

PHYTOCHEMICAL STUDY, ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF THE LEAVES EXTRACTS OF CITRUS PARADISI

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ABSTRACT

Chemical investigation of the crude methanolic extract of the leaves of Citrus Paradisi resulted in the isolation of two known compounds (**isolated for the first time from the leaves**) namely citracridone II and apigenin. The structures of these compounds were established by using various spectroscopic methods including 1D NMR (1H NMR and 13C NMR) and 2D NMR (COSY, HMQC and HMBC) in conjunction with mass spectrometry and by the comparison with literature data. Acridone alkaloids isolated from Citrus medica and others species of genus Citrus appear to be useful as chemotaxonomic markers in Citrus. The crude extract was evaluated for its antibacterial activity against nine strains and antifungal activity against four yeasts using the MHA and SDB methods. The tested treatments showed Significant antibacterial activity against 2 bacterial strains tested with MBC/MIC (Ratio used to determine bactericidal or bacteriostatic activity) equal to 2 (< 4), and Significant antifungal activity against 2 yeast strains tested with MFC/MIC (Ratio used to determine fungicidal or Fungistatic activity) equal to 2 (≤ 4).

I. Introduction

The genus Citrus (Rutaceae) is known to produce a variety of biologically active extracts and metabolites such as flavonoids (Tripoli et al., 2007), (Zhang et al., 2004) Papas et al., en 2010., limonoïdes (Soladoye et al., 2010; Segun et al., 2018), alcaloïdes (Lv et al., 2015 (Atangana et al., 2017 (Essombe Malolo et al., 2023)) as well as Coumarines (Nallely et al., 2012 ; (Chan et al., 2017)). In the continuation of our search for bioactive substances from Citrus species, we have investigated the methanolic extract of the leaves of Citrus Paradisi. Citrus Paradisi is a hybrid plant, a tree up to 6 - 12 m on high. It produces large, green, glossy and leathery leaves that are persistent. Its flowers are white with five sepals and petals and 20-25 stamens. The species is found in Cameroon, Nigeria, Kenya, Morocco and in many other countries in the whole World (Heywood,1996).

We describe herein the isolation and structure elucidation of minor constituents from Citrus Paradisi, which included one alkaloid, citracridone II and one Flavonoid, apigenin. The methanolic extract of this species was tested for antibacterial and antifungal activities.

II. Materials and Methods

2.1 Plant Material

The leaves of Citrus Paradisi was harvested at Penja, littoral region of Cameroon. It was identified by Mr NGANSOP Eric, botanist

at the National Herbarium in Yaoundé, where a voucher specimen (ref. 67 471/HNC) was deposited.

2.2 Extraction and isolation of compounds

1 Kg of powder of the leaves of *Citrus Paradisi* species was obtained after cutting, drying and grinding. This resulting powder was extracted by maceration in 8.5 liter of MeOH at room temperature. After concentration in a rotary evaporator, 25g of crude extract was obtained. A mass of 21 g of the extract was fixed to 45 g of silica gel (0.63-200 mm) in a chromatographic column and subjected to gradient elution with the solvent hexane, a mixture of hexane/ethyl acetate gradients, 100 % ethyl acetate and finally ethyl acetate/Methanol system of increasing polarity. A total of 356 fractions of 100 ml each were collected and then regrouped into 9 Fractions indexed from F1 to F24 based on analyses made on the TLC profiles.

Fraction F10 (6 g) was subjected to CC over silica gel, eluted with n-hex/EtOAc mixtures (80/20) and yielded compound 1 FCP5 (citracridone II, 19.2 mg, yellow amorphous powder,). Fraction F24 (4 g) was subjected to CC over silica gel eluted with AcOEt/MeOH mixture of polarity (90/10) and yielded compound 2 FCP11 (Apigenine, 18.2 mg, yellow powder).

