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InVitro Antimicrobial Activity of *Conocarpus* spp Leaf Crude Extract

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Abstract

The plants represent one of the important sources that can produce antimicrobial agents. So, this study was designed to evaluate the ability of the local *Conocarpus* spp leaves to detect their bioactivity against some microbial pathogens. The filtrate produced by soaking the dried methanol pulverized leaves in the distilled water revealed a bioactivity against three clinical pathogenic isolates were *Staphylococcus aureu*s, *Escherichia coli* and *Candida albicans* recorded 25, 15 and 31 mm of the inhibitory zones respectively. The isolates besides *Streptococcus mutans* were inhibited by the crude chloroform extract of the filtrate in which the inhibitory zones were 16, 19, 28 and 15 mm respectively. The GC-MS analysis revealed that the crude chloroform extract contains seven compounds besides DMSO as solvent were [ethanone,1,1'-(1,4-phenylene)bis-], [benzene, 4-methoxy-1-nitro-2-(trifluoromethyl)-], [cholan-24-oic acid, 3,6-bis(acetyloxy)- methyl ester (3,alpha., 5.beta., and 6.alpha)], [terephthalic acid, isobutyl 2-phenylethyl ester], [1,3,4-oxadiazol-2-amine, 5-(4-bromophenyl)-], [1H-indole, 5-methyl-2-phenyl-], and [benzo [h]quinolone, 2,4-dimethyl-].

Keywords: *Conocarpus* spp, Bioactivity, GC-Mass.

Introduction

Plants belong the biological sources that can produce secondary metabolites including, the antimicrobial agents that some of them serve as therapeutic compounds as medicines for human and animals (Kalaivani *et al*., 2013; Huda *et al*., 2015; Kumaradevan *et al*., 2015). Two-thirds of the world population still depend on the plants as traditional drugs for the care their health against the diseases (Neldner, 2000; Leonti *et al*., 2009). For these

reasons, it is most important to evaluate the plant extracts for the discovering compounds which may have a novelty to be drugs for treating the diseases. The previous studies showed that 74% of 119 of extracted substances from plants used as traditional drugs (Alves and Rosa 2007; Saroya, 2011). *Conocarpus* spp considered the two species belong the *Conocarpus* genus of family Combretaceae.

The origin of this tree is the coastal and riverine areas in Somalia, Djibouti, and Yemen. Also, East Africa, the Arabian Peninsula, and South Asia have the areas for flourishing the tree. The *Conocarpus* spp grow and flourishes even the semiarid conditions are available. Therefore, the majority of the research studies focused on the ability of the tree for response and resistance against the extreme environmental conditions such as stress, drought, and salinity (Al-Kandari *et al*., 2009; Redha *et al.*, 2011). It has also been shown that plant extracts used as inhibitors against plasmodia, leishmania, and trypanosoma(Al-Musayeib *et al*.,2012). .

This study aimed to evaluate the ability of *Conocarpus* spp leaf crude extract against some species of the pathogenic bacteria and one species of the yeasts, as well as chemical analysis of the crude extract by using gas chromatography-mass spectrometry (GC-MS).

Materials and Methods

Sample Preparation

The healthy leaves of *Conocarpus* spp (Fig. 1) were obtained and washed with tap water then by distilled water. The leaves left at the room temperature for 3 days until they dried. The dried leaves chopped and pulverized, soaking by a volume of the methanol, and left 24 hours until the methanol evaporated.

Preliminary Bioactivity of Dried Methanol leaf Filtrate

One liter of the distilled water was added to the fifty grams of the dried pulverized methanol leaves, left at the room temperature for 3 hours, the filtrate separated from the residue by a filter paper, and a volume of the filtrate sterilized by syringe Millipore (µM 0.45) in which 100 µL placed in a well (7 mm diameter) of the Petri dish center containing potato dextrose agar (PDA) inoculated by 0.1 ml (spread through sterile L-shaped rod) of *Candida alibicans*, while *Staphylococcus aureus* and *Escherichia coli* were tested by the using Petri dishes containing a nutrient agar (NA) at the same

conditions. All dishes incubated at 37 °C for 2 days to examine the filtrate bioactivity. The bioactivity test repeated more than three times and by triplicate for each microorganism.

Fig.1: *Conocarpus* spp tree.

