at a temperature of 60°C until completely dried. (Shahidi and Synowiecki, 1991).

Decolouration and Dewatering

The demineralized crab material was treated with 300ml acetone for 10 minutes and dried for 2 hours at an ambient temperature and the residues were removed to achieve decolourization. The decolourized sample was washed in running water, filtered and dried at 60°C until it was completely dried to obtain crab chitin (Shahidi and Synowiecki, 1991).

Deacetylation of Chitin

Deacetylation of chitin was carried out using the method of Yen *et al.* (2009). The obtained chitin was treated with 40% w/v aqueous sodium hydroxide in the ratio of chitin to the solution 1:15 w/v at 105°C in a water bath for 2 hours. Thereafter, the chitin was filtered with filter pump and washed with deionized water until pH was neutral to obtain chitosan. The obtained chitosan was then dried at 60°C for 2 hours in the oven. The dried chitosan was preserved in a well labelled bottle and kept for the experiment.

Extract concentration Preparation

The extract solution for the study was prepared by dissolving 0.5g of the extract in 1ml of di-methylsulfoxide (DMSO) solvent to have a stock concentration of 500mg/ml.

Oral Toxicity Testing to Determine LD₅₀

The Bruce method of 1985 was used to determine the LD₅₀ in this study. Following this method, Swiss mice

were dosed one at a time beginning from 1000mg/kg of the extract from the crab because since the extract was from an edible source, there might not be low toxic doses. This was increased by a factor of 1.3 thus after this dose which produced no death, higher doses used were 1300mg/kg, 1690mg/kg, 2197mg/kg, 2856mg/kg, 3713mg/kg, 4827mg/kg and 6275mg/kg. With these doses there was no observed death of the mice and it was concluded that the extract is safe for the study based on pharmacology rule.

Experimental Procedure

This was based on the guinea pig cough model of Nadig (2005) with minor alterations. A day before the test, guinea pigs were placed individually in a transparent chamber (60 × 36 × 60 cm) for 5 minutes before cough was induced by exposure to 15% citric acid, delivered using an Omron (Omron Health Care Ltd, Japan) compressor nebulizer (rate of 0.4 ml/min and particle size 5µm) for 10 minutes. The animals were then monitored visually within this exposure time for cough; the latency and counts, of which, were taken as the basal values. The animals exhibiting 10 - 20 bouts of cough were selected for the study and fasted overnight but with access to water. The selected animals were randomly allotted to 5 groups (n=5 per group). The animals were treated orally thus: Group 1 was the control group and received 2 ml/kg distilled; group 2 received 25 mg/kg dihydrocodeine; group 3 received 150 mg/kg extract; group 4 received 300 mg/kg extract and group 6 received 600 mg/kg of the extract. An hour after administration, they were re-exposed to citric acid aerosol (as earlier described) and the latency of cough and cough count were recorded. The procedure was repeated at hours 2 and 3 after treatment. Antitussive activity was then evaluated in each guinea-pig as the percentage reduction in the number of coughs also known as percentage suppression of cough and percentage increase in latency of cough in comparison with the previously established control basal value, calculated as below:

$$\text{Percentage reduction in cough count} = \left[1 - \left(\frac{C_2}{C_1} \right) \right] \times 100 \quad (1)$$

Where;

C1 is basal values and

C2 is the total number of coughs after treatment.

$$\text{Percentage increase in latency of cough} = \left[1 - \left(\frac{L_2}{L_1} \right) \right] \times 100 \quad (2)$$

Where;

L1 is basal values, and

L2 is the latency of coughs after treatment.

Data analysis

All results are expressed as means ± SEM. An analysis of variance was performed on the different treatment groups to determine significant effects of the treatments. Post-

hoc analysis between the different groups was performed with a Dunnett's t-test. A value of $p \leq 0.05$ was accepted as the level of statistical significance.

