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Phytochemical Screening and Biological Activity Studies of the Extract from the Bark of *Ricinodendron heudoletti*, Euphorbiaceae

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Abstract: The fractions and pure compounds isolated from the bark of *R. heudoletti* were tested for their antibacterial properties on negative and positive gram bacteria using the disc diffusion method. Phytochemical analysis of the fractions and pure compound isolated was also conducted. The inhibitions parameters of the fractions and pure compound were determined using macro dilution method. The results showed that fraction and isoflavonoid exhibited a significant antibacterial effect against twelve strains isolated from patients. These micro-organisms were Gram-negative (*Escherichia coli, Salmonella typhi, Shigella flexneri*, *Pseudomonas aeroginosa, Enterobacter cloacae, Klebsiella pneumoniae, Klebsiella oxytoca, Morganella morganii, Citrobacter freundi, and Proteus vulgaris)* and positive gram (*Staphylococcus aureus, and Streptococcus feacalis)*. The ratio of minimal bactericidal concentration (MBC) over the minimal inhibitory concentration (MIC) indicated a promising bactericidal effect of fractions and pure compounds isolated. These results support the current common use of the bark of *Ricinodendron heudoletti* and flavonoids in the treatment of some infectious diseases.

Keywords: Biochemistry Properties, Flavonoids, Infectious Diseases, *Ricinodendron heudoletti*

1. Introduction

Medicinal plants constitute an important aspect of study for the development of phytomedicine. Studies on traditional medicine has become an important priority of the World health organization since almost 80% of the population in developing economies use medicinal plants as alternative source of treatment of diseases [1, 2]. The search for new chemical entities from herbal materials has increase the interest of Cameroonian scientist to exploit the rich cocktail of plant biodiversity for phytochemical screening of potential compounds of pharmaceutical importance and test of activities [3, 4]. The current problem of bacterial resistance and nosocomial infection has led to more research on the use of medicinal plant research for potential new drugs in the drug development pipeline. [1, 5]. The bark extract of *R. heudoletti* is used against cough, as poison antidote and for the treatment of intestinal diseases. The bark is also used to

treat yellow fever, malaria, headache, stomach pains and in some cases it can help pregnant women [6]. Infusion of barks of *R. heudelotii* has been shown to possess diuretic and aphrodisiac effects. The leaves are used to treat dysentery and the fruits can be used as spices [6, 7]. *R. heudelotii* is widely used for the above mentioned biological activities; no toxicological study of the plant has been reported previously as well as its in vitro antibacterial and antioxidant activities in Cameroon [8]. As a contribution to the search for non-toxic, novel antibacterial properties from medicinal plants of Cameroon.

2. Materials and Methods

2.1. Plant Material

The bark of *R. heudoletti* was collected at Minlamizibi in the South Region of Cameroun in January, 2009. The biological identification of the plant was done by the national herbarium in Yaoundé where the voucher identification was assigned under the reference number 16610/SFR/CAM

2.2. Chemicals

The antibiotics gentamicin was purchased from local community pharmacy. Methanol ethanol, ethyl acetate, hexane chloroform, nutrient agar and nutrient broth were purchased from Merck Company and the other chemicals used were from Sigma Company

2.3. Antibacterial Assay Microorganisms

Twelve bacterial strains isolated from patients at the "Centre Pasteur of Cameroon", a reference center for disease diagnostic and identification were used for the evaluation of antibacterial activity. These micro-organisms were Gram-negative **(***Escherichia coli, Salmonella typhi, Shigella flexneri*, *Pseudomonas aeroginosa, Enterobacter cloacae, Klebsiella pneumoniae, Klebsiella oxytoca, Morganella morganii, Citrobacter freundi, and Proteus vulgaris)* and positive gram (*Staphylococcus aureus, and Streptococcus feacalis)*.

