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Research Article

Phytochemical screening and antibacterial activity of hydroalcoholic extracts from cloves of Cola nitida Schott &Endl.

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ABSTRACT

Infectious diseases are a real public health problem in the world. One of the strategies is to explore the traditional medicine virtue. *Cola nitida is* a medicinal plant used locally in Cameroon as traditional medicine for the treatment of these diseases. The cloves of *Cola nitida* has submitted for chemical screening by conventional techniques focusing on color reactions and chemical precipitation. The extracts were tested against eleven strains mainly *Salmonella typhimurium, Esherichia coli, Klebsiella pneumonia, Enterococcus faecalis, Staphylococcus aureus, Proteus mirabilis* and *Shigella as clinic strains* and *Staphylococcus aureus, Escherichia coli, Pseudomonas* and *Klebsiella pnuemoniae* as reference strains, using macrodilution in liquid medium. The phytochemical analysis showed the presence of alkaloids, polyphenols, flavonoids, saponins, tannins, quinones, coumarins and terpenoids. The Minimum Inhibitory Concentration (MIC) value of the hydroalcoholic extract was lowest for *Staphylococcus aureus* (0.78 mg/ml) and the lowest Minimum Bactericidal Concentration with *Shigella sp.* (1.56 mg/ml). Those values varied from 0.78 to 25 mg/ml in MIC and from 1.56 to 400 mg/ml in MBC. The most inhibition diagram was obtained at 28.5 mm with *Klebsiella pneumoniae.* These results suggested the use of *Cola nitida cloves* for the treatment of infectious diseases.

Keywords: *Cola nitida,* phytochemical screening, hydroalcoholic extract and antibacterial activities.

INTRODUCTION

Many plants were used in traditional to treat various diseases. Some of them possess antimicrobial properties. They have been used in different parts of the World to treat human diseases and infections as preservatives and as means of preventing microorganism development[1].*Cola* Schott &Endl. (Sterculiaceae) is a genus of about 125 species of trees indigenous to the tropical rain-forest African region[2]. Indeed, *C. nitida* is a plant native to tropical West Africa and belongs to Sterculiaceae family[3]. *Cola* is commonly used to counteract hunger and thirst, in some cases it is used to control vomiting in pregnant women. Also it is used as a principal stimulant to keep awake and with stand fatigue by students, drivers, and other menial workers. *Cola*

nitida is not advised for individuals with stomach ulcers due both to its caffeine and its tannin content[4].
The plant is used locally in Cameroon as traditional medicine for the treatment of infectious diseases where infectious diseases are among the most commonly reported and the largest cause of death[5]. These diseases are the leading cause of death in the world with nearly 17 million deaths each year[6].
Antibiotic resistance has become a serious and widespread problem in developing countries, causing high mortality each year[7]. The emergence of drug-resistant bacteria appears to be a major limitation of the use of antibiotics[8]. In order to resolve these health problems (resistance of microorganisms) the track of medicinal plants deserves to be explored[9] because many of them plants are without any scientific evidence of efficacy. Anti-bacterial compounds with herbal sources have a wide range of therapeutic use. These compounds are not only efficient for the treatment of infectious diseases, but also concurrently diminish existing side effects[10]. This implies that the search for alternative antimicrobial compounds is an urgent area of biomedical research and extracts derived from plants have long held interest as potential sources of new therapeutic agents[11].
In our research group, the investigation of the pharmacological properties of such plants, used in Cameroonian traditional medicine includes the evaluation of the activity of the crude organic extracts as well as that of the isolated compounds.
Therefore, the objective of this study was to evaluate the antibacterial activities of the hydroalcoholic clove extract *Cola nitida* against various microbial strains.

MATERIALS AND METHODS

Plant material

The cloves of *Cola nitida* were collected in April 2014 at Baboaté; village localized about 5 kilometers from Bafang City (Cameroon West Region). The botanical identification of the plants was done by the National Herbarium in Yaoundé, where the voucher specimens were conserved under the reference number 14 590 SRF.

