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Phytochemical Screening and Antibacterial Activity of *Cnidoscolus aconitifolius* and Associated Changes in Liver Enzymes in Wistar Rats.

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ABSTRACT

Cnidoscolus aconitifolius (Euphorbiaceae) is used traditionally to combat disease causing organisms which have prevail in Nigeria and some other developing countries where lip service is paid to matters concerning the health and welfare of the people and where public health rules and regulations made for preservation and promotion of well-being of everybody are not adequately monitored and enforced. To this end, this research was conducted to study the phytochemical constituents and antibacterial activity of *Cnidoscolus aconitifolius* and associated changes in liver enzymes in wistar rats. The phytochemical screening of the leaf extracts revealed the presence of alkaloids, tannins, phlobatannin, phenol, saponins and cardiac glycosides. The ethanolic extract was found to contain anthraquinones and flavonoids. The antibacterial activity of the plant leaves showed broad spectrum antibacterial activity at 200mg/ml, on *Staphylococcus aureus* (19.7±1.5mm), *Shigella* species (7.0±0.6mm), *Salmonella* species (5.0±0.1mm), *Streptococcus pneumoniae* (17.1±0.2mm). The minimum inhibitory concentrations of *Cnidoscolus aconitifolius* were determined against the clinical isolates that showed positive inhibition, *S. aureus* and *S. pneumoniae* had 10mg/ml while *Shigella* species and *Salmonella* species had 20mg/ml. The result showed no difference (P>0.05) between *Cnidoscolus aconitifolius* administered rats (at 100, 150 and 200 mg /kg body weight for 3 weeks) and control with respect to changes in body weight as well as in the liver enzymes in serum. From this study, it may be concluded that *Cnidoscolus aconitifolius* showed broad spectrum antibacterial activity and absence of cumulative toxicity as reflected by the non-significant changes in the parameters studied.

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INTRODUCTION

The communicability and contagiousness of communicable diseases are responsible for their transmission to other people, due to the extent to which endemic pathogenic organisms, or contagious diseases prevail in Nigeria and some other developing countries where lip service is paid to matters concerning the health and welfare of the people and where public health rules and regulations made for preservation and promotion of well-being of everybody are not adequately monitored and enforced is alarming (Onwuchekwa, 2004). Consequence upon this fact, many people have been exploring so many health care methods and have made vast number of people still stick to traditional medicine which employ the usage of herbs and plants in curing one disease or the other. This however is done without ascertaining the active ingredients of such vis-à-vis the right / appropriate dosage. The abuse of medicinal plants product especially in the wrong dosage has led to various health complications and premature death especially in Nigeria.

One of the plant genera widely used traditionally for the treatment of many diseases is *Cnidoscolus aconitifolius* (family :Euphorbiaceae) . It has continued to be used as food, medicine and ornamental plant till date. Due to its ease of cultivation, potential productivity and above all its substantial nutritional value, the plant has spread all over the world including the tropics. Colloquially the plant is referred to as Chaya (*Donkoh et al.*, 1990). In the Western part of the Nigeria, it is called different names such as efo-Iyana-Ipaja and efo-Jerusalem,

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while in the Niger Delta of Nigeria, it has been nick-named “Hospital Too Far” or “Get Well Quick” because of its numerous traditional claims. Usually herbal medicines are widely perceived by the public as being natural, helpful and free from side effects. A number of studies exist reporting the toxic effect of herbal medicines (Calixto, 2000).

The liver is an organ involved in many metabolic functions and is prone to xenobiotic induced injuries because of their central role in xenobiotic metabolism (Sturgill and Lambert, 1977). Liver contains a host of enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), acid phosphatase (ACP) and alkaline phosphatase (ALP). Under normal circumstances, these enzymes reside within the cells of the liver. But when the liver is injured for any reason, these enzymes are spilled into the blood stream, raising the enzyme levels in the blood and signaling liver disease. Other blood tests pertaining to the liver are measurements of some of the other enzymes found the liver. In addition to AST and ALT, alkaline phosphatase 5' nucleotidase, and gamma-glutamyltranspeptidase (GGT) are other enzymes located in the liver.. Among the most sensitive and widely used liver enzymes are the aminotransferases. They include aspartate aminotransferase (AST or SGOT) and alanine aminotransferase (ALT or SGPT). The activities of these enzymes are used to assess the functional status of the liver and as the biochemical markers of liver damage (Moss & Ralph, 1999). This work evaluated the antibacterial activity of *Cnidioscolus aconitifolius* on bacterial strains and associated changes in liver enzymes in wistar rats.