2.3 Apparatus.

2.3.1. General

The mass of substances were determined using an electronic scale of type ACCULAB startorius TE 612. Solvent evaporation was done with a rotary evaporator manufactured by JANKE & JUNNEL (of type IKA-WERK, RV05, N°70799).

Analytical thin-layer chromatography (TLC) was carried out on silica gel plates on aluminum sheets (precoated MERCK plates) with dimensions of 20 × 20 cm² and a thickness of 0.2 mm, coated with silica gel 60 F254.

The development of the chromatographic plates was performed in cylindrical chromatographic chambers.

Eluent systems such as Hex/AcOEt, ethyl acetate/Methanol of increasing polarity, and AcOEt were used as the mobile phase depending on the nature and polarity of the compounds to be analyzed.

Column chromatography was carried out on a column with a diameter of 6.2 cm and a length of 85 cm. The silica used was Merck silica gel type 60 with particle sizes of 70–230 mesh and 230–400 mesh.

A rotary evaporator (LAUKE & JUNNEL, IKA-WERK), type RV05 and N°70799, was used for solvent concentration.

Detection was carried out using a universal UV lamp, model CAMAG TL 9001, with wavelengths between 254 and 350 μm, and by spraying with sulfuric acid (50%).

The melting points were determined using an Electrothermal melting point apparatus, model IA9100, with a temperature range up to 300 °C, and without correction.

1D and 2D Nuclear Magnetic Resonance spectra of proton and carbon were recorded on a Varian NMR System AS 600 and 150 MHz, with chemical shifts being represented in ppm, coupling constant in Hz and tetramethylsilane used as an internal standard. High Resolution Mass Spectra were obtained with an OTOF Spectrometer (Bruker, Germany) equipped with a HESI source.

2.3.2. Antibacterial assay

. Preparation of Mueller Hinton Agar (MHA): Bacterial culture medium

A total of 6.5 g of Mueller Hinton Agar was introduced into 100 ml of distilled water. The mixture was sterilized in an autoclave at 37°C for 24 hours. Once prepared, the solution was allowed to cool.

. Bacterial inoculation

The broth microdilution method was used for testing the sensitivity of bacterial species in sterile 96-well microtiter plates, as described by Newton et al. (2002). For this purpose, crude extracts were dissolved in a 5% dimethyl sulfoxide (DMSO) solution and diluted in Mueller Hinton broth to obtain a concentration of 500 μg/ml for isolated compounds.

Serial two-fold dilutions of the tested substances were carried out in Mueller Hinton broth to obtain a final volume of 100 μl per well. This produced a concentration range from 250 to 0.97 μg/ml, respectively.

One hundred microliters of each bacterial suspension (containing approximately 1.5×10^6 CFU/ml) were added to the wells, followed by the respective tested samples and mixed carefully to obtain final concentrations ranging from 500 to 0.48 μg/ml for the extracts and from 125 to 0.48 μg/ml for the isolated compounds.

The solvent control (5% DMSO) did not show inhibitory effects on bacterial growth. Ciprofloxacin® (Bayer, Germany) at a concentration ranging from 4 to 0.5 μg/ml was used as the standard reference. The microtiter plates were incubated at 37°C for 24 hours.

The inhibitory concentrations of the extracts were determined after the addition of 50 μl of 0.2 mg/ml p-iodonitrotetrazolium chloride (INT) (Sigma-Aldrich, South Africa) and incubation at 37°C for 30 minutes (Mativandle et al., 2006). Viable bacteria transforming the yellow dye p-iodonitrotetrazolium chloride into a pink-colored product.

The test is based on the ability of living cells to convert the tetrazolium salt (INT) into a pink-colored formazan structure under the action of mitochondrial enzymes and other dehydrogenases inside the cell. The lowest concentration at which no visible color change was observed was considered as the MIC (minimum inhibitory concentration).