Extraction and Bioactivity of the Crude Substance

The extraction carried out by mixing methanol leaf filtrate with chloroform (200ml: 50ml) in which the organic layer (lower layer) was selected, and evaporated by air until getting residue was dissolved in the dimethyl sulfoxide (DMSO) to form a solution. Aseptically, the Petri dishes of potato dextrose agar (PDA) were prepared for testing *C. albicans* while nutrient agar (NA) dishes used to *S. aureus*, *S. mutans*, and *E. coli*. After the dishes dried, each one inoculated by 0.1 ml of 1.5 x 10^9 cell/ ml obtained from each tested pathogen, the well (7mm diameter) was made in a center of each dish, 100 µl of the crude chloroform extract solution placed in the well, and incubated at 37 °C for 2-3 days until the zones of inhibition were seen.

Gas Chromatography-Mass Spectrometry Analysis

The crude chloroform extract was dissolved in DMSO and filtered by syringe Millipore (µM 0.45) that the filtrate submitted to the GC-Mass spectrometry which carried out by gas chromatography-mass spectrometry, MSDCHEM\1\METHODS\MUAFAQ.M for the determination of negative ions(m/z) through using column characterized by HP-5MS, 5% phenyl methyl Sillox(1629.5), 30m × 0.250 µm I.D. x 0.25 µm, SS. then application of the parameters in (Table 1).

Table 1: GC-MS analysis parameters used to detect the compounds within crude chloroform extract produced by the filtrate of the dried methanol leaves of *Conocarpus* spp.

Analysis Parameters		
1	EMV mode	Gain Factor (1.00)
2	Resulting EM voltage	1306
3	Power capacity	70 EV
4	Low Mass	28.0
5	High Mass	441
6	Threshold	150
7	Minimum quality for all narcotics	90-97%
8	Flow rate	1ml/min
9	Runtime	24 min
10	Hold up time	1.5288 min
11	Solvent delay	3.00 min
12	Average velocity	36.796 cm/sec
13	Temperature	Initial 70 ^{o}C to Maximum 375 ^{o}C
14	Pressure	8.81 Psi

Results

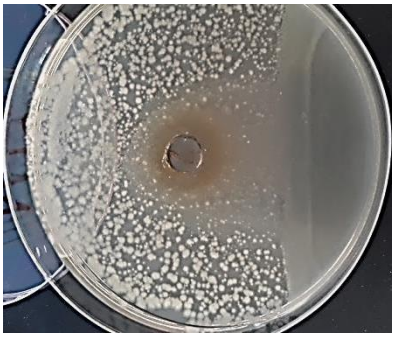
Bioactivity of The Dried Methanol-Water Leaf Filtrate

The sterile filtrate of the dried pulverized methanol *Conocarpus* spp leaves exhibited the values of the inhibition which the highest inhibition zone was observed against *C.albicans* followed by *S .aureus* while less value was against *E. coli*(Table 2) and (Fig. 2).

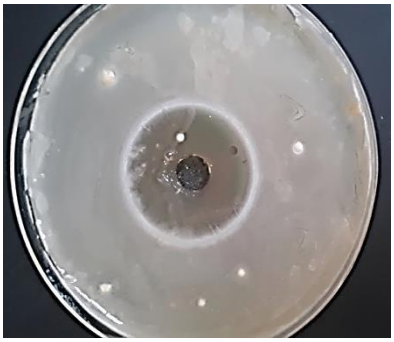
Table 2: Antimicrobial activity of the *Conocarpus* spp leaf filtrate by agar well diffusion method against three clinical pathogenic isolates at 37 °C for 2 days.

Zones of Inhibition Measured by Millimeter (mm)		
S .aureus	*E. coli*	*C. albicans*
25	15	31

A



B



C

Fig. 2: Antimicrobial activity of the *Conocarpus* spp leaf filtrate by agar well diffusion method against three clinical pathogenic isolates at 37 °C for 2 days. A: *S .aureus*, B: *E. coli*, and C: *C.albicans*.

Bioactivity of The Crude Chloroform Extract

Very small amount as a crude creamy residue was extracted by the evaporated organic layer (lower residue) of the used chloroform which mixed with the filtrate of the plant dried pulverized methanol leaves. The solution of the crude chloroform extract revealed the highest value of the

inhibitory zones against *C. albicans* followed by *E. coli*, *S.aureus*, and *S. mutans* respectively. (Tabel 3) and (Fig. 3).

