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Original Research Article

Airway Smooth Muscles Relaxant and Mast Cells Stabilizing Activity of Some Medicinal Plants Used in Managing Asthma in North-Western Nigeria

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ABSTRACT

Cassia occidentalis whole plant (C.O.W.P), *Jatropha curcas* leaves (J.C.L.), *Ximenia americana* Santalales leaves (X.A.L.), and *Eucalyptus citriodora* leaves (E.C.L.) have been revealed to be widely used by locals of north-western Nigeria for the management of asthma. This study was aimed at providing a pharmacological rationale for the ethnomedical use of these plants in the management of asthma.

The four extracts were screened for their effects on the spontaneous contraction of isolated rabbit ileum strip; also on pre-contracted isolated guinea Pig ileum strip; on pre-contracted isolated Guinea Pig trachea chain; and on ovalbumin-induced peritoneal mast cell degranulation in Wistar rats. Ethanol extracts of C.O.W.P., E.C.L., J.C.L., and X.A.L. at 100 mg/mL remarkably relaxed spontaneous contractions of isolated rabbit ileum. Greatest relaxation was obtained with X.A.L. Ethanol extracts of C.O.W.P., E.C.L., J.C.L., and X.A.L. statistically significantly ($p \leq 0.05$) inhibited histamine-induced contractions of isolated guinea pig ileum. In addition, the four extracts statistically significantly inhibited ($p \leq 0.05$) histamine-induced contraction of isolated guinea pig trachea. The ethanol leaves extracts of *C. occidentalis* and *Jatropha curcas* exhibited mast cell stabilizing effect in ovalbumin-induced rat peritoneal mast cell degranulation. However, *Eucalyptus citriodora* and *Ximenia Americana* did not protect against ovalbumin-induced rat peritoneal mast cell degranulation.

C.O.W.P., E.C.L., J.C.L., and X.A.L all possess broncho-relaxant activity, while only C.O.W.P. and J.C.L. exhibited mast cell stabilizing activity in laboratory animals thus supporting the traditional use of the plant in inflammatory and allergic conditions including asthma.

Keywords: Medicinal plant, North-western Nigeria, Airway smooth muscles, Broncho-relaxant, Mast cell stabilizer.

Introduction

Asthma is a heterogeneous disease usually associated with airway hyperresponsiveness and airway inflammation and defined by the history of respiratory symptoms (e.g. wheeze, shortness of breath, chest tightness, and cough) that vary over time and in intensity, together with variable expiratory airflow limitation.[1] Nigeria, with a population of about 200 million, the number of persons with clinical asthma is approximately 13 million, is likely to rank among the highest in Africa.[2] The prevalence of clinical asthma increases with age from about 3% in children to about 10% in adults[2]. Asthma has been an area of considerable unmet medical need.[3] Adverse effects from the existing medications and a plateau in dose response[4] lead to poor compliance and increase the severity of the disease. Adverse effects also brought about by orthodox medicines make the search for "non-drug" strategies clinically attractive and relevant.[5] An increasing tendency for utilization of medicinal plants in developing and industrialized countries has been reported.[6-9] Herbal preparations have been cited as the third most popular complementary treatment modality by asthma sufferers.[10]

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Cassia occidentalis whole plant (C.O.W.P), *Jatropha curcas* leaves (J.C.L.), *Ximenia americana* leaves (X.A.L.), and *Eucalyptus citriodora* leaves (E.C.L.) have been widely mentioned to be used traditionally in managing asthma and other airway related illnesses by inhabitants of North-western Nigeria.

Cassia occidentalis leaves paste is applied topically to wounds, sores, itch, cutaneous diseases, bone fracture, fever, ringworm, skin diseases, and throat infection and to cure sore eyes. It was also used in hematuria, rheumatism, typhoid fever, tuberculosis, and asthma and hemoglobin disorders, to cure leprosy, and treat diabetes.[11] Its roots, leaves, flowers, and seeds were used as laxatives and purgatives. The plant was also used as febrifuge, vermifuge, anticonvulsant, and against chicken pox, guinea worm, and black quarter.[12]

The decoction of *Jatropha curcas* leaves is used traditionally for the management of cough, diarrhoea, dysentery, and as an antiseptic.

The roots and leafy twigs of *X. americana* have been used in different African countries as an antiseptic to treat fever, edema, diarrhea, febrile cold, cough, sexually transmitted diseases, and as an antidote for poisons.[13] Leaves, barks, peeling and roots of *X. americana* are used in different African countries for treating toothaches, mumps, and conjunctivitis in frontal applications.[13]

Eucalyptus leaves are a traditional Aboriginal herbal remedy. The essential oil found in the leaves is a powerful antiseptic and is used all over the world for relieving coughs and colds, sore throats, and other infections.

However, there is a paucity of data on scientific validation of the ethnobotanical claim of the use of these plants in the management of asthma. This research was therefore undertaken to scientifically evaluate the ethanol extracts of C.O.W.P, J.C.L., X.A.L., and E.C.L.

for airway smooth muscles relaxant and mast cells stabilizing activities in laboratory animals.

