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Original article

Antitussive activity of *Triclisia dictyophylla* of the family Menispermecae

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Abstract

Objective: To verify through a scientific mean the therapeutic use of the plant as an anti-tussive agent, by traditional medicine healers and also aid in the search for new drugs developments from plants. **Methods**: The roots of Triclisia dictyophylla were investigated for their antitussive properties. The plant was uprooted in the month of June 2003 at Igbodo, Delta State, Nigeria and was taken to the University of Nigeria Nsukka for taxonomy. The roots were chopped, ground and immersed in pure drinking water for 24 hours. After filtration, extraction was carried out using a Rotary evaporator. preliminary phytochemistry and acute toxicity studies were carried out. Antitussive study was carried out using a total of 42 young rats of average weight of 72.2 g. The rats were housed in standard animal house of the University and were allowed access to feeds and water but, were fasted for 12 hours prior to commencement of experiment. Specific and appropriate dosage of the crude extract and Codeine re – dissolved in water were administered orally 30 minutes prior to induction of cough. Cough was induced by exposing the animals to Sulphure dioxide gas for 3- minutes. Coughing was taken as number of Head-nods per minute, Stethoscope aided audible sounds and, or tears secretion. Percentage cough inhibition for crude extract and, or Codeine treated rats were compared with reference to control animals. Results were subjected to statistical analysis using SPSS 13.0. **Results**: A 10.2% extraction yield was got from a starting root initial weight of 320 g. The preliminary phytochemistry of the aqueous root extract revealed the presence of alkaloids, saponins, flavonoids, proteins, reducing sugars, steroids, resins fats/oils and glycosides. The Median lethal dose (LD_{50}) based on Lorke's 1983 method was 548 mg/kg The aqueous root extract at concentrations of 10 mg/kg, 50 mg/kg, 100 mg/kg and 200 mg/kg orally administered, inhibited cough in rats induced by sulphure dioxide gas by 16.67%, 33.33%, 50.00% and 83.33% respectively. While Codeine phosphate, a standard antitussive agent, at oral concentrations of 10 mg/kg and 20 mg/kg inhibited cough in rats induced by sulphure dioxide gas by 33.33% and 60.67% respectively. **Conclusion**: Earlier works by some authors had led to isolation of Morhinian Alkaloids from *Triclisia dictyophylla* thus most probably linking its mechanism of antitussive activity to that likable of Morphine. This study justifies the use of the plant in treatment of cough by Traditional Medicine Healers.

Keywords: Antitussive; Cough; Rats/Mice; *Triclisia dictyophylla*; Menispermaceae; Moonseed; Sulphure dioxide gas; Codeine

INTRODUCTION

Africa is endowed with abundant plant resources, many of which are of medicinal value and their uses in the treatment of various ailments are widely prac-

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ticed despite over a century of introduction of western medicine[1].

Cough can be defined as a reflex or a deliberate (voluntary) act of violently and noisily discharge of air through the throat. Coughing (act of producing cough) is mediated by the vital centres of the medulla oblongata. Coughing occurs when the special stretch receptors in the mucosa of the large respiratory passages (Tracheobronchial passages) are irritated[2, 3].

It has been found that the root extract of Asparagus racemosus is equally as effective antitussive agent as codeine phosphate[4].

Datura alba, a member of Solenaccae is a strong agent against whooping cough[5].

Most cough medicines do not have clear cut mechanism of action and it is worse with medicinal plants[6], Fritillaria pallidiflora and Fritillaria delavayi appear to fall into this case. Perhaps as expectorant, but they do have anti cough property[7].

Chen and colleagues found Jin´an Kechuan granules to have both antitussive and expectorant effects[8]. In the same way, a polypharmaceutical preparation, Joshina idia has both antitussive and expectorant activities[9].

Passiflora incarnata leaves as well as the Apamarga plant are greatly antitussive[10].

In another group work by Srikanth, et al; 2002, it was found that the entire plant, Trichodesma indicum is antitussive[11].

Vinegar-processed genkwanin has both antitussive and expectorant action[12]. While wild sichuan Fritillaria (*F. unibracteata*) is a more powerful antitussive than its cultured Fritillary wabueasis co-specie[13]. In the same manner the antitussive effect of the rhizome of Bergenia ciliata stem has been evaluated for its anti cough potentials[14].