2.4. Inocula Preparation

An inoculum for each micro-organism was prepared from broth cultures containing approximately 5.10^5 to 9.10^6 colony forming units per mililiter (CFU/ml). Each diluted into (1:50) inoculum, was applied as a lawn with a micropipette calibrated to deliver 50 µl containing around 9.10^6 CFU [9]. The discs impregnated with extract and pure methanol only to show if there was any activity using pure methanol solvent, were evaporated for 24 hours at sterile condition. The inoculated plates were incubated at 37°C for 24 h. Each assay in this experiment was repeated 3 times.

2.5. Determination of the Diameters of Inhibition Zone

The extracts were tested *in vitro* for antibacterial activity by the standard disc diffusion method against the micro-organisms at a concentration of 80 mg/ml with pure methanol. Gentamycin used as standard antibiotic (positive control) was tested at a concentration of 1 µg/µl. The diameters of inhibition zones produced by these extracts and disc impregnated with pure methanol were then compared to standard antibiotic (Gentamycin).

2.6. Minimum Inhibitory Concentration (MIC) Determination

For MICs determination, only the most sensitive microorganisms were tested with the extract. Serial dilutions were from 75, 93 µg/ml to 3000 µg/ml of extract in the nutrient broth medium. 100 µl of the suspension of each pathogenic bacterium (10^6 cells/ml) were added and incubated at 37°C for 24 h. Standard antibiotic (positive control) was tested in the concentration between 2.5 to 80 µg/ml. The lowest concentration which did not show any macroscopic growth of tested microorganism was identified as the MIC.

2.7. Minimum Bactericidal Concentration (MBC) Determination

For MBC determination, 100 µl of each tube of bacterial strain was subculture in the nutrient broth medium at 37°C for 24 h. The absence of any macroscopic growth of the nearest MIC tube was identified as the MBC [9].

2.8. Phytochemical Analysis

Phytochemical tests were carried out as described by Odebiyi and Sofowara [10]. The phytochemical analyzes were performed, focusing on the color reaction and precipitate. The phytochemical screening has been summarized in table 1A and 1B.

Table 1a. *Phytochemical Screening of the hydroalcoholic extract and its fractions [11, 12, 13].*

Métabolite	Reagent Methods	Indicator
Polyphenols	Ferric chloride	Greenish color.
	Lead acetate	Whitish precipitate
Flavonoids	Iso amyl alcohol/Magnesium + hydrochloric acid	Pink color or red color
Alkaloids	Hodger	Reddish precipitate
	Wagner	Whitish precipitate
	Mayer	Whitish-yellow creamy precipitate
Cardiac glycosides	Glacial acetic acid/ Ferric chloride + Sulfuric acid	Green-blue color
Saponosides	Frothing test	Persistent frothing

Métabolite	Reagent Methods	Indicator
Tannins	Ferric chloride	Green, blue or black color
Anthocyanins	Sulfuric acid/Ammonia	Purple-blue
Quinones	Sodium hydroxyde	Red or purpe color
Mucilages	Ethanol 95°	Air bubbles
Resins	Glacial acetic acid/ Sulfuric acid	Yellow color
Betalains	Sodium hydroxyde	Yellow color
Terpenoids and steroids	Liebermann-Burchard	Pink, blue or grey color
Coumarins	Ferric chloride/nitric acid	Green or blue color
Oxalates	Glacial acetic acid	Black or green color

2.9. Extraction and Purification

The bark of R. heudoletti was air dried and pulverized. The resulting powder (5 kg) was macerated in methanol for 72 hours; the concentrated methanolic extract was treated with hydrochloric acid (5%). The aqueous solution was made alkaline with ammonia and extracted with acetate. The methanolic extract was screened for antibacterial activity. The active fractions were subjected to a bio-guided fractionation by flash chromatography on silica column. Gradient elution using hexane- ethyl acetate (Hex-AE). The active fractions F1 (6.05 g) obtained with methanol-ethyl acetate (Me-EA) (20%) was purified by sephadex chromatography gradient eluted with hexane ethyl acetate to afford 14 fractions (FA1-FA14). The active fraction FA1 and FA10 was crystallized from acetone to yield 422 mg of shiny yellow crystals and 300 mg of shiny white crystals. Those 422 mg yellow crystals were identified as flavonoids (Iso flavonoids) on the basis of spectrophotometric data