Materials and Methods

Collection, identification, and drying of plant material

Cassia occidentalis was collected from a roadside location in Zangon Shanu, *Jatropha curcas*, *Aimenia emericana* and *Eucalyptus citriodora* at Area BZ, all in Samaru Zaria during the month of May, 2019 in Kaduna State – Nigeria. They were identified and authenticated by a taxonomist in the Herbarium Section of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Kaduna State – Nigeria by comparing with the existing specimens (Voucher specimen number 4771 for C.O.W.P., 1531 for J.C.L., 004 for X.A.L. and 2201 for E.C.L.).

The basic operation included steps, such as drying of plant materials under the shade with intermittent weighing until constant weights were obtained, size-reduction to obtain a homogenous sample using mortar and pestle to improve the kinetics of analytic extraction and also to increase the contact of the sample surface with the solvent system at the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Science, ABU, Zaria, Kaduna - Nigeria.

Extraction of plant material

The powdered materials of C.O.W.P. (750 g), J.C.L. (750 g), X.A.L. (750 g) and E.C.L. (500 g) were each extracted with 7.5 L of 70% ethanol (except for E.C.L. which 5.0 L was used) by cold maceration for seventy-two (72) hours with intermittent shaking. C.O.W.P., J.C.L., X.A.L., and E.C.L. extracts were concentrated by evaporation on a water bath maintained at 40 – 60ºC, then stored in desiccators until needed for the work. Solutions were freshly prepared for each study using distilled water.

Phytochemical screening

Phytochemical screening was carried out on both the aqueous and methanol leaf extracts using simple chemical tests to detect the presence or absence of chemical constituents as described by Evans in 2009[14] and Tin Layer Chromatography as described by Ghani in 1990.[15]

Experimental animals

Guinea pigs, rabbits, and rats of either sex, weighing 300 – 350 g, 1.3 kg, and 180 – 200 g, respectively, bred in the Animal House Facility of the Department of Pharmacology and Therapeutics, ABU, Zaria - Nigeria were used for the experiment. They were kept in the Animal House for some days at standard conditions under a normal phase light-dark cycle for acclimatization. All experimental procedures followed the ethical guidelines for the care and use of laboratory animals as provided by Ahmadu Bello University Research Policy (Revised, 2010) and accepted internationally (National Institutes of Health, 1985; Publication no. 85-25, Revised 1996). Ethical approval (Approval No: ABUCAUC/2021/068) was obtained from committee on animals use and care, Ahmadu Bello University, Zaria, Kaduna State.

Acute toxicity studies in rats (LD_{50})

The oral median lethal doses of both aqueous and methanol extracts were determined using Lorke's method.[16] The study was carried out in two phases. Before the commencement of the studies, rats were deprived of food overnight. In phase 1, three groups of three animals were used. The extract was administered orally in geometrically increasing doses (10 mg/kg, 100 mg/kg, and 1000 mg/kg). The treated animals were observed for twenty-four hours for signs and symptoms of toxicity and death. In phase 2, three groups of one animal each were given the extract orally at doses of 1600 mg/kg, 2900 mg/kg, and 5000 mg/kg (since none of the phase 1 animal died). The animals were then observed for signs of toxicity for the first 4 hours and mortality for 24 hours. The geometric mean of the lowest lethal dose (for which the animals died) and highest non-lethal dose (for which the animals survived) was taken as the median lethal dose (LD_{50}).

Pharmacological Studies

Determination of the effects of ethanol extracts of C.O.W.P., J.C.L., X.A.L., and E.C.L. on spontaneous contractions of isolated rabbit ileum

Overnight fasted rabbit weighing 1.4 kg was sacrificed using the cervical dislocation method. 3 cm long ileum was quickly dissected out and mounted in an organ bath maintained at 30 ± 0.5°C and containing 20 mL Tyrode's solution under basal tension of 500 mg. The solution was continuously aerated with air. Spontaneous contractions of the ileum were recorded on a macro dynamometer using an isotonic transducer, which exerts a basal tension equivalent to 500 mg load on tissues. The tissue was allowed to equilibrate for 30 minutes, during which, the bathing solution was changed every 10 minutes. The contractile / Relaxant responses of the ileum to graded doses of J.C.L. was observed for 10 mins with washing off (three times) and an equilibration period of another 10 mins allowed after each administration. Responses were recorded and maximum contractile / Relaxant response was plotted to generate a dose-response curve of spontaneous contraction against a contraction in the presence of J.C.L. extract. The same procedure was repeated for C.O.W.P., X.A.L., and E.C.L extracts.[17, 18]

Determination of the effects of the ethanol extracts of C.O.W.P., J.C.L., X.A.L., and E.C.L. on histamine-induced contraction of isolated guinea pig ileum

Overnight fasted guinea pigs weighing 300 – 500 g were sacrificed using the cervical dislocation method. 3 cm long ileum was quickly dissected out and mounted in an organ bath maintained at 30 ± 0.5°C and containing 20 mL Tyrode's solution under basal tension of 500 mg. The solution was continuously aerated with air. The drug responses were recorded on a macrodynanometer using an isotonic transducer, which exerts a basal tension equivalent to 500 mg load on tissues. The tissue was allowed to equilibrate for 30 minutes, during which, the bathing solution was changed every 10 minutes. The contractile responses of the ileum to 0.1, 0.2, 0.4, 0.8, and 1 mL of agonists (histamine 10 µg/mL) were observed and recorded for 30 seconds with washing off (three times) and an equilibration period of another 10 mins allowed after each administration. Responses to histamine were recorded in presence of graded concentrations of the extracts (0.4, 0.8, 1.6, and 3.2 mg/mL). Maximum contractile response was plotted to generate dose-response curve of histamine, in the presence and absence of the extracts.[19, 20]