RESULTS AND DISCUSSION

Bronchoconstriction is significant in cough induction since the process stimulates intrapulmonary rapidly adapting receptor (RAR), a type of cough receptor to cause or enhance the sensitivity of the cough (Pavord, 2004). RAR activation initiates bronchospasm and mucus secretion via parasympathetic reflexes. This study investigated antitussive properties of the *Uca tangeri* in guinea pigs. Guinea pigs were used in the antitussive investigation because their airways possess the needed afferent nerves and can produce cough, just like in humans (Agrawal et al., 1991). Cough was detected with a characteristic sound and by stretching of limbs accompanied by inspiration and then expiration similar to that described by Morice and co-workers (Morice et al., 2007). These criteria were adopted so as to distinguish it

from other respiratory reflexes like sneezing and expiratory reflex. As a tussigenic agent when inhaled, citric acid is known to stimulate transient receptor potential vanilloid1 on the C-fibers. This then causes the release of tachykinins to mediate bronchoconstriction and mucus secretion, which in turn stimulates RAR (Bonham et al., 1996 and Canning et al., 2001), a widely studied cough receptor. The impulse is then conveyed through the vagus nerve to the CNS and then back to respiratory muscle through the efferent pathway to cause cough. Dihydrocodeine was used as the positive control because it is the second most specific antitussive of the commonly used opioids (Eddy et al., 1969). Dihydrocodeine acts on the μ opioid receptors to suppress the cough reflex (Kamei 1996).

Number of cough in guinea pigs pre-treatment with Dihydrocodeine, and *Uca tangeri* and thereafter exposure to citric acid

Table 1. Effect of *Uca tangeri* on latency period (in seconds) in animals treated with acetic acid in a tussive protocol.

Group	BLP (seconds)	OLP (seconds)	TLP (seconds)	THLP (seconds)
Normal Saline (NS)	43.60±0.25	44.60±0.25	44.80±0.37	45.40±0.40
Dihydrocodeine (DH)	43.60±0.25	59.80±0.20	60.00±0.01	60.60±0.25
Low Dose (<i>Uca tangeri</i>) (LDUT)	43.60±0.25	48.00±0.01	48.20±0.20	48.60±0.24
Medium Dose (<i>Uca tangeri</i>) (MDUT)	43.60±0.24	51.80±0.20	52.00±0.01	52.60±0.24
High Dose (<i>Uca tangeri</i>) (HDUT)	43.60±0.24	55.40±0.24	56.20±0.20	56.60±0.24

BLP = Basal Latency Period; **OLP** = One Hour Latency Period; **TLP** = Two Hours Latency Period; **THLP** = Three Hour Latency Period; **Low Dose (*Uca tangeri*) (LDUT)** = 150mg/kg; **Medium Dose (*Uca tangeri*) (MDUT)** = 300mg/kg; **High Dose (*Uca tangeri*) (HDUT)** = 600mg/kg

Table 1 presents the results of the number of cough bouts produced by the guinea pigs before and after pre-treatment with normal saline, 25mg/kg of dihydrocodeine, 150mg/kg, 300mg/kg and 600mg/kg of the extract from *Uca tangeri*. Although, there were no significant differences, observed between the basal i.e., pre-treatment cough bouts (17.60±0.24) and those of normal saline for the various time frames (14.60±0.24, 13.80±0.37 and 13.40±0.24), there were significant differences between the basal and those of 25mg/kg of dihydrocodeine, 150mg/kg, 300mg/kg and 600mg/kg of the extract from *Uca tangeri*. ANOVA comparison of number of cough bouts between normal saline (14.60±0.24, 13.80±0.37 and 13.40±0.24), and dihydrocodeine (4.00±0.32, 3.20±0.20 and 3.00±0.01) respectively also showed significant differences. Also, ANOVA comparison of the number of cough bouts between normal saline (14.60±0.24, 13.80±0.37 and 13.40±0.24) and low dose of *Uca tangeri* (8.20±0.37, 8.40±0.24 and 8.00±0.32) revealed a significant difference. This was also true for both medium dose and high dose. These results implies that normal saline does not reduce the number of cough bouts induced with citric

acid, since its administration did not produce any significant difference from animals induced with citric acid without treatment. Thus, it could be stated that normal saline does not have any effect on the intrapulmonary rapidly adapting receptor (RAR). It neither activates nor inhibits its ability to cause or enhance the sensitivity of the cough. On the contrary, dihydrocodeine and all the different doses of the extract significantly reduced the number of cough bouts in the different time frames, implying that both the standard drug and the extract exerted inhibitory effect on the stimulatory process on the intrapulmonary rapidly adapting receptor (RAR). Therefore, this inhibitory effect also prevents the frequency of bronchospasm and mucus secretion caused by citric acid. These, antitussive properties were greater for dihydrocodeine, followed by high dose of the extract, medium dose of the extract and low dose of the extract. Also, it is known that dihydrocodeine acts on the μ opioid receptors to suppress the cough reflex (Kamei 1996), hence, this reduction in the number of cough bouts is associated with a stimulation of μ opioid receptors in the CNS. In a similar manner the effect produced by the *Uca tangeri* extract,

could be associated with CNS stimulation of μ opioid receptors. Finally, it was noted that the effect of *Uca tangeri* extract on the number of cough bouts was dose

dependent, implying that the number of cough bouts decreased as the dose of the extract increased.