3. Results and Discussions

3.1. Phytochemical Screening

The results of phytochemical screening as shown in table 1, showed the presence of alkaloids, poly phenol, flavonoids, saponins, cardiac glycoside, triterpenes. The poly phenols group constitutes the principal component of *R. heudoletti* extract with three bioactive subgroup: tannins flavonoids and saponins. Alkaloids and steroids whose anti micro bacterial activities have been demonstrated were also found in this extract. Earlier studies have associated high accumulation of alcaloids [2], flavonoids [14], cardiac glycoside and saponins [3, 17], all linked to promising antibacterial and antioxidant activities in studied medicinal plants.

Table 1b. *Phytochemical screening of R. heudoletti extract.*

Components	Hexane extract	Ethyl acetate Extract	Ethanolic Extract of.	Methanol Extract
Phenol	+	+	-	-
Polyphenol	+	+	++	++
Flavonoids	-	+	++	++
Saponins	-	+	+	++
Cardiac Glycosides	-	+	++	++
Triterpens	-	+	+	+
alkaloids	+	+	+	+

(+) Presence of component; (++) Abundance of component; (-) Absence of component

The results of thin layer chromatography table 2 showed that methanol and ethyl acetate 10% were the best eluted system that separated the compound for the crude extract of *R. heudoletti* and that explicate the differential solubility of phenolics component in the high polar system. This result was collaborated the work of [1] methanol and ethyl acetate were good solvent of elution of plant extracts separation

Table 2. *Chromatogram of the methanol extract from R. heudoletti.*

Chromatogram of the methanol's extract	S_1: Hex-AE 10%	S_2: Hex-AE 40%	S_3: AE pur	S_4: AE-MeOH 5%	S_5: AE-MeOH 10%
UV: 365 nm	1 blue stain	-1 blue fluorescent strain -1 green stain	-1 green stain -1 blue and fluorescent strain -1 green strain	-1 violet strain -2 green and clear strains -1 bleu fluorescent strain	-1 violet strain -2 green and clear strains -1 blue fluorescent strain
UV: 254 nm	1 stain	2 blue and dark stains	2 blue and dark stains	-1 green strain 4 Blue and dark stains	-1 green strain 4 blue and dark stains
After pulverization at H_2SO_4 50%, Δ=105°C	No migration	Two stains	3 stains	5 stains	5 stains

The methanol acetate ethyl system (5 and 10%) fraction of the methanolic extract of *R. heudoletti* released 5 components coloured in green clear, blue fluorescent violet. These characteristics indicated the presence of flavonons, flavons flavonols and glycosids [15].

The results of flash chromatography showed the fractions from methanolic 100% elution given 0.84 g. The methanol-ethyl acetate system (10, 20, 30, 50%) produced fractions

weight of 7 g.

Table 3. *Sephadex chromatography of plant extract fractions.*

Elution solution	Fractions	Observations and results
Hex-EA 50%	1-6	Oily product
	7-20	flow
	21-26	Flow and stain
	27-34	flow
	35-44	Flow and stain
	45-74	flow
	75-82	flow
	83-99	nothing
Hex-EA 60%	100-109	nothing
Pure ethyl acetate	110-111	Nothing
	112-113	flow
	114-115	Flow and strain
	116-122	Flow and strain
	123-137	nothing
	138-147	flow
AE-MeOH 5%	148-150	Nothing
	151-160	Yellow shiny crystals
	161-176	Yellow shiny crystals
AE-MeOH 10%	177-200	Yellow shiny crystals
	201-210	Yellow shiny crystals
	211-234	Yellow crystals
AE-MeOH 15%	235	White crystal
	236-244	White crystal
	245-254	White crystal
	255-263	White crystal
AE-MeOH 20%	264-275	Nothing
AE-MeOH 25%	276-282	Nothing
AE-MeOH 30%	283-288	Nothing
AE-MeOH 35%	289-293	Nothing

The flash and sephadex chromatography showed 2 pure components were isolated with 422 mg of the shiny yellow crystals and 300 mg of white crystals (table 4). Many fractions were expressed with the different elution solution, most especially with the EA/MeOH 10%, EA/MeOH 20%, EA/MeOH 30%.

