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Comparison of in vitro Antibacterial Effects of Cnidoscolus aconitifolius and OcimumgratissimumHydromethanolic Leaf Extracts

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Abstract: *The study provided evidence for the ethno-medicinal claims of Chaya and scent leaves. The in vitro antibacterial effect ofCnidoscolusaconitifolius(Chaya) and Ocimumgratissimum (Scent) hydromethanolic leaf extracts were determined and compared. They were tested against pure cultures of clinical isolates of Escherichia coli, Klebsiellaoxytoca,Shigellaflexneri, Salmonella typhi, Staphylococcusaureus, Pseudomonas aeruginosa, Bacillus subtilisandVibrocholerausing agar well diffusion method.Methanol (80%) was used for theSoxhlet extraction of the active constituents of the plants. The susceptibility patterns of the test isolates to the hydromethanolic extracts of Cnidoscolusaconitifolius and Ocimumgratissimum leaves were determined at concentrations of 0.25g/10ml, 0.5g/10mland 1g/10ml. The results showed that the antibacterial activities of the extracts were concentration dependent and vary with each isolate. The zones of inhibition of the extracts were compared with a standard antibiotic (Chloramphenicol).It was observed that the total percentage bacterial inhibitionof Cnidoscolusaconitifoliuscompared with Chloramphenicol was higher (67.39%) than that ofOcimumgratissimum (45.61%).This affirmed the traditionaluse of both leaves for prophylaxis or treatment of infectious diseases. The work suggested further identification and isolation of the bioactive antibacterial compounds fornewer drug development.*

Key words: *Antibacterial, Hydromethanolic Extracts, Cnidoscolus aconitifolius, Ocimumgratissimum*

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I. Introduction

Traditional medicine is the use of plants or animals for the treatment of diseases or for the amelioration of human health within an organized indigenous system[1]**.** It is often referred to as the bedrock for discovering new drugs from plants and animals for human treatment[2]. The breakthrough in the efficacious use of plant and animal products for human treatment had led to the discovery of most pharmaceuticals. It is therefore pertinent to every nation to scientifically ascertain the therapeutic claims of its traditional medicine and pharmacologically or physiologically explain their bases for greater socio-economic development.

Rich storehouses of medicinal plants exist everywhere especially in Africa which offers a vast reservoir of plants that has been categorized[3].The search for new antibacterial agents from natural sources has been intensified due to the limitation of currently available therapy and the emergence of drug resistance strains. For a very long time, herbal medications have been used for relief of symptoms of some diseases[4].

In spite of the great advances achieved in modern medicine, plants still make an important contribution to health care[5]. This underlined value of traditional medical systems.World Health Organization describes a medicinal plant as any plant in which one or more of its organs contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs[6].

Medicinal plants are of great importance to the health of individuals and the community. The medicinal value of these plants lies in some chemical substances that produce definite physiological actions on the human body. The most important of these bioactive constituents of plant are the alkaloids, tannins, flavonoids,saponins and phenolic compounds [7] and the diverse range of these bioactive molecules therefore make them a rich source of different type of potential drugs[8].

Today, almost 88percent of the global population turn to plant derived medicines as their first line of action for maintaining health and combating diseases [9] and also because of their efficacy and cost

effectiveness.Thus, many plants that are of medicinal important have been investigated by various researchers [10, 11, 12, 13, 14, 15, 16] and the investigations of certain indigenous plants for their antibacterial properties yielded useful results[17, 18].

In last few decades, there has been an upsurge in demand and delivery of various herbal products for multifaceted health benefits. In some developed countries like the United States, herbal remedies still continue as dietary supplements. A variety of herbal remedies have stood the test of time because of their efficacy and potency in the treatment of various bacterial infections with a few having scientific evidences of significant usefulness [19, 20, 21, 22].

This study is focused on the comparison of the antibacterial effects of hydromethanolic extracts of *Cnidoscolusaconitifolius* (Chaya) and *Ocimumgratissimum* (scent) leaf extracts.

