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# Evaluation of the anti-asthmatic and antitussive effects of concurrently administered extracts of *Bryophyllum pinnatum* and *Andrographis paniculata* in rodents

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**Abstract**
We evaluated the anti-asthmatic and antitussive effects of concurrently administered doses of aqueous *Bryophyllum pinnatum* (BP) and methanol *Andrographis paniculata* (AP) extracts in rodents. Doses of 200 and 400 mg/kg of BP were variously administered concurrently with 4 or 8 mg/kg of AP. In the anti-asthmatic evaluation, ovalbumin-sensitized guinea pigs were given oral doses of both extracts for seven days and then exposed to 0.2% histamine aerosol in a glass chamber. Latency to preconvulsive dyspnea (PCD), tracheal fluid volume and viscosity were measured. In guinea pigs given acute doses of the extract, bouts of cough were counted after exposure to 7.5% citric acid aerosol. Phenol red expectoration was estimated in mice after 7 days of treatment with the extracts. Doses of BP administered alone significant increased latency to PCD ($P < 0.05$); and reduced the number of cough bouts ($P < 0.0001$). The concurrent administration of 400 mg/kg BP with the doses of AP significantly increased latency to PCD compared to 400 mg/kg BP alone. Doses of AP significantly altered the antitussive effect of BP but significantly reversed the reduction of phenol red release by 400 mg/kg BP. We conclude that while concurrent administration of 400 mg/kg BP with doses of AP may be beneficial in asthma**,** this is not the case in cough. This has implication on the label indications following possible co-formulation of the extracts.

*Keywords*: Concurrent extract administration, Asthma, cough, expectoration

## INTRODUCTION

Medicinal plants have continued to play important roles in the effective delivery of health care in developed and less developed countries. It has been estimated that 25% of all prescribed orthodox medicines are substances derived from plants [1]. A survey by the US National Asthma Campaign found that 60% of people with moderate asthma and 70% with severe asthma had used complementary and alternative medicine to treat their condition [2,3].

*Andrographis paniculata* Nees (Acanthaceae) has been used since ancient times in Asian traditional medicine [4]. The extract and its constituents possess many medicinal properties some of which include anti-inflammatory [5], anti-tumour [6], vasorelaxant and cardioprotective [7], and uterine relaxant [8]. The tracheal smooth muscle relaxant effect has been studied [9].

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The plant is cultivated in Nigeria for herbal medicinal purposes [10] and the standardized methanol extract of the plant is marketed as KalmCold® by Natural Remedies Private Ltd (Bangalore, India) for the treatment of common cold. Constituents of the plant include flavonoids, and tannins but andrographolide and its closely related diterpenes such as neoandrographolide and 14-deoxy-11, 12-didehydroandrographolide have been mostly associated with its biological activities [11].

*Bryophyllum pinnatum* Lam. (Crassulaceae) is used by herbalists in Nigeria for the prophylaxis of asthma and treatment of cough. Reports have shown antihypertensive [12], tocolytic [13,14], anti-inflammatory and analgesic properties [15]. In addition, the aqueous leaf extract possesses anti-asthmatic properties and is able to attenuate responses of isolated tracheal smooth muscles to spasmogens such as histamine and carbachol [16-18]. Phytoconstituents identified in the leaves include flavonoids, saponins, tannins and alkaloids [19] and a cytotoxic bufadienolide [20].

Combination of different herbal extracts (polyherbal formulation) has become routine in ethnomedicine [21,22]. Although the aim is to have a product with better effectiveness than each of the ingredients, it is often not backed by scientific evidence. Expectedly, evidence of enhanced effectiveness of concurrently administered formulations should precede their co-formulation into one product. In this report, we investigated the anti-asthmatic and antitussive effects of concurrent administration of aqueous extract of BP and standardized AP in rodents, in order to evaluate the potentials of this combination in the treatment of asthma and cough.

## EXPERIMENTAL

**Plant extracts.** Sachets of standardized dried methanol extract of *A. paniculata* (AP) were donated by Natural Remedies Ltd (Bangalore, India). The label characteristics on the sachets include batch number: RD/2114; date of manufacture: November 2008; colour: green to brownish green powder; taste: bitter; and bulk density: 0.20 to 0.80. The moisture content was 5.0% and it contained ≥ 10% andrographolide. The sachets were kept airtight and stored in a refrigerator.