Bactericidal concentrations were determined by adding 50 μl of aliquots of preparations (without INT), which did not show a visible color change after the subculture of MIC assays, into 150 μl of Mueller Hinton broth without extract. These preparations were incubated at 37°C for 48 hours, and bacterial growth was revealed by the addition of INT as described above.

The lowest concentration at which no visible color change was observed was considered as the MBC. The tests were performed in duplicate. The MBC/MIC ratio was calculated to determine bactericidal ($MBC/MIC \leq 4$) and bacteriostatic ($MBC/MIC > 4$)

effects.

2.3.3. Antifungal Assay

Preparation of yeast inocula

Yeast inocula were prepared from 48-hour-old cultures by collecting numerous colonies and suspending them in sterile saline solution (NaCl, 0.9%). The absorbance was read at 530 nm and adjusted with saline solution to match that of a 0.5 McFarland standard, corresponding to approximately 10^6 yeast cells/ml (Husein et al., 2010).

Determination of minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC)

The minimum inhibitory concentration (MIC) of each extract was determined using the broth microdilution technique in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI, formerly the National Committee for Clinical and Laboratory Standards, NCCLS) for yeasts (M27-A2) (Husein et al., 2010).

Stock solutions of the tested extracts were prepared in an aqueous solution of dimethyl sulfoxide (DMSO) at 5% and diluted with Sabouraud dextrose broth (SDB) to obtain a concentration of 1 mg/ml. This solution was serially two times diluted to obtain concentrations ranging from 500 to 0.24 $\mu\text{g/ml}$ for the extracts and from 125 to 0.24 $\mu\text{g/ml}$ for the compounds.

The final concentration of DMSO in the wells was less than 1% (a preliminary analysis with 1% DMSO did not inhibit the growth of the tested organisms). The negative control consisted of 195 μl of SDB and 5 μl of inoculum (containing approximately 10^6 cells/ml for yeast).

The plates were covered with a sterile lid and incubated on a shaker at 37°C for 48 hours (for yeasts). MIC values were visually evaluated after the corresponding incubation period and were considered as the lowest product concentration at which no growth or almost no growth was observed.

Each assay was repeated three times. For the determination of MFC, aliquots of 5 μl from each well that showed no microbial growth were subcultured and showed no growth of the microorganism.

Aliquots were inoculated into 195 μl of SDB and incubated at 37°C for 48 hours (yeasts). The lowest concentration that did not show any growth after subculturing was considered as the MFC. Nystatin (for yeasts) was used as the positive control.

III. Results and Discussion

3.1 Identification of Compounds.

The crude methanolic extract of the leaves of *Citrus Paradisi* was subjected to repeated column chromatography (CC) on silica gel and two compounds were isolated: One known alkaloid, citracridone II (FCP5) (1) (Essombe Malolo et al., 2023) and one known flavonoid, apigenin (2) (FCP11) (Sapkota et al., 2022).

Compound 1 (19.2 mg) was isolated as yellow amorphous powder substance in n-hex/EtOAc mixtures (80/20) and was soluble in chloroform. It reacted positively to the Dragendorff test for alkaloids.

Different spectroscopic techniques were applied for the structure assignment of our isolated compound 1 including ^1H NMR, ^{13}C NMR, COSY, HSQC and HMBC.

Its mass spectrum under electron impact (Figure 8) shows the molecular ion peak at $m/z = 390.2$.

The ^1H NMR spectrum (500 MHz, CDCl_3) (Table 1) of FCP5 shows:

