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# Antitussive properties of the root extract and fractions of *Acanthospermum hispidum* (L)

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## Abstract

*Acanthospermum hispidum* (Asteraceae) is plant widely used in South Eastern Nigeria to treat cough. This study investigated the antitussive properties of the root extract and fractions of *A. hispidum*. The antitussive effects of oral administration of 400 and 800 mg/kg of *A. hispidum* root methanol extract (ME), hexane (HF), ethyl acetate (EF) and methanol fractions (MF) were evaluated using sulphur (iv) oxide and ammonia induced cough models in mice. The extract and fractions were subjected to acute toxicity test, phytochemical analysis and HPLC finger printing. The extract and fractions of *A. hispidum* exhibited strong and significant antitussive effects in both cough models. The MF and EF exhibited significant ($p< 0.01$) antitussive effect when compared to the control. The inhibition of ammonia induced cough (86.6 %) was greater than sulphur (iv) oxide induced cough (47.2 %). An oral $LD_{50}$ value >5000 mg/kg in mice was established for the extract. Phytochemical analysis revealed the presence of saponins and flavonoids which may be responsible for its antitussive effects. Results revealed the antitussive property of *A. hispidum* and root which correlate to its ethnomedicinal use to relieve cough.

**Keywords:** Antitussive, *Acanthospermum hispidum*, sulphur (iv) oxide, ammonia.

## 1. Introduction

Cough is a nonspecific feature of most respiratory and a number of non-respiratory conditions such as inflammation in the respiratory tract, bronchial asthma [1] or by neoplastic processes [2]. Cough is an essential protective and defensive mechanism for clearing the upper airways hence can be considered to be an innate inbuilt defence mechanism. However, impairment or absence of the coughing mechanism can be harmful and even fatal in some disease conditions [3]. Health care statistics placed cough as the most common reason for patients seeking medical help [4]. Despite the efficacies of widely used antitussives agents, they are still associated with a relatively high rate of undesirable side effects such as sedation, depression of the respiratory centre, decreased mucus secretion in bronchioles, as well as inhibition of ciliary activity [5]. The increasing need for more effective and safe antitussive medication calls for the search for novel antitussive natural product [6].

*A. hispidum* (Asteraceae) is an annual plant widely used in South Eastern Nigeria. It is used traditionally for the treatment of jaundice, malaria, vomiting, cephalgias, headache, abdominal pain, convulsions, stomach ache, constipation, eruptive fever, snake bites, epilepsy, blennorrhoea, hepatobiliary disorders, microbial infection, cough and bronchitis [7].

This study was design to investigate the antitussive activity of *Acanthospermum hispidum.*

## 2. Materials and Methods

### 2.1 Animals

Conventional grade UN-FERH: NS outbred strain of albino mice (15–25 g) of either sex bred in the Laboratory Animal Facility of the Department of

Pharmacology and Toxicology, University of Nigeria, Nsukka, and Nnamdi Azikiwe University, Awka were used for the study. The animals were maintained *ad libitum* on standard pellets and water. All animal experiments were in compliance with National Institute of Health Guide for Care and Use of Laboratory Animals (Pub no. 85-23, revised 1985) and with prior permission from the National Health Research Ethics Committee (NHREC) of the University of Nigeria, with protocol ethical clearance number NHREC/05/01/2012A.

**2.2 Preparation of extract**

Fresh root barks of *Acanthospermum hispidum* were collected from Nsukka, Nigeria, in October and November, 2010. The plant materials was identified and authenticated by Mr. Alfred Ozioko, a taxonomist at the International Centre for Ethnomedicine and Drug Development (InterCEDD), Nsukka, where voucher specimens were deposited. The root bark was cleaned, dried under shade for 1 month and pulverized to coarse powder using a milling machine. The powdered plant material (1 kg) was extracted by maceration in methanol at room temperature (28 ± 1 $^{o}$C) for 48 h, and the mixture was filtered. The plant material was repeatedly washed with fresh solvent until the filtrate became clear. The filtrate was concentrated using a rotary vacuum evaporator under reduced pressure at 40 $^{o}$C to obtain 84.29 g of the methanol extract (8.429% w/w).

**2.3 Solvent-guided fractionation of methanol extract (ME)**

The ME (84.29 g) was subjected to solvent-guided fractionation in a silica gel (60–120 mesh size) column, (length 60 x 7.5 cm) successively eluted with n-hexane, ethyl acetate and methanol in order of increasing polarity, the eluents were concentrated using a rotary evaporator under reduced pressure at 40 $^{o}$C to yield hexane (HF; 5.70 g; 6.76% w/w), ethyl acetate (EF; 9.55 g; 11.33% w/w) and methanol (MF; 34.20 g; 40.57 % w/w) fractions, respectively. The extract and fractions were subjected to phytochemical analysis using standard procedures [8].