MATERIALS AND METHODS

Collection of Plant Material:

Cnidioscolus aconitifolius leaves were collected from Obowo, Imo State and authenticated at College of Natural Resources and Environmental Management, Federal University of Agriculture, Umudike.

The leaves were separated from stems and washed in clean water and dried at room temperature. The dried plant leaves were then ground using a blender. The powdered samples were transferred into an air tight container and stored at room temperature.

Preparation of the Aqueous Plant Extract:

Two hundred grams (200g) of the powdered plant were measured into a conical flask and 500ml of hot water were added and left at room temperature for 48 hours. The extracts were filtered. The filtrate was evaporated to dryness on a water bath to give a crude extract. The extraction efficiency were qualified by determining the weight of the extract. The dried extract was stored in desiccators until required for use. The extract was dissolved in appropriate volume of distilled hot water to the desired concentration (Gidado *et al.*, 2005).

Preparation of the Ethanolic Plant Extract:

Two hundred grams (200g) of the powdered plant were measured into a conical flask and 500ml of 70% ethanol were added and left at room temperature for 48 hours. The extract was filtered. The filtrate was evaporated to dryness on a water bath (50°C) to give the crude extract, which the mass was determined.

Phytochemical Screening:

The methods described by Odebiyi and Sofowora (1978) were used to test for the presence of saponins, tannins, phenolics and alkaloids, Lieberman Burchard reaction as described by Herburne (1973) was used for steroids, while the Salkowski test was used to test for the presence of glycosides.

Sterility Test of the Plant Leave Extracts:

Each of the extracts (aqueous extract and ethanolic extract) was tested for contaminants. This was carried out by inoculating them on nutrient agar and incubation at 37°C for 24 hours. The plates were observed for growth. No growth in the extracts after incubation indicated that the extracts were sterile. The extracts were then assessed for antibacterial activity (Arekemase *et al.*, 2011).

Collection and Maintenance of Test Organisms:

The test organisms were clinical isolates from New General Hospital Umuguma, Owerri, Imo States. The organisms were *Staphylococcus aureus*, *E. coli*, *Shigella* species, *Salmonella* species and *Streptococcus pneumoniae*. The bacteria were maintained on nutrient agar slant and stored in the refrigerator at a temperature of 4°C. The bacteria were sub-cultured onto fresh media at regular intervals until they were used for the test.

Antibacterial Testing:

The antibacterial activity of the extracts (hot water and ethanol) was determined by agar well-diffusion method according to Amed *et al.* (1998). 0.5 McFarland standard (approx. 10⁸cfu/mL) was prepared using the

test organisms and 0.1ml of the bacterial species were mixed in Mueller Hinton Agar medium and poured in pre-sterilized Petri plates. A cork borer (6mm diameter) was used to punch wells in solidified medium and which was filled with extracts of 45 μ l of 200mg/ml final concentration of extracts. Selected solvent (i.e. hot water) was used as negative control. The efficacy of extracts against bacteria was compared with the broad spectrum antibiotic Ciprofloxacin (positive control). The plates were incubated at 37^oC for 24hrs in an incubator and the diameter of the zones of inhibition was measured in millimeter. Each sample was assayed in triplicate and the mean values were observed. The antibacterial activity was interpreted from the size of the diameter of zone of inhibition measured to the nearest millimeter (mm) as observed from the clear zones surrounding the wells.

Determination of Minimum Inhibitory Concentrations (MICS):

The MIC of the extracts (aqueous and ethanol) were performed by agar well diffusion method for all selected test pathogens. Concentrations of 200mg/ml, 150mg/ml, 100mg/ml, 50mg/ml, 20mg/ml of the extracts were prepared. The MIC of the extracts for the test micro-organisms were regarded as the lowest concentration that inhibited visible growth of the test organisms on the agar plate after 24hrs incubation at 37^oC.