Lippia alba is equally a good antitussive agent[15]. Asada-ame extract containing Plantycodi radix, Ephedrae herba and ipecacuanhae has both antitussive and anti-expectorant effect which stem perhaps mainly from the radix plant[16]. In a recent work by Boominathan and colleagues, the leaves of Ionidium suffraticosam was found to be antitussive[17]. And in another very recent study by Guan and Zhao, it was found that Chenodeoxycholine acid extracted from plants do possess both antitussive and expectorant effect; Perhaps a way into the elucidation of the mechanism of actions of some herbal plants[18].

MATERIALS AND METHODS

Thirteen mice out of which 9 were used for an initial stage of LD_{50} study while the balance 4 was used at the secondary stage. They were bought from the department of pharmacology and therapeutics, Nnamdi Azikiwe University Nnewi while a total of 42 adult White, Wister Albino rats were bought from the animal house of the University of Nigeria Nsukka. There was no selection or discrimination based on sex but, animals of very close weight and age range were used. Codeine Phosphate (8 mg tabs) of TLc, former Roots Pharmaceuticals, Isolo Industrial Estate Lagos. Nigeria, were bought from pharmacy shops while, Copper metal powder (100 g) bottle by Griffin & George, Bishop Meadow, Southborough, Laics. England and Concentrated Sulphuric acid (2.5 litres) were bought from a chemical shop at Onitsha, Anambra State, Nigeria.

Collection, taxonomy and description of plant

The plant was uprooted during the month of June 2003 from Idumu Ozei Village of Igbodo in Ika N/East L. G. A of Delta state Nigeria. The root portion was immersed in a water soaked – sand bottle to retain freshness. The plant was then taken to Mr. Ozioko of the department of Botany of the University of Nigeria, Nsukka, Nigeria who identified the plant as *Triclisia dictyophylla*, a member of the family Menispermaceae (moonseed family)[19].

Triclisia dictyophylla is a member of the family Menispermaceae (Moonseed). Its local names are derived from its uses thus "Akwukwo oji" as its leaves are used in the preservation of fresh kola-nuts and "okwuite" as the leaves were used as lid to provide for tight covering during the early days of cooking with clay pots. *Triclisia dictyophylla* is a big lianne and can climb up to 60 feet usually on old woods. Earlier works by Kronlaud, et al; 1970, isolated Morphinan alkaloids from the plant[20]. The plant is indigenous in Africa and has been used in the treatment of various ailments like leg oedema, anaemia and spasm[21].

Preparation of aqueous root extract

Since the root of the plant is eaten or chewed with the liquid extract being swallowed for the treatment of cough, the aqueous extract was prepared by air-drying the uprooted root parts. The roots were then chopped into small short pieces and pounded in a woody mortar. The fine powder from the root back, the hoarse root fibres and the other particles of fibres were weighed and soaked overnight in 3 litres of pure drinking water. The mixture was sieved, removing the root wood and fibres. Then it was filtered using Whatman No I filter paper[22]. The brownish filtrate was then poured into the conical flask of the rotary evaporator. The PH was taken while the boiling point was taken at boiling. The filtrate was evaporated to dry semisolid black-brawn viscous residue before being poured into an evaporating dish and kept in the incubator at 60 ℃ for the next three days. The solid extract was then scraped out of the evaporating dish weighed and refrigerated until required.

Preliminary phytochemistry

Methods described by Trease and Evans[19] were employed for this work. Using the required quantity of the extract re-dissolved in water vehicle, preliminary phytochemistry was carried to test for the presence of reducing sugar, alkaloids, glycosides, saponins, tannins, proteins, fats/oils flavonoids, resins, steroids, carbohydrates, starch and terpenoids.

Animal experiment median lethal dose LD_{50} study

The median lethal dose was based on Lorke's 1983 method[19], and as used by Akah, et al; 2003. White Wister albino mice were used as animals. The animals were of average weight of 24.5 g they were housed in 3 groups in a standard Laboratory environment, fed on standard rat/mice pellets (Pfizer Nigeria) and allowed access to drinking water through out the experiment. A preliminary study using three different doses of the extract in water-vehicle were administered intraperitoneally to 9 animals. The doses of extract per group of animals at the first stage were 10 mg/kg, 100 mg/kg and 1 000 mg /kg. The second stage involved administration of 500 mg/kg, 600 mg/kg, 700 mg/kg and 800 mg/kg each and separately to one further animal per group.