Preparation of extract

The clove of *Cola nitida* were collected, dried at room temperature then crushed using a mortar and pestle to obtain a powder. The powder (2000 g) of *Cola nitida* was soaked at room temperature in 6000 mL of hydroalcoholic solution (70:30 v/v in water). During maceration, mixtures were stirred 3 times/ day. It was made in two stages that is, during two successive days with change of solvent every 48 hours (6000 = 3000 x 2). The first macerate was filtered using Whatman® N°1. The resulting filtrate was collected; and then 3000 mL of solvent were added to the residue for a new extraction. The two filtrates were combined into one single volume. The ethanol was evaporated at Rotavapor. The resulting solution was freeze-dried to remove residual water and get the crude extract. The extraction yield was 2.8 %.

Chemical screening

The phytochemical analyzes were performed, focusing on the color reaction and precipitate[12,13]. The phytochemical screening was summarized in the table 1.

Determination of minimum inhibitory concentrations (MIC)

Macrodilution technique in liquid medium was used[14]; Mueller Hinton broth (1000 µL) was introduced into 14 tubes making a range of dilution. The first volume of the extract was from a stock solution S, previously filtered on sterile membrane. Then 1000 µL of the stock solution S, being concentrated at 400 mg/mL, were introduced into the first tube of the dilution range. As a result, serial dilutions were in Mueller Hinton broth, so as to obtain a concentration range between 400 mg/mL and 195.10^{-3}mg/ml of plant extract. Then 15 µL of bacterial inoculum was added to each tube of the dilution range (except the controls), and then incubated at 37 °C. After 18 to 24 hours, the turbidity was visually evaluated, and the tubes were centrifuged at a speed of 5000 revolutions/minute for 5 minutes. The MIC of each test sample was derived from the first tube of the range within which any visible growth has not occurred.

Determination of minimum bactericidal concentrations (MBC)

The minimum bactericidal concentration (MBC for bacteria) is the minimum concentration corresponding to the lowest concentration of a substance capable of killing more than 99.9% of bacterial inoculum or initial (less than 0.1% of survivors) after 18 to 24 .hours of incubation at a temperature of 37 °C[15]; and MBC determination was based on the subculture of bacterial inoculum from the MIC on nutrient agar[16].
In each of the tubes in which visible growth was not observed and the control tube used in determining the MIC[15] samples were taken and then streaked on Mueller Hinton agar plates which were then incubated for 18-24 hours at 37 °C. MBC of each extract was derived from the first dilution (concentration) at which no culture was observed on Mueller Hinton agar.

Sensibility Test

The method of Cheesbrough (2000) cited by Shiriki[17] was used. The various isolates were each inoculated and incubated at temperatures ranging from 28˚C to 42˚C and their growth and radial growth diameter were observed and measured.

RESULTS

The results in the Table 2 represent the phytochemical analysis. According it, the table showed le present of many compounds (secondary metabolites) mainly alkaloids, polyphenols compounds, saponosides, quinones, terpenoids, coumarins, flavonoids and tannins. However, the method used did not show steroids, mucilage and anthocyanins.

According the Table 3 and Figure 1, the MIC value of *Cola nitida* was lowest in the extract against *Staphylococcus aureus* (clinical strain) with MIC value 0.78 mg/mL; than 1.56 mg/mL for *Shigella sp*. however it was greater for *Proteus mirabilis* (25 mg/mL). We found the same value with *Klebsiella pneumonia, Staphylococcus aureus, Enterococcus faecalis* and *Enterococcus faecalis* (3.125 mg/mL). The MBC value varied from 12.5 mg/mL (*Klebsiella pneumonia, Klebsiella pneumonia* CS, *Staphylococcus aureus* and *Staphylococcus aureus* CS). The greatest value was 400 mg/mL with *Echerichia coli; Proteus mirabilis* CS and *Salmonella sp.*

The Table 4 showed the largest diameter of inhibition (28.5 mm) of extract was obtained on *Klebsiella pneumonia*, while the lowest (11.5 mm) was recorded with *Proteus mirabilis.* The other largest values were 22.5 mm (*Escherichia coli)*, 21 mm (*Shigella sp*) and 19.5 (*Enterococcus faecalis*).