Determination of the effects of the ethanol extracts of C.O.W.P., J.C.L., X.A.L., and E.C.L. on histamine-induced contraction of isolated guinea pig tracheal chain

A guinea pig weighing 300 g was killed by a blow on the head and the throat was immediately cut. The entire trachea was dissected out and transferred to a dish containing Kreb's solution, cleaned from connective tissue and was cut into individual rings. Twelve rings were tied together with silk threads and mounted in an organ bath containing oxygenated Kreb's solution and maintained at 37°C.[21,22] The tissue was placed under an initial tension of 0.5 g and was washed three times at 15 min intervals. It was then equilibrated under a 1 g tension and after a 30–60 min equilibration period, an experiment was initiated[23]. Isometric contractions in response to 0.1 mL histamine (30 µg/mL) alone were recorded using a macrodynamometer at a speed of 6 mm/min; the responses were allowed to plateau and then recorded. The tissue was rinsed thoroughly and contractions were induced again by adding 0.1 mL of 30 µg/mL histamine and the individual extracts (0.1 mL of 100 mg/mL) concurrently; the responses were allowed to plateau then then recorded. The tissue was rinsed thoroughly and the process was repeated for 0.2 mL, 0.4 mLs, and 0.8 mL. The procedure was repeated again but with the tissue being pre-treated with the individual extracts before addition of the spasmogen.[17,18]

Assessment of mast cells stabilizing effect of the ethanol extracts of C.O.W.P., J.C.L., X.A.L., and E.C.L. on ovalbumin-induced rat peritoneal mast cell degranulation

This procedure was carried out by adopting the model described by Anita and Babita in 2008.[24] Five (5) rats were sensitized by administering three doses of 20 mg/kg of egg albumin adsorbed on 60

mg/kg of aluminium hydroxide, the doses being given on the first, third, and fifth days subcutaneously. The animals were kept on standard feed and water until the tenth day. On the tenth day of sensitization, 5 mL of normal saline containing 5 unit/mL of heparin was injected into the peritoneal cavity of the rats which were initially lightly anaesthetized in a chamber containing cotton wool wetted with chloroform. After a gentle abdominal massage, the peritoneal fluid containing mast cells was collected using a pasture pipette in centrifuge tubes and placed over ice. This was achieved by using scissors and forceps to cut the outer skin of the peritoneum and gently pull it back to expose the inner skin lining the peritoneal cavity.

A 25G needle bevel up, attached to a 5 mL syringe was inserted in the peritoneum to collect the fluid while moving the tip of the needle gently to avoid clogging with fat tissue or other organs. As much fluid as possible was collected and the collected cell suspension was deposited in tubes kept on ice after removing the needle from the syringe. An incision in the inner skin was made and while holding up the skin with forceps a plastic pasture pipette was used to collect the remaining fluid from the cavity. Any sample with visible blood contamination was discarded.

The collected peritoneal fluid was centrifuged at 2000 rpm for 5 minutes. The supernatant solution was discarded and the cells were washed twice with saline and re-suspended in 1 mL of saline.

0.1 mL of the peritoneal cell suspension was transferred to 6 test tubes and treated as follows;

Test tubes 1 and 2: saline
Test tube 3: 0.1mL of 0.5 mg/mL of extract in saline
Test tube 4: 0.1mL of 1.0 mg/mL of extract in saline
Test tube 5: 0.1mL of 2.0 mg/mL of extract in saline
Test tube 6: 0.1mL 10 µg/mL of Ketotifen fumarate

Each test tube was incubated for 15 minutes at 37°C and then ovalbumin (1 mg/mL) was added to each test tube except test tube no. 1. After further incubation for 10 minutes, the cell was stained with toluidine blue solution made in distilled water and was examined under a light microscope.

Percentage protection of the mast cells in the control and the treated group were calculated by counting the number of degranulated mast cells from the total of at least 100 mast cells counted. The process was performed with each of the four extracts.

Statistical analysis

Data were expressed as mean ± SEM and analysis was done using Statistical Package for the Social Sciences (SPSS) Version 19. One-way analysis of variance (ANOVA), followed by Dunnett's post hoc test, was used where appropriate. Mean differences will be considered to be significant when $p \leq 0.05$. Values will be represented as tables, charts, and figures.

Results and Discussion

Yield of ethanol soluble extractives of C.O.W.P., J.C.L., X.A.L., and E.C.L

The total weight of *Cassia occidentals* (whole plant), *Jatropha curcas* leaves, *Ximenia americana* leaves, and *Eucalyptus citriodora* leaves extracted were 750, 750, 750, and 500 g respectively. The weights of the extracts obtained were 124.63, 75.91, 176.5, and 120.74 g respectively and percentage of ethanol soluble extractives were calculated with reference to air-dried plant material and the yield was determined to be 16.62, 10.12, 23.5, and 24.1 % respectively (Table 1).