Leaves of *B. pinnatum* that had never been exposed to herbicides were collected from a suburb of Benin City, Nigeria, in August 2010. A herbarium specimen of the plant with voucher number FHI 107762 exists at the Forest Research Institute of Nigeria. As reported previously [16,17], adulterants were picked out and the leaves were thoroughly rinsed in tap water. The leaves (2 kg) were boiled for one hour in 4 L of distilled water, allowed to cool and then filtered two times with a clean white cloth. The resulting extract was concentrated in a rotary evaporator before drying in an oven at 40$^{o}$C over 24 h (yield = 3.6 % w/w). The extract (BP) was stored in amber-coloured bottles in a refrigerator.

**Animals.** Guinea pigs of either sex (300-400 g) were procured from the Animal House, Department of Physiology, Ambrose Alli University, Ekpoma, Nigeria. Adult mice (20-35 g) of either sex were obtained from a private animal farm in Benin City, Nigeria. The guinea pigs were kept in standard plastic cages in the Animal House Unit, Department of Biochemistry, University of Benin, while the mice were kept in the animal facility of the Department of Pharmacology and Toxicology, University of Benin, Benin City. All animals were allowed two weeks acclimatization before they were randomly assigned to groups comprising of both sexes. They were allowed free access to pellets and tap water, and were exposed to natural lighting condition, and room temperature. Standard protocols for the use of laboratory animals [23] were followed.

**Anti-asthmatic and antitussive experiments**

*Evaluation of latency to preconvulsive dyspnea.* Guinea pigs were randomly allotted to nine groups and treated daily as follows: DW-NS (non-sensitized and given 2 ml/kg distilled water); DW-S (sensitized and given 2 ml/kg distilled water); BP200 (sensitized and given 200 mg/kg BP); BP400 (sensitized and administered 400 mg/kg BP); BP200 + AP4 (sensitized and given 400 mg/kg BP + 4 mg/kg AP); BP400 + AP4 (sensitized and given 400 mg/kg BP + 4 mg/kg AP); BP400 + AP8 (sensitized and given 400 mg/kg BP + 8 mg/kg AP); and SAL0.5 (sensitized and given 0.5 mg/kg salbutamol). Animals were sensitized by modifying the method of Bramley *et al.* [24]. Briefly, a single dose of 100 mg/kg (i.p.) ovalbumin was given on the first day and another 50 mg/kg (i.m.) the following day. A final dose of 50 mg/kg was administered on the 7$^{th}$ day. Treatment with water, extract or salbutamol was *per os* for 7 consecutive days.

One hour after the 7$^{th}$-day treatment, response to an allergen was measured in the guinea pigs. The animals were exposed to 0.2% histamine dihydrochloride aerosol using Omron compressor nebulizer (Omron®, USA) at a rate of 0.4 ml/min and particle size 5 µm, in a glass chamber (60 x 36 x 60 cm) until preconvulsive dyspnea (PCD) was observed. The latency to PCD was recorded.

*Measurement of tracheal fluid volume and viscosity.* A piece of trachea measuring 2 cm from the point of thoracic bifurcation towards the pharynx was isolated after the guinea pigs were sacrificed and 2 ml of deionized water was used to clear each trachea of fluid. The trachea fluid volume was taken as the difference between the volume of the deionized water used for flushing and the volume of the final fluid.

The effluent fluid was subjected to viscosity test using the method of Reid and Ugwu [25]. In brief, the fluid was shaken thoroughly and 1 ml was withdrawn with a 1 ml syringe, which was then held in place with a retort stand. The plunger of the syringe was carefully withdrawn and the time it took for the whole fluid to drain was recorded. The flow rate expressed in ml/s was used as an index for viscosity.

