Study of the Biological Activity of Aqueous Extract of *Cuminum cyminum* L. and *Hibiscus sabdariffa* L. and Detection of Some Active Groups in them.

دراسة الفعالية الحيوية للمستخلصات المائية لنباتي الكمون والكجرات والكشف عن بعض المجاميع الفعالة فيها

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Abstract:

The studies about the effect of plant extract against different types of bacteria are still one of the most important fields of researches because they are available, cheep, safe, and easy to use by not professional populations. In this work, two plant extracts (*Hibiscus sabdariffa*) and *Cuminum cyminum*) were tested for there possible biological activity against bacteria. Boiled aqueous extract of both plants at the following concentrations (1, 5, 10, and 20%) were used after cooling. Agar well diffusion was used to examine the biological activity of each extract and the results expressed as zone of inhibition in (mm). The bacteria used in this study are (Gram positive: *Streptococcus faecalis* and *Staphylococcus aureus)* and *(*Gram negative: *Pseudomonas pyogens, Escherichia coli, and Klebsiella pneumonia*e).The tests for functional groups that can be extracted by water were carried out. Hence Alkaloids, Saponines, Tannins, Glycosides were screened using a suitable method while oils noticed as upper layer (if present). At 1% concentration, there are no detectable inhibition zones of both aqueous extracts. The inhibition zones appeared at 5% and further inhibition showed as concentration increased. In general, the aqueous extract of *Hibiscus sabdariffa* has shown more biological activity than the corresponding concentration of *Cuminum cyminum*. The lowest activity was shown against *Escherichia coli* bacteria while the other bacteria showed different zone diameters as a response for the same concentration. In *Cuminum cyminum* there are detectable amounts of essential oils, glycosides and high amounts of tannins while alkaloids and saponines are not detectable using the described methods. In *Hibiscus sabdariffa,* there is a detectable amount of saponines, glycosides, and high amounts of tannins while there are no positive results for essential oils and alkaloids.In conclusion, the aqueous extracts of *H. sabdariffa* and *Cuminum cyminum* have antibacterial activity at concentrations ≥5%. *Hibiscus sabdariffa* has shown more biological activity than the corresponding concentration of *Cuminum cyminum.* This fact may be due, in part, to the presence of saponines *in Hibiscus sabdariffa.* The results can be explained through the presence of different active substances in the aqueous extract of both plants.

Keywords: Biological activity, Aqueous extracts, *Cuminum cyminum* L., *Hibiscus sabdariffa* L., Alkaloids, Tannins, Saponines, Glycosides, oils.