**2.4 Acute toxicity and lethality ($LD_{50}$) test**

The acute toxicity and lethality ($LD_{50}$) of ME of *A. hispidum* was estimated in mice as described earlier [9]. The test was done in two stages. In stage one, mice were randomly grouped into 3 groups (n=3) to receive oral administrations of 10, 100 or 1000 mg/kg of ME dissolved in 10 % Tween 80, and the animals were monitored for 24 h for signs of toxicity and death. No death was recorded after 24 h. Since no death occurred in any of the 3 dose levels in stage one, three higher doses, 1600, 2900 and 5000 mg/kg of ME were administered to a fresh batch of animals at one dose per animal (n=1) in stage two of the test, and the animals were observed for 24 h for signs of toxicity and death.

**2.5 Antitussive Studies**

**2.5.1 Ammonia-induced Cough**

The method described by Xu *et al* [9] was used with slight modification. Albino mice of either sex (15-25 g) were randomly grouped (n=4) to receive an oral administrations of 400 and 800 mg/kg of ME, HF, EF and MF of *A. hispidum* respectively, 10 % Tween 80 (0.3 ml) and 6 mg/kg of codeine phosphate. Before and after thirty minutes of drug administrations, cough was induced by exposing the mice to 5 % ammonium hydroxide (1 ml) cotton ball placed in a petri dish at the base of the 1000 ml glass chamber for two minutes. The frequency of the cough was observed and recorded for five minutes.

Time course studies for ammonia induced cough were also investigated using the same procedure above. Albino mice of either sex were weighed and randomly divided and grouped into six (n=6). Before and after different time interval of drug administration, cough was induced by ammonia according to the time interval of each group as follows: group i (30 mins), group ii (60 mins), group iii (90 mins), group iv (120 mins), group v (180 mins) and group vi (240 mins) respectively. The frequency of the cough was observed and recorded for five minutes in mice.

**2.5.2 Sulphur (iv) oxide-induced Cough**

The method described by Miyagoshi *et al*[10] was used with slight modification. Albino mice of either sex were randomly grouped (n=4) to receive an oral administrations of 400 and 800 mg/kg of ME, HF, EF and MF of *A. hispidum* respectively, 10 % Tween 80 (0.3 ml) and 6 mg/kg of codeine phosphate. Before and after thirty minutes of drug administrations, cough was induced by placing the mice on a wire gauze platform with a 2 ml vial within a desiccators of 2000 ml capacity, then 2 ml of 500 mg/ml solution of Sodium Hydrogen Sulphite ($NaHSO_3$), in double distilled water was introduced into the vial and 0.2 ml of Sulphuric acid ($H_2SO_4$) was introduced also using a pipette. After 15 sec, the mice were exposed to Sulphur (iv) oxide ($SO_2$) for 45 sec. The number of bouts of cough was observed and recorded for five minutes.

Time course studies for sulphur (iv) oxide induced cough was also carried out using the same procedure above.

**2.6 Statistical analysis**

Results were presented as mean ± SEM, and analysed using one-way ANOVA in SPSS 16 and subjected to Dunnett's multiple comparison test. Differences between means of treated and control groups was considered significant at $p<0.05$.

## 3. Results

### 3.1Phytochemical constituents

Preliminary phytochemical analysis showed that ME contained all the typical phytoconstituents assayed. The HF gave positive reactions for steroids and fats and oils, EF tested positive to alkaloids, steroids, tannins and terpenoids, while MF gave positive reactions for alkaloids, flavonoids, saponins, terpenoids and tannins (Table 1).

### 3.2 Acute toxicity and lethality ($LD_{50}$)

Administration of ME of *A. hispidum*(10–5000 mg/kg) orally did not elicit signs of acute toxicity, and none of the animals died. The oral $LD_{50}$value of ME in mice was thus established to be greater than 5000 mg/kg.

### 3.3 Effect of extract and fractions on ammonia induced cough

Administration of methanol extract and fractions (400 and 800 mg/kg) confer varying degrees of protection against cough induced by ammonia. The inhibition produced by ME and MF were significant ($p< 0.01$) from pre-treatment value Table 2.

### 3.4 Effect of extract and fractions on sulphur (iv) oxide induced cough

Administration of methanol extract and fractions (400 and 800 mg/kg) inhibited cough induced by sulphur (iv) oxide. The inhibition produced ME and MF were significant ($p< 0.05$) from pre-treatment value Table 3.

### 3.5 Effect of time course studies

The methanol fraction at 800 mg/kg produced maximum inhibition of cough at 120 min post treatment in both cough models (Table 4 & 5).