The presence of a singlet at $\delta\text{H} = 14.28$ ppm, integrating for one proton, attributable to a chelated hydroxyl proton characteristic of acridones (Antagana et al., 2017); A singlet at $\delta\text{H} = 3.76$ ppm integrating for three protons, assigned to the N-methyl group of acridones; An AB system of two protons at $\delta\text{H} = 8.14$ ppm (1H, d, $J = 8.8$ Hz) and $\delta\text{H} = 7.28$ ppm (1H, d, $J = 8.8$ Hz), indicating aromatic protons adjacent to an α,β -unsaturated carbonyl group, attributable respectively to H-8 and H-7 protons of acridones; The presence of a singlet at $\delta\text{H} = 6.26$ ppm, attributable to an aromatic proton; The presence of another AB system of two protons appearing as doublets at $\delta\text{H} = 5.58$ ppm (1H, d, $J = 9.7$ Hz) and $\delta\text{H} = 6.62$ ppm (1H, d, $J = 9.7$ Hz), each integrating for one proton, characteristic of protons of the dimethylpyran ring, together with an intense singlet at $\delta\text{H} = 1.53$ ppm integrating for six protons, attributable to the geminal methyl protons of the dimethylpyran ring (Essombe Malolo et al., 2023); The presence of two methoxy group signals at $\delta\text{H} = 3.92$ (3H, s) and 4.03 ppm (3H, s) in the spectrum.

The broadband-decoupled ^{13}C NMR spectrum of FCP5 (125 MHz, CDCl_3) (Table1) shows signals for 19 carbons distributed as follows: A signal at $\delta\text{C} = 181.5$ ppm, attributable to an α,β -unsaturated ketone carbonyl and characteristic of the carbonyl at position C-9 of acridones (Wansi et al., 2006); Two signals corresponding to olefinic carbons at $\delta\text{C} = 122.7$ and 108.2 ppm, attributable respectively to methine carbons C-8 and C-7, and two others at $\delta\text{C} = 120.8$ and 124.2 ppm, attributable respectively to methine carbons C-1' and C-2'; Four methyl signals, including one at $\delta\text{C} = 27.18$ ppm attributable to the geminal dimethyl group corresponding to carbons C-4'/C-5', one N-methyl carbon resonating at $\delta\text{C} = 49.04$ ppm, and two at $\delta\text{C} = 56.31$ and 60.28 ppm attributable to methoxy methyl carbons.

Analysis of the COSY spectrum shows vicinal correlations between protons borne by neighboring carbons. Thus, we observe: A correlation between the proton at $\delta\text{H} = 5.58$ ppm and that at $\delta\text{H} = 6.62$ ppm, confirming the presence of the AB system of the dimethylpyran ring; Another correlation between the protons at $\delta\text{H} = 7.01$ ppm and $\delta\text{H} = 8.14$ ppm, confirming the second AB system of aromatic protons adjacent to an α,β -unsaturated carbonyl group.

Analysis of the NOESY spectrum reveals: A correlation between the protons at $\delta\text{H} = 7.28$ ppm and those of the methoxy group at $\delta\text{H} = 4.02$ ppm, suggesting that they are spatially on the same side; Another correlation between the N-methyl protons and one of the olefinic protons at $\delta\text{H} = 6.61$ ppm of the dimethylpyran ring, indicating spatial proximity; A correlation between the

chelated proton and the proton at $\delta H = 6.26$ ppm.

Analysis of the HMBC spectrum (Figure 16) shows: A correlation between the proton at $\delta H = 8.14$ ppm and the ketone carbonyl. This information, combined with COSY analysis, allows assignment of this proton to C-8 and its neighboring proton to C-7; Correlations between proton H-7 and the methoxy protons at $\delta H = 4.02$ ppm with a carbon at $\delta C = 157.5$ ppm (an oxidized carbon). This information, combined with NOESY data, allowed assignment of this methoxy group to C-6; Correlations between the proton at $\delta H = 6.26$ ppm and two oxidized carbons at $\delta C = 165.2$ and 161.2 ppm, where the carbon at $\delta C = 165.2$ corresponds to C-1 and the other participates in the dimethylpyran ring. These data, combined with NOESY analysis, allowed assignment of this proton to C-2 and the dimethylpyran ring to positions C-3 and C-4.