Animal and Experimental Designs:

Forty male Wistar rats (107-167g) were used for this study. Forty rats were divided into two sets of twenty rats each for the assessment of the effect of the aqueous (Hot Water) and ethanolic extracts. Each set was divided into four experimental groups of five rats per group. Members of each group were housed in a standard rat cage and allowed to acclimatize to laboratory condition for one week. All rats were allowed free access to drinking water and rat feed.

Treatment of Animal for Chronic Study:

Rats in groups I, II, and III were administered with *Cnidoscopus aconitifolius* extracts at the doses of 100, 150 and 200mg/kg body weight per day for 3 weeks by gavages, respectively. Rats in group IV (control) received distilled water for a period of 3-weeks. The animals were observed daily for any signs of morbidity and mortality and their body weights were measured every one week during the experimental period.

Collection of Serum Samples for Analysis:

At the end of the experimental period (3wks) after an overnight fasting, all rats were sacrificed by decapitation. Blood was collected in tubes without anticoagulant to separate serum for various biochemical estimations. The samples were stored frozen until required for use.

Biochemical Analysis:

The total protein and the activities of some liver enzymes such as alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and acid phosphatase (ACP) in the serum collected were assayed using commercial kit (ALT, AST, ALP Randox kits–Rocwe Diagnostics, GmbH, Germany). Total protein was determined by following the method of Lowry *et al.* (1951) using bovine serum albumin (BSA), at 660nm.

Statistical Evaluation:

The results of the biochemical analysis were expressed as mean \pm SD for five animals in each group. The difference between the *Cnidoscopus aconitifolius* extract administered groups and control were analyzed by student's t-test. P-value <0.05 was considered as significant while tables and figures were used to express the results of the bacterial analysis.

Results:

The phytochemical screening carried out with *Cnidoscopus aconitifolius* leaves using hot water and ethanol showed the presence of some bioactive compounds – alkaloids, tannin, phlobatannin, phenol, saponins and cardiac glycosides while only the ethanolic extract of *C. aconitifolius* showed the presence of anthraquinones and flavonoids (Table 1).

The antibacterial activity was assessed by the measurement of zones of inhibition of various extracts of *C. aconitifolius* and the plant showed broad spectrum antibacterial activity at 200mg/ml, on *S. aureus* (19.7 \pm 1.5mm) *Shigella* species (7.0 \pm 0.6mm), *E. coli* (0.0mm), *Salmonella* species (5.0 \pm 0.1mm), *S. pneumoniae* (17.1 \pm 0.2mm) (Table 2). The mean zones of inhibition following exposure of both Gram positive (*S. aureus*, *S. pneumoniae*) and Gram negative (*Salmonella* species and *Shigella* species) bacteria to extracts of *C. aconitifolius* (200mg/ml) and ciprofloxacin (200mg/ml) are shown in fig.1 and fig 2, respectively.

Furthermore, the minimum inhibitory concentration was determined against the four clinical isolates that showed positive inhibition. The minimum inhibitory concentration of the ethanolic extract against *S. aureus* and *S. pneumoniae* was 10mg/ml while *Shigella* species and *Salmonella* species was 20mg /ml (Tables 3).

The mean body weight gain of the aqueous extract of *C. aconitifolius* administered on Wistar rats (groups) when compared with the control after 3 weeks duration of the study are group I (9.0%), group II (8.5%), group III (7.6%) and group IV-control (8.7%) and the results revealed that there was no significant difference ($P>0.05$) from control (Table 4). Also, after 3 weeks treatment with *C. aconitifolius* ethanolic extract, the mean body weight gain are group I (7.8%), group II(7.7%), group III (8.0%) and group IV-control (7.7%) this shows that there was no significant difference between the administered groups and the control group at $p>0.05$ (Table 5) .