Table 4. *Flash chromatography.*

Elution solution	Fractions	TLC	Observations and results
Hex/EA 20%	1-6	One strain with flow	Fatty products
	7-15		
Hex/EA 30%	16-20	Flows with one strain	Impure products
	21-25		
Hex/EA 40%	26-35	flow	
Hex/EA 50%	36-40	flow	
Pure EA	41-50	Flow with 1 strain	Group of Fractions regrouped for purification
EA/MeOH 10%	51-54		Group of Fractions regrouped for purification Wt=2.9 g
EA/MeOH 10%	55-60	1 strain and flows	Group of Fractions regrouped for purification Wt=3.15 g
EA/MeOH 20%	61-70		
EA/MeOH 30%	71-80		Fractions regrouped for purification Wt=0.97 g
EA/MeOH 50%	81-90	Strain and flow	Group of Fractions regrouped for purification
MeOH 100%	91-93	washing	Group of Fractions regrouped for Wt=0.84 g

The first compound with 422 mg with shiny yellow crystal was identified as glycosyl flavonoids the TLC demonstrated that it was a polar compound that showed a violet fluorescence at 365 nm UV figure 1. Other works of Nawel et al [16] and Lahouel [12] had used TLC for the identification of glycosyl flavonoids.

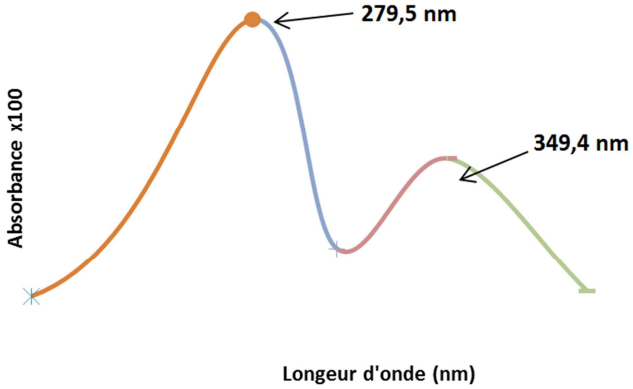

Figure 1. *UV spectrum of the yellow crystal compound.*

This compound was identified as a glycosyl flavonoid with substitution at the 3 position using IR, SM, RMN1H, ^{13}C and RMN 2D as shown in figure 2.

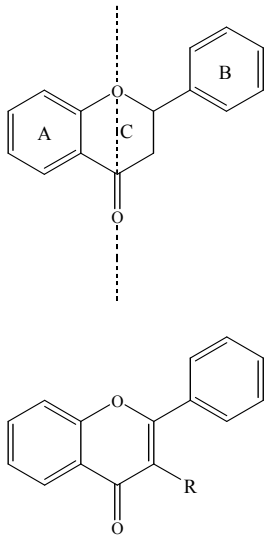

Figure 2. *Glycosyl flavonoids isolated.*

3.2. Antibacterial Activity of Crude Extract of R. Heudoletti

The results of the *in vitro* antibacterial activity of methanol extracts determined by diameters of inhibition zones are presented in Table 5.

