Evaluation of the Anti-asthmatic and Antitussive Effects of Aqueous Leaf Extract of *Ocimum gratissimum* in Rodents

Ozolua RI**, Umuso DI†, Uwaya DO‡, Modugu AA†, Oghuwuu SO† and Olomu JM†

**1**Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, 300001, Nigeria
†Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City 300001, Nigeria
‡Department of Internal Medicine, University of Benin Teaching Hospital, PMB 1111, Benin City, Nigeria

Abstract

**Background:** *Ocimum gratissimum* Linn (Lamiaceae) is an aromatic plant popular for its culinary uses. The aqueous leaf extract is used in ethnomedicine for the treatment of various diseases including asthma and cough. The purpose of this study was to evaluate the anti-asthmatic, antitussive and muco-suppressant effects of the aqueous leaf extract (OGE) in rodent species.

**Methods:** Ovalbumin-sensitized guinea pigs were exposed to 0.2% histamine aerosol in a glass chamber. Latency to preconvulsive dyspnea (PCD), tracheal fluid volume and viscosity were measured. For the antitussive screening, guinea pigs were exposed to 7.5% citric acid aerosol in a glass chamber and the bouts of cough pre and post-acute exposure were recorded. Mucus expectoration was estimated in mice after seven-day treatment with OGE.

**Results:** Latency to preconvulsive dyspnea was not significantly increased by doses of 100, 200 and 400 mg/kg of OGE when compared with distilled water-treated sensitized guinea pigs. Tracheal fluid volume but not viscosity was significantly (p<0.0001) reduced by all the doses of OGE when compared with distilled water-treated sensitized guinea pigs. Also, the doses of OGE significantly reduced (p<0.0001) the number of cough bouts when compared with distilled water control. All the doses of extract significantly (p<0.003) reduced phenol red dye expectoration from mice tracheae.

**Conclusion:** The aqueous leaf extract of *O. gratissimum* does not protect against acute bronchospasms but possesses antitussive and muco-suppressant effects that may be helpful in asthmatics. Central mechanism may be contributory to the antitussive effect. These results lend credence to its ethnomedicinal use in the treatment of these diseases.

Keywords: *Ocimum gratissimum*; Aqueous extract; Asthma; Cough; Muco-suppression

Introduction

Herbal medicine remains relevant in meeting the healthcare needs of many people. In Nigeria, about 80 percent of the population uses herbal medicine almost exclusively while about 95 percent use it concurrently with Western medicine [1]. The interest in herbal medicine is growing in the Western world as well [2]. Asthma and cough are some of the diseases for which herbal medicines are often sought [3-5].

*Ocimum gratissimum* Linn (Lamiaceae) is a shrub found in Africa, South Asia, and South America [6]. It is variously called clove basil, African basil or wild basil [6]. It is commonly planted around homes because the leaves are very popular as food spice and condiment. Herbalists use the leaf extract for the treatment of gastrointestinal problems such as stomach aches, diarrhea, and dysentery [7,8]. Some of its ethnomedical uses have been reviewed [9]. In Benin City, Nigeria, the extract is used for the treatment of airway diseases such as cough and asthma. The medicinal properties that have been investigated include antibacterial [10-12], antifungal [13], anti-trypanosomal [14] and larvicidal effects [15]. Others include smooth muscle contractile effect [16], anti-diabetic effect [17-19], anti-hepatotoxic effect [20], analgesic effect [21], and anticonvulsant and anxiolytic effects [22].

Phytoconstituents found in *O. gratissimum* leaves include tannins, steroids, terpenoids, flavonoids, cardiac glycosides, anthraquinones and essential oil [23]. The essential oil contains different compounds but eugenol has been identified as the chief constituent [24-27]. Other constituents in the oil include the monoterpenes-1, 8-cineole [26], camphor and methyl eugenol [27,28], and thymol [29].

Our laboratory has in recent times been evaluating the scientific basis for the ethnomedical uses of some plants in the treatment of cough and asthma [30-32]. In the present study, we evaluated the anti-asthmatic and anti-tussive effects of the aqueous leaf extract of *O. gratissimum* in rodent species.