The liver enzymes such as alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP) and acid phosphatase (ACP) were assessed to check the functional status of the liver and as the biochemical markers of liver damage. The levels of total proteins and activities of serum liver enzymes for *C. aconitifolius* aqueous extract on wistar rats (Group I-III) and control (Group IV) showed no significant difference when compared at $p>0.05$, while the mean of ALT, AST, ALP and ACP for *C. aconitifolius* aqueous extract of wistar rats (Groups I-III) and control (Group IV) showed no significant difference when compared (Table 6). Furthermore, *C. aconitifolius* ethanolic extract administered wistar rats (Group I-III) and control (Group IV) had levels of total proteins ($15.1\text{mg} \pm 0.1$, $15.1\text{mg} \pm 0.1$, $15.3\text{mg} \pm 0.0$ and $15.0\text{mg} \pm 0.2$) and the comparison of the mean levels of total proteins for *C. aconitifolius* ethanolic extract of Wistar rats and control showed no significant difference at $p>0.05$, the mean ALT, AST, ALP & ACP for *C. aconitifolius* ethanolic extract of wistar rats (Group I-III) was compared with the control (Groups IV) (Table 7).

Table 1: Phytochemical Constituents of *Cnidioscolus aconitifolius*

Phytochemical	Hot water extract	Ethanolic extract
Alkaloids	++	+++
Tannins	+	+++
Phlobatannin	++	+
Phenol	++	+++
Saponins	+++	++
Anthraquinones	-	+
Flavonoids	-	+
Cardenolides	-	-
Cardiac glycosides	+	+

+++ = appreciable amount, ++ = moderate amount, - = not detected

Table 2: Antimicrobial susceptibility pattern of extracts of *C. aconitifolius*

Organisms	Diameters of the inhibition zone (mm)		
	Hot water extract	Ethanolic extract	Ciprofloxacin
<i>S. aureus</i>	0.0	19.7±1.5	20.0±0.0
<i>E. coli</i>	0.0	0.0	20.7±0.9
<i>Shigella species</i>	0.0	7.0±0.6	30.2±0.0
<i>Salmonella species</i>	0.0	5.0±0.1	30.0±0.0
<i>S. pneumoniae</i>	0.0	17.1±0.2	21.0±0.8

Values are mean of three replicates and expressed as mean±SD, Cork borer diameter: 6mm.

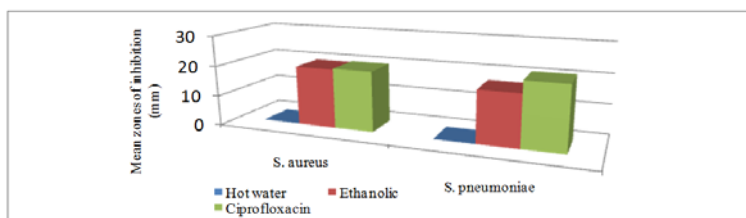


Fig. 1: Mean zones of inhibition following exposure of Gram positive bacteria to extracts of *C. aconitifolius* (200mg) and Ciprofloxacin (200mg).

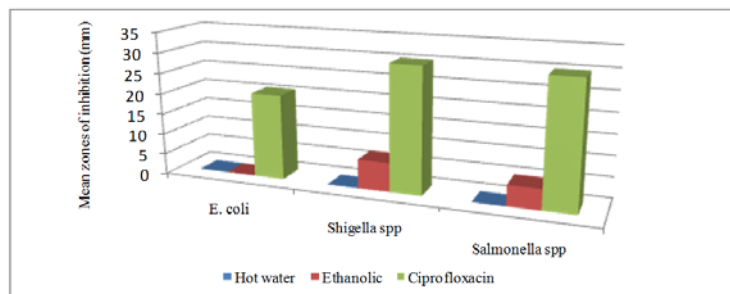


Fig. 2: Mean zones of inhibition following exposure of Gram negative bacteria to extracts of *C. aconitifolius* (200mg) and Ciprofloxacin (200mg).

Table 3: Determination of MIC Value of Ethanolic Extract of *C. aconitifolius* against tested clinical isolates.

Concentration Mg/ml	Test organisms			
	<i>S. aureus</i>	<i>Shigella sp.</i>	<i>Salmonella sp.</i>	<i>S. pneumoniae</i>
200	-	-	-	-
150	-	-	-	-
100	-	-	-	-
50	-	-	-	-
20	-	+	+	-
10	+	+	+	+

(-) = Absence of growth, (+) = Presence of growth

Table 4: Mean body weight changes before and after the 3 weeks treatment with *C. aconitifolius* aqueous extract.