Assessment of antibacterial activity was made by the disc diffusion method with 80 mg / ml concentration. Assay done on 12 bacteria strains. *In vitro* studies of the crude extract of R. *heudoletti* on most germs showed antibacterial inhibition diameter from 12 mm to 20 mm. These results indicated that the diameters of inhibition zones varied from 12- 19.67 mm and 26 – 34.67 mm for the extracts and gentamycin respectively. Gentamycin used as a standard antibiotic at the concentration of 1 µg/µl exhibited higher diameters of inhibition than other extracts. No diameters of inhibition

zones was obtained with dics containing pure methanol. Among the twelve isolates, eight bacteria (*Staphylococcus aureus*; *Klebsiella pneumonia*; *Streptococcus feacalis*; *Escherichia coli*; *Pseudomonas aeruginosa*; *Morganella morganii*; *Citrobacter freundi*; *Enterobacter Cloacae*) were sensitive to the extract. However, *P. aeruginosa* was the most sensitive with 19.67 mm. *S. typhi; K. oxytoca; S. flexneri*; *P. vulgaris;* were the most resistant bacteria isolate with no diameters of inhibition zones. Studies conducted by Nawel et al [16] have shown that plant extracts with active phytochemical compounds like alkaloids, saponins and cardiac glycosides to be potent antibacterial and antimutagenic activities and Suh et al [18] also confirmed anticancer and antioxidant activities associated with alkaloids, coumarins and flavonoids.

Table 5. *Antibacterial activities of R. heudolotti from the disc diffusion method.*

Extracts	Bacterial strains											
	S. aureus	***Kb. pneumo***	***S. feacalis***	***S. typhi***	***Kb. oxy***	***S. flexneri***	***E. Coli***	***P. vulgaris***	***P. aeruginosa***	***M. morganii***	***C. freundi***	***E. cloacae***
Gentamicin	34.33±1.53	26±2.65	31±2	27.33±6.51	32±1	27.33±1.53	26±1	26±3.61	34.67±1.53	30.33±1.53	30.67±2.52	28±1.73
R. heudelotii	12±0	15.33±0.58	12.67±0.58	/	/	/	12.33±0.58	/	19.67±1.59	14.67±0.58	16±1	12.33±1.15

S. aureus =Staphylococcus aureus; Kb. pneumo =Klebsiella pneumonia; S. feacalis =Streptococcus feacalis; S. typhi =Salmonella typhi; Kb. oxy =Klebsiella oxytoca; S. flexneri = Shigella flexneri; E. coli =Escherichia coli; P. vulgaris =Proteus vulgaris P. aeruginosa =Pseudomonas aeruginosa; M. morganii = Morganella morganii; C. freundi =Citrobacter freundi; E. cloacae = Enterobacter Cloacae;

The results of the antibacterial activity of the extract determined by Minimal Inhibitory Concentration (MIC) and Minimal Bactericide Concentration (MBC) are represented in Table 6. The MICs ranged between 188 and 750 µg/ml and The MBC ranged between 375 and 1500 µg/ml. These results revealed that *P. aeruginosa* was the most sensitive with values of 188 µg/ml (Table 6). The MIC of the extract of *R. heudelotii* was less active than standard antibiotic (gentamycin) ranged between 5 and 10 µg/ml. No result of MIC and MBC was determined for *Salmonella typhi*, *Klebsiella oxytoca, Shigella flexneri*, *Proteus vulgaris* isolates.

Table 6. *MIC, MBC values (µg/ml) of R. heudolotti extract in the macro dilution assay comparable to gentamicin.*

Extract/gentamicin	Parameters (ug/ml)	Bacterial strains										
		SA	**KP**	**AB**	**ST**	**KO**	**SF**	**EC**	**PV**	**PA**	**MM**	**CF**	**EC***
R. *heudelotii*	MIC	750	375	750	/	/	/	750	/	188	375	375	750
	MBC	1500	750	1500	/	/	/	1500	/	375	750	750	1500
	MBC/MIC	2	2	2	/	/	/	2	/	2	2	2	2
Gentamicin	MIC	10	10	10	20	10	10	10	10	10	5	10	10
	MBC	20	20	20	40	20	20	20	40	20	10	20	20
	MBC/MIC	2	2	2	2	2	2	2	2	2	2	2	2

S. aureus =Staphylococcus aureus; Kb. pneumo =Klebsiella pneumonia; S. feacalis =Streptococcus feacalis; S. typhi =Salmonella typhi; Kb. oxy =Klebsiella oxytoca; S. flexneri = Shigella flexneri; E. coli =Escherichia coli; P. vulgaris =Proteus vulgaris P. aeruginosa =Pseudomonas aeruginosa; M. morganii = Morganella morganii; C. freundi =Citrobacter freundi; E. cloacae = Enterobacter Cloacae;

The results of antibacterial activity of the fraction were determined in the table 6. The fraction of methanol showed the best antibacterial activity than the fraction of acetone and methanol-ethyl acetate. The inhibitor diameter of the methanolic fraction varied from 12±0.6 to 14±0.66. The different fractions isolated given a good significant ratio MBC/MIC with values between 4 and 1. (Table 6).