All physicochemical and spectroscopic data, compared with those reported in the literature, allowed the structure of compound FCP5 to be assigned as Citracridone II, previously described in the literature and recently isolated from the trunk of *Citrus paradisi* (Essombe Malolo et al., 2021).

3.2 Previous work

Previous phytochemical investigations on *Citrus paradisi* have resulted in the isolation of a variety of secondary metabolites which mainly included 5-hydroxynoracridone (Essombe Malolo et al., 2023), decycloxycomicosine (Essombe Malolo et al., 2023), apigenin (Sapkota et al., 2022), naringénine (Papas et al., en 2010), Narirutine (Sapkota et al., 2022), Neohesperidine (Sapkota et al., 2022), Quercétine (Niassy et al., 2004), Grandisinine (Wu et al., 1996), Citracridone I (Wu et al., 1996), Citracridone II (Atangana et al., 2017).

3.3 Chemotaxonomic significance

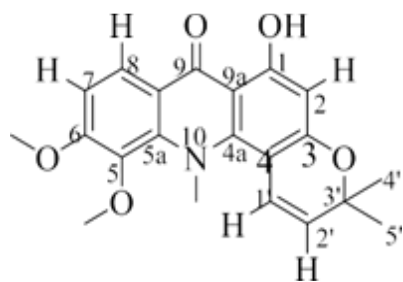
Previous work on this species have permitted to isolate apigenin, naringénine, Citracridone I, Grandisinine and the same natural products have been isolated in the species belonging to the Rutaceae family. The same natural product, Citracridone II has been isolated also in the species *Citrus medica* (Atangana et al., 2017). acridone Alkaloids appear to be useful as chemotaxonomic markers in *Citrus*.

Table 1: Données de RMN1H (500 MHz, CDCl₃) et RMN13C (125 MHz, CDCl₃) du composé FCP5 comparées à celles de la citracridone II (fanny Essombe Malolo 2021).

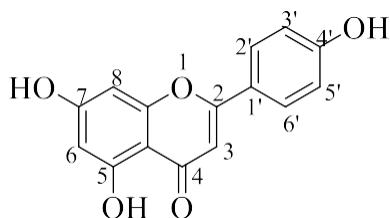
POSITION	Données du composé FCP5		Citracridone II	
	RMN 13C δ_c	RMN 1H δH ; m, J(Hz)	RMN 13C δ_c	RMN 1H δH ; m, J(Hz)
1	165,2	14,28 (s)	165,4	14,20 (s)
2	98,3	6,26 (s)	98,3	6,20 (s)
3	161,2	/	164,0	/
4	102,5	/	102,6	/
4a	147,7	/	148,7	/
5	142,3	/	146,3	/
5a	138,3	/	138,9	/
6	157,4	/	154,6	/
7	108,2	7,28 (1H, d)	108,4	6,92 (1H, t, J= 8,8Hz)
8	122,6	8,14 (1H, d)	123,2	8,0(1H, dd, J= 8,8Hz)
8a	118,8	/	118,5	/
9	181,5	/	182,0	/
9a	106,7	/	100,9	/
5-OMe	60,3	3,92 (3H, s)	60,0	3,92 (3H, s)
6-OMe	56,3	4,03 (3H, s)	60,0	4,04 (3H, s)

N-CH3	49,1	3,75 (3H, s)	48,6	3,75 (3H, s)
1'	120,8	6,62 (1H, d)	120,3	6,65(1H, d, J=9,7 Hz)
2'	124,2	5,58 (1H, d)	124,08	5,55(1H, d, J= 9,7Hz)
3'	/	/	77,2	/
4'	27,2	1,53 (3H, s)	27,2	1,45 (3H, s)
5'	27.2	1,53 (3H, s)	27,2	1,45 (3H, s)

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Compound 1 : Citracridone II



Compound 2 : Apigenin

Figure: Structure of some isolated compounds



3.4 Evaluation of biological activities by the microdilution method in liquid medium

3.4.1. Evaluation of antibacterial activity

Determination of minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC)

The methanolic extract of *Citrus paradisi* leaves was subjected to antibacterial testing. The liquid microdilution method using microplates allowed the determination of inhibitory parameters of sensitive species, namely the MIC, the MBC, and the MBC/MIC ratio.