Table 3: Antimicrobial activity of the crude chloroform extract against four clinical isolates of the microbial pathogens at 37°C for 2 days.

Zones of Inhibition Measured by Millimeter (mm)			
S.aureus	*S.mutans*	*E.coli*	*C.albicans*
16	15	19	28

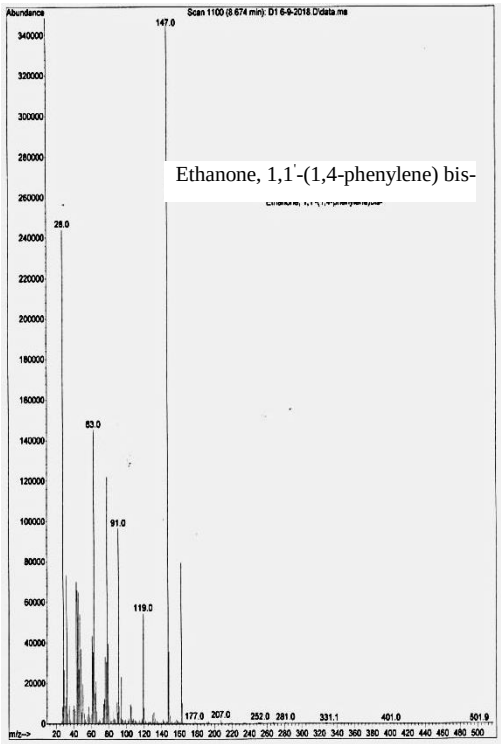
Fig.3: Antimicrobial activity of the crude chloroform extract by agar well diffusion method against *C.albicans* at 37°C for 2 days.

GC-Mass Spectrometry Analysis of Crude Chloroform Extract

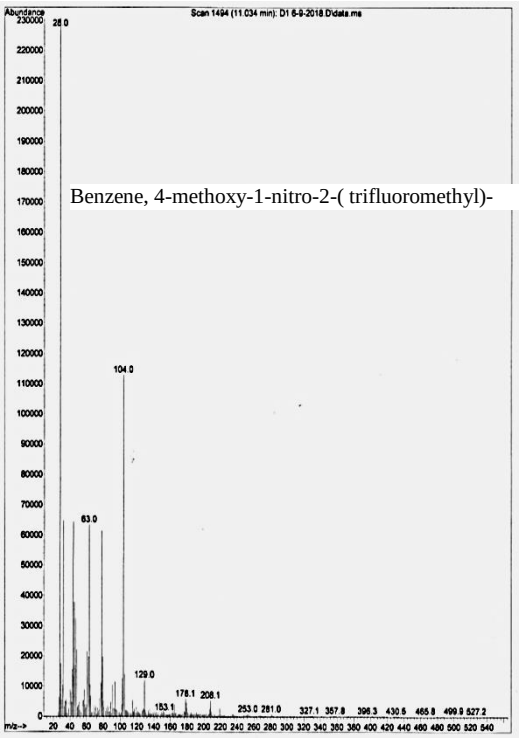
The GC-MS analysis revealed that the crude chloroform extract contains seven compounds besides DMSO as solvent were [ethanone,1,1'-(1,4-phenylene)bis-], [benzene, 4-methoxy-1-nitro-2-(trifluoromethyl)-], [cholan-24-oic acid, 3,6-bis(acetyloxy)- methyl ester (3,alpha., 5.beta., and 6.alpha)], [terephthalic acid, isobutyl 2-phenylethyl ester], [1,3,4-oxadiazol-2-amine, 5-(4-bromophenyl)-], [1H-indole, 5-methyl-2-phenyl-], and [benzo [h]quinolone, 2,4-dimethyl-].(Table 4). (Figures: 4a and 4b).

Table 4: The compounds detected by GC-MS analysis of the crude chloroform extract produced by dried methanol pulverized *Conocarpus* spp leaves.