Treatment	Body weight changes (g)		
	Initial	Final	Change (%)
Group I (100mg/kg)	131.0 ± 19.2	149 ± 18.3	9.0
Group II (150mg/kg)	139.8 ± 18.9	156.8 ± 16.7	8.5
Group III (200mg/kg)	137.0 ± 24.5	152.2 ± 22.5	7.6
Group IV (control)	137.2 ± 22.5	154.6 ± 15.2	8.7

N=5; values were expressed as mean ± SD; P> 0.05; not significantly different from control.

Table 5: Mean body weight changes before and after 3 weeks treatment with *C. aconitifolius* ethanolic extract.

Treatment	Body weight changes (g)		
	Initial	Final	Change (%)
Group I (100mg/kg)	140.0 ± 16.2	155.6 ± 13.5	7.8
Group II (150mg/kg)	139.4 ± 13.1	154.8 ± 11.6	7.7
Group III (200mg/kg)	139.8 ± 14.2	155.8 ± 10.7	8.0
Group IV (control)	139.6 ± 15.0	155.0 ± 13.6	7.7

N=5; values were expressed as mean ± SD; P> 0.05; not significantly different from control.

Table 6: Levels of total protein and activities of serum liver enzymes for *C. aconitifolius* aqueous extract of wistar rats and control.

Treatment	Total protein (mg/100g)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	ACP (IU/L)
Group I (100mg/kg)	25.7 ± 0.1	60.6 ± 0.2	27.3 ± 1.1	76.7 ± 0.3	136.4 ± 1.0
Group II (150mg/kg)	24.7 ± 0.1	60.1 ± 0.5	26.7 ± 1.1	79.3 ± 0.3	137.8 ± 0.9
Group III (200mg/kg)	26.3 ± 0.0	58.4 ± 0.6	26.0 ± 1.1	78.5 ± 0.3	137.2 ± 1.2
Group IV (control)	27.2 ± 0.2	57.0 ± 0.7	27.6 ± 0.5	80.2 ± 0.2	136.2 ± 1.1

N=5; values were expressed as mean ± SD; p> 0.05; not significantly different from control.

Table 7: Levels of total protein and activities of serum liver enzymes for *C. aconitifolius* ethanolic extract of wistar rats and control.

Treatment	Total protein (mg/100g)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	ACP (IU/L)
Group I (100mg/kg)	15.1 ± 0.1	31.8 ± 0.3	13.8 ± 0.7	100.3 ± 0.2	150.3 ± 1.1
Group II (150mg/kg)	15.1 ± 0.1	31.5 ± 0.4	15.6 ± 0.3	100.1 ± 0.1	151.9 ± 1.3
Group III (200mg/kg)	15.3 ± 0.0	30.1 ± 0.3	14.0 ± 1.0	102.8 ± 0.4	152.1 ± 1.0
Group IV (control)	15.0 ± 0.2	29.2 ± 0.3	13.5 ± 0.5	101.0 ± 0.4	150.9 ± 1.4

N=5; values were expressed as mean ± SD; p> 0.05; not significantly different from control.

Discussion:

The phytochemical screening carried out with *Cnidocolus aconitifolius* leaf showed the presence of bioactive compounds in the plant. An appreciable amount of alkaloids and tannins were obtained from the ethanolic extract than the aqueous extract. The presence of alkaloids has been implicated in its detoxifying and antihypertensive properties as a result of its stimulatory effects (Trease and Evans, 1989; Zee-chang, 1997), while the presence of tannins suggests the ability of this plant to play a role as anti-diarrhoea and anti-haemorrhagic agents (Asquith and Butler, 1986).

A moderate amount as well as appreciable amount of phenol was observed in the aqueous and ethanolic extracts, respectively. This indicates that the plant might play an important role as dietary antioxidants. Phenolic compounds prevent oxidative damage in living systems (Block, 1992; Hertog and Feskens, 1993).

Saponins though positive for both extracts, persistent frosting was intense in the aqueous extract (appreciable amount) than the ethanolic extract (moderate amount), this compound has been shown to have immense significance as antihypercholesterol, hypotensive and cardiac depressant properties (Trease and Evans, 1989; Price *et al.*, 1981).

Antraquinones, flavonoids were negative for the aqueous extract but positive for the ethanolic extract, the main reason that can be adduced from this is the mode of extraction. On this premise it will be advisable to extract the leaf of *C. aconitifolius* with ethanol in an attempt to exploit its antioxidant and antitumor properties (Olayinka *et al.*, 1992).