II. Materials and Methods

Sources and identification of Plant Materials

Fresh samples of *Cnidoscolusaconitifolius* (chaya) leaves were collected from OgbekeObibiezena in Owerri-North of Imo State, Nigeria from 06:56am to 8:00am in the month of March.

The fresh samples of*Ocimumgratissimum*(scent) leaves were collected from Imo state river basin Agbala in Owerri-North of Imo State, Nigeriafrom 06:00am to 8:45am in the month of March.

The leaves were identified by Prof. Tunde Joseph Ogunkunle, a Taxonomist in the Department of Pure and Applied Biology,LadokeAkintola University of Technology, Ogbomoso, Oyo state.

Plates A and B show the pictures of the medicinal plants used.

Plate A: *Cnidoscolus aconitifolius* (Chaya);Plate B: *Ocimumgratissimum* (Scent)

Preparations of the Plant Hydromethanolic Extracts.

It wasensured that the leaves were collected in the early hours of the day in its fresh state. The leaves were air-dried in a normal room temperature. The air-dried leaves were ground to its powdered state.

The method used for hydromethanolic extract of the leaves was described [23]. Five hundred grams (500g) of the powdered leaves of *Ocimumgratissimum* was measured into a conical flask, afterwhich 2000ml of methanol and 500ml of distilled water was added (4:1of methanol to water).

For *Cnidoscolusaconitifolius*, one hundred and fifty grams (150g) of the powdered plant was measured into a conical flask and 600ml of methanol and 150ml of water was added (4:1 of methanol to water).

Solvent Extraction

Sigma Aldrich Soxhlet extractor (model Z556203, 2005)and 80% methanol were used. The liquid extract was dehydrated using an electric oven at the temperature of 6ºC. The *Ocimumgratissimum*dried leaves yielded 77.31g (26.71%) while that of *Cnidoscolusaconitifolius*yielded 40.07g (15.46%)gel-like extracts.

Collection and Maintenance of Test Organisms

The 8 test organisms used were clinical isolates from New Concept Laboratories,Obinze, Owerri West, Imo State Nigeria. The organisms were *Staphylococcus aureus, E. coli, Bacillus subtilis, Salmonella typhi, Klebsiellaoxytoca, Pseudomonas aeruginosa, Shigellaflexneriand Vibro cholera.* These bacteria were chosen based on their clinical and pharmacological importance and were maintained in nutrient agar plate at 37^0C and stored in the refrigerator at a temperatureof 4^0C and Chloramphenicol was used as the standard control.

Nutrient Agar Media Preparation

28.0g of nutrient agar powder was dissolved in 100ml of distilled water. It was gently heated to dissolve the medium completely. After which it was sterilize by autoclaving at 15 Psi (121^0C) for 15 minutes. The medium was dispensed as desired in the Petri dishes and allowed to cool and solidify.

Determination of Antibacterial effects of the leaf extracts

The antibacterial effects were determined using modifiedCollins agar well-diffusion method [24]. 0.2ml of each of the standardized old culture of the tested organism in nutrient broth was inoculated unto the sterile nutrient agar plates or Mueller Hinton agar plate. These were then allowed to set. With the aid of a sterile Cork borer, four (4) wells of about 5mm diameter were bored on the solidified medium. About 0.2ml of each concentration of the extracts (i.e. 0.25g/10ml, 0.5g/10ml and 1g/10ml) were dispersed into the three wells and the control (Chloramphenicol) was dispensed into the fourth hole and was allowed to stand for about 15 minutes for diffusion of the extract to occur. These plates were then incubated at 37^0C for 24hours.

Evaluations of the Zone of Inhibition, MIC and MBC

At the end of the period, the inhibition zones formed on the nutrient agar plates were evaluated in "mm" [25]. The diameter of the zones of inhibition was determined by calculating the difference between the diameter of the cork borer (5mm) and the area or zone of inhibition.