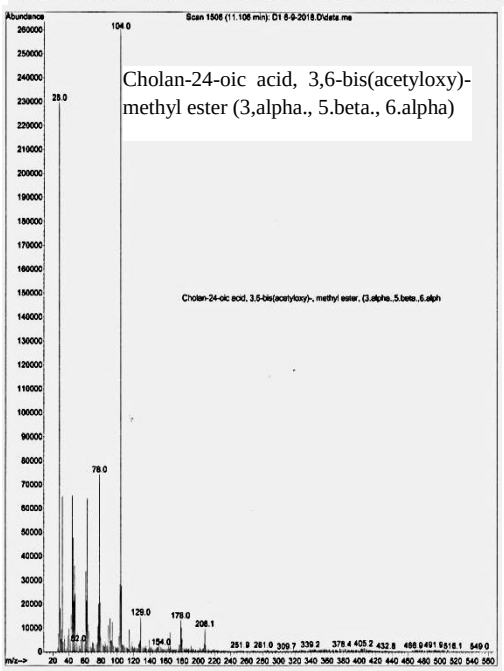
	Compounds	Retention Time (min.)
1	Dimethyl sulfoxide (DMSO)	3.464
2	Ethanone, 1,1'-(1,4-phenylene) bis-	8.674
3	Benzene, 4-methoxy-1-nitro-2-(trifluoromethyl)-	11.034
4	Cholan-24-oic acid, 3,6-bis(acetyloxy)- methyl ester (3,alpha., 5.beta., 6.alpha)	11.106
5	Terephthalic acid, isobutyl 2-phenylethyl ester	11.112
6	1,3,4-Oxadiazol-2-amine, 5-(4-bromophenyl)-	14.429
7	1H-indole, 5-methyl-2-phenyl-	18.735
8	Benzo [h]quinolone, 2,4-dimethyl-	19.089



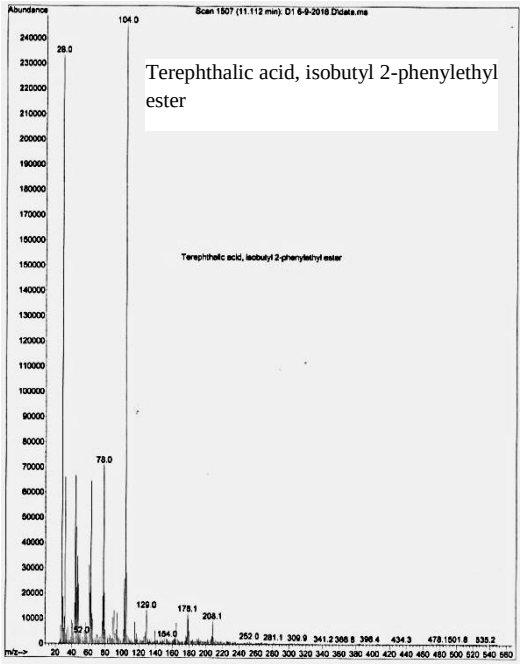
Ethanone, 1,1'-(1,4-phenylene) bis-



Benzene, 4-methoxy-1-nitro-2-(trifluoromethyl)-



Cholan-24-oic acid, 3,6-bis(acetyloxy)-methyl ester (3,alpha., 5.beta., 6.alpha)



Terephthalic acid, isobutyl 2-phenylethyl ester

Fig.4a: The compounds of crude chloroform extract produced by the dried methanol pulverized *Conocarpus* leaves detected by GC-MS analysis.