Latency period of cough in guinea pigs pre-treatment with Dihydrocodeine and *Uca tangeri* and exposure to citric acid

Table 2. Effect of *Uca tangeri* on number of cough bouts in animals treated with acetic acid in a tussive protocol.

Group	PCB	OCB	TCB	THCB
Normal Saline (NS)	17.60±0.24	14.60±0.24	13.80±0.37	13.40±0.24
Dihydrocodeine (DH)	17.60±0.24	4.00±0.32	3.20±0.20	3.00±0.01
Low Dose (<i>Uca tangeri</i>) (LDUT)	17.60±0.24	8.20±0.37	8.40±0.24	8.00±0.32
Medium Dose (<i>Uca tangeri</i>) (MDUT)	17.60±0.24	7.00±0.32	6.60±0.24	6.40±0.24
High Dose (<i>Uca tangeri</i>) (HDUT)	17.60±0.24	5.80±0.37	5.40±0.24	5.20±0.20

PCB = Pre-treatment cough bouts; **OCB**= Cough bouts after one hour of drug administration; **TCB** = Cough bouts after hour of drug administration; **THCB** = Cough bouts after 3 hours of drug administration

Table 2 presents the results of the latency period caused by *Uca tangeri* and dihydrocodeine in comparison to normal saline. It was observed that the basal latency period (43.60±0.25) was not significantly lower than that produced by normal saline, after one hour (44.60±0.25), two hours (44.80±0.37) and three hours (45.40±0.40) pre-treatment, but was significantly lower than those produced by dihydrocodeine (25mg/kg) after one hour, two hours and three hours (59.80±0.20, 60.00±0.01 and 60.60±0.25) pre-treatment respectively. This was also true for 150mg/kg, 300mg/kg and 600mg/kg of the extract from *Uca tangeri*. It was also observed that the latency periods of normal saline were significantly lower than those for 25mg/kg of dihydrocodeine and 150mg/kg, 300mg/kg and 600mg/kg of the extract from *Uca tangeri* for the different time frames of one, two and three hours. ANOVA comparison revealed significant differences. This implies that there was no cough suppression associated with normal saline, while on the contrary; significant cough suppression is

associated with dihydrocodeine and the extract from *Uca tangeri*. This could be associated with activation of μ opioid receptors, since dihydrocodeine acts on the μ opioid receptors to suppress the cough reflex (Kamei 1996). The effect produced by *Uca tangeri* extract was similar to that of dihydrocodeine, hence a similar assumption could be made for the *Uca tangeri* extract. It is also worth stating here that *Uca tangeri* extract produce a dose dependent increase in the cough latency period, i.e., the cough latency period increasing as the dose increased. Also, since cough induction is associated with significant stimulation of the cough receptor ‘intrapulmonary rapidly adapting receptor (RAR)’, it can be deduced that both dihydrocodeine and the extract from *Uca tangeri* significantly inhibited the cough receptor ‘intrapulmonary rapidly adapting receptor (RAR)’ and could be use in acute or chronic conditions of bronchial constriction, since significant bronchio-constriction is associated with cough induction.

Effect of dihydrocodeine and *Uca tangeri* on percentage reduction in cough counts in animals treated with acetic acid in a tussive protocol

Table 3. Effect of *Uca tangeri* on percentage reduction in cough counts in animals treated with acetic acid in a tussive protocol.

Group	OPRCC	TPRCC	THPRCC
Normal Saline (NS)	17.06±0.24	21.63±1.28	23.86±1.02
Dihydrocodeine (DH)	77.19±2.03	81.83±1.04	82.94±0.24
Low Dose (<i>Uca tangeri</i>) (LDUT)	51.24±2.34	52.29±1.05	54.51±1.87
Medium Dose (<i>Uca tangeri</i>) (MDUT)	60.19±1.85	62.48±1.41	63.66±1.10
High Dose (<i>Uca tangeri</i>) (HDUT)	66.93±2.52	69.28±1.53	70.46±1.02

OPRCC = Percentage reduction in cough count after one hour of drug administration; **TPRCC** = Percentage reduction in cough count after two hours of drug administration; **THPRCC** = Percentage reduction in cough count after three hours of drug administration

Table 3 presents the results of the percentage reduction in cough counts produced by normal saline,