Physicochemical properties of the ethanol extracts of C.O.W.P., J.C.L., X.A.L., and E.C.L

Physicochemical properties revealed the colour of the ethanol extracts of *Cassia occidentals* (whole plant), *Jatropha curcas* leaves, *Ximenia americana* leaves, and *Eucalyptus citriodora* leaves were brown, dark green, green, and brown respectively. They had odours that were pleasant, odorless, pleasant, and pleasant respectively, and tasted bitter, bitter, slightly bitter, and tasteless respectively. Their pH was determined to be 6.38 at 31.8°C, 7.16 at 30.8°C, 6.65 at 31.4°C, and 4.50 at 31.5°C respectively. Their specific gravities were calculated to be 1.336, 1.199, 1.38, and 1.27 respectively (Table 2).

Phytoconstituents of ethanol extracts of C.O.W.P., J.C.L., X.A.L., and E.C.L

Preliminary phytochemical screening of the ethanol whole plant extract of *Cassia occidentalis* using various detecting reagents revealed the presence of flavonoids, alkaloids, terpenes, saponin, glycoside, steroids, and tannins. Anthraquinones were found to be absent (Table 3). That of the ethanol leaves extract of *Jatropha curcas* revealed the presence of flavonoids, alkaloids, terpenes, saponin, glycosides, steroids, anthraquinone, steroids, and carbohydrates (Table 3). Preliminary phytochemical screening of the ethanol leave extract of *Ximenia americana* revealed the presence of carbohydrates, flavonoids, Alkaloids, Terpenes, Saponin, cardiac Glycoside, phenolic compounds, steroids, and tanins. The extract of *Eucalyptus citriodora* also tested positive for alkaloids, terpenes, saponins, glycosides, anthraquinones, steroids and carbohydrates (Table 3).

Table 1: Yields of the Ethanol Extracts of Some Traditionally Used Anti-asthmatic Plants in North Western Nigeria

Properties	**Plants**			
	C. occidentalis	***E. citriodora***	***J. curcas***	***X. americana***
Plant parts	Whole plant	Leaves	Leaves	Leaves
Weight of powdered plant Used (g)	750	500	750	750
Volume of Solvent (70% Ethanol) (L)	7.5	5.0	7.5	7.5
Yield (g)	124.63	120.74	75.91	176.5
Percentage Yield (% w/w)	16.62	24.2	10.12	23.5

Table 2: Physicochemical Properties of the Ethanol Extracts of Some Traditionally Used Anti-asthmatic Plants in North Western Nigeria

Properties	**Plants (Ethanol extract)**			
	***C. occidentalis* Whole Plant**	***E. citriodora* Leaves**	***J. curcas* Leaves**	***X. americana* Leaves**
Plant part	Whole plant	Leaves	Leaves	Leaves
Specific gravity	1.34	1.27	1.20	1.38
Colour	Brown	Brown	Dark green	Green
Taste	Bitter		Bitter	
Odour	Pleasant	Pleasant	Odourless	Almond-like
pH	6.38 at 31.8°C	4.5 at 31.5°C	7.16 at 30.8°C	6.65 at 31.4°C

Basic phytochemical screening is designed to detect the presence or absence of some classes of plant metabolites by subjecting them to reaction with reagents that could yield observable colored products. Some of the reactions involve the formation of complexes between the organic metabolites and heavy metals resulting in an appearance of coloured precipitate.[14,25] Flavonoids are found to be active at both phases of allergic response.[26] The presence of commonly occurring phytochemicals in anti-asthmatic plants like flavonoids, alkaloids, and terpenes among others in *Cassia occidentals (whole plant), Jatropha curcas* leaves, *Ximenia americana leaves,* and *Eucalyptus citriodora* leaves extracts supports the use of these plants in the treatment of asthma.

Oral median lethal doses (LD_{50}) of ethanol extracts of some medicinal plants (C.O.W.P., J.C.L., X.A.L., and E.C.L) used for Asthma in North Western Nigeria

The oral LD_{50} of ethanol extracts of C.O.W.P., J.C.L., X.A.L., and E.C.L in rats was found to be greater than 5000 mg/kg (Table 4).

Safety tests begin with acute toxicity testing, where the animals are given a single dose of the test compound. The tests aim is to determine the range between the dose that causes no adverse effect and the life-threatening dose. They give information about the dosage that should be used, and how toxicity may occur.[27] The high oral LD_{50} of ethanol extracts of *C. occidentalis, E. citriodora, J. curcas,* and *X. Americana* which were found to be greater than 5000 mg/kg in rats implies the relative safety of the extracts if used orally.

Effects of ethanol extracts of C.O.W.P., E.C.L., J.C.L., and X.A.L. on spontaneous contractions of isolated rabbit ileum

Ethanol extracts of C.O.W.P., E.C.L., J.C.L., and X.A.L. at 100 mg/mL remarkably relaxed spontaneous contractions of isolated rabbit ileum. The greatest relaxation was obtained with X.A.L. (Figures 1 to 5).