The Minimum Inhibition Concentration (MIC) was determined by measuring about 5ml of nutrient broth into empty sterile test tubes. 1ml of the different concentrations of the different leave extracts (0.25g/10ml, 0.5g/10ml and 1g/10ml) were added to 0.5ml of the different test organisms. This was then incubated for 24hours at 37^0C. The tubes were then observed for visible growth with the help of a spectrophotometer. The tubes with the least concentration of the extract that showed lesser growth was determined as "MIC" while the negative tubes were pour plated on nutrient agar and incubated for 24hours at 37^0C.

The tube with the least concentration of the extract that showed NO growth at all was reported as the Minimum Bactericidal Concentration "MBC".

III. Results and Discussion

Tables 1 and 2 show the zones of inhibition (antibacterial activities) of different hydromethanolic extract concentrations of *Cnidoscolusaconitifolius* and*Ocimumgratissimum* leaves and the standard drug (Chloramphenicol) against the test organisms.

Table 1: Zones of inhibition of different hydromethanolic extract concentrations of *Cnidoscolus aconitifolius* and control Chloramphenicol

Micro organisms	0.25g/10ml (mm)	0.5g/10ml (mm)	1g/10ml (mm)	Control (mm)
Staphylococcus aureus	11	12	21	26
Escherichia coli	12	14	17	35
Salmonella typhi	7	12	17	22
Klebsiellaoxytoca	8	11	18	32
Pseudomonas aeruginosa	9	12	14	36
Shigellaflexneri	12	17	26	34
Vibro cholera	11	15	25	27
Bacillus subtilis	12	15	17	18

Table 2: Zones of inhibition of different hydromethanolic extract concentrations of *Ocimumgratissimum*and standard drug (Chloramphenicol)

Micro organisms	0.25g/10ml (mm)	0.5g/10ml (mm)	1g/10ml (mm)	Control (mm)
Staphylococcus aureus	10	15	17	27
Escherichia coli	9	11	17	32
Salmonella typhi	7	12	14	34
Klebsiellaoxytoca	9	10	13	32
Pseudomonas aeruginosa	9	11	15	31
Shigellaflexneri	10	12	16	35
Vibro cholera	10	12	18	30
Bacillus subtilis	6	8	9	18

Tables 3 and 4 show Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal concentration (MBC) of different hydromethanolic extract concentrations of *Cnidoscolusaconitifolius* and *Ocimumgratissimum*leaves.

Table 3: Minimum Inhibition Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) of hydromethanolicleaf extract of *Cnidoscolusaconitifolius* (Chaya).

Micro organisms	0.25g/10ml	0.5g/10ml	1g/10ml	MIC (%)	MBC (%)
Staphylococcus aureus	+	+	-	1g/10ml	>1g/10ml
Escherichai coli	+	-	-	0.5g/10ml	>0.5g/10ml
Bacillus subtilis	+	-	-	0.5g/10ml	>0.5g/10ml
Salmonella typhi	++	+	-	1g/10ml	>1g/10ml
Klebsiellaoxytoca	+	+	-	1g/10ml	>1g/10ml
Pseudomonaaeruginosa	+	+	-	1g/10ml	>1g/10ml
Shigellaflexneri	+	-	-	0.5g/10ml	>0.5g/10ml
Vibro cholera	+	-	-	0.5g/10ml	>0.5g/10ml

- =No Growth, ++ =Very turbid with Growth, +=Slightly turbid

- The higher the zone of inhibition the lesser the turbidity.
- The lower the zone of inhibition the higher the turbidity

Table 4: Minimum Inhibition Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) of hydromethanolicleaf extract of *Ocimumgratissimum*(Scent).