*Exposure to citric acid aerosol.* The acute cough model of Kotzer *et al.* [26] was used. Guinea pigs were initially exposed to 7.5% citric acid aerosol using the Omron compressor nebulizer (rate 0.4 ml/min and particle size 5 µm) in the glass chamber (described above) for 5 min. Animals with cough bouts of 10-20 were randomly allotted into seven groups and treated orally thus: DW (given 2 ml/kg distilled water); BP200 (given 200 mg/kg BP); BP400 (given 400 mg/kg BP); BP200 + AP4 (given 200 mg/kg BP + 4 mg/kg AP); BP400 + AP4 (given 400 mg/kg BP + 4 mg/kg AP); BP200 + AP8 (given 200 mg/kg BP + 8 mg/kg AP); BP400 + AP8 (given 400 mg/kg BP + 8 mg/kg AP); and DF118-25 (given 25 mg/kg of a standard antitussive, DF118). The animals were re-exposed to 7.5% citric acid aerosol after 1 h and the number of cough bouts recorded.

*Phenol red expectoration.* In the phenol red expectorant method [27], mice were randomly allotted into nine groups and were treated daily: DW (given 2 ml/kg distilled water); BP200 (given 200 mg/kg of BP); BP400 (given 400 mg/kg BP); BP200 + AP4 (given 200 mg/kg BP + 4 mg/kg AP); BP400 + AP4 (given 400 mg/kg BP + 4 mg/kg AP); BP200 + AP8 (given 200 mg/kg BP + 8 mg/kg AP); BP400 + AP8 (given 400 mg/kg BP + 8 mg/kg AP); BROM (given 15 mg/kg bromhexine); and SC50 (given 50 mg/kg of sodium cromoglycate only on the 7$^{th}$ day). Except for sodium cromoglycate, which was administered i.p., treatment was *per os*. Ammonium chloride (5 mg/kg p.o) was administered 1 h after the 7$^{th}$-day dose and then followed with phenol red dye (0.5 g/kg i.p.) after 30 min. Each trachea (2 cm) was excised after cervical dislocation of each

mouse and then placed in a solution of 1 ml normal saline + 0.1 ml 1 N NaOH. Absorbance of dye secreted from each trachea was measured at 460 nm with a UV-Visible spectrophotometer (Cecil Instrument Limited, Milton Technical Centre, England).

**Drugs and chemicals.** All the chemicals and reagents used in the study were of analytical grade and obtained from internationally known suppliers such as Sigma (UK) and BDH (UK). Histamine, ovalbumin and citric acid crystals were manufactured by Sigma (UK). Salbutamol was manufactured by GlaxoSmithKline Nigeria Plc. Sodium cromoglycate was a kind gift by Dr. S.O. Okpo of the Department of Pharmacology & Toxicology, University of Benin, Benin City. Dihydrocodeine phosphate (DF118) was purchased from University of Benin Teaching Hospital, Benin City. Bromhexine hydrochloride was manufactured by Nigeria German Chemicals Plc (Nigeria). Drug and extract solutions were freshly prepared before administration.

**Statistics.** Data are presented as mean ± SEM (standard error of mean) and "n" represents the number of guinea pigs or mice per experimental group. Data were compared by use of one way ANOVA with Student-Newman Keul's post hoc test. All data were analysed using GraphPad Prism version 6 software (USA). Statistically significant difference was set at $P < 0.05$.

## RESULTS

**Anti-asthmatic effects.** Sensitized guinea pigs, which were not given any treatment (DW-S), had the lowest latency to preconvulsive dyspnea (PCD). BP at doses of 200 and 400 mg/kg significantly ($P < 0.05$) increased PCD when compared with DW-S. The concurrent administration of 4 and 8 mg/kg AP with 200 mg/kg of BP did not significantly increase latency to PCD but the concurrent administration of 400 mg/kg with the doses of AP resulted in significant increase ($P < 0.05$) in the latency to PCD compared to doses of BP alone. For example while the value of latency to PCD was 228.7 ± 23.5 s for guinea pigs given only 400 mg/kg BP, it increased significantly to 419.0 ± 97.0 s and 450.4 ± 103.0 in the groups that were in addition given 4 and 8 mg/kg of AP (Figure 1).

In Table 1, the volume of tracheal fluid was not significantly altered by treatment with the extract. Similarly, concurrent administration of the extracts did not significantly alter the viscosity of tracheal fluid in the guinea pigs.