Antimicrobial activity of fractions of the extracts of *R heudelotti* from the disc diffusion method showed for methanol extract lowest diameter of 12±0.6 for SA followed by 13 for AB (Table 7). Acetone extract showed the lowest diameter of 10 with respect to the methanol and methanol ethyl acetate.

Table 7. *Antibacterial activity of fractions of the extracts of R. heudelotti from the disc diffusion method.*

Fxtract/Bac	SA	KB	AB	ST	KO	SF	EC	PV	PA	MM	CF	EC*
Methanol (M)	12±0.6	14±0.3	13±0	/	/	/	13±0.33	/	14±0.66	14±0	14±0	0
Acetone (A)	10±0	11±0.66	11±0	/	/	/	11±0.7	/	11±0.6	12±0	11±0	0
Methanolethyl acetatel	13±1.3	13±0. 66	12±0.66	/	/	/	12±0	/	14±0.33	12±1.7	14±1.33	0

SA=Staphylococcus aureus; KB=Klebsiella pneumonia; AB=Streptococcus feacalis; ST=Salmonella typhi; KO=Klebsiella oxytoca; SF= Shigella flexneri; EC=Escherichia coli; PV=Proteus vulgaris PA=Pseudomonas aeruginosa; MM= Morganella morganii; CF=Citrobacter freundii; EC*= Enterobacter Cloacae; /= not determined

The MIC, MBC of *R heudolotti* fraction in the micro dilution assay showed the lowest MIC of 6.25 ug/ml recorded on bacterial strain KB with the methanol and methanol ethyl acetate. Lowest MBC was recorded for the methanol ethyl acetate of 6.25 on the bacterial strain MM. The MBC/MIC ration was lowest for the methanol extract treatment for AB

and MM, followed by SA and PA respectively.

Table 8. *MIC, MBC values (µg/ml) of R. heudolotti fraction in the micro dilution assay.*

Fract/Bac		SA	KB	AB	ST	KO	SF	EC	PV	PA	MM	CF	EC*
methanol	MIC	12.5	6.25	12.5	/	/	/	6.25	/	12.5	12.5	/	/
	MBC	25	25	25	/	/	/	25	/	25	12.5	/	/
	MBC /MIC	2	4	1	/	/	/	4	/	2	1	/	/
Acétone	MIC	12.5	12.5	12.5	/	/	/	12.5	/	12.5	12.5	/	/
	MBC	50	50	50	/	/	/	50	/	50	50	/	/
	MBC /MIC	4	4	4	/	/	/	4	/	4	4	/	/
Methanol-ethyl acetate	MIC	25	6.25	12.5	/	/	/	12.5	/	12.5	12.5	/	/
	MBC	100	25	50	/	/	/	50	/	25	6.25	/	/
	MBC /MIC	4	4	4	/	/	/	4	/	4	4	/	/

SA=Staphylococcus aureus; KB=Klebsiella pneumonia; AB=Streptococcus feacalis; ST=Salmonella typhi; KO=Klebsiella oxytoca; SF= Shigella flexneri; EC=Escherichia coli; PV=Proteus vulgaris PA=Pseudomonas aeruginosa; MM= Morganella morganii; CF=Citrobacter freundii; EC*= Enterobacter Cloacae; / = not determined

After purification two pure components were isolated the first named C22 with yellow shiny crystals and the second CJ with white shiny crystals. These compounds were tested and released a good anti bacterial activity with antibacterial inhibitor diameter between 11 to 15 mm for C22 and CJ from 13 to 14 mm. (table 9). We noticed that when these two component are test both they produced an additional effect concerning antibacterial activity.