These results show that the MIC values ranged from 125 to 62.5 $\mu\text{g/ml}$ for the methanolic extract and from 1 to 0.5 $\mu\text{g/ml}$ for ciprofloxacin used as the reference drug.

Two of the eight strains sensitive to the extract (*Escherichia coli* and *Staphylococcus aureus*) showed MIC values of 62.5 $\mu\text{g/ml}$ and 125 $\mu\text{g/ml}$, respectively. According to the classification of Kuete (2010), when the MIC is between 1 and 100 $\mu\text{g/ml}$, the extract exhibits good (significant) activity; when the MIC is between 100 and 500 $\mu\text{g/ml}$, the extract shows moderate activity; and when the MIC is greater than 500 $\mu\text{g/ml}$, the extract shows weak activity.

Our results indicate that the crude extract possesses significant antibacterial activity against *Escherichia coli* and moderate antibacterial activity against *Staphylococcus aureus* (Kuete, 2010).

Inoculation of the contents of the microplates showing discoloration on Mueller Hinton agar (MHA) did not show any recovery of bacterial growth. The MBC/MIC ratio allowed us to determine whether the extract had a bactericidal or bacteriostatic effect on the sensitive isolates. According to Marmonier (1990), when the MBC/MIC activity ratio of an antibacterial substance is less than four (< 4), it is considered bactericidal; if the MBC/MIC ratio is greater than or equal to four (≥ 4), it is considered bacteriostatic.

Our extract showed a ratio lower than 4, indicating that it has a bactericidal effect.

Table 2. Antibacterial activity of the crude methanolic extract of Citrus paradisi and the reference antibiotics against bacterial strains.

Treatment	Parameter	SP	SARM	SA	STM	SF	ST	EC	PA	KP
CPF (Citrus paradisi extract)	MIC (µg/mL)	>125	>125	125	>125	>125	>125	62.5	>125	>125
	MBC (µg/mL)	ND	ND	250	ND	ND	ND	125	ND	ND
	MBC/MIC	ND	ND	2	ND	ND	ND	2	ND	ND
Amoxicillin	MIC (µg/mL)		0.5							
	MBC (µg/mL)		1							
	MBC/MIC		2							
Ciprofloxacin	MIC (µg/mL)	1		0.5	0.25	0.5	0.5	0.5	0.5	0.5
	MBC (µg/mL)	2		1	2	2	2	1	2	2
	MBC/MIC	2		2	0.125	4	4	2	4	4

MIC (µg/ml): Minimum Inhibitory Concentration, MIC ≤ 100 µg/mL: significant activity; MIC 100-500 µg/mL : moderate activity ; MIC>500µg/mL : weak activity ; MBC (µg/ml): Minimum Bactericidal Concentration ; MBC/MIC: Ratio used to determine bactericidal or bacteriostatic activity, Bactericidal if MBC/MIC ≤ 4 ; Bacteriostatic if MBC/MIC > 4 ; Reference antibiotics: Amoxicillin and Ciprofloxacin (Cipro) ; Bacterial strains: [SP: Streptococcus pneumoniae (ATCC 49619) ; SARM: Methicillin-resistant Staphylococcus aureus (ATCC 43300) ; SA: Staphylococcus aureus (ATCC 25923) ; STM: Salmonella typhi (ATCC 6539) ; SF: Shigella flexneri (NRS) ; ST: Salmonella typhimurium (ATCC 14028) ; EC: Escherichia coli (ATCC 25922) ; PA: Pseudomonas aeruginosa (ATCC 27853) ; KP: Klebsiella pneumoniae (ATCC 700603)]. Notes: Values are expressed as mean ± SD. ; ND: Not determined.