**Antitussive effects.** Figures 2 and 3 show the effect of the two extracts on citric acid induced cough in guinea pigs and phenol red secretion in mice, respectively. Doses of BP given singly or concurrently with doses of AP significantly ($P < 0.0001$) reduced the number of cough bouts when compared with distilled water but administration of doses AP concurrently with BP did not significantly alter the reduction in cough bouts produced by the BP alone (Figure 2).

Phenol red excretion (Figure 3) was only significantly reduced by 400 mg/kg of BP when compared with distilled. Concurrent administration of 4 mg/kg/day of AP with 400 mg/kg/day of BP significantly ($P < 0.05$) reduced phenol red concentration. The higher dose (8 mg/kg/day) of AP significantly ($P < 0.05$) reversed the reduction in phenol secretion observed with 400 mg/kg dose of BP. Percentage inhibition of phenol red secretion was 53% at 400 mg/kg BP alone. This value was reduced to 43% with 4 mg/kg/day AP and further reduced to 24% with 8 mg/kg/day AP.

## DISCUSSION

This study has shown that concurrent administration of aqueous leaf extract of *B. pinnatum* (BP) with standardized methanol extract of *A. paniculata* (AP) does significantly enhance the anti-asthmatic effect

of BP alone. Previous *in vitro* studies have shown that BP possesses tracheal smooth muscle relaxant effect and increased the latency to PCD in guinea pigs [16-18]. Also, the standardized extract of *A. paniculata* (AP) relaxed isolated tracheal smooth muscles [9]. In ovalbumin-sensitized guinea pigs, airways become hyper responsive to spasmogens such as histamine resulting in preconvulsive dyspnea (PCD) and this mimics the human form of the disease [28].

Bronchosecretion and mucus plugging of the airways are often symptoms of asthma [29,30]. Therefore, agents that reduce tracheal fluid volume and viscosity are helpful in the management of the diseases. In this present study, neither BP alone nor its concurrent administration with AP significantly reduced tracheal fluid volume and viscosity. In a previous study [18] in which same doses of BP were administered daily for 21 days, tracheal fluid viscosity was significantly reduced. It is possible that the 7-day treatment protocol used in the present study accounted for the lack of consistency with the previous report.

**Table 1.** Tracheal fluid volume and viscosity of guinea pigs treated concurrently with extracts of *B. pinnatum* and *A. paniculata*.

| Groups | Tracheal Fluid Volume (x $10^{-3}$ ml) | Tracheal Fluid Flow Rate (x $10^{-4}$ml/s) |
|---|---|---|
| DW-NS | 0.04 ± 0.01 | 30.77 ± 0.65 |
| DW-S | 0.10 ± 0.03 | 32.68 ± 1.29 |
| BP200 | 0.03 ± 0.01 | 31.88 ± 0.60 |
| BP400 | 0.05 ± 0.01 | 33.74 ± 0.40 |
| BP200 + AP4 | 0.04 ± 0.02 | 32.88 ± 1.87 |
| BP400 + AP4 | 0.03 ± 0.01 | 31.96 ± 1.63 |
| BP200 + AP8 | 0.05 ± 0.02 | 29.76 ± 1.08 |
| BP400 + AP8 | 0.07 ± 0.02 | 30.49 ± 0.97 |
| SAL0.05 | 0.06 ± 0.01 | 32.12 ± 1.48 |

Values for tracheal fluid volume and viscosity are not significantly different. DW-S. DW-NS, distilled water, non-sensitized group; DW-S, distilled water, sensitized group. Values after abbreviations represent doses (mg/kg). n = 5-7 per group.