### 3.6 Results of HPLC Fingerprint of methanol fraction of *Acanthospermum hispidum*

The HPLC finger print detected two compounds: 4, 5-dicaffeoyl quinic acid and procyanidine B2. These compounds were identified by comparing the retention times and UV spectral with inbuilt library (Figure 1).

**Table 1: Phytochemical constituent's of *A. hispidum* root extract**

| Phytoconstituent | ME | HF | EF | MF |
|---|---|---|---|---|
| Alkaloids | +++ | – | +++ | +++ |
| Flavonoids | +++ | – | – | +++ |
| Saponins | +++ | – | – | +++ |
| Steroids | ++ | ++ | ++ | – |
| Tannins | ++ | – | ++ | ++ |
| Terpenoids | +++ | – | ++ | +++ |
| Fats and oil | ++ | ++ | – | – |

ME: methanol extract; HF: hexane fraction; EF: ethyl acetate fraction; MF: methanol fraction; –: absent; +: mildly present; ++: moderately present; +++: highly present.

**Table 2: Effect of extract and fractions on ammonia induced cough**

| Treatment | Dose (mg/kg) | Pre-treatment | 30 mins post-treatment | % Inhibition |
|---|---|---|---|---|
| ME | 400 | 27.0±1.9 | 11.6±0.8** | 57.0 |
| | 800 | 26.6±0.5 | 5.40±0.7** | 79.7 |
| HF | 400 | 24.2±1.3 | 23.4±1.2 | 3.3 |
| | 800 | 26.2±0.9 | 25.6±1.0 | 2.3 |
| EF | 400 | 25.4±0.9 | 26.4±0.9 | - |
| | 800 | 23.6±0.9 | 25.6±0.7 | - |
| MF | 400 | 25.2±1.3 | 10.2±1.2** | 59.5 |
| | 800 | 23.8±1.5 | 3.2±0.4** | 86.6 |
| Codeine Phosphate | 20 | 23.8±1.0 | 13.8±1.0** | 42.0 |

n=5. ME: methanol extract; HF: hexane fraction; EF: ethyl acetate fraction; MF: methanol fraction. **P<0.01 compared with control (Dunnett's multiple comparison test).

**Table 3: Effect of extract and fractions on sulphur (iv) oxide induced cough**

| Treatment | Dose (mg/kg) | Pre-treatment | 30 mins post-treatment | % Inhibition |
|---|---|---|---|---|
| ME | 400 | 44.0±2.7 | 30.8±1.2* | 31.5 |
| | 800 | 46.5±1.9 | 31.7±2.1* | 31.8 |
| HF | 400 | 45.2±2.4 | 48.7±1.7 | - |
| | 800 | 47.3±1.8 | 45.7±0.9 | 3.4 |
| EF | 400 | 43.7±2.2 | 58.7.4±5.5 | - |
| | 800 | 45.6±3.4 | 58.0±8.7 | - |
| MF | 400 | 40.5±2.8 | 26.3±3.3* | 35.0 |
| | 800 | 42.3±1.7 | 22.3±4.2** | 47.2 |
| Codeine Phosphate | 20 | 40.4±2.1 | 22.3±5.2** | 44.7 |

n=5. ME: methanol extract; HF: hexane fraction; EF: ethyl acetate fraction; MF: methanol fraction.*p< 0.05 or **P<0.01 compared with control (Dunnett's multiple comparison test).

**Table 4: Time required for maximum inhibition of cough in ammonia induced cough by ME.800mg/kg**

| Time (m) | Mean frequency of cough ± SEM | | % inhibition |
|---|---|---|---|
| | Pre-treatment | Treatment | |
| 30 | 21.2 ± 0.6 | 14.8 ± 0.8 | 30.19 |
| 60 | 21.6 ± 0.4 | 10.8 ± 0.9 | 50.00 |
| 120 | 22.0 ± 0.8 | 9.2 ± 0.6 | 58.18 |
| 180 | 20.5 ± 0.4 | 11.0 ± 0.5 | 46.34 |
| 240 | 21.4 ± 0.5 | 12.2 ± 0.6 | 42.99 |