3.4.2 Evaluation of antifungal activity

Determination of minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC)

The antifungal activity of the methanolic extract of Citrus paradisi leaves was evaluated against four yeasts: Candida albicans (CA), Candida krusei (CK), Candida parapsilosis (CP), and Cryptococcus neoformans (CN). The method used was the broth microdilution method (Husein et al., 2010).

Table 10 presents the results of the inhibition test of the extract within the tested concentration range. It can be observed that:

The methanolic extract of Citrus paradisi leaves showed significant activity against the strain Cryptococcus neoformans, with an MIC of 62.5 µg/ml, and moderate activity against Candida albicans, with an MIC of 125 µg/ml (Kuetze, 2010).

The antifungal activity of the methanolic extract of Citrus paradisi leaves (CPF) was evaluated against four pathogenic yeasts using the broth microdilution method. CPF exhibited significant antifungal activity against Cryptococcus neoformans, with an MIC of 62.5 µg/mL and an MFC of 125 µg/mL. A moderate activity was observed against Candida albicans, with MIC and MFC values of 125 and 250 µg/mL, respectively. No significant inhibitory effect was detected against Candida krusei or Candida parapsilosis at the tested concentrations. The MFC/MIC ratios for C. albicans and C. neoformans were equal to 2, indicating a fungicidal effect of the extract against these strains. Fluconazole, used as the reference antifungal, showed strong activity against all tested yeasts, with MIC values ranging from 0.25 to 1 µg/mL.

Overall, these results demonstrate that Citrus paradisi leaf extract possesses promising fungicidal activity, particularly against Cryptococcus neoformans.

Table 3: Antifungal activity of Citrus paradisi leaf extract and fluconazole against yeast strains

Treatment	Parameter	Yeast strains			
		CA	CK	CP	CN
CPF	MIC (µg/mL)	125	>500	>500	62.5
	MFC(µg/mL)	250	ND	ND	125
	MFC/MIC	2	ND	ND	2
Flu	MIC (µg/mL)	0.5	0.25	1	0.25
	MFC (µg/mL)	2	1	2	1

MFC/MIC	4	0.25	2	0.25
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Notes : ND: Not determined ; CPF: Citrus paradisi leaf methanolic extract ; Flu: Fluconazole ; MIC: Minimum Inhibitory Concentration ; MFC: Minimum Fungicidal Concentration ; CA: *Candida albicans* ; CK: *Candida krusei* ; CP: *Candida parapsilosis* ; CN: *Cryptococcus neoformans*. Activity interpretation (Kuete, 2010): MIC \leq 100 $\mu\text{g}/\text{mL}$: significant activity ; MIC 100–500 $\mu\text{g}/\text{mL}$: moderate activity ; MIC > 500 $\mu\text{g}/\text{mL}$: weak activity ; Fungicidal effect: MFC/MIC \leq 4; Fungistatic effect: MFC/MIC > 4;

IV. Conclusion

One known acridone alkaloid, citracridone II (1) and one known flavonoid, apigenin (2) have been isolated for the first time from the leaves of *Citrus paradisi*. According to the previous works done on some species of genus *Citrus* and others species belonging to the Rutaceae family, we finally conclude that acridone alkaloids appear to be useful as chemotaxonomic markers in *Citrus*. The methanolic extract of the leaves of this species were tested to examine its antibacterial and antifungal activity. The extract exhibited a significant antibacterial activity with MBC/MIC less than four (< 4) for the bacterial strains *Escherichia coli* and *Staphylococcus aureus*. Furthermore the extract exhibited also a significant Antifungal activity with MFC/MIC \leq 4 for the yeast strains *C. albicans* and *C. neoformans*. These results suggest that *Citrus paradisi* leaf extract possesses promising antibacterial and antifungal activities and can be developed into standardized herbal medicines used to treat bacterial and fungal infections.

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