The antibacterial activity of the pure compounds isolated on the disc diffusion method table 9 showed the lowest records of 11 on strain SA and EC expressed by compound C22 treatment.

Table 9. *Antibacterial activity of pure compounds isolated on the disc diffusion method.*

Fract/Bac	SA	KB	AB	ST	KO	SF	EC	PV	PA	MM	CF	EC*
C22	11	13±0.33	13±0.33	/	/	/	12±0.66	/	15±0.0	14±0.6	/	11
CJ	13±0	12±0.3	13±0.3	/	/	/	13±0.33	/	14	13±	12±0.6	13±0.3
C22+ CJ	14±0.6	14±0.33	13	/	/	/	14±0.5	/	14±0.25	15±0.66	15±	14±0.06

SA=Staphylococcus aureus; KB=Klebsiella pneumonia; AB=Streptococcus feacalis; ST=Salmonella typhi; KO=Klebsiella oxytoca; SF= Shigella flexneri; EC=Escherichia coli; PV=Proteus vulgaris PA=Pseudomonas aeruginosa; MM= Morganella morganii; CF=Citrobacter freundii; EC*= Enterobacter Cloacae; nd= not determined

The MIC's and MBC results witnessed a best *in vitro* antibacterial activity of *R. heudoletti* (table 10) pure components isolated named C22 and CJ also demonstrated a very good ratio MBC/MIC. When these two components C22 and CJ tested both the results of ratio MBC/MIC decreased, confirmation of the antibacterial synergetic affect (table 10). The study conducted by Eyoh et al, [9]; Ngoupayo et al, [17] showed that MBC/MIC ratio less than 4 is an indication of bactericidal activity of a plant extract.

Table 10. *MIC, MBC values (µg/ml) of R. heudolotti pure component in the micro dilution assay.*

Fract/Bac		SA	KB	AB	ST	KO	SF	EC	PV	PA	MM	CF	EC*
C22	MIC	0.5	0.5	0.5	/	/	/	0.25	/	0.5	0.5	/	0.5
	MBC	0.5	0.5	0.5	/	/	/	0.25	/	0.5	0.5	/	0.5
	MBC /MIC	1	1	1	/	/	/	1	/	1	1	/	1
CJ	MIC	0.25	0.5	0.5	/	/	/	0.25	/	0.5	0.25	0.25	0.5
	MBC	0.25	0.5	0.5	/	/	/	0.25	/	0.5	0.25	0.25	0.5
	MBC /MIC	1	1	1	/	/	/	1	/	1	1	1	1
C22-CJ	MIC	0.12	0.25	0.12	/	/	/	0.25	/	0.11	0.12	0.12	0.12
	MBC	0.12	0.25	0.12	/	/	/	0.25	/	0.11	0.12	0.12	0.12
	MBC /MIC	1	1	1	/	/	/	1	/	1	1	1	1

C22: Yellow shiny crystals (422 mg) identified and characterized as glycosyl flavonoid
CJ: White shiny crystals (300 mg) not yet identified and characterized

4. Conclusion

At the end of this study we observed that the crude extract of R. *heudoletti* contained high levels of alkaloids, polyphenols, saponins and cardiac glycosides in both methanol and ethanolic extracts. The extract fractions and pure compounds showed promising antibacterial properties. The crude extract contains many phytochemical secondary metabolites. The chromatographic separation and elucidation of the crude extract from the fraction of methanol, acetone, and the fraction mixed methanol and ethyl acetate led to the selection of the fraction of methanol for purification. This fraction showed the best antibacterial activity.

Two pure compounds were isolated from the methanolic extract of *R. heudeloti*. The fist with yellow shiny crystal were characterized as iso-flavonoid, However further research needs to be carried out to identify the second pure

molecule then a follow up study for safety by evaluating the sub-acute or chronic toxicities.

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