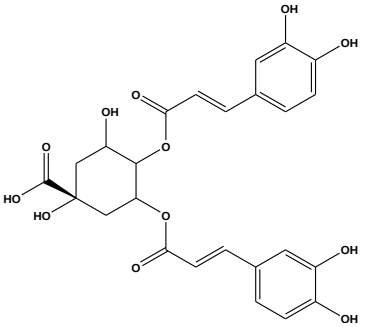
n=5

**Table 5: Time required for maximum inhibition of cough in sulphur (iv) oxide induced cough by ME.800mg/kg**

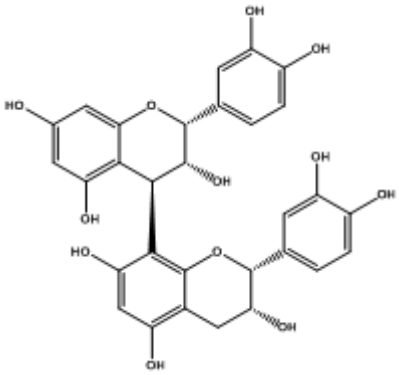
| Time (m) | Mean frequency of cough ±SEM | | % inhibition |
|---|---|---|---|
| | Pre-treatment | Treatment | |
| 30 | 42.3±1.7 | 22.3 ± 4.2 | 47.2 |
| 60 | 44.6 ± 1.1 | 16.0 ± 3.7 | 64.1 |
| 120 | 39.7 ± 2.8 | 3.3 ± 2.5 | 91.6 |
| 180 | 36.2 ± 3.0 | 21.3 ± 5.5 | 41.1 |
| 240 | 38.3 ± 3.4 | 28.7 ± 6.2 | 25.1 |

n=5

**Fig 1: Compound A: 4, 5-dicaffeoyl quinic acid**



**Compound B: Procyanidine $B_2$**



## 4. Discussion

The results of these studies revealed that the root barks of *Acanthospermum hispidum* are endowed with some pharmacological properties that may account for its traditional use in treating cough. Phytochemical analysis revealed the presence of alkaloids, tannins, flavonoids, saponins, steroids, terpenoids, fats and oils. The methanol extract also exhibited high content of saponins and flavonoids (phenolic compounds). The antitussive activity of the root extracts of *A. hispidum* could be attributed to the high saponins and flavonoid contents which have been documented to possess the ability to suppress the cough reflex in patients with chronic bronchitis [11].

Saponins can modulate the cough parameters and phlegm quality [11]. After oral administration of therapeutic doses, saponins irritate vagal nerves reflexively [12]. This leads to increased phlegm secretion in the airways [13]. Additionally, the breathing and cough centres are irritated resulting in more frequent expectoration [11].

Also, the high content of flavonoids may contribute to its antitussive activity. Flavonoids (e.g. Catechins) can inhibit oxidative and reductive processes and decrease the activity of cholinesterase and xanthineoxidase [11]. Rutin and quercertin (flavonoids) inhibit the metabolism of catechol-o-methyl transferase (COMT), and so prolong the pharmacodynamics effects of norepinephrine [11]. This effect could be of value in asthma management since there is a strong link between cough and asthma [14, 15]. Rutin slows down the ascorbic acid oxidation and protract the effect of Vitamin C in organisms [16]. Additionally, in the respiratory systems, flavonoids have been reported to show spasmolytic activity [11,17]. Antiflogistic and antiallergic effect of flavonoids is enhanced by concomitant administration of Vitamin C[18]. This spasmolytic activity is of great value in the management of cough due to asthma and bronchospasm.

The extract showed a very low toxicity profile with an oral $LD_{50}$ of above 5000 mg/kg body weight. This shows that the plant is relatively safe when orally administered in high doses.

The extract and fractions were more effective in inhibiting cough produced by ammonia than that produced by sulphur (IV) oxide. This effect could be explained in terms of the mechanism of receptors stimulated to elicit the cough by the two tussinogens.

The cough reflex can be elicited by the stimulation of three major types of receptors in both the larynx and trachea; rapidly adapting receptors, slowly adapting receptors [19] and C- fibre receptors and acid sensing ion channels (ASICS) [20]. Some of these receptors are selective in the type of stimuli they respond to, while some respond to every tussive stimuli. The rapidly adapting receptors respond to almost every tussive stimuli while the C-fibre receptors are strictly selective [21].

Hence ammonia could have elicited cough by stimulating specific adapting receptors only, whereas sulphur (IV) oxide could have elicited cough by stimulating multiple receptors. The stimulation of multiple receptors have led to the enhanced cough response which was not successfully suppressed by the extracts [18,22].

The result of the time course studies showedan effect at 30 min which peaked at 120 min.

Results of the HPLC analysis revealed two active compounds which are likely contribute to its anti-inflammatory, antioxidant, astringent and consequently antitussive properties. The compounds isolated from the methanol fraction are 4, 5-dicaffeoyl quinic acid and procyanidine B2.

The compound 4, 5 -dicaffeoylquinic acid is a chlorogenic acid derivative (just as aspirin) which has been reported to have antiflammatory and antibacterial properties [23]. Procyanidine B2 is an oligomeric procyanidine or condensed tannin, reported to be a potent anti-oxidant [24], anti-inflammatory and consequently antitussive agent [24,25].

## 5. Conclusion

The results of these studies justified the use of the stem and root extract of *Acanthospermum hispidum* in the traditional treatment of cough, asthma and bronchitis. However, further experiments are required to establish and

elaborate the molecular mechanisms of its antitussive activity before initiation of clinical trials.

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