DISCUSSION

The phytochemical analysis performed on clove from *Cola nitida* revealed the presence of several secondary metabolites (Table 2), of which alkaloids, polyphenols compounds, saponosides, quinones, terpenoids, coumarins, flavonoids and tannins. However, the method used did not show steroids, mucilage and anthocyanins. Various studies have shown that plants that are rich in these secondary metabolites. Our results are the same as those found by Dah-Nouvlessouno[9] when they showed in their study the presence of tannins, saponosides, anthocyanins, flavonoids and cardiac glycosides in the bark of *Cola nitida*. The presence of the secondary metabolites (alkaloids, saponins and tannins) in *Cola millenii* both seed and pulp are also confirmed and recognized as criteria in the classification of *Cola* species[18]. Many studies reported several metabolites in different parts and extract of *Cola nitida* including alkaloids, tannins, terpenes, steroids, phenols, cardiac glycosides, alkaloids and flavonoids[19,20].

The presence of phytochemical components that exhibit inhibitory activity on microbes is an indication that such plants may contain bioactive components that are useful for preparation of pharmaceuticals[21]. Previous studied have shown antibacterial properties of these secondary metabolites, including flavonoids, saponins, steroids and terpenes[15].

The antibacterial activity of plant extracts can be attributed not only to a single bioactive principle but also in concert action with other compounds. A number of phytochemicals have been studied for their antimicrobial activity and found potentially useful against infectious diseases. The chemical structure of the antimicrobial agents found in higher plants belong to most commonly encountered classes of higher plants secondary metabolites such as flavonoids, terpenes, terpenoids and phenolic acids[22].

The tannins have an antibacterial effect by precipitating their proteins making unavailable their nutritional proteins[5]. Flavonoids, triterpenoids, flavonoids, alkaloids, saponins, Tannins and quinones have been reported to be used by plants for protection against bacterial and are responsible for antimicrobial activity[23]. Generally, the phenolic compounds have been found to be quite active, both as bactericidal and bacteriostatic, the effect being contingent upon a medium pH, although some authors have found low efficiency for individual compounds[24].

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were variable depending on the strains. The MIC value of *Cola nitida* was lowest in the extract against *Staphylococcus aureus* and greater for *Proteus mirabilis* (25 mg/mL).

According to a one study[25], the *in vitro* antimicrobial evaluation of ethanol extracts of four species of *Cola* Schott & Endl. was done using human isolated strains of *Staphylococcus aureus, Staphylococcus albus, Bacillus subtilis, Klebsiella pneumonia, Pseudomonas aeruginosa, Candida albicans, Aspergillus niger* as test organisms. The leaf ethanol extracts of the plants were found to be more effective against the tested fungi than the bacteria at high concentrations.

One study reported that the seeds aqueous extract of *G. kola* had an antimicrobial activity against clinical isolates of *S. aureus*, *P. aeruginosa* and *E. coli* at 50 mg / ml with a mean inhibition diameter of 3.66 ± 0.28 mm[26].

According to Abalaka[27], methanol and water soluble fractions of *Cola nitida* possess antibacterial activity, *G. kola* was more active against some members of Enterobacteriaceae, namely, *Escherichia coli* and *Salmonella typhi,* whereas, methanol extracts of *Colanitida* showed grater activity on *S. aureus.* Thus, the plants possess potentials for the manufacture of potent drugs for the treatment of infections caused by the test organisms, such as typhoid fever, gastroenteritis, urogenital tract infections and boils.

The result of this study revealed that the extract are more effective against test organisms compared to some standard antibiotic (Gentamycin, Nalixic acid) with a MIC value of 62.5 mg/ml cited by Itoandon[27]. The MBC value varied from 12.5 mg/mL (*Klebsiella pneumonia, Klebsiella pneumonia* CS, *Staphylococcus aureus* and *Staphylococcus aureus* CS) to 400 mg/mL (*Escherichia coli, Proteus mirabilis* and *Salmonella sp*).