The animals were observed for a maximum of 24 hours separately for both stages. The animals were observed for withdrawal from feed or water, withdrawal from active mice/rats movements, respiratory distress and, or death.

Anti tussive study using SO_2 gas

Cough ensures when the stretch receptor of the respiratory air passage are irritated. A noxious gas (SO_2) if well inhaled by rats /mice irritates the stretch receptors and induces cough. The degree of inhibition of coughing by a drug or extract is taken as the antitussive potency of the drug or the extract[4].

The method employed a comparative study using codeine treated and non treated rats with the extract treated rats. While codeine treated was taken as positive, non drug/crude extract treated was taken as negative control. Appropriate amount of drug/crude extract were dissolved in water vehicle. A total of 42 young white Wister albino rats of average weight of 72.72 g were used. The animals were grouped into seven (A-G) of six animals per group. They were placed in cages, provided with feed (Rats pellets-Pfizer Nigeria plc) and pure drinking water. They were housed to acclimatize in the University Pharmacology Laboratory for four days prior to the experiment. The rats were deprived access to feed and water for 12 hours prior to the commencement of the experiment and were then weighed. Group A was used as negative control thus was only given the vehicle, water. (10 mL/kg orally). Group B, C, D, E were separately given 10 mg/kg, 50 mg/kg, Triclisia100 mg/kg and 200 mg/kg of the extract orally. Group F was given 10mg/kg of Codeine phosphate orally. Group G was given 20 mg/kg of Codeine phosphate orally. The animals were then exposed to sulphure dioxide in a semi closed chamber for 4 minutes. The gotten LD_{50} of 548 mg/kg, informed our decision on dosage ranges of 10-200 mg. Precaution was taken during the exposure as to avoid suffocating the animals. Since sulphure dioxide gas is very soluble in water, exposure was done with dispatch. Coughing in rats was taken as visible tears secretion from the eyes, gnawing, audible cough sound (with the aid of Stethoscope) and repeated forceful nodding of the head with forceful up and downward movement of the intercostals muscle. Tears secretions were best ob-

served with the aid of a hand microscopic lens. Cough frequency was practically taken as the count of forceful nods of head per minute. Animals that did not nod were taken as not coughing. While those that nodded were taken as coughing. Percent inhibition of coughing and percent non inhibition of coughing were got by multiplying by 100%.

RESULTS

Result of aqueous extraction of *Triclisia Dictyophylla*

The vehicle used for extraction is water. A volume of 3 – litres was started with at the immersion of the pounded or ground root back/fibres, but 2.80 litres of filtrate was eventually obtained post filtration. The PH of the filtrate was gotten at 5.00 suggestive of acidic (weak) filtration extract. This may account for its boiling (94.5℃) and evaporation (98.0 ℃); points being below that of pure water. The weight of semisolid residue is 42.8 g. The final extract yield was got as 32.52 g, that is 10.20% (32.52/320) of the starting weight, 320 g.

Table 1 Preliminary phytochemistry of the aqueous root extract of *Triclisia dictyophylla*

Substance	Inference
Alkaloids	+ + +
Glycosides	+ +
Saponins	+ + +
Reducing sugar	+
Resins	+
Fats/Oils	+ +
Starch	Neg
Tannins	+
Flavonoids	+
Terpenoids	+ +
Proteins	+ +
Carbohydrate	Neg
Steroids	+ +

+ - Present
+ + - Moderately present
+ + + - Abundantly present
Neg - Absent

Result discussion on preliminary phytochemistry and LD_{50} of *Triclisia dictyophylla*

As indicated from the key and on Table 1, the plus sign indicates presence of the test substance and scoring is done with increase or decreases on the number of plus sign to indicate the degree of the availability of the test substance.

Neg., indicates, not present or absence of the test substance and was not used for scoring.

The richly presence of alkaloids is in line with Krolaund[20] that isolated two morphinian alkaloids from the plant. The presence of proteins may also be in line with the account of Spiff[21] in the use of this plant in the treatment of anaemia and leg oedema.

The acute toxicity study was based on Lorke´s method of 1983 and as used by Akah[23]. The animals after receiving the dose extract showed withdrawal from feeds and water. This withdrawal was however very short lived for the 10 mg/kg dose (Group 1).

The 100 mg/kg dose (Group 2) exhibited withdrawal for several hours but returned gradually back to normal active movement, drinking and feeding.