الخلاصة

لازالت دراسة الفعالية البايولوجية للمستخلصات النباتية واحدة من الحقول البحثية المهمة كون هذه المستخلصات متوفرة و رخيصة و أمينة و سهلة الاستعمال من قبل الناس غير المتخصصين. في هذا البحث تمت دراسة الفعالية البايولوجية للمستخلصات النباتية لنباتي الكجرات (*Hibiscus sabdariffa*) والكمون (*Cuminum cyminum*) والبحث عن بعض المواد الفعالة فيها. استعملت المستخلصات المائية المغلية لكلا النباتين عند التراكيز الآتية (١%، ٥%، ١٠%، و ٢٠%) بعد تبريدها. واستعملت طريقة الحفر بالاكار لاختبار الفعالية البايولوجية لكل مستخلص وحسبت الفعالية بقياس قطر منطقة التثبيط بوحدة المليمتر. اختبرت فعالية المستخلصات ضد البكتيريا الاتية الموجبة لصبغة گرام (*Streptococcus faecalis* و *Staphylococcus aureus*) والسالبة لصبغة گرام (*Pseudomonas pyogens* و *Escherichia coli* و *Klebsiella pneumoniae*). كما أجريت بعض الاختبارات للكشف عن وجود المجاميع الفعالة فيهما حيث أجريت الكشوفات عن القلويدات و التانينات والصابونينات و الكلايكوسيدات بينما تم استنتاج وجود الزيوت من ملاحظة وجودها من عدمه على سطح المستخلص.اظهرت النتائج انه عند تركيز ١% لم تلاحظ أي منطقة للتثبيط لكلا المستخلصين لكنها ظهرت عند تراكيز أعلى من ٥% وازداد قطر منطقة التثبيط بزيادة التركيز. بصورة عامة أظهرت النتائج أن مستخلص الكجرات له فعالية أعلى من فعالية مستخلص الكمون. كما أظهرت النتائج ومن خلال قطر منطقة التثبيط أن المستخلصين لهما اقل فعالية تجاه بكتيريا *Escherichia coli* بالنسبة للمجاميع الفعالة فقد اظهر البحث وجود كميات محسوسة من الكلايكوسيدات و الدهون وكميات كبيرة من التانينات بينما لم يلاحظ وجود القلويدات و الصابونينات في مستخلص الكمون. أما الكجرات فقد وجدت كميات من الكلايكوسيدات و الصابونينات وكميات كبيرة من التانينات بينما لم يلاحظ وجود الدهون الأساسية و القلويدات.يستنتج من البحث أن المستخلصات المائية لكلا النباتين تمتلك فعالية بايولوجية عند تراكيز تساوي أو أعلى من ٥%. كما أن مستخلصات نبات الكجرات له فعالية أعلى من نظيراتها من نبات الكمون وربما يعود السبب الى وجود الصابونينات في الكجرات. من الممكن تفسير النتائج من خلال طبيعة ونوعية المواد الفعالة الموجودة في المستخلصات المائية لكلا النباتين.

مفاتيح الكلمات: الفعالية البايولوجية، المستخلصات النباتية، الكجرات (*Hibiscus sabdariffa*)، الكمون (*Cuminum cyminum*) القلويدات، التانينات، الصابونينات، الكلايكوسيدات، الزيوت.

Introduction:

A lot of antimicrobial screening evaluations have been published based on the traditional use of Chinese, African, and Asian plant drugs during this period. The studies about the effect of plant extract against different types of bacteria are still one of the most important fields of researches [1, 2, 3, 4].

Many new antibacterial agents submitted to analysis by the American food and drug agency (FDA) and some of them were natural products while others were semisynthetic products modeled on a natural product lead [5].

Hibiscus sabdariffa L. (Rosella) water extract is a local soft drink material and medicinal herb. The *Hibiscus sabdariffa* extract consider to be save knowing that LD(50) of roselle calyx extract. The LD(50) was found to be above 5000 mg kg^{-1} [6]. Though the average consumption of 150-180 mg/kg per day appears safe, the extract should be taken with caution bearing in mind that higher doses could affect the liver [7]. *Hibiscus sabdariffa* exhibits antihypertensive and cardioprotective effects in vivo and supports the public belief that *Hibiscus sabdariffa* may be a useful antihypertensive agent [6, 8, 9, 10]. In one study [11], the results suggested that *Hibiscus* extract blocks adipogenesis, in part, by its suppression on the expression of adipogenic transcription factors [11].These results suggest that *Hibiscus sabdariffa* L. inhibits serum lipids and shows an antiatherosclerotic activity [12].

Administration of a crude extract of *Hibiscus sabdariffa* have protected erythrocytes against lipid peroxidation [13]. Water extract of the dried flowers of *Hibiscus sabdariffa* L. reduces the hepatotoxicity caused by different agents [14, 15, 16]. The results indicate that rosella has antimutagenic activity [17]. The data suggested that *Hibiscus* protocatechuic acid is an apoptosis inducer in human leukemia cells [18]. These results indicate that *Hibiscus* protocatechuic acid (PCA), a phenolic acid isolated from *Hibiscus sabdariffa* L., possesses potential as a cancer chemopreventive agent against tumor promotion [19].