Materials and Methods

Plant materials and extraction

Fresh leaves of *Ocimum gratissimum* which had never been exposed to herbicides were collected from Ekosodin community in Benin City, Nigeria, in August 2014. Adulterants were carefully picked out of the leaves before rinsing them thoroughly in tap water. The leaves (764.28 g) were chopped into small pieces and blended with 4 l of tap water, allowed to stand for 12 h and then filtered twice with a clean white cloth. The final filtrate which was free from particles was dried in an oven.
at 40°C over 24 h (yield=1.2% w/w) and then stored in amber-colored bottle in a refrigerator. The extract (OGE) was reconstituted in distilled water and administered according to the experimental protocols.

**Animals**

Antitussive and anti-asthmatic experiments were performed using 55 adult guinea pigs of either sex weighing 460-600 g. Mucosuppressant effect was evaluated using 30 mice of either sex weighing 20-30 g. The guinea pigs were obtained from the animal facility of the Department of Physiology, Ambrose Alli University, Ekpoma, Nigeria. The mice were obtained from a private animal farm in Ibadan, Oyo State, Nigeria. All animals were allowed two weeks acclimatization in the animal facility of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City. They were all allowed free access to pellets 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 [33] and the experiments were overseen by members of Ethics Committee of the Faculty of Pharmacy, University of Benin, Nigeria.

**Anti-asthma screening protocols**

Guinea pigs were randomly allotted into six groups (n=5 per group) comprising of: (1) non-sensitized control (given 2 ml/kg of distilled water); (2) ovalbumin (OA)-sensitized+2 ml/kg of distilled water-treated; (3) OA-sensitized+100 mg/kg OGE; (4) OA-sensitized+200 mg/kg OGE; (5) OA-sensitized+400 mg/kg OGE; and (6) OA-sensitized+0.5 mg/kg salbutamol.

The animals were sensitized by modifying the Bramley et al. method [34]. They were given intraperitoneal injections of 100 mg OA, a booster dose of 50 mg OA (i.m.) 24 h later and a final dose of 50 mg (i.p) 24 h before exposure to histamine aerosol. The administration of extract and distilled water was done daily per os by use of an orogastric tube (CU.FNC-16-3) for 14 days.

On the 14th day, the extract, distilled water and salbutamol were administered as appropriate. One hour later, the animals were exposed to 0.2% histamine dihydrochloride (dissolved in normal saline) aerosol in glass chamber (60 × 56 × 60 cm) using Omron (Omron Healthcare Ltd, Japan) compressor nebulizer (rate of 0.4 ml/min and particle size 5 µm) for 10 min. 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: (1) control group given 2 ml/kg distilled; (2) 100 mg/kg day OGE; (3) 200 mg/kg day OGE; (4) 400 mg/kg OGE; and (5) 25 mg/kg dihydrocodeine. An hour after administration, they were re-exposed to citric acid aerosol (as earlier described) and the numbers of cough bouts were recorded. Percentage suppression of cough was calculated using the formula:

\[
\text{Percentage suppression of cough} = \left(1 - \frac{\text{Number of Pre-cough Bouts} - \text{Number of Post-cough Bouts}}{\text{Number of Pre-cough Bouts}}\right) \times 100
\]

**Mucus expectoration (phenol red) experiment**

This was based on the method first described by Engler and Szelenyi [37]. Six groups of mice were used treated as follows: (1) 2 ml/kg/day distilled; (2) 100 mg/kg/day OGE; (3) 200 mg/kg/day OGE; (4) 400 mg/kg/day OGE; (5) 15 mg/kg/day bromhexine hydrochloride; and (6) 50 mg/kg of sodium cromoglycate. All treatment was per os except for sodium cromoglycate that was administered intraperitoneally.