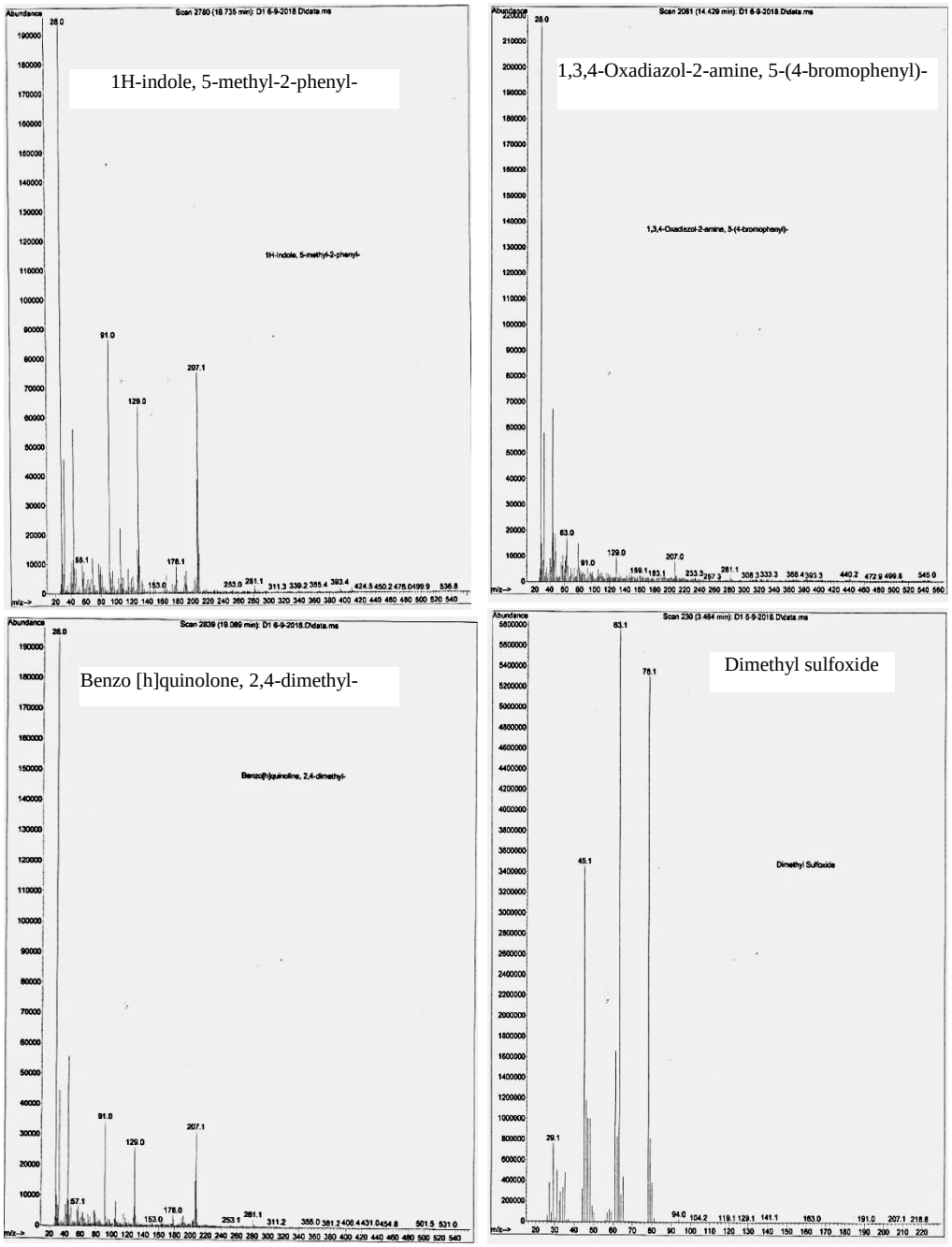


Fig.4b: The compounds of crude chloroform extract produced by the dried methanol pulverized *Conocarpus* leaves detected by GC-MS analysis besides DMSO (sample solvent).

Discussion

Conocarpus spp., including *C. lancifolius* Engl. have the ability to produce antiplasmodial, anti-leishmania, anti-trypanosoma, and antibacterial agents (Al-Kandari *et al*., 2009; Redha *et al.,* 2011; Al-Musayeib *et al*., 2012; Ali *et al*.,2013). The leaves of *C. lancifolius* were used to be extracted by aqueous methanol for obtaining the alkaloids which exhibited the antimicrobial activity against some human microbial pathogens (Ali *et al*.,2013). Generally, our study had an agreement with the mentioned researchers in which the leaves of the plant contain the compounds possess the antimicrobial activity that the results of this study showed the water filtrate of the dried methanol pulverized leaves (*Conocarpus* spp tree) exhibited the activity against *C. albicans, S. aureus,* and *E. coli* (Table 2) and (Fig. 2).

The extraction by solvents considered as one of the known and most common methods by which the extracts are given. If a solid sample liquid can be extracted; the sample must be homogenized through chopping or pulverizing to mix it with an appropriate organic solvent, then separate the liquid from a solid substance by filtration or centrifugation that a filtrate can be given to extract it with a suitable organic solvent. This method is named liquid-liquid extraction- LLE-(Novakova and Vlckova, 2009; Zhang *et al*., 2012). Water is one of the solvents which can extract the fixed and essential oils, and sterones while chloroform has an ability for extracting alkaloids (Saroya, 2011). Based on the principle of the mentioned words, our study used the chopping and pulverizing leaves of *Conocarpus* spp. for getting crude extract which had antimicrobial activity against some human microbial pathogens (Tabel 3) and (Fig. 3).