Cuminum cyminum L. (Cumin) is a small annual herb native to the Mediterranean region. India and Iran are the largest cumin exporters. The valued portion of the plant is the dried fruit called cumin seed, which is esteemed as a condiment. The odor and flavor of cumin is derived largely from the essential oil, which contains cuminaldehyde as the main constituent. Other ingredients of the oil are dihydrocuminaldehyde, d, 1-pinene, d--pinene, para-cymene, -pinene, dipentene, and cuminyl alcohol. The dried seeds of cumin have 2.5 to 5 percent essential oil on a dry weight basis as obtained by steam distillation. As a medicinal plant, cumin has been utilized as an antispasmodic, carminative, sedative, and stimulant. Cumin oil has been reported to has antibacterial activity (1.8-130). Distinct phototoxic effects have been reported from undiluted cumin oil (8.2-79) Cumin is generally recognized as safe for human consumption as a spice/flavoring and plant extract [20]. The main essential oils of *Cuminum cyminum* L. oil were p-mentha-1,4-dien-7-al, cumin aldehyde, gamma-terpinene, and beta-pinene [21].

The essential oils extracted from the seeds of *Cuminum cyminum*, have been studied for antibacterial activity against different eight pathogenic bacteria, causing infections in the human body. It has been found that the oil of *Carum capticum* is very effective against all tested bacteria. The oil of *Carum cyminum* and *Anethum graveolens* also gave effective against all tested bacteria [22].

The present study has been designed to identify antibacterial activity in extracts obtained from against type cultures and organisms that were isolated form various infections. Some tests for active groups in these aqueous plant extract also studied.

Materials and Methods

1. Collection and Extraction of Plant Material:

Whole dried calyces of the flower of *Hibiscus sabdariffa* and dried seeds of *Cuminum cyminum L.* were collected from the market and classified by expert taxonomist. The plant materials were carefully sorted out to remove any debris or possible contaminating substances. Then they were pulverized in a mill. The following quantities of the dried plant parts (1, 5, 10, and 20 grams) were then covered with 100 milliliters of distilled water and thoroughly extracted by boiling for 15 minutes. The evaporated water was substituted by adding an extra volume of distilled water to obtain a final volume of 100 milliliters of filtered extract.

2. Identification of Active groups in aqueous plant extract:

The tests that can be carried out to screen functional groups that can be extracted by water were carried out. While those tests that required alcoholic extract were not performed because this work deals with water soluble compounds in both plants. Hence tests about flavones, coumarins, and terpenes were not done while oils noticed as upper layer (if present). Alkaloids measured using Dragendroff's reagent according to Harborn (1973) method [(23)]. Saponines screened by two methods. The first method was carried out by mercuric chloride method. The second method by vigorous mixing of a tube containing the extract; if suds formed and keep on for 5-10 minutes this indicates the presence of saponines [(24)]. Tannins screened by ferric chloride method as described by Harborn (1984) [(25)]. Glycosides screened by Fehling's reagent as described by Adeday *et al* (2001) [(26)].

3. Antibacterial Activity:

The agar-well diffusion method (Perez *et al* 1990) [(27)] was used to screen the antibacterial activity of each extract. Aliquots of 20mL of Müeller Hinton agar (Oxoid®, Basingstoke, Hampshire, England) were poured into 9 cm Petri dishes in order to evaluate bacterial growth. The agar inoculated with 100 µL of 18-24 hour nutrient broth cultures of the following bacteria (Gram positive: *Streptococcus faecalis* and *Staphylococcus aureus) and (*Gram negative: *Pseudomonas pyogens, Escherichia coli, and Klebsiella pneumonia*e). Four holes, equidistant from each other and from the edge of the plate, were bored into the solidified seeded agar using sterile glass borers (8 mm in diameter).The plates were incubated at 37°C for 30 minutes before introducing 50 µL of each extract (at 1%, 5%, 10%, and 20%) into each of two holes using micropipette. Holes containing distilled water alone were included on each plate as control. The plates were refrigerated at 4°C for 30 minutes to allow for diffusion before incubating at 37 °C for 24 hours. The produced zones of inhibition were measured for each organisms screened.