The case was different for Group 3 (1 000 mg/kg). One out of the three animals died within the first 20minutes post crude drug administration. The second died after about an hour post crude drug administration. The last of the three died in the night, which is after about 19 hours post crude drug.

At the second stage, the animals of 700 mg/kg and 800 mg/kg died in the night. The animal of 600 mg/kg however also died a few minutes exceeding 24 hours of crude drug administration while the mouse of 500 mg/kg remained healthy and hearty. Sequence of events prior to death followed this order: Withdrawal from feeds and water, to absolute inactivity with a seeming – like sleeping state, to decrease in respiratory rate, to backward twisting of neck, to convulsive – like kind of fits, then finally death.

The sequence of events leading to death appears to be logical as the richly presence of morphine has been demonstrated[20,21] also appears to correlate with the lethal dose of pure Morphine as 400 mg/kg[23].

The LD_{50} of *Triclisia dictyophylla* in mice was then taken as approximately 548 mg/kg being the geometry mean of 500 mg/kg that caused no death and 600 mg/kg dose that caused death (Figure1).

Table 2 Coughing frequency in four minutes per rat.

Dose (mg/kg)	1	2	3	4	5	6	Mean ± SD
Extract 10	60	61	60	59	62	Nil	60.40 ± 1.00
50	59	60	60	60	Nil	Nil	59.75 ± 0.86
100	59	59	40	Nil	Nil	Nil	52.67 ± 11.05
200	40	Nil	Nil	Nil	Nil	Nil	40.00 ± 0.00
Codeine 10	50	52	Nil	Nil	Nil	Nil	51.00 ± 1.00
20	36	40	Nil	Nil	Nil	Nil	38.00 ± 2.00

Table 3 % Coughing inhibition and non inhibition.

Dose	No of rats	Coughing	Not coughing	% Inhibition	% Non inhibition
Extract 10 mg/kg	6	5	1	16.67	83.33
50 mg/kg	6	4	2	33.33	66.67
100 mg/kg	6	3	3	50.00	50.00
200 mg/kg	6	1	5	83.33	16.67
Codeine 10 mg/kg	6	4	2	33.33	66.67
20 mg/kg	6	2	4	66.67	33.33
Water only 10 mL/kg	6	6	0	0.00	100.00

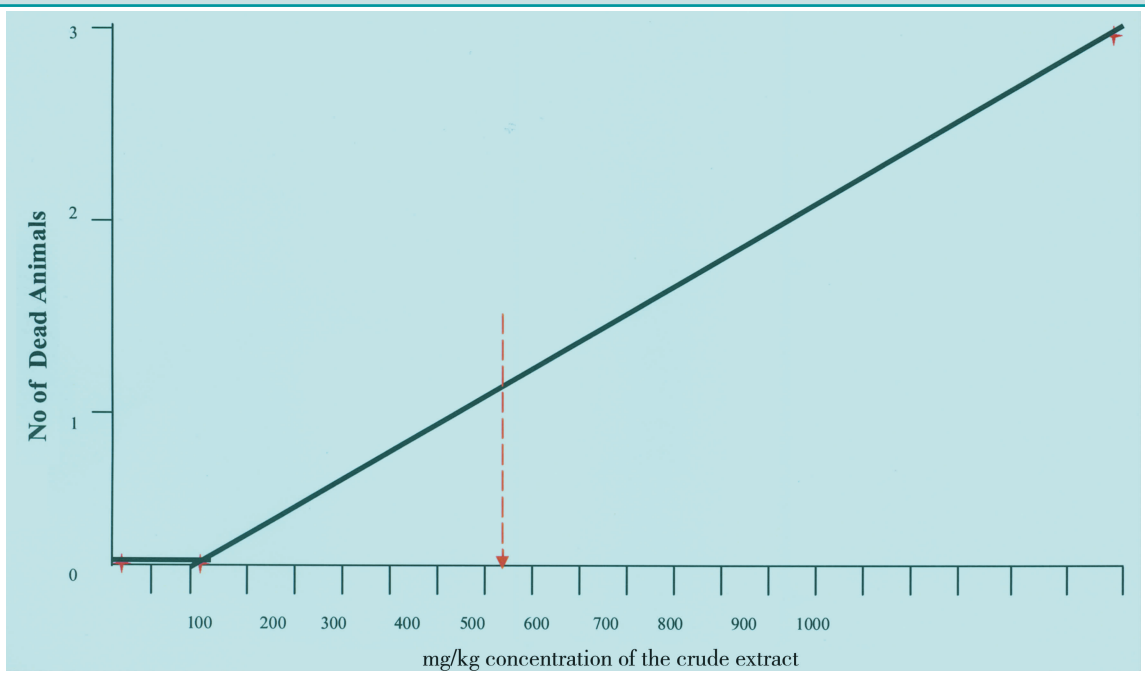

Figure 1 LD_{50} Graph of *Triclisia dictyophylla* in mice.