On the 8th day, after an overnight fast, treatment was done as usual and the animals in group 6 were given 50 mg/kg (i.p.) sodium cromoglycate 30 min prior to the administration of the secretagogue, ammonium chloride (5 mg/kg p.o.). Thirty minutes later, each mouse was injected with phenol red (500 mg/kg i.p.). All the mice were sacrificed by cervical dislocation 30 min after phenol red injection. The 2 cm of trachea was removed from the thyroid cartilage to the main stem bronchi. Each trachea was kept for 30 min in 2 ml normal saline. Sodium hydroxide (0.1 ml, 1 M) was added to the fluid to stabilize the pH. The absorbance of phenol red released from the trachea was read at 460 nm using a T80+UV/VIS spectrophotometer (PG Instruments Ltd, Beijing, China). A standard curve (graph of absorbance against concentration, \(R^2=0.947\) was plotted from which the concentrations of phenol red were extrapolated.

**Drugs and chemicals**

Chemicals and reagents were of analytical grade. They were 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, University of Benin, Benin City. Dihydrocodeine phosphate was purchased from University of Benin Teaching Hospital, Benin City. Bromhexine hydrochloride was manufactured by Germany Chemicals Plc (Nigeria). Drug and extract solutions were freshly prepared before administration.

**Statistics and data presentation**

Data are presented as mean ± SEM (Standard Error of Mean) and “n” represents number of guinea pigs or mice per group. Data were compared by use of one-way ANOVA with Tukey post hoc. All data were analyzed using GraphPad Prism version 6 software (GraphPad Software Inc. UK). P<0.05 indicates statistically significant difference.
Results

Effect of extract on latency to preconvulsive dyspnea

Figure 1 shows that the administration of 100, 200 and 400 mg/kg did not significantly inhibit the bronchospasm induced by histamine aerosol as indicated by the latency to preconvulsive dyspnea. Salbutamol (0.5 mg/kg) significantly (p<0.001) increased the latency when compared to other groups.

Effect of extract on tracheal fluid volume and viscosity

The tracheal fluid volumes of the various groups are shown in Figure 2. All doses of the extract and salbutamol significantly (p<0.0001) reduced tracheal fluid volume when compared with sensitized but distilled water-treated group. Tracheal fluid volume was reduced to levels comparable with that of non-sensitized group.

The extract and salbutamol did not significantly alter the viscosity of the tracheal fluid of guinea pigs (Figure 3). For example while the flow rate was 44.4 ± 1.3 × 10^{-3} ml/s in the non-sensitized group, it was 40.0 ± 0.5 × 10^{-3} ml/s and 41.4 ± 0.6 × 10^{-3} ml/s in the sensitized but distilled water-treated, 400 mg/kg OGE and salbutamol groups respectively.

Effect of extract on citric acid induced cough

Figure 4 shows that all doses (100, 200 and 400 mg/kg) of the extract significantly (p<0.0001) suppressed the number of cough bouts by 78.6 ± 10.3%, 79.6 ± 2.9% and 80.4 ± 4.6% respectively when compared with control (27.4 ± 9.5%). The standard drug, dihydrocodeine, significantly suppressed cough bouts by 83.9 ± 2.5% when compared to control.

Effect of extract on phenol red dye expectoration

In Figure 5, all the doses of OGE significantly reduced phenol red dye secretion in mice. *p<0.003, **p<0.001, ***p<0.0001, and †p<0.05 compared with control; BH, bromhexine; SC, sodium cromoglycate. n=5 per group.

Citation: Ozolua RI, Umuso DI, Uwaya DO, Modugu AA, Oghuvwu SO, et al. (2016) Evaluation of the Anti-asthmatic and Antitussive Effects of Aqueous Leaf Extract of Ocimum gratissimum in Rodents. Med Aromat Plants 5: 235. doi:10.4172/2167-0412.1000235
dye secretion in mice compared with control. The highest reduction was obtained with 200 mg/kg (p<0.0001) of the extract. Bromhexine and sodium cromoglycate also significantly (p<0.001, p<0.05) reduced dye secretion in mice but sodium cromoglycate was less effective than bromhexine.