Syndrome of bronchial asthma is characterized by widespread narrowing of the bronchial tree due to contraction of the smooth muscles in response to multiple stimuli resulting in the release of chemical mediators such as histamine and leucotrienes.[28] Isolated tracheal chain and isolated ileum strip of rodents can be used to test compounds that inhibit bronchospasms. They are used to detect β-sympathomimetic, cholinergic receptor blocking, H_1-receptor blocking, cAmp-phosphodiesterase inhibitors, and leukotriene receptor blocking properties of test drugs. Stimulation of muscarinic M_3 receptor and/or histamine H_1 receptor of isolated rabbit ileum by endogenous acetylcholine and histamine respectively which causes an increase in intracellular Ca^{2+} and other variety of events consequently resulting in its spontaneous contractions. Therefore, the potential of ethanol extracts of C.O.W.P., E.C.L., J.C.L., and X.A.L. to relax spontaneous contractions of isolated rabbit ileum supports their traditional claim of bronchorelaxant activities.

Effect of the ethanol extracts of C.O.W.P., E.C.L., J.C.L., and X.A.L. on histamine-induced contraction of isolated guinea pig ileum

Ethanol extracts of C.O.W.P., E.C.L., J.C.L. and X.A.L. statistically significantly ($p \leq 0.05$) inhibited histamine-induced contractions of isolated guinea pig ileum. X.A.L. however, produced the highest inhibition (Table 5 and Figure 6).

Guinea pigs ileum contains abundant histamine H_1 receptors. Stimulation of the H_1 receptors by histamine produces graded dose related contractions of isolated guinea pig ileum preparation (Pandit *et al*., 2008; Saraf and Patwardhan, 1998). Therefore, the potential of ethanol leaves extracts of C.O.W.P., E.C.L., J.C.L., and X.A.L. to significantly inhibit histamine induced contractions of isolated guinea pig ileum confirms their smooth muscles relaxant and potential broncho-relaxant activities.

Table 3: Phytoconstituents of Some Traditionally Used Anti-asthmatic Plants in North Western Nigeria

Chemical Constituents	Methods	Remarks			
		***C. occidentalis* Whole plant**	***E. citriodora* leaf**	***J. curcas* Leaf**	***X. Americana* Leaf**
Carbohydrate	Molish test	+	+	+	+
Steroids and terpenoids	Liebermann-Burchard's test	+	+	+	+
Anthracene (free)	Bontragers test	_	+	+	
Cardiac glycoside	Keller killiani test	+	+	+	+
Saponins	Frothing test	+	+	+	+
	Haemolysis test				
Tannins	Ferric chloride test	+			+
	Lead subacetate test	+			
Flavonoids	Shinoda's test				
	Sodium hydroxide test	+		+	+
Alkaloids	Mayer's test				
	Dragendoff test	+	+	+	+

Table 4: Oral Median Lethal Doses (LD_{50}) of Ethanol Extracts of Some Traditionally Used Anti-asthmatic Plants in North Western Nigeria

Parameters	Plants Extracts			
	***C. occidentalis* Whole Plant**	***E. citriodora* Leaf**	***J. curcas* Leaf**	***X. americana* Leaf**
Sign(s) of Toxicity	Nil	Nil	Nil	Nil
LD_{50} Values (mg/kg)	> 5000	> 5000	> 5000	> 5000

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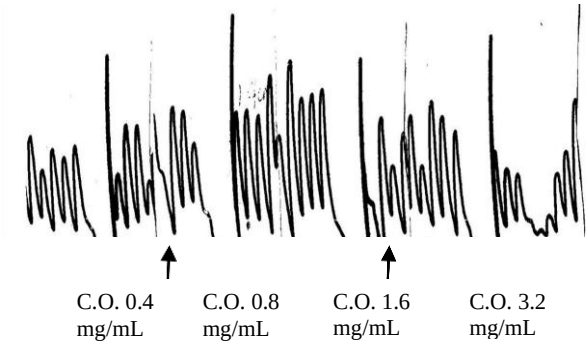

Figure 1: Effects of Ethanol Extracts of *C. occidentalis* Whole Plant (C.O.W.P.) on Spontaneous Contractions of Isolated Rabbit Ileum

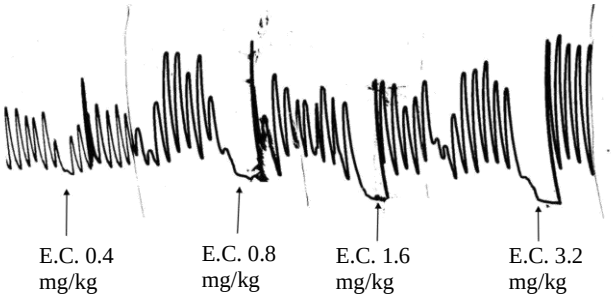

Figure 2: Effects of Ethanol Extracts of *E. citrodura* Leaf (E.C.L) on Spontaneous Contractions of Isolated Rabbit Ileum

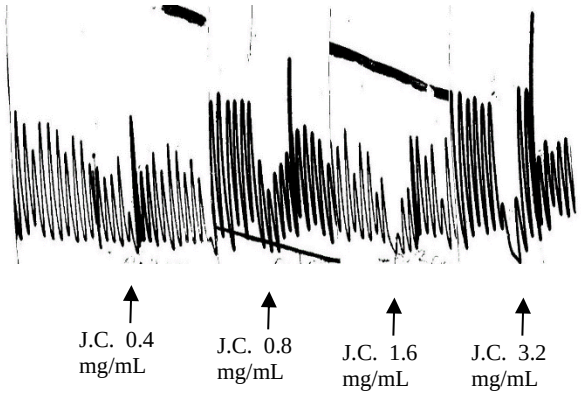

Figure 3: Effects of Ethanol Extracts of *Jatropha curcas* Leaf (J.C.L.) on Spontaneous Contractions of Isolated Rabbit Ileum