Table1
Phytochemical Screening of clove from Cola nitida

Métabolite	***Reagent/Methods***	***Indicator***
Polyphenols	Ferricchloride	Greenishcolor.
	Lead acetate	Whitishprecipitate
Flavonoids	Sodium hydroxyde	Yellow-orange color
	Sulfuricacid	Yellowcolor
	Hodger	Reddishprecipitate
	Wagner	Whitishprecipitate
Alkaloides	Mayer	Whitish-yellowcreamyprecipitate
Saponosids	Frothing test	Persistent frothing
Tannins	Copper sulphate/ammonia	Green, purple, blue or black color
Anthocyanins	Sulfuricacid/Ammonia	Purple-blue
Quinones	Sulfuricacid	Redcolor
Mucilages	Ethanol 95°	Air bubbles
Anthocyanins	Sulfuricacid/Ammonia	Purple-blue
Terpenoids	Chloroform/sulfuricacid	Reddish-brown coloration
Steroids	Liebermann-Burchard	Green color
Coumarins	Ferricchloride/nitricacid	Green or bluecolor

Table 2
Result of Phytochemical Screening of clove from Cola nitida

Métabolite	***Reagent/Methods***	***Result***
Polyphenols	Ferricchloride	+
	Lead acetate	+
Flavonoids	Sodium hydroxyde	+
	Sulfuricacid	+
	Hodger	-
	Wagner	+
Alkaloides	Mayer	+
Saponosids	Frothing test	+
Tannins	Copper sulphate/ammonia	+
Anthocyanins	Sulfuricacid/Ammonia	-
Quinones	Sulfuricacid	+
Mucilages	Ethanol 95%	-
Terpenoids	Chloroform/sulfuricacid	+
Steroids	Liebermann-Burchard	-
Coumarins	Ferricchloride/nitricacid	+

Legend: ***+: Positive test*** ***-: Negative test***

Table 3
Antimicrobial activity of clove from Cola nitida

Microbialstrains	*MIC*(mg/mL)	*MBC*(mg/mL)	*MBC/MIC*
Escherichia coli	12.5	400	32
Escherichia coli CS	6.25	100	16
Klebsiella pneumoniae	3.125	12.5	4
Klebsiella pneumoniae CS	1.56	12.5	8
Proteus mirabilis CS	25	400	16
Staphylococcus aureus	3.125	12.5	4
Staphylococcus aureus CS	0.78	12.5	16
Enterococcus faecalis	3.125	100	32
Enterococcus faecalis CS	3.125	200	64
Shigella sp	1.56	1.56	1
Salmonella sp	12.5	400	64

Legend. CS: Clinical Strain

Table 4
Zone of inhibition of Cola nitida extract with test organisms in mm

Microbialstrains	*Inhibition diameter*	*Inhibition diameter*	*Average*
Escherichia coli	25	20	22.5
Klebsiella pneumoniae	15	15	15
Klebsiella pneumoniae CS	30	27	28.5
Proteus mirabilis CS	13	10	11.5
Staphylococcus aureus	16	15	15.5
Staphylococcus aureus CS	12	12	12
Enterococcus faecalis	19	20	19.5
Enterococcus faecalis CS	14	11	12.5
Shigella sp	20	22	21
Salmonella sp	14	14	14

The ratio MBC/MIC shows the kind of effect exerted by the extracts on the tested strains. The results of this ratio show that the extracts have bactericidal effects on reference strains. Despite the weak antibacterial property of *Cola nitida*, its inhibitory effect on *Staphylococcus aureus* advocates for its broad spectrum antimicrobial properties against both Gram negative and positive organisms. The antimicrobial studies revealed that the extract of *Cola nitida* showed inhibitory effects on *Klebsiella pneumoniae CS* (28.5 mm), *E. coli* (22.5 mm) and *Shigella sp* (21 mm).