Discussion

Results from this study show that the aqueous leaf extract of *O. gratissimum* did not significantly increase the latency to preconvulsive dyspnea in guinea pigs exposed to histamine aerosol. Increase in the latency to preconvulsive dyspnea is an indication of the inhibitory action of substances on bronchospasm induced by spasmogens such as histamine and acetylcholine [30,34]. In the absence of protection against bronchoconstriction, the guinea pigs suffer dyspnea which may result in death. Previous studies have demonstrated that *O. gratissimum* has immunomodulatory effect, hence, preventing inflammatory response to allergens in an asthmatic lung [38]. This means that its usefulness in asthma is not likely due to bronchodilatation. Eugenol and thymol constituents of the essential oil of the plant have been reported to possess anti-inflammatory properties [39-41]. This glucocorticoid-like effect may be useful for prophylaxis and long term treatment of asthma [42].

Symptoms of asthma often include bronchoconstriction and mucus plugging of the airways [43,44]. Sensitization with ovalbumin is a model of obstructive airway disease such that the airways become hyper-responsive and inflamed [45]. Therefore, the therapy of asthma also aims at reducing tracheal fluid viscosity and volume. Increased tracheal fluid volume was seen in the sensitized guinea pigs that were not given the extract but this parameter was significantly reduced in the extract treated groups. The extract contains tannins [23]. These secondary plant metabolites are known to have astringent/antisecretory effects [46,47] which may have been responsible for the decrease in tracheal fluid volume as well as the reduction in the secretion of phenol red dye in mice. Such reduction in dye secretion indicates lack of expectorant property by the extract. Plants containing tannins have been shown to possess antitusive effects [48,49]. The presence of secretions in the airway is often a trigger of the cough reflex [50]. Thus, although the extract may lack a bronchodilator property, the antisecretory effect may be helpful in cough and asthma.

The extract significantly reduced the number of cough bouts in guinea pigs. Although reduction of tracheal fluid volume may be contributory, it is possible that the extract possesses central nervous system effect similar to that of dihydrocodeine (DF118). Cough induction by citric acid is possibly by activating C-fibers in the airways or through activation of rapidly adapting receptors by tachykinnins released from activated C-fibres [51,52]. The mechanisms by which opioids such as codeine and dextromethorphan act as antitussive are not well understood but they act by stimulating mu and kappa receptors in the cough centre [53]. In addition, nasal administration of thymol (constituent of the essential oil of *O. gratissimum*) has been associated with reduction in the urge to cough by olfactory mechanism [54].

Conclusion

The results from this study have shown that the aqueous leaf extract of *O. gratissimum* suppresses coughing by reducing tracheal fluid secretion in addition to a possible central effect of inhibiting the cough centre. Although acute doses of the extract did not protect guinea pigs from histamine induced bronchospasm, the antisecertory effect may be helpful in chronic asthma. The results lend credence to the ethnomedicinal use of the extract in the treatment of cough and asthma.

Competing Interests

The authors have no competing interests in the conduct of the study.

Authors’ Contributions

The study was conceptualized by JMO. It was designed and supervised by RIO who also drafted the manuscript. DOU, AAM and SOO were all actively involved in the experiments. All authors read and approved the manuscript before its submission.

Acknowledgements

The authors wish to thank the laboratory staff of the Departments of Pharmacology and Toxicology, University of Benin, Benin City, Nigeria. The study was the outcome of final year project dissertation of DITU for the Pharm D degree of the University of Benin, Benin City, Nigeria.