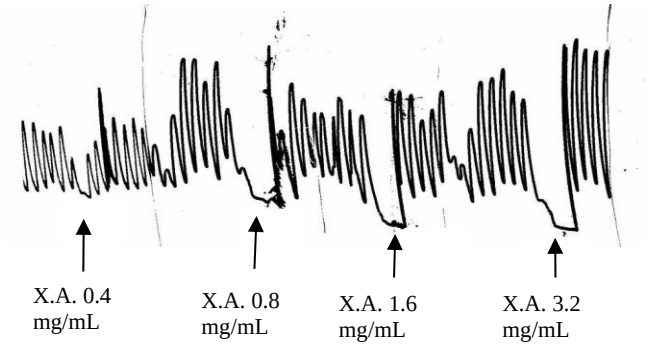

Figure 4: Effects of Ethanol Extracts of *X. americana* Leaf (X.A.L.) on Spontaneous Contractions of Isolated Rabbit Ileum

Effect of the ethanol extracts of some medicinal plants (C.O.W.P., E.C.L., J.C.L., and X.A.L.) commonly used in treating asthma in North-Western Nigeria on histamine induced contraction of isolated guinea tracheal chain

H_1 receptors mediate most of the effects of histamine that is related to asthma (Tripathy, 2003). Histamine is known to bind to H_1 histamine receptor in the airways of rodents and men resulting in a dose-dependent increase in contraction of the trachea. This is further strengthened in the result in Table 6 and Figure 7 below, showing an increase in tracheal contraction with an increase in the concentration of histamine until receptor saturation was achieved at 4.0 µg/mL.

This experiment also revealed the potentials of J.C.L. and X.A.L. extracts to statistically significantly inhibit ($p \leq 0.05$) histamine-induced contraction of isolated guinea pig trachea which is directly related to an increase in the concentration of the extracts up to the highest concentration used (3.2 mg/mL). E.C.L. and C.O.W.P. extracts also statistically significantly inhibited histamine-induced contraction at lower concentrations. However, at higher concentrations, they were only able to slightly inhibit such effect without any statistical significance ($p > 0.05$) (Table 6 and Figure 7).

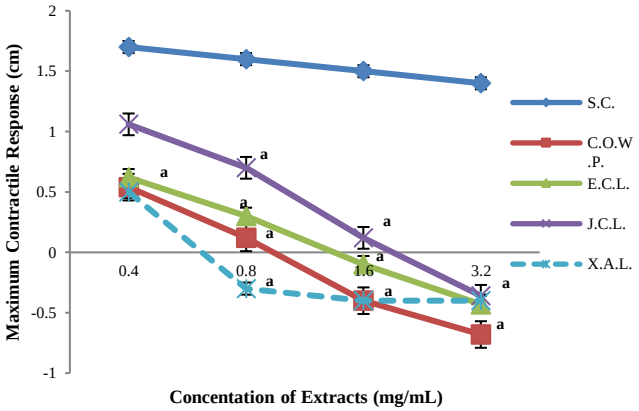

Figure 5: Effects of Ethanol Extracts of Some Traditionally used Anti-asthmatic Plants on Spontaneous Contractions of Isolated Rabbit Ileum

Values are Mean ± SEM, n=5. One-way ANOVA followed by Dunnett's *post hoc* test. "a" is significantly different from control at $P \leq 0.05$. S.C. = Spontaneous contraction (Control), C.O.W.P. = *Casia occidentalis* whole plant, E.C.L. = *E. citriodora* Leaf, J.C.L. = *J. curcas* Leaf and X.A.L. = *X. americana* Leaf

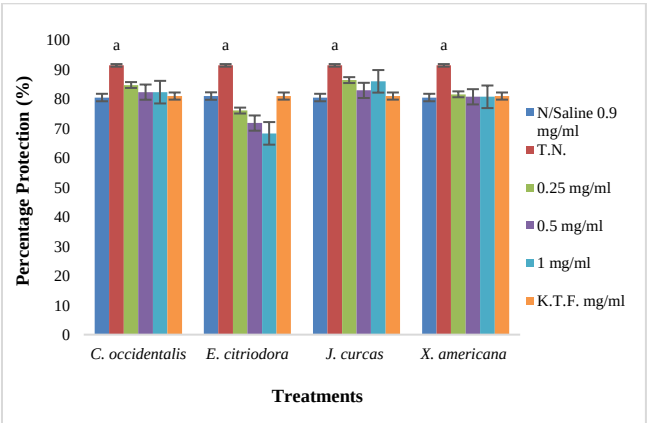

Figure 6: Protective Effect of Selected Medicinal Plants used for Treating Asthma in North-west Nigeria on Ovalbumin-Induced Rat Peritoneal Mast Cell Degranulation

Values are expressed as mean ± SEM (n = 5). "a" is significantly different from control (N/Saline) at $P \leq 0.05$ using One Way ANOVA followed by Dunett's *post hoc* tests. K.T.F. = Ketotifen, T.N. = Treatment naïve.