Cardenolides was absent in both extracts, thus the absence may not be a minus for the medical efficacies of *Cnidoscopus aconitifolius*. Cardiac glycosides showed positive results (minute amount) for both the aqueous and ethanolic extracts with no clear intensity in both extracts. The cardiac glycosides have been used for centuries as stimulants in cases of cardiac failure (Olayinka *et al.*, 1992). This perhaps justifies the already locally established function of the plant in the treatment and management of hypertension.

The growth of bacterial resistance to antibiotics is a threat to the world population with an increasing recurrence of infectious diseases due to the emergence of multidrug resistant bacteria that hinder chemotherapy (Aqil *et al.*, 2005). Therefore, in accordance with the established standards, the ethanolic extract showed the highest activity against Gram-positive bacteria (*S. aureus* and *Streptococcus pneumoniae*) because the wells are considered excellent tools to determine the antimicrobial susceptibility of organisms while the aqueous extract produced no inhibition on the organisms. This work is in accordance with Kuete *et al.* (2010), who have reported the antimicrobial properties for species of the family Euphorbiaceae. However, the ethanolic extract produced less inhibition on Gram negative bacterial species (*Shigella* sp. and *Salmonella* sp.) while the aqueous extract produced no inhibition on the organisms. Moreover, both the aqueous and ethanolic extracts showed no inhibition on *E. coli*. This result is consistent with Tadeu *et al.* (2012), who reported the antibacterial activity of four *Cnidoscopus* species against standard strains and clinical isolates.

Furthermore, minimum inhibitory concentration (MIC) was determined against the four pathogens (clinical isolates) that showed positive inhibition and was found that the lowest value was exhibited by *S. aureus* and *S. pneumoniae* at 20mg/ml, *Shigella* species and *salmonella* species at 50mg/ml concentrations, respectively.

Despite having shown promising results, the extract was inactive to *E. coli*, but less active against other Gram negative bacteria. This result may have occurred for two reasons: the absence or low relative concentration of potentially active compounds and structural differences of the Gram-negative bacteria has in relation to gram-positive that can hinder the action of the active components of the extracts (Ayres *et al.*, 2008). However, the ability of *C. aconitifolius* showing sensitivity to Gram positive and Gram negative bacteria shows its application as a broad spectrum antibacterial agent with the highest efficacy being the ethanolic extract in this study.

The mean body weight gain of the aqueous and ethanolic extract of *C. aconitifolius* administered groups showed no appreciable difference when compared with the control after 3 weeks duration of the study. This result agrees with the work done by Mordi and Akanji (2011), where *C. aconitifolius* extracts (aqueous & ethanolic) were administered to wistar rats for weeks and compared with the control and no appreciable difference was recorded in terms of body weight.

According to Sturgill and Lambert (1977), liver is an organ involved in many metabolic functions and is prone to xenobiotic induced injuries because of their central role in xenobiotic metabolism. Liver contains a host of enzymes such as AST, ALT, ACP and ALP. The activities of these enzymes are used to assess the functional status of the liver and as the biochemical markers of liver damage (Moss & Ralph, 1999). The results from this study showed that there were no increased activities of AST, ACT, ACP and ALP between *C. aconitifolius* extracts administered groups and the control group. This work is in accordance with the findings of Mordi and Akanji (2011).

Cnidoscopus aconitifolius administration at doses of 100, 150 and 200mg/kg body weight, may be safe from this study. The non-significant changes in the parameters studied, is suggestive to say that *C. aconitifolius* showed absence of cumulative toxicity.

Conclusion:

The qualitative phytochemical screening of the plant revealed the presence of alkaloids, tannins, saponins and phenol among others, both in aqueous extract and to greater quantity in the ethanolic extract. These bioactive agents may contribute to the medical efficacy of the plant. The mechanism of resistance of bacteria (Gram positive) to current antibiotics does not confer resistance to the compound present in the leaves of *C. aconitifolius*. *Ad hoc*, identification of the chemical constituents responsible for the antibacterial activities of the plant species may lead to the identification of new antibacterial drugs against these pathogens. Finally, results

obtained from the liver marker enzyme assay did not show any significant difference between the extracts administered groups and the control group.

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