References

1. Adeosujo T (2014) Traditional and Orthodox medical systems in Nigeria. The imperative of a synthesis. Am J Health Res 2: 118-124.
2. Lu W, Lu DP (2014) Impact of chinese herbal medicine on american society and health care system: perspective and concern. Evid Based Complement Alternat Med 2014: 251891.
3. Li J, Zhang F, Li J (2015) The Immunoregulatory Effects of Traditional Chinese Medicine on Treatment of Asthma or Asthmatic Inflammation. Am J Chin Med 43: 1059-1081.
4. Zhang T, Zhou X (2014) Clinical application of expectorant therapy in chronic inflammatory airway diseases (Review). Exp Ther Med 7: 763-767.
5. Miyata T (2007) Pharmacological basis of traditional medicines and health supplements as curatives. J Pharmacoal Sci 103: 127-131.
6. Orwa C, Mutua A, Kindt R, Jamnadsa R, Anthony S (2009) Agroforestree Database: A tree reference and selection guide. Version 4.0.
7. Ezekwesili CN, Obiora KA, Uguw OP (2004) Evaluation of anti-diarrhoeal property of crude aqueous extract of Ocimum gratissimum L. (Labiatae) in rats. Biokemistri 16: 122-131
8. Iwu MM (1993) Handbook of African medicinal plants. Florida: CRC Press Inc., Boca Raton.
9. Prabhu KS, Lobo R, Shirwaiker AA, Shirwaiker A (2009) Ocimum gratissimum: A Review of its chemical, pharmacological and ethnomedicinal properties. The Open Complement Med J 1: 1-15.
10. Ahonkhai I, Ba A, Edogun O, Mu U (2009) Antimicrobial activity of the volatile oils of Ocimum baccicum L. and Ocimum gratissimum L. (Lamiaceae) against some aerobic dental isolates. Pak J Pharm Sci 22: 405-409.
11. Mbata IT, Saikko A (2009) Antibacterial activity of essential oil from Ocimum gratissimum on Listeria monocytogenes. Internet J Food Safety 5: 15-19.
12. Ilori M, Shetoeul AO, Omonigbehin EA, Adeneye AA (1996) Antidiarrhoeal activities of Ocimum gratissimum (Lamiaceae). J Diarrhoeal Dis Res 14: 283-285.
13. Lemos Jde A, Passos XS, Fernandes Ode F, Paula JR, Ferri PH, et al. (2005) Antifungal activity from Ocimum gratissimum L. towards Cryptococcus neoformans. Mem Inst Oswaldo Cruz 100: 55-58.
14. Kpovessi BG, Kpovessi SD, Ladekan EY, Gbaguidi F, Frédéric M (2014) In vitro antitypanosomal and antiplasmodial activities of crude extracts and essential oils of Ocimum gratissimum Linn from Benin and influence of vegetative stage. J Ethnopharmacol 155: 1417-1423.
15. Kamara C, Abdul Rahuman A (2010) Larvicidal and adulticidal potential of medicinal plant extracts from south India against vectors. Asian Pac J Trop Biomed 285.
16. Onabolu TB, Ojuade OA, Osho O, Ayeni MB, Akinlabi OK, et al. (2008) In vitro antidiabetic, antifungal and antibacterial activities of Ocimum gratissimum. Afr J Biotechnol 7: 2087-2090.
18. Egesie UG, Adelaiye AB, Ibu JD, Egesie OJ (2006) Safety and hypoglycaemic properties of aqueous leaf extract of Ocimum gratissimum in streptozotocin induced diabetic rats. Niger J Physiol Sci 21: 31-35.

19. Aguiyi JC, Obi CI, Gang SS, Igweh AC (2000) Hypoglycaemic activity of Ocimum gratissimum in rats. Filatropia 71: 444-446.

20. Chiu CC, Huang CY, Chen TY, Kao SH, Liu JY, et al. (2012) Beneficial Effects of Ocimum gratissimum Aqueous Extract on Rats with CCI(4)-Induced Acute Liver Injury. Evid Based Complement Alternat Med 2012: 736752.

21. Paula-Freire L, Andersen ML, Molea GR, Köhn DO, Carlini EL (2013) Evaluation of the antiinfective activity of Ocimum gratissimum L. (Lamiaceae) essential oil and its isolated active principles in mice. Phytother Res 27: 1220-1224.

22. Okoli CO, Ezike AC, Agwagah OC, Akah PA (2010) Antiinflammtant and antiinflammatory properties of Ocimum basilicum L. leaves. Pharmacochemistry 2: 36-40.