The stimulation of H_1 receptors produces graded dose-related contractions of isolated guinea pig trachea preparation. Histamine acts by binding to G-protein coupled histamine receptors (H_1, H_2, H_3, and H_4), in this case, H_1 receptors are found on smooth muscles of the bronchial tree. The H_1 receptor is linked to an intracellular G-protein (Gq) that activates phospholipase C and phosphatidylinositol (PIP2) signalling pathway thus leading to smooth muscle contraction.[29]

In the present study, the ethanol extracts of C.O.W.P., E.C.L., J.C.L., and X.A.L were involved in inhibition of the contractions produced by histamine either by directly preventing the binding of histamine to its receptor, specifically by acting as a receptor antagonist of peripheral histamine H1 receptors[30] thus inhibiting the contractions; or by acting as beta 2 adrenergic receptor agonist, stimulation of beta 2 receptors leads to activation of enzyme adenylcyclase that form cyclic adenosine monophosphate (AMP) from adenosine triphosphate (ATP). This high level of cyclic AMP relaxes bronchial smooth muscles and decreases airway resistance. Also, high levels of cyclic AMP inhibit bronchoconstricting mediators such as histamine and leukotriene from the mast cell in the airway, or the extracts acted as anticholinergic agents which causes relaxation by specifically blocking the M3 type muscarinic acetylcholine receptors in the smooth muscle of the bronchial tree;[31] or by acting as phosphodiesterase 4 inhibitors preventing cyclic AMP. Consequently, the tracheal relaxing activity elicited by C.O.W.P., E.C.L., J.C.L., and X.A.L extracts provides additional evidence for the traditional use of these plants in asthma.

Protective effect of ethanol extracts of C.O.W.P., E.C.L., J.C.L. and X.A.L. on ovalbumin induced rat peritoneal mast cell degranulation

The ethanol leaves extract of *C. occidentalis* and *Jatropha curcas* exhibited a mast cell stabilizing effect on ovalbumin-induced rat peritoneal mast cell degranulation but it was not statistically significant ($p \geq 0.05$). However, *Eucalyptus citriodora* and *Ximenia Americana* did not protect against ovalbumin-induced rat peritoneal mast cell degranulation (Figure 6 and Plate II).

In the classic immunologic model, asthma is a disease mediated by reaginic (IgE) antibodies bound to mast cells in the airway mucosa. On re-exposure to an antigen, antigen-antibody interaction takes place on the surface of mast cells, triggering mast cell degranulation; the central event of an allergic reaction.[32,33] Ovalbumin induces degranulation of mast cells through an IgE-dependent mechanism. Consequently, the inhibition of antigen-antibody reaction by ethanol leaves extracts of C.O.W.P. and J.C.L. prevented the degranulation of mast cells in ovalbumin-induced rat peritoneal mast cells degranulation, thus depicting their anti-allergic properties.

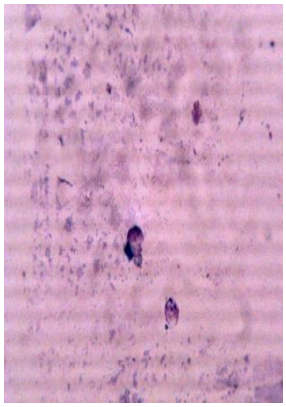

Plate I: Degranulated Mast Cell (DMC) of N/saline Group (x100, T.

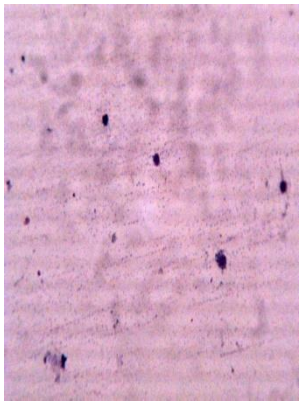

Plate II: Normal Mast cell (NMC) after treatment with 0.5 mg/ml J.C. (x100, T. B.

Table 5: Effect of the Ethanol Extracts of Some Selected Medicinal Plants on Histamine-induced Contraction of Isolated Guinea Pig Ileum

Conc of Extracts (mg/mL)	Conc of Hist (µg/mL)	Maximum Contractile Response (cm)				
		Control (Histamine)	Hist in the presence of C.O.W.P.	Hist in the presence of E.C.L.	Hist in the presence of J.C.L.	Hist in the presence of X.A.L.
0.4	1.0	0.60 ± 0.06	0.100 ± 0.04[a]	0.24 ± 0.51[a]	0.10 ± 0.04[a]	0.22 ± 0.37[a]
0.8	2.0	0.80 ± 0.03	0.300 ± 0.04[a]	0.42 ± 0.37[a]	0.10 ± 0.05[a]	0.48 ± 0.37[a]
1.6	3.0	1.38 ± 0.04	0.580 ± 0.04[a]	0.70 ± 0.45[a]	0.66 ± 0.04[a]	0.38 ± 0.37[a]
3.2	4.0	1.52 ± 0.08	0.620 ± 0.04[a]	1.08 ± 0.37[a]	0.90 ± 0.04[a]	0.36 ± 0.81[a]

Values are Mean ± SEM, n=5. One-way ANOVA followed by Dunnett's Post HOC Test. "a" is significantly different from control at $P \leq 0.05$. C.O.W.P. = *Casia occidentalis* whole, Hist = Histamine, Conc = Concentration, Hist = Histamine.