Micro organisms	0.25g/10 ml	0.5g/10ml	1g/10ml	MIC%	MBC%
Staphylococcus aureus	+	-	-	0.5g/10ml	>0.5g/10ml
Escherichai coli	+	+	-	1g/10ml	>1g/10ml
Bacillus subtilis	++	+	+	≥1g/10ml	>1g/10ml
Salmonella typhi	++	+	-	1g/10ml	>1g/10ml
Klebsiellaoxytoca	+	+	-	1g/10ml	>1g/10ml
Pseudomonaaeruginosa	+	+	-	1g/10ml	>1g/10ml
Shigellaflexneri	+	+	-	1g/10ml	>1g/10ml
Vibro cholera	+	+	-	1g/1ml	>1g/10ml

- =No Growth, ++ =Very turbid with Growth, +=Slightly turbid

- The higher the zone of inhibition the lesser the turbidity
- The lower the zone of inhibition the higher the turbidity.

Fig 1.0 represents the concentration dependentantibacterial effects of hydromethanolic leaf extract of *Cnidoscolusaconitifolius*and Chloramphenicol (Control)against the test isolates.

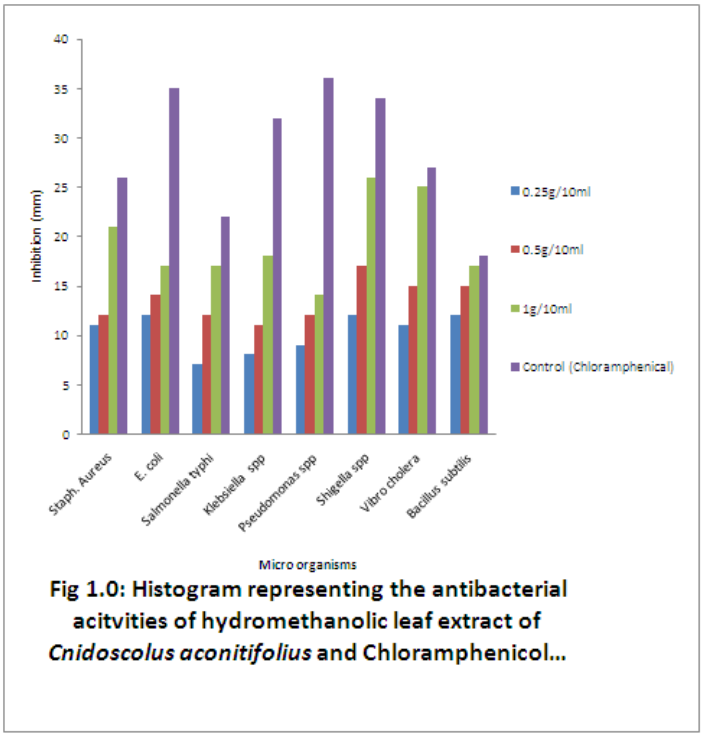

Fig 1.0: Histogram representing the antibacterial acitvities of hydromethanolic leaf extract of *Cnidoscolus aconitifolius* and Chloramphenicol...

Similarly,Fig 2.0.Histogram represents the antibacterial activities of hydromethanolic leaf extract of *Ocimumgratissimum* against the bacterial isolates.