It was clearly observed from Table 2 and Table 3 that both *Triclisia dictyophylla* and codeine, inhibited cough in dose dependent version with higher dose of extract of *Triclisia dictyophylla* displaying higher ac-

tivity (83.33% inhibition of cough frequency at 200 mg/kg dose) than Codeine. Antitussive activity of the plant cannot be unconnected to the richly presence of Morphinian alkaloids.

DISCUSSION

Mechanism of coughing stems from; when the lining of the respiratory passages are irritated, the glottis closes leading to bronchoconstriction. Bronchoconstriction in turn leads to increase in intra – pulmonary pressure which leads to activation of stretch receptors (cough receptors) in the tracheobronchial passages. Activation of these receptors is rapidly followed by afferent vagal neuron conduction of these impulses to the brain (CNS), a feedback mechanism leads to opening of the closed glottis and whereupon the glottis suddenly opens, an explosive discharge of air (coughing), occurs[2].

Disorders that result in inflammation, Airway constriction, infiltration or compression of the air way passages can be associated with cough. Inflammation commonly results from air- ways infections, ranging from viral, bacterial or fungal to bronchiectasis[24-26]. Microbial infection or infiltration of the airway tract are associated with pneumonia common cold[27], pertussis (Whooping cough)[28-30], bronchitis[31], bronchiolitis obliterans[32-34], HIV/AIDS[35, 36] and Tuberculosis[37-39]. Cough can also occur in some other diseases like Asthma/Allergy[3, 40, 41] Neoplasm and Emphysema[42, 43]. Cough also occurs in patients on agiotensin-converting enzyme inhibitors (ACEI) due to activation of bradykinins[2, 3].

Cough can broadly be classified into productive and non productive (dry) cough and definitive treatment depends essentially on determining the underlying cause and initiating specific therapy. Elimination of an exogenous inciting agent like cigarette smoke, cold or drugs like ACE-inhibitors or endogenous agents like postnasal drips or gastroesophegeal reflux are appreciable first steps where necessary.

Productive coughs are treated with cough expectorant while non-productive coughs are treated with suppressant or antitussive agents. Cough suppressants are therefore drugs or agents that act on this reflex pathway to suppress coughing mechanism and are of great value in dry or non - productive cough. Cough suppressants are however contraindicated in productive cough.

Antitussive agents could be peripherally or centrally acting and could be narcotics or non narcotics. Expectorants on the other hand comprise a group of drugs that facilitate the removal or coughing up sputum in productive cough. Their mechanism of action involves reducing the viscosity of pulmonary secretions and increasing the volume of these pulmonary secretions. Factors that enhance expectoration include increased liquefactions of the pulmonary secretion and reduction in the viscosity of sputum, movement of ciliated epithelia, peristaltic movement of the bronchial muscle and intact cough reflex arc. Expectorants exert their actions by acting directly on mucous glands or indirectly via the cough reflex pathways. Unlike these orthodox drugs with known mechanism of actions, most herbal products do not have known clear cut mechanism of action[6]. They however, exhibit less unwanted effects hence their popular usage around the world [44].

Inflammation could be induced by conditions that bring about the release of inflammatory mediators such as Histamine, Prostaglandins, Nitric oxide, Serotonin, Cytokines, Leukotrienes, Platelet Activating Factor and substance P[45]. Prostaglandins and leukotrienes are released by a host of mechanical, thermal, chemical, bacterial and other insults, and they contribute importantly to the signs and symptoms of inflammation[46]. Mast cell which is very rich in histamine has membrane receptors both for special class of antibody, (IgE) and for Complements Components – C3a and C5a. Mast cell can be activated to secrete inflammatory mediators through these receptors and also by direct physical damage[3].

Airway obstruction/bronchoconstriction or airway hyper responsiveness result in coughing and is believed to be a direct consequence of airway inflammation which can possibly follow the above described mechanism.