Table 6: Effect of the Ethanol Extracts of Some Selected Medicinal Plants on Histamine Induced Contraction of Isolated Guinea Pig Trachea

Conc of Extract (mg/mL)	Conc of Hist (µg/mL)	Maximum Contractile Response (mm)				
		Control (Histamine)	Hist in the presence of C.O.W.P.	Hist in the presence of E.C.L.	Hist in the presence of J.C.L.	Hist in the presence of X.A.L.
0.4	1.0	3.80 ± 0.87	2.30 ± 0.50[a]	1.30 ± 0.90[a]	1.10 ± 0.55[a]	1.30 ± 0.60[a]
0.8	2.0	4.20 ± 0.51	2.85 ± 0.80[a]	2.55 ± 1.30[a]	1.30 ± 0.51[a]	1.35 ± 0.31[a]
1.6	3.0	4.80 ± 0.89	3.30 ± 0.40[a]	2.70 ± 0.49[a]	1.60 ± 0.44[a]	1.31 ± 0.45[a]
3.2	4.0	5.30 ± 0.58	4.75 ± 0.95	4.40 ± 0.63	1.75 ± 0.52[a]	1.79 ± 0.62[a]
0.4	1.0	5.30 ± 0.51	4.60 ± 0.50	5.10 ± 0.50	1.82 ± 0.40[a]	1.85 ± 0.50[a]

Values are Mean ± SEM, n=5. One-way ANOVA followed by Dunnett's Post *Hoc* Test. "a" is significantly different from control at $P \leq 0.05$. C.O.W.P. = *Casia occidentalis* whole plant, E.C.L. = *E. citriodora* Leaf, J.C.L. = *J. curcas* Leaf and X.A.L = *X. americana*Leaf, Hist = Histamine

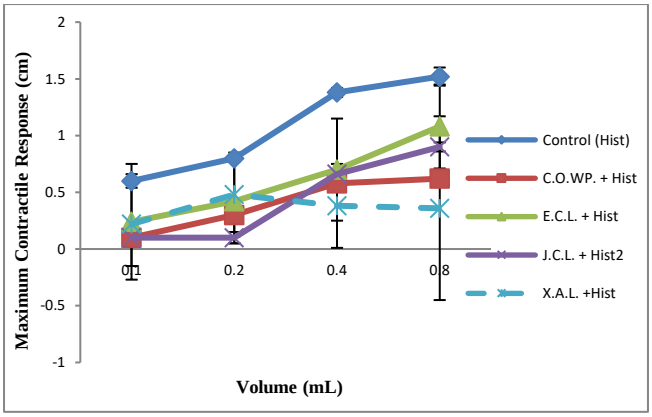

Figure 7: Effect of the Ethanol Extracts of Some Selected Medicinal Plants on Histamine-induced Contraction of Isolated Guinea Pig Ileum

Values are Mean ± SEM, n=5. One-way ANOVA followed by Dunnette's Post HOC Test. "a" is significantly different from control at $p \leq 0.05$. cm is centimeter, mL is milliliter, C.O.W.P. is *Casia occidentalis* whole plant, E.C.L. is *Eucalyptus citriodora* Leaves, J.C.L. is *Jatropha curcas* Leaves and X.A.L is *Ximenia Americana* Leaves.

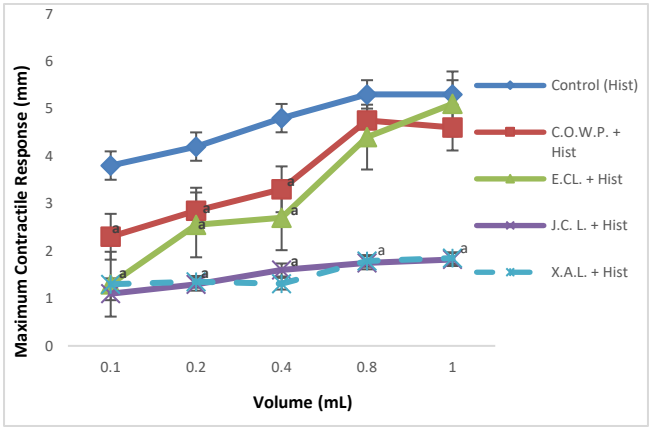

Figure 8: Effect of the Ethanol Extracts of Some Selected Medicinal Plants on Histamine Induced Contraction of Isolated Guinea Pig Trachea

Values are Mean ± SEM, n=5. One-way ANOVA followed by Dunnett's Post *Hoc* Test. "a" is significantly different from control at $P \leq 0.05$. C.O.W.P. = *Casia occidentalis* whole plant, E.C.L. = *E. citriodora* Leaf, J.C.L. = *J. curcas* Leaf and X.A.L = *X. americana* Leaf, Hist = Histamine

Conclusion

C. occidentalis whole plant, *E. citriodora* leaves, *J. curcas* leaves, and *X. Americana* leaves all possess bronchorelaxant activity, while, in addition, *C. occidentalis* and *Jatropha curcas* exhibited mast cell stabilizing effect in laboratory animals hence support the traditional use of the plant in inflammatory and allergic conditions including asthma.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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