23. Mann A (2012) Phytochemical constituents and antimicrobial and grain protectant activities of clove basil (Ocimum gratissimum L.) grown in Nigeria. Int J Plant Res 2: 51-58.

24. Nakamura CV, Ueda-Nakamura T, Bando E, Melo AO, Cortez DA, et al. (1999) Antibacterial activity of Ocimum gratissimum L. essential oil. Mem Inst Oswaldo Cruz 94: 675-678.

25. Inatokan BA, Gberle GO, Adeowo TA, Akinsinde KA (2001) Shigellotoxic properties of three Nigerian medicinal plants: Ocimum gratissimum, Terminalia avicennoides and Monordia balsamia. J Health Popul Nutr 19: 331-335.

26. Veira RF, Grayer RJ, Paton A, Simon JE (2001) Genetic diversity of Ocimum gratissimum L. based on volatile oil constituents, flavonoids and RAPD markers. Biochem Syst Ecol 29: 287-304.

27. Matsayoh LG, Matsayoh JC, Wachira FN, Kinyua MG, Muigai AW, et al. (2008) Antimicrobial activity of essential oils of Ocimum gratissimum L. From different populations of Kenya. Afr J Tradit Complement Alter Med 5: 187-193.

28. Pino Benitez N, Melendez León EM, Stashenko EE (2009) Eugenol and methyl eugenol chemotypes of essential oil of species Ocimum gratissimum L. and Ocimum campechianum Mill. from Colombia. J Chromatogr Sci 47: 800-803.

29. Keita SM, Vincent C, Jean-Pierre S, Belanger A (2000) Essential oil composition of the protective and ameliorative properties of Garcinia kola on histamine-induced bronchial obstruction in guinea pigs. Pharmacognosy Res 4: 203-207.

30. Salami EO, Ozolu RI, Okpo SO, Eze GI, Uwaya DO (2013) Studies on the antiasthmatic and antitussive properties of aqueous leaf extract of Bryophyllum pinnatum in rodent species. Asian Pac J Trop Med 6: 320-328.

31. Braga PC, Da Sasso M, Cucchi L, Bianchi T, Bordoni L, et al. (2006) Anti-inflammatory activity of thymol: inhibitory effect on the release of human neutrophil elastase. Pharmacochemistry 77: 130-136.

32. D’Urzo A, Donohue JF, Kardos P, Miravitlles M, Price D (2015) A re-evaluation of the role of inhaled corticosteroids in the management of patients with chronic obstructive pulmonary disease. Expert Opin Pharmacother 16: 1845-1860.

33. Rogers DF (2007) Mucoactive agents for airway mucous hypersecretory diseases. Respir Care 52: 1176-1193.

34. Fanta CH (1985) Clinical aspects of mucus and mucous plugging in asthma. J Asthma 22: 295-301.

35. Shin MS, Takeda K, Gelfand EW (2009) Understanding asthma using animal models. Allergy Asthma Immunol Res 1: 10-18.

36. de Jesus NZ, de Souza Falcão H, Gomes IF, de Almeida Leite TJ, de Morais Lima GR, et al. (2012) Tannins, peptic ulcers and related mechanisms. Int J Mol Sci 13: 3203-3228.

37. Engler H, Szelenyi I (1984) Tracheal phenol red secretion, a new method for screening mucosecretolytic compounds. J Pharmacol Methods 11: 151-157.

38. Costa RS, Carneiro TC, Queiroza-Lima AT, Queiroz NV, Alcântara-Neves NM, et al. (2012) Ocimum gratissimum Linn. and rosmarinic acid, attenuate eosinophilic airway inflammation in an experimental model of respiratory allergy to Blomia tropicalis. Int Immunopharmacol 13: 126-134.

39. Riella KR, Marinho RR, Santos JS, Pereira-Filho RN, Cardoso JC, et al. (2012) Anti-inflammatory and cicatrizing activities of thymol, a monoterpenic of the essential oil from Lippia gracilis, in rodents. J Ethnopharmacol 143: 656-663.