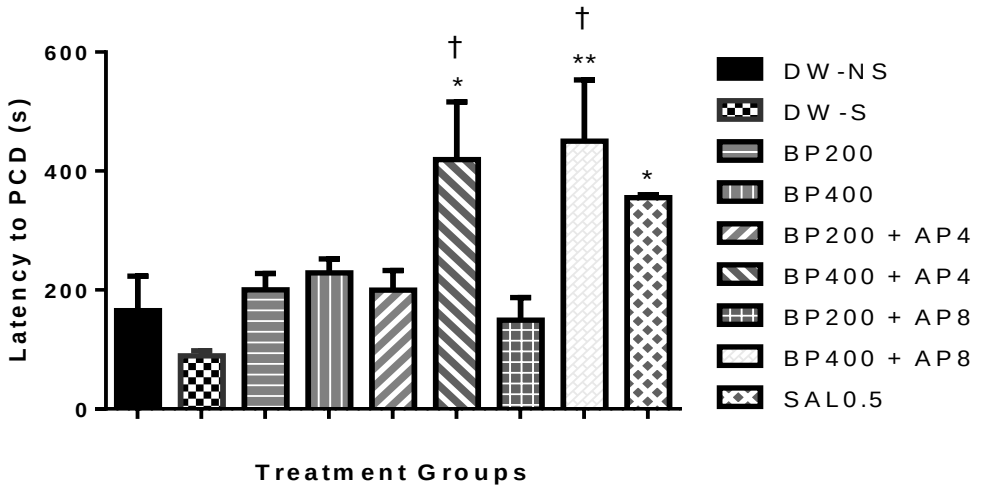


**Figure 1.** Effect of concurrent administration of aqueous extract of *B. pinnatum* (BP) and methanol extract of *A. paniculata* (AP) on latency to preconvulsive dyspnea (PCD) in guinea pigs. *P<0.03, **P<0.004 versus DW-S; †P<0.05 versus other groups except SAL0.5. DW-NS, distilled water, non-sensitized group; DW-S, distilled water, sensitized group; SAL, salbutamol, sensitized group. Values after abbreviations represent doses (mg/kg). n = 5-7 per group.

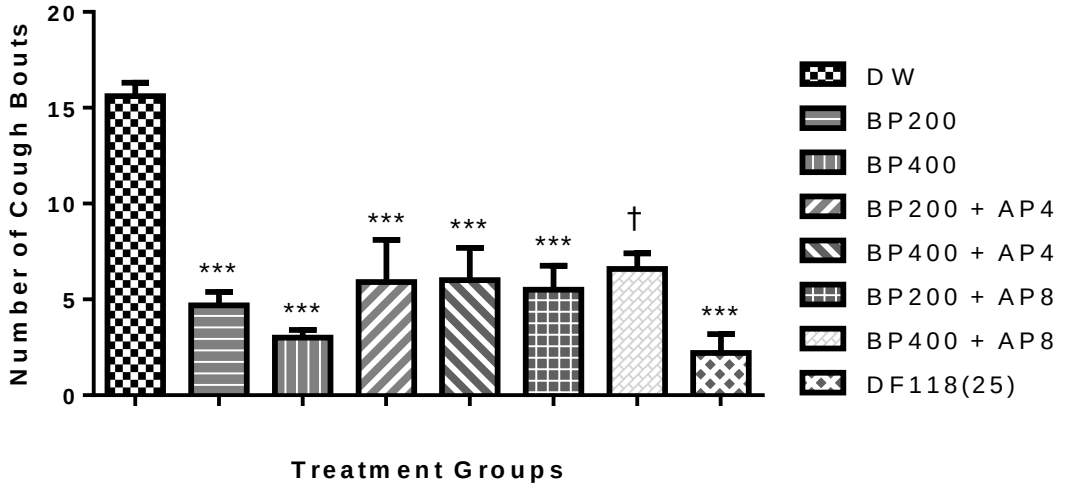


Figure 2. Antitussive effect of concurrent administration of aqueous extract of *B. pinnatum* (BP) and methanol extract of *A. paniculata* (AP) in guinea pigs. ***P < 0.0001, †P < 0.003 versus distilled water (DW). Values after abbreviations represent doses (mg/kg). n = 5-7 per group.