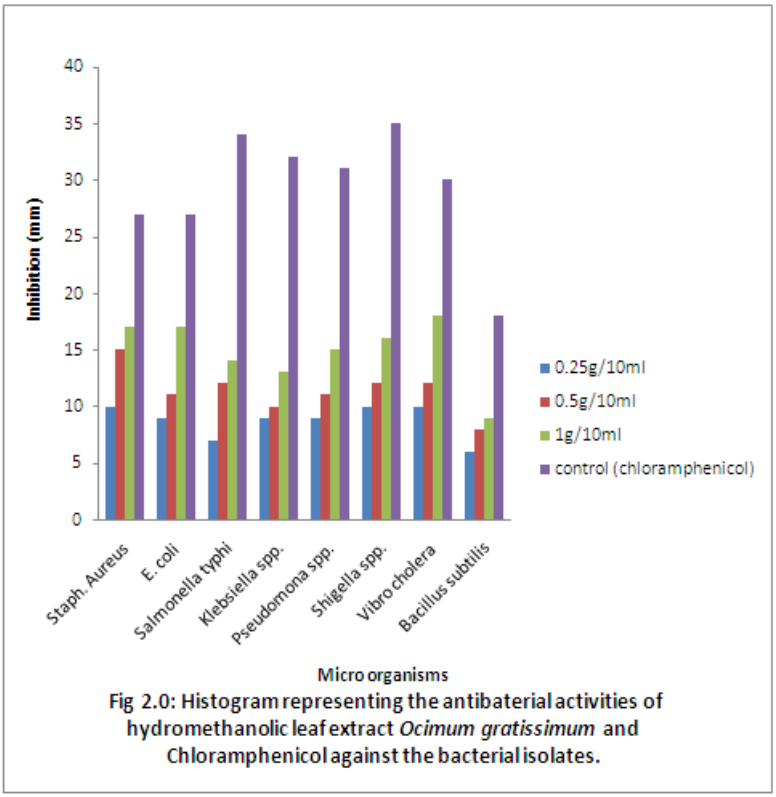

Fig 2.0: Histogram representing the antibaterial activities of hydromethanolic leaf extract *Ocimum gratissimum* and Chloramphenicol against the bacterial isolates.

The comparison (in percentage (%) of the antibacterial activities of hydromethanolicleaf extracts of *Cnidoscolusaconitifolius*, *Ocimumgratissimum*and the standard drug against bacterial isolates are presented in Tables 5-6.

Table 5: Shows the comparison(in percentage) of the antibacterial activity of 1.0g/10ml hydromethanolic extract of*Cnidoscolusaconitifolius leave* and the standard drug (Chloramphenicol).

Micro organisms		A (1g/10ml) (mm)	B (1g/10ml) (mm)	C (%)	D (%)
1.	*Staphylococusaureus*	21	26	80.7	14.29
2.	*Escherichie coli*	17	35	8.57	8.59
3.	*Salmonella typhi*	17	22	77.27	13.67
4.	*Klebsiellaoxytoca*	18	32	56.25	9.9
5.	*Pseudomonas aeruginosa*	14	36	38.88	6.88
6.	*Shigellaflexneri*	26	34	76.47	13.53
7.	*Vibro cholera*	25	27	92.59	16.38
8.	*Bacillus subtilis*	17	18	94.44	16.71

Keys: A and B means zones of inhibition of 1g/10ml leaf extract(in mm) and the control, respectively. C is the extract percentage (%) inhibition compared with Control and D is the individual Sensitivity of isolates to control

The total percentage (%) bacterial inhibition of*Cnidoscolusaconitifolius*comparedwith the Controlyielded 67.39%.

Table 6: Shows the comparison (in percentage) of the antibacterial activity of 1.0g/10ml hydromethanolic extract of *Ocimumgratissimum* (Scent) leave and the standard drug (Chloramphenicol).

Micro organisms	A (1g/10ml) (mm)	B (1g/10ml) (mm)	C (%)	D (%)
Staphylococusaureus	17	27	62.96	15.86
Escherichie coli	17	32	53.12	13.38
Salmonella typhi	14	34	41.17	10.37
Klebsiellaoxytoca	13	32	40.62	10.23
Pseudomonas aeruginosa	15	31	43.38	10.93
Shigellaflexneri	6	35	45.71	11.52

Vibro cholera	18	30	60	15.11
Bacillus subtilis	9	18	50	12.59

Keys: A and B means zones of inhibition of 1g/10ml leaf extract (in mm) and the control, respectively. C is the extract percentage (%) inhibition compared with Control and D is the individual Sensitivity of isolates to control

The total percentage (%) bacterial inhibition of *Ocimumgratissimum*comparedwith the Control yielded 45.61.