Results:

Antibacterial activity of *H.sabdariffa* and *Cuminum cyminum* was measured as diameter of the inhibition zone (mm) as shown in Tables (1 and 2). The results of both plants showed that the inhibitions of bacterial growth are concentration dependent.

Table (1): Biological activity of the aqueous extract of *Hibiscus sabdariffa* L.
expressed as zone of inhibition (mm) using agar well diffusion method.

Bacteria type	Zone of Inhibition (mm)			
	1%	5%	10%	20%
Streptococcus faecalis	0	16	18	22
Staphylococcus aureus	0	24	24	24
Pseudomonas pyogens	0	14	18	26
Escherichia coli	0	14	18	18
Klebsiella pneumonia	0	3	18	20

Table (2): Biological activity of the aqueous extract of *Cuminum cyminum* L. expressed as zone of inhibition (mm) using agar well diffusion method.

Bacteria type	Zone of Inhibition (mm)			
	1%	5%	10%	20%
Streptococcus faecalis	0	14	18	18
Staphylococcus aureus	0	12	12	18
Pseudomonas pyogens	0	12	12	18
Escherichia coli	0	3	3	14
Klebsiella pneumonia	0	3	16	20

At 1% concentration, there are no detectable inhibition zones of both aqueous extracts. The inhibition zones appeared at 5% and further inhibition showed as concentration increased. In general, the aqueous extract of *H.sabdariffa* has shown more biological activity than the corresponding concentration of *Cuminum cyminum*. The lowest activity was shown against *E.coli* bacteria while the other bacteria showed different zone diameters as a response for the same concentration.

The active components were studied in 10% of aqueous plant extract to detect the active components in both plants (Tables (3)).

Table (3): Biological activity of the aqueous extract of *Cuminum cyminum* L. expressed as zone of inhibition (mm) using agar well diffusion method.

Plant Extract	Presence of detectable active components in 10% of Plant Extract				
	Essential Oils	Alkaloids	Tannins	Glycosides	Saponines
Cumin	+	-	++	+	-
Sabdhariffa	-	-	++	+	+

In *Cuminum cyminum* there are detectable amounts of essential oils, glycosides and high amounts of tannins While alkaloids and saponines are not detectable using the described methods. In *H.sabdariffa*, there is a detectable amount of saponines, glycosides, and high amounts of tannins while there are no positive results for essential oils and alkaloids.

Discussion:

The search for antibiotics in plant extract still an attractive field of study because they are available, cheep, safe, and easy to use by unspecialized populations [28, 29, 30].

Gram-negative organisms tend to have a higher intrinsic resistance to most antimicrobial agents. In the study by Ndukwe *et al* (2005) [1] the Gram-negative organisms were less susceptible than Gram-positive organisms to the activity of the different plant extracts. Antibacterial activity, determined with the agar diffusion method, was observed against Gram-positive and Gram-negative bacterial species in this study. The lower activity was observed against bacteria belonging to the genus *Pseudomonas*. These results suggested the potential use of the above essential oils for the control of bacterial diseases [21]. The essential oil of different plant sources showed different antibacterial activity [31, 32], and many types of bacteria did not affected by essential oils of some plants [31].

Hibiscus protocatechuic acid (PCA), a phenolic compound found in the dried flowers of *Hibiscus sabdariffa L.* (Malvaceae), was demonstrated to have an antioxidant effect in vitro and *in vivo*, and an antitumor property in previous study [15]. These results indicate that protocatechuic acid, a polyphenolic compound from *Hibiscus sabdariffa* L. protects against tert-butylhydroperoxide-induced hepatotoxicity by its antioxidant [33, 34] and anti-inflammatory characteristics accompanied by blocking of stress signal transduction [34].