25mg/kg of dihydrocodeine and 150mg/kg, 300mg/kg and 600mg/kg of the extract from *Uca tangeri*. It was

observed that there was significant difference in the percentage reduction of number of coughs produced by 25mg/kg of dihydrocodeine (77.19±2.03, 81.83±1.04 and 82.94±0.24), as compared to normal saline (17.06±0.24, 21.63±1.28 and 23.86±1.02) in the respective time frames of one hour, two hours and three hours. Similar significant differences were observed between normal saline and the different doses (150mg/kg, 300mg/kg and 600mg/kg) of *Uca tangeri* in the various time frames as shown in table 3. Thus, normal saline clearly does not decrease the percentage number of cough, as observed in the study, but this is contrary to the effect observed with 25mg/kg of dihydrocodeine and 150mg/kg, 300mg/kg and 600mg/kg of the extract from *Uca tangeri*, which

implies that both the standard drug and the test extract actually suppressed the stimulatory process of the intrapulmonary rapidly adapting receptor (RAR), which are responsible for the cough or enhancement of the sensitivity of the cough. The fact that dihydrocodeine acts on the μ opioid receptors to suppress the cough reflex (Kamei 1996) obviously implies that, the decrease in the percentage number of cough is related to the stimulation of μ opioid receptors in the CNS. A similar deduction could be made for the *Uca tangeri* extract, since similar and dose dependent pattern was observed with the extract. Meaning the extract could possess some CNS effect similar to dihydrocodeine.

Effect of dihydrocodeine and *Uca tangeri* on percentage increase in cough latency in animals treated with acetic acid in a tussive protocol

Table 4. Effect of *Uca tangeri* on percentage increase in cough latency in animals treated with acetic acid in a tussive protocol.

Group	OPICL	TPICL	THPICL
Normal Saline (NS)	2.29±0.01	2.75±0.45	4.13±0.45
Dihydrocodeine (DH)	37.13±0.58	37.56±0.73	38.92±0.17
Low Dose (<i>Uca tangeri</i>) (LDUT)	10.61±0.62	10.61±0.62	11.92±0.43
Medium Dose (<i>Uca tangeri</i>) (MDUT)	18.83±0.95	19.28±0.67	20.65±0.81
High Dose (<i>Uca tangeri</i>) (HDUT)	27.07±0.54	28.91±0.68	29.83±0.85

OPICL = Percentage increase in cough latency after one hour of drug administration; **TPICL** = Percentage increase in cough latency after two hours of drug administration; **THPICL** = Percentage increase in cough latency after three hours of drug administration

Table 4 presents the results of percentage increase in cough latency. ANOVA comparison revealed that there were significant differences between normal saline (2.29±0.01, 2.75±0.45 and 4.13±0.45) and 25mg/kg of dihydrocodeine (37.13±0.58, 37.56±0.73 and 38.92±0.17) respectively, for the various time frames of one hour, two hours and three hours. Similar significant differences were observed between normal saline and the different doses (150mg/kg, 300mg/kg and 600mg/kg) of *Uca tangeri* for the various time frames. This implies that both 25mg/kg of dihydrocodeine and 150mg/kg, 300mg/kg and 600mg/kg of the extract from *Uca tangeri* cause inhibition to the cough reflex or the stimulation of the intrapulmonary rapidly adapting receptor (RAR), leading to a reduction of in the urge for cough, thereby causing a significant increase in the latency period of the cough. Dihydrocodeine acts on the μ opioid receptors to suppress the cough reflex (Kamei 1996). Thus, the suppression of the cough leading to an increase in latency period is related to μ opioid receptors. It was also noted that the increase in cough latency produced by *Uca tangeri* was dose dependent, meaning a greater suppression was observed with the highest dose, while the smallest suppression was produced by the lowest dose. Also, since this suppression was similar to that caused by dihydrocodeine, it could be deduced that

extract possesses central nervous system effect similar to that of dihydrocodeine.

CONCLUSION

Dihydrocodeine and *Uca tangeri* were found to be effective antitussive agents. Dihydrocodeine was used as the positive control because it is the second most specific antitussive of the commonly used opioids (Eddy et al., 1969). Dihydrocodeine acts on the μ opioid receptors to suppress the cough reflex (Kamei 1996). *Uca tangeri* also exhibited a dose-dependent antitussive effect reducing both the cough count and latency of cough, similar to dihydrocodeine. Thus, it is possible that the extract possesses central nervous system effect similar to that of dihydrocodeine on cough.

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