The plant extracts varied for getting the antimicrobial susceptibility according to the species and strains of the bacteria (Karou *et al*., 2006). The current study gave the various zones of the inhibition around the tested microorganisms either by water filtrate and chloroform extract of

Conocarpus spp leaves. Additionally, water filtrate and crude chloroform extract gave more antimicrobial activity against *C.albicans* than bacteria (Tables: 2 and 3).It may be said that the activity resulted in due to leaves of the plant contain compounds are more effective against the mentioned yeast than bacteria.

Medicinal plants have bioactive compounds, including the antibacterial and antifungal agents such as alkaloids, phenols, and terpenoids (Saxena *et al*., 2013). Quinolones are alkaloids and bactericidal agents which can kill the pathogenic bacteria. Examples of the quinolones are ciprofloxacin, gemifloxacin, levofloxacin, and moxifloxacin that block synthesis of the bacterial DNA through destroying topoisomerase II and topoisomerase IV (Katzung and Trevor, 2015). Alkaloids represent the antimicrobial and antioxidant agent (Erdemoglu *et al*., 2007; Ali *et al*., 2013). In the present study, GC-MS analysis of the crude chloroform extract detected that the extract contains benzo [h]quinolone, 2,4-dimethyl- (Table 4) and (Fig.4b). It may be thought the tested microorganisms were inhibited due to the quinolones in the crude chloroform extract (Table 3) and (Fig.3).

Conclusions

The current study concluded that the leaves of *Conocarpus* spp tree contain the bioactive compounds have an ability to inhibit the microbial pathogens. It is very important to separate, purify and characterize these compounds, then separately testing them against many pathogenic isolates, normal cell lines, laboratory animals, and volunteers. After performing these tests, the bioactive compound can be implemented to apply as medicines for treating the diseases in the hospitals of human and animals.

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الفعالية الميكروبية خارج جسم الكائن الحي للمستخلص الخام لأوراق نبات الكاربس *Conocarpus* spp

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المستخلص

تعتبر النباتات واحدة من اهم المصادر الحيوية لإنتاج المضادات الميكروبية بما فيها المستخلصات المضادة للجراثيم والفطريات. هذه الدراسة اوضحت بأن الراشح المائي للأوراق المطحونة الجافة لنبات الكاربس *Conocarpus* spp. التي نقعت في الميثانول المطلق المتطاير منها, اعطى فعالية حيوية ضد ثلاثة انواع من الاحياء المجهرية المرضية هي *S.aureus*, *E.coli*, and *C.albicans* بمناطق تثبيط هي ٢٥, ١٥ و ٣١ وبالترتيب حيث انجزت بطريقة الانتشار بالحفر ومقاسة بالمليمتر.

ان المستخلص الخام الذي تم الحصول عليه من خلال مزج الكلوروفورم مع الراشح المائي لأوراق نبات الـ *Conocarpus* spp انتج ايضا فعالية حيوية ضد الاحياء المجهرية المذكورة وبنفس الطريقة اذ ان مناطق التثبيط هي ١٦, ١٩ و٢٨ ملم وبالترتيب فضلا عن تثبيطه لجرثومة الـ *S.mutans* بمنطقة تثبيط مقدارها ١٥ ملم. ان تحليل كروماتوغرافيا الغاز مع الطيف الكتلي اظهر بأن مستخلص الكلوروفورم الخام يتكون من [ethanone,1,1'-(1,4-phenylene)bis-], [benzene, 4-methoxy-1-nitro-2-(trifluoromethyl)-], [cholan-24-oic acid, 3,6-bis(acetyloxy)- methyl ester (3,alpha., 5.beta., and 6.alpha)], [terephthalic acid, isobutyl 2-phenylethyl ester], [1,3,4-oxadiazol-2-amine, 5-(4-bromophenyl)-], [1H-indole, 5-methyl-2-phenyl-], and [benzo [h]quinolone, 2,4-dimethyl-].

الكلمات المفتاحية: نبات الـ *Conocarpus* spp , الفعالية الحيوية, GC.MS.