These results are near to those obtained with *E. coli. P. aeruginosa, S. aureus* and *K. pneumonia* with zone of inhibition of 20.8, 25.3, 29.9 and 27.5mm respectively with the crude extract of *Combretodendron macrocarpum* Stem Bark[28]. They are compared to the results obtained by Lateef[25] where the antimicrobial analysis showed the total obliteration of *E. coli* and *P. aeruginosa*, as well as of *A. niger*, *A. fumigates* and *A. flavus*, in AgNPs-treated paint compared to the abundant growth observed in the control. The results of the sensitivity of the organisms to aqueous *Cola* nut extracts at *Escherichia coli* and the diameter of zone of inhibition around *Staphylococcus aureus* for dilution 10^{-1} was 20±0.1mm while that of dilution 10^{-2} was 13±0.3mm[27]. Our results were close toofObey[29] where an antibacterial activity of *Cola nitida* methanol extract against selected pathogenic microorganisms showed an inhibition zone of 13±0.577 mm (*Proteus vulgaris) against* 26.00±0.577 for penicillin as drug reference.

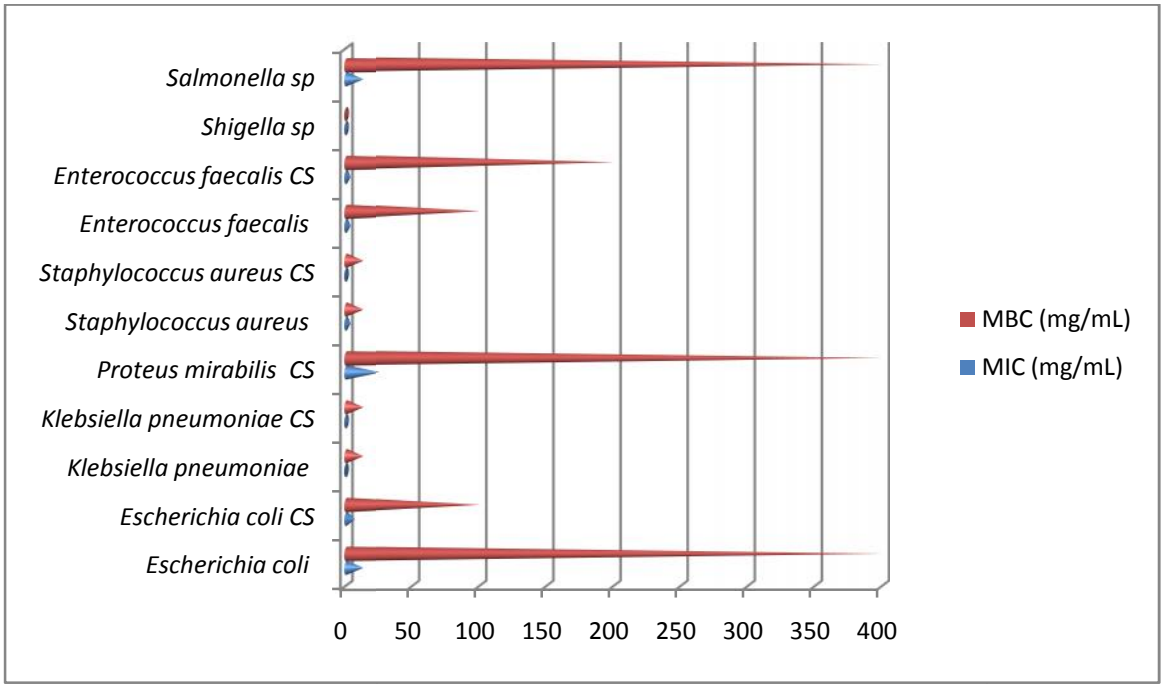

Figure 1
Evolution of minimum inhibitory concentrations (MIC) and bactericidal (MBC).

CONCLUSION

The result of this present study showed that the extract of *Cola nitida* clove possessed antimicrobial properties. The phytochemical analysis suggested that this plant contains secondary metabolites which will be used against pathogenic microorganisms. Other extraction methods should be carried out in future in view of enhancing its antimicrobial potentials. Therefore, further investigation needs to be carried out on the toxicity of the plant.

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