The crude extract of Triclisia dictyophlla exhibited varying degree of anti-tussive activity in dose dependent version and was comparable to that of Code-

ine, a standard antitussive agent. Mechanism that possibly underline this anti-tussive activity include inhibition of the actions of inflammatory mediators such as Histamine, Serotonin, Prostaglandins, Nitric oxide, Cytokines, Platelet Activating Factor and substance P, effect on adrenocorticoid hormone and immunosuppression.

Morphine is an established antitussive as well as an antiinflammatory drug and since its like-alkaloids are found in Triclisia dictyophylla, activation of opioid receptors is the exert mechanism of its antitussive activity.

Opioids of μ – receptors are the most efficacious antitussive agents but the adverse effects of sedation, drowsiness, nausea, constipation and physical dependences are their draw back[47]. Thus in recent studies, researchers are fighting to block the cough reflex with drugs that constitute less-nuisance. Novel drugs such as Opioids of K- and δ- receptors agonist have been developed and non-opioids like nociceptin have been developed. (Neuropeptides - Neurokinin receptors antagonist, bradykinin receptor antagonist, vanilloid receptor (VR - 1) antagonist and may be beneficial in blocking the affects of tachykinins and sensory nerve activating agents thus may be good antitussive. Local anaesthetic blockers of sodium dependent channels and maxi-K-Ca + +-dependant channel activators of efferent nerves are inhibitors of cough.

Some of these novel agents may act peripherally or centrally or both sites as antitussives. Large-scale trails of these novel compounds have not been carried out on cough in man but, there is a serious need for more effective antitussive drugs devoid of side effects[47,48]. Medicinal plants show less unwanted effects hence may indeed be our best bet.

Earlier works by some authors had led to isolation of Morphinian Alkaloids from Triclisia dictyophylla thus most probably linking its mechanism of antitussive activity to that likable of Morphine. This study justifies the use of the plant in treatment of Cough by Traditional Medicine Healers.

REFERENCES

1 **Ugwu OF**, Unekwe PC, Ogbonnaya EC. The effects of Aqueous extract of Landophia owariensis on whole blood clotting time *in – vitro*. *Jorunal of Health & Visual Scie* 2002; 4 (3): 179 - 182.

2 **Fauci AS**, Braunward E, Isselbacher KJ, Wilson JD, Martin JB, Kasper DL, et al. *Harrison's principle of internal medicine*. 15th edition. McGraw – Hill: Health Professions Division; 2002. 75, 194 – 197, 736 – 747 1004 – 1022, 870 – 873, 973, 933 – 936, 1417 – 1423.

3 **Rang HP**, Dale MM, Ritter JM. Asthma and inflammatory conditions. In: *Pharmacoloy*. 5th ed. Edinburgh: Churchll livingstane; 2002. 579-603.

4 **Mandal SC**, Kumare KA, Mohana Lakshmi S, Sinha S, Marugesan T, Pakum Saha BP. Antisussive effect Asparagus racemosus against sulfur dioxide induced cough in mice. *Fitoterapia* 2000; 71:686 - 689.

5 **Priya KS**, Gnanamai A, Radhakrishnan N, Babu M. Healing potential of Datura alba on burn wounds in albino rats *Journal of Ethnopharmacology* 2002; 83 (3): 193 - 199..

6 **Ziment I.** Herbal antitussive. *Pulmonary – Pharmacology and Therapeutics* 2002;15 (3): 327-333.

7 **Xu HB**, Sun XB, Wen FC, Zhou JH, Ding T, Sun LW, et al. A preliminary comparative study on physiological activity of Fritillaria pallidifolia schrek and F. delavayi French. *Zhonggou Zhong Yao Za Zhi* 2000;25 (7): 391 - 394.

8 **Chen GL**, Wang Y, Wang QM, Liu C, Cheng HJ. Studies on antitussive, antiasthmatic, expectorant and anti-inflammatory effects of Jin'an Kechuan granules. *Zhonggou Zhong Yao Za Zhi* 2000; 25 (2): 107 - 110.

9 **Kheterpal R**, Khanna TO, Arora RB, Siddiqui HH. Study of a Poly-poyharmaceutical preparation: Joshina as antitussive & expectorant in experimental models. *Indian J Pharm* 1998;19: 187 – 202.

10 **Dhalvan K**, Sharma A. Antitussive activity of the methanol extract of Passiflora incarnata leaves *Ind J Pharmacol* 2002; 29 (4):220-291.