A roselle (*Hibiscus sabdariffa* Linn.) tea extract was found to have high inhibitory activity against porcine pancreatic α-amylase [35] and the crude hydroalcoholic extract showed in vitro an appreciable enzyme-inhibiting activity towards the Angiotensin I Converting Enzyme (ACE), attributable to flavones, but weak inhibiting activities towards elastase, trypsin and alpha-chymotrypsin. The angioprotective activity in vivo, also important, was due to flavones and anthocyanins [36]. This ability to inhibit enzyme activity may be the reason about the antibacterial activity (i.e. may inhibit some enzymes necessary for bacterial biological activity. Anthocyanins are natural plant dyes present as glycosides and water soluble. The concentration of anthocyanins in the cold extract of *H.sabdariffa* found to be 5% while the hot extract of *H. sabdariffa* contained the second highest concentration of anthocyanins (31%). The heat aided the extraction of the anthocyanins in *H. sabdariffa*, despite their susceptibility to rapid loss of color because of the acidified methanol used for extraction. It is recommended that extracts of both *H. sabdariffa* great commercial value as natural colorants in Nigeria [37]. Aqueous extracts of the calyces are concentrated with anthocyanins [38]. *H. sabdariffa* contain different anthocyanins in addition to their colourful characteristics possess antioxidant properties [39, 40]. *Hibiscus sabdariffa* also contain lignin [41].

Hibiscus sabdariffa extract had an inhibitory effect on yeast induced pyrexia. Among the phytoconstituents found in both plants, flavanoids, polysaccharides, and organic acids may be mainly responsible for their pharmacological activities [42].

Raw polysaccharides were isolated from the flowers of *Hibiscus sabdariffa* L. and fractionated by ion exchange chromatography into one neutral and three acidic subfractions. Raw polysaccharides and all acidic subfractions caused a strong induction of proliferation of human keratinocytes of up to 40 %, while the neutral polymers were ineffective. While mitochondrial activity was not influenced, raw polysaccharides induced early differentiation of primary natural human keratinocytes [43]. The neutral polysaccharides (HIB 1 and 2) are composed of arabinans and arabinogalactans of low relative molecular mass [44].

The results also indicated that hibiscus contained low levels of aluminum and greater amounts of iron and copper [45]. The possible effect of these ions in the extract on biological activity needs more investigation.

Among the 49 compounds of volatile oil was extracted from *Cuminum cyminum* L. identified, there were 16 hydrocarbons and 32 oxygenated compounds. The main components were cuminal and safranal (accounting for 32.26% and 24.46% respectively in the components identified). The other nine compounds with contents all over 1% were monterpenes, sesquiterpenes, aromatic aldehydes and aromatic oxides etc. The other components with relatively small amounts were chiefly terpenes, terpenols, terpenals, terpenones, terpene esters and aromatic compounds [46]. From the water-soluble portion of the methanol extract of cumin (fruit of *Cuminum cyminum* L.), which has been used as a spice and medicine since antiquity, sixteen monoterpenoid glucosides, including twelve new compounds, were isolated. Their structures were clarified by spectral investigation [47]. Many spices including cumin were found to contain the following elements: Mg, Al, Si, P, S, Cl, K, Ca, Ti, Mn, Fe, Cu, and Zn, with varying concentrations. Mutagenic studies using different *Salmonella typhimurium* strains showed a very weak oxidative mutagenic action has been revealed by cumin [48]. Ethanolic extracts of *Cuminum cyminum* L. expressed minimum

inhibitory concentration (MIC90) values of 0.075 mg/mL. The results showed a significant *in vitro* effect of plant extracts against *H. pylori* that could be considered a valuable support in the treatment of the infection and may contribute to the development of new and safe agents for inclusion in anti-*H. pylori* regimens [49].

In conclusion, the noticed biological activity in the present work showed that the activity depends on the chemical constituents of each plant extract and the activity is dependent on the concentration of the water soluble chemicals from each plant.

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