According to the World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. [26] Bacterial infections are of great concern to health care [27, 28] The success story of chemotherapy lies in the continuous search for new drugs to counter the challenge posed by resistant strains of microorganisms.[29,30,31, 32] Medicinal plants are being scientifically screened for their usefulactivities. [33, 34, 35, 36, 37] Various methods of extraction were considered to ensure optimum extraction yield. [38, 39, 40] The extracts of *Cnidoscolus aconitifolius* (Chaya) and *Ocimumgratissimum* (Scent) leaves had been reported to have some prophylactic and therapeutic potential. [12, 13, 41,42, 43,44, 45]Assessment of the antibacterial activities of the leaf extracts considered several reported procedures. [46, 47, 48, 49, 50, 51, 52]

The results of the present study on the antibacterial potential of hydromethanolic extracts of *Cnidoscolusaconitifolius* (Chaya) and *Ocimumgratissimum* (Scent) leavesagainst *Staphylococcusaureus, Escherichia coli, Salmonella typhi, Shigellaflexneri, Pseudomonas aeruginosa, Klebsiellaoxytoca, Basillussubtilis*and *Vibrocholera* showed that the hydromethanolic extracts of the plantsinhibited the growth of majority of the test isolates.

The inhibition zones of the extracts are less than that of the control (Chloramphenicol) which indicated that Chloramphenicol was more effective than the 1g/10ml extracts. There was also an indicationthat the extracts possessed substances that might inhibit the growth of some microorganisms. More so, the observed inhibitory effects through the zones of inhibition increased with increased concentrations of the extracts for both hydromethanolic extracts of *Cnidoscolusaconitifolius* and *Ocimumgratissimum*(0.25g/10ml, 0.5g/10ml and 1g/10ml).

The hydromethanolic extract of *Cnidoscolusaconitifolius*gave higher zones of inhibition on some microbes (such as *Shigellaflexneri, Salmonella typhi, Pseudomonas aeruginosa, Vibro cholera* and *Staphylococcus aureus)* when compared with the hydromethanolic extract of*Ocimumgratissimum* but*Escherichia coli*have the same zone of inhibition for the both leaf extracts.

The zones of inhibition of1g/10ml *Cnidoscolusaconitifolius* extract per inhibition zone of control and the sensitivity of the microbes were in decreasing order:*Shigellaoxytoca*(26mm) >*Vibro cholera*(25mm) 0>*staphylococcus aureus*(21mm)>*Klebsiellaoxytoca* (18mm) >*Echerichie coli*(17mm)> *Salmonella typhi*(17mm) >*Bacillus subtillis* (17mm) >*Psuedomonasaeruginosa* (14mm) and that of hydromethanolicleaf extract of *Ocimumgratissimum*were also in decreasing order: *Vibro cholera*(18mm) >*staphylococcus aureus*(17mm) >*Escherichia coli*(17mm)>*Shigellaoxytoca*(16mm) >*Psuedomonasaeruginosa* (15mm) >*Salmonella typhi*(14mm) >*Klebsiellaoxytoca* (13mm) >*Bacillus subtillis* (9mm).These were in accordance with the earlier workswhich reported that the increase in inhibitory effects wereas the concentrations of theextract increases and might be as a result of the presence of higher concentrationactive antimicrobial phytochemicals. [53, 54]

The phytochemicals in the extracts, such as alkaloids and flavonoids, could be responsible for the antimicrobial activities which made the plants medicinal. [55, 56, 57, 58, 59, 60]

II. Conclusion

This study supported the traditional treatment of some bacterial diseases using crude extracts of *Cnidoscolusaconitifolius* and*Ocimumgratissimum*leaves. It was shown that the extracts of both leaves were good sources of antibacterial phytochemicals for the inhibition of the growth of *staphylococcus aureus, Echerichia coli, Bacillus subtilis, Salmonella typhi, Klebsiellaoxytoca, Pseudomonas aeruginosa, Shigellaflexneri and Vibro cholera.*This indicated that both leaf extracts could be used against any bacterial infection caused by these microorganisms.

Consequently, the extracts of *Cnidoscolusaconitifolius* and *Ocimumgratissimum* could be used locally as a source of raw materials by pharmaceutical industries in producing newer drugs.

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