11 **Srikanth K**, Murugesan T, Kwmar Cha, Suba V, Das AK, Sinha S, et al. Effect of Trichodesma indicum extract on cough reflex induced by sulphure dioxide in mice. *Phytomedicine* 2002; 9(1): 75 -77.

12 **Zhao Y**, Yuan S, Li A, Zhang B, Wang Z. Effects of Vinegar – processed genkwanin on toxicity and pharmacological actions in animal models. *Zhongguo Zhong Yao Za Zhi*1998; 23 (6): 344 – 347.

13 **MO Zitang X**, Sun Z, Li WComparison on Sichuan Fritillary bulb. *Zhonguo Zhong Yao Za Zhi* 1998; 23(1): 14-16

14 **Sinha S**, Murugesan T, Pal M, Saha BP, Saha BP. Evaluation of antitussive activity of Bergenia cilliata sternb: Rhizome extract in mice. *Phytomedicine* 2001;8 (4): 298 - 301.

15 **Pascual – Slowing K**, Carretero ME, Villar A. Antiulcerogenic activity of lippia alba (mill) N. E. Brown (verbenaceae). *Farmaco* 2001; 56 (5-7):501-504.

16 **Imamura N**, Misawa M, Kitagawa H, Yanuaras, Istizone H. Antitussive & expectorant effect of Asada-ame extract. *Nippon Yakuigaku Zasslie* 1986;(4): 497-505.

17 **Boominathan R**, Parimala B, Devi, Subhash C, Mondes K. Evaluation of antitussive potential of lonidium suffruticosam Ging (Violaceae) extract. *Phyto Therapy Research* 2003; 17 (7):838-839.

18 **Guan H**, LI P, Zhao H. Antitussive, antiasthmatic and Expectorant actions of Chenodeoxycholine acid. *Zhong Yao Cai* 2004; 27(3): 206 – 208

19 **Akah PA**, Ezike AC, Nwafor SV, Okoli CO, Enwerem NM. Evaluation of the antiasthmatic property of Asystasia angetic leaf extracts. *J Ethnopharmacology* 2003; 89 (2003): 25 – 30.

20 **Kronlund A**, Kristiana K, Sandberg F. The occurrence of Pheceanthine and N. NI-dimethylphaentine in Triclisia dictyophylla and T. patens: A new simple method for estimation of muscle relaxant effect. *Acta Pharm* 1970; 7(3): 299-284.

21 **Spiff AL**, Zabel V, Watson WH, Zemaitis NA, Ateya AM, Slatkin DJ, et al. Constituents of West African medicinal Plants XXX. Tridictyophyllin: A new morphinan alkaloid from Triclisia dictyophylla. *J Nat Products* 1981; 44(2): 160 – 165.

22 **Sadiq Y**, Mohammed A , Omotosho WT. The effects of aqueous root extract of Watherian indica on gastro intestinal smooth muscle activity, Nigerian. *J Health & Biomedical Sciences* 2003; 2 (20): 27 – 30.

23 **Okenwa MO.** *Current book of medical pharmacology & therapeutics*. Benin City, Nigeria: Parkland Medical Publications; 1997. 244 – 251.

24 **Woodhead M.** Community acquired pneumonia in Europe: Causative pathogens and resistance patterns. *Eur Respire Suppl* 2002; 36(2): 20-27.

25 **Montalto NJ.** An office – based approach to influenza: clinical diagnosis and Laboratory testing. *American Family Physician* 2003; 67(1): 111-118.

26 **Lombardi G**, Farina C, Andreani S, Antonio D, Faggie D, Manso. E, et al. A multicentre evalution of an enzyme immunoassay (platelia aspergillus) for the detection of Aspergillus antigen in serum. *Mycopathological* 2003; 155 (3): 129 – 133.

27 **Dautin N**, Karimova G, Ladent D. Bordetella pertussis adenylate cyclase toxin: A versatile screening tool. *Toxicon* 2002 ; 40(10): 1383-1387.

28 **Yamamoto Y**, Osanai S, Fusiuchi S, Yamazaki Nakano H, Ohsaki Y, Kikuchi K. Extrinsic allergic alveolitis induced by the yeast Debaryomyces hanseni. *Eur Respir Journal* 2000; 20(5): 1351-3.

