

The Activity of Aqueous Extract of Cuminum cyminum L. and Hibiscus sabdariffa L. against Trichophyton mentagrophytes and Trichophyton rubum and Detection of Some their Active Chemical Groups.

دراسة قدرة المستخلصات المائية لنباتي الكمون والكرجات على تثبيط نمو الفطريين الممرضين

Trichophyton rubum و *Trichophyton mentagrophytes* وكشف عن بعض مجاميعها الكيميائية الفعالة

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Abstract:

The development of more effective and less toxic antifungal agents is required for the treatment of dermatophytosis. The studies about the effect of plant extract against different types of fungi are still one of the most important fields of researches because they are available, cheap, and safe. Dermatophytes are fungi that