40. Magalhães CB, Riva DR, DePaula LJ, Brando-Lima A, Koatz VL, et al. (2010) In vivo anti-inflammatory action of eugennol on lipopolysaccharide-induced lung injury. J Appl Physiol (1985) 108: 842-851.

41. Vitalini S, Beretta G, Iriti M, Basilio N, et al. (2011) Phenolic compounds from Achillea millefolium L. and their bioactivity. Acta Biochim Pol 58: 203-209.

42. Fontana GA (2008) Before we get started: what is a cough? Lung 186 Suppl 1: S3-6.

43. Akram M (2013) Minireview on Achillea millefolium Linn. J Membr Biol 246: 661-663.

44. Akram M (2013) Minireview on Achillea millefolium Linn. J Membr Biol 246: 661-663.

45. Vitalini S, Beretta G, Iriti M, Orsenigo S, Basilico N, et al. (2011) Phenolic compounds from Achillea millefolium L. and their bioactivity. Acta Biochim Pol 58: 203-209.

46. Fontana GA (2008) Before we get started: what is a cough? Lung 186 Suppl 1: S3-6.

47. Iwalokun BA, Gbenle GO, Adewole TA, Akinsinde KA (2001) Shigellocidal and protease-selective activities of essential oils of Ocimum gratissimum L., O. basilicum L. and O. suave L. grown in Nigeria. Afr J Tradit Complement Alter Med 5: 187-193.

48. Inatokan BA, Gberle GO, Adeowo TA, Akinsinde KA (2001) Shigellotoxic properties of three Nigerian medicinal plants: Ocimum gratissimum, Terminalia avicennoides and Monordia balsamia. J Health Popul Nutr 19: 331-335.

49. Keita SM, Vincent C, Jean-Pierre S, Belanger A (2000) Essential oil composition of the protective and ameliorative properties of Garcinia kola on histamine-induced bronchial obstruction in guinea pigs. Pharmacognosy Res 4: 203-207.

50. Salami EO, Ozolu RI, Okpo SO, Eze GI, Uwaya DO (2013) Studies on the antiasthmatic and antitussive properties of aqueous leaf extract of Bryophyllum pinnatum in rodent species. Asian Pac J Trop Med 6: 421-425.

51. Matsayoh LG, Matsayoh JC, Wachira FN, Kinyua MG, Muigai AW, et al. (2008) Antimicrobial activity of essential oils of Ocimum gratissimum L. From different populations of Kenya. Afr J Tradit Complement Alter Med 5: 187-193.

52. Pino Benitez N, Melendez León EM, Stashenko EE (2009) Eugenol and methyl eugenol chemotypes of essential oil of species Ocimum gratissimum L. and Ocimum campechianum Mill. from Colombia. J Chromatogr Sci 47: 800-803.

53. Keita SM, Vincent C, Jean-Pierre S, Belanger A (2000) Essential oil composition of the protective and ameliorative properties of Garcinia kola on histamine-induced bronchial obstruction in guinea pigs. Pharmacognosy Res 4: 203-207.

54. Salami EO, Ozolu RI, Okpo SO, Eze GI, Uwaya DO (2013) Evaluation of the protective and ameliorative properties of Garcinia kola on histamine-induced bronchial obstruction in guinea pigs. Pharmacognosy Res 4: 203-207.

55. Matsayoh LG, Matsayoh JC, Wachira FN, Kinyua MG, Muigai AW, et al. (2008) Antimicrobial activity of essential oils of Ocimum gratissimum L. From different populations of Kenya. Afr J Tradit Complement Alter Med 5: 187-193.

56. Pino Benitez N, Melendez León EM, Stashenko EE (2009) Eugenol and methyl eugenol chemotypes of essential oil of species Ocimum gratissimum L. and Ocimum campechianum Mill. from Colombia. J Chromatogr Sci 47: 800-803.

57. Keita SM, Vincent C, Jean-Pierre S, Belanger A (2000) Essential oil composition of the protective and ameliorative properties of Garcinia kola on histamine-induced bronchial obstruction in guinea pigs. Pharmacognosy Res 4: 203-207.