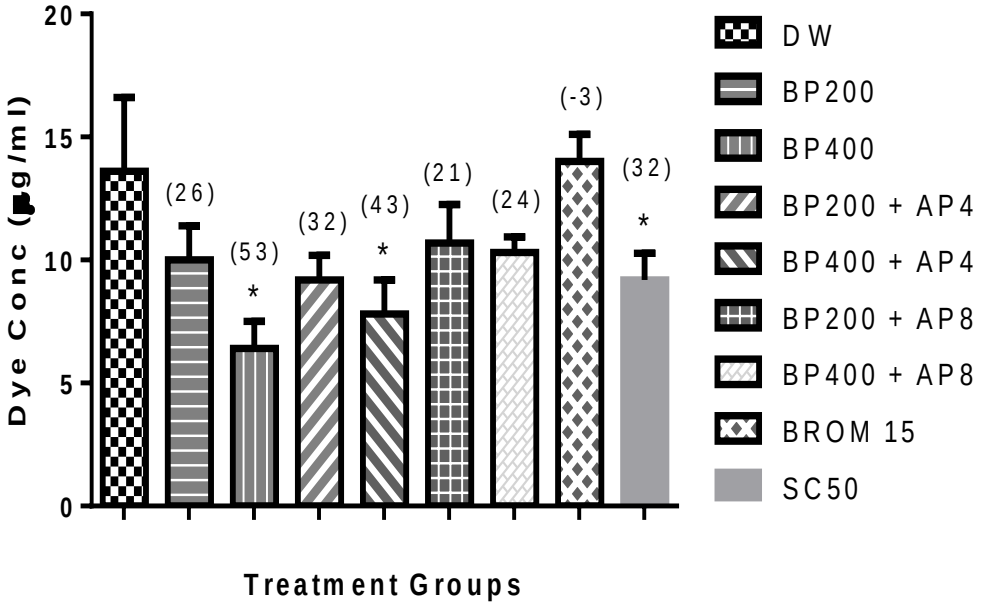


Figure 3. Effect of concurrent administration of aqueous extract of *B. pinnatum* (BP) and methanol extract of *A. paniculata* (AP) on phenol red dye secretion from mice tracheae. *P < 0.05 versus distilled water (DW), **P < 0.05 versus BP400. Values in parentheses are percentage inhibition of dye secretion while values after abbreviations represent doses (mg/kg). n = 5-7 per group.

We have previously reported [18] the antitussive property of BP but its concurrent administration with AP in the present study did not significantly enhance this effect. Although there has been no study on the antitussive potential of AP, its antibacterial and antiviral properties may underlie its use for the treatment of common cold [31-33] for which cough is often a symptom [34]. Cough induction by citric acid is possibly by

activation of C-fibers in the airways or through activation of rapidly adapting receptors by tachykinins released from activated C-fibres [35,36]. Therefore, while BP may possess either or both peripheral and central antitussive mechanisms, AP may not. Since the presence of mucus in the airway is a trigger of the cough reflex [37], reduction of mucus secretion should enhance the antitussive effect of a drug or herbal extract. We observed that the higher dose (400 mg/kg/day) of BP significantly reduced phenol dye secretion, an effect that was reversed with the concurrent administration of 8 mg/kg AP/day. This suggests that while BP inhibits mucus secretion (dye release), AP tends to facilitate it. Reduction in dye release (reduction in mucus secretion) may also explain the lack of significant increase in the tracheal fluid volume of ovalbumin-sensitized guinea pigs. Reduction in mucus secretion has been associated with tannin phytoconstituents of extracts [38,39]. *B. pinnatum* contains tannins [19], which effect is reversed by AP in the present study.

The dose of AP used in the present study is critical. In the absence of previous whole animal anti-asthmatic and antitussive studies involving AP, we chose the doses for the present study by giving multiples of human equivalent dose (2.86 mg/kg/day) indicated for treatment of common cold by the manufacturer of KalmCold®. In rodents, a high dose (5000 mg/kg) of the extract has not been associated with any adverse effects [40]. This suggests that the doses used in the present study are safe and higher than what is used for the treatment of common cold.

Studies have shown that herbal medicines can interact on the basis of pharmacokinetics mechanisms [41,42]. Due to their multicomponent nature, pharmacodynamic mechanisms are difficult to predict [43]. These interactions may either increase or decrease the effectiveness of the individual medicines. It is therefore imperative to investigate the outcome of concurrently administered herbal medicines before attempting to co-formulate them for therapeutic purposes. Our study addresses the concerns in attempting to co-formulate BP and AP for the treatment of asthma and cough.

In conclusion, this study has shown that while the concurrent administration of the methanol extract of *A. paniculata* with the aqueous extract of *B. pinnatum* may enhance anti-asthmatic property of *B. pinnatum* alone, the reverse may be the case for the antitussive effect. This study suggests that label indication must be carefully addressed in the event of co-formulation of both extracts.

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