29 **Gent JF**, Ren P, Belanger K, Triche E, bracken MB, Holford TR, et al. Level of house hold mold associated with respiratory symptoms in the First year of life in a community at risk for asthma. *Enviro Health Perspect* 2002; 110 (12): 781-786.

30 **McLeod R**, Bolser DC, Jia Y, Parra LE, Mutter JC, Wang X, et al. antitussive effects of nocicetirorphanin in experiment cough models. *Pulm Pharmeol Then* 2002; 15 (3): 213-216.

31 **Karnak D**, Beder S. Treatment of Chlamydia pneumoniae infections and chronic obstructive pulmonary disease. *Expert-Opinion in Pharmacotherapy* 2002; 3 (10): 1461-1470.

32 **Bisgaard H.** A randomized trial of montelukast in respiratory syncytial virus postbronchiolitis. *Am J Respir Care Med* 2003; 167 (3): 379-383.

33 **Kreiss K**, Gomaa A, Kullman G, Fedan K, Simoes EJ. Enright P. L. Clinical bronchiolitis obliterans in workers at a microwave-popcorn plant. *New Eng J Med* 2002; 347 (5): 330-338.

34 **Cheng B**, Wheeler S. *Qualitative research for nurses*. London: Blackwell Science; 1996.

35]**Aligbe JU**, Akhiwu WO, Nwosu SO. Prospective study of coroner´s autopsies in Benin City. Nigeria. *Med Sci Law* 2002; 94(2): 318-324.

36 **Adesunkanmi AR**, Akinkuobie AA, Badru OS . Five years analysis of death in accident and emergency room of a semi-urban hospital. *West Afri J Medicine* 2002; 21 (2): 99-104.

37 **Jones TF**, Woodley CL. Fountain FF, Schaftner W. Increased incidence of out break of strain of Mycobacterium tuberculosis surrounding communities after an outbreak in a jail. *South Med* 2003; 96 (2): 155 – 7.

38 **Idoko JA**, Akinsete L, Abalaka AD, Keshinro LB. Dutse L, Onyenekwe B, et al . A multicentre study to determine the efficacy and tolerability of a combination of nelfinavir, Zalcitabine and Zidovudine in the treatment of HIV infected Nigerian patients. *West Afr J Med* 2002; 21(2) :83 – 86.

39 **Ekwcani CN**, Chylous ascites, tuberculosis and HIV/AIDS: A case report. *West African J Med* 2002; 21(2): 170 – 172.

40 **Esamai F**, Ayaya S, Nyandiko W. Allergic rhinitis and dermatitis among School Children in Uasin Gishu district, Kenya. *East African Med Journal* 2002; 79 (10): 514 – 518.

41 **Erhabor GE**, Kutey IF, Obembe F. Asthma: the psychosocial impact among a sample of South Western Nigeria. *J Nigerian Med Association* 2002; 94; 987 – 993.

42 **Sequerzati A**, Basteri V, Antinolfi G, Dagostino F, Scutellari PN, Ravenna F, et al. Bronchial carcinoid tumors: Clinical and radiological correlation. *Radiological Medicine* 2002; 104 (4): 273 – 284.

43 **Omasa M**, Kobayashi T, Takahashi Y, Tamada J. Surgically treated pulmonary inflammatory Pseudotumors Jan-Jun. *Cardiovascular Surgery* 2002; 50 (7): 305 – 308.

44 **Bielory L.** Complementary and alternative interventions in asthma, allergy and immunology. *Ann of Allergy, Asthma & Immunol* 2004; 93 (2 suppl) S – 45 – 54.

45 **Payne DO.** Nitric oxide in allergic air way inflammation*Current Opinion in Allergy & Clinical Immunology* 2005; 3(2): 133-137.

46 **Jason D**, Marrow L, Jackson Roberts. Lipid-Derived Autacoids, Eicosanoids and platelet – activating factor. In : Laurence L. Brunton ed. *Goodman & Gilman´s The Pharmacological Basis of Therapeutics* USA: McGRAW – HILL, Medical Publishing Division; 2001. 669-680.

47 **Chung KF**, Chang AB . Therapy for cough active agents. *Pulm Pharmacology and Therapy* 2002; 15 (3): 335-338.

48 **Puject JC**, Keddad K, Sevenier F, Jolivet Lanreau. Comparative study of two antitissive drugs in the treatment of cough of infections origin (Randomized prospective trial). *Therapies* 2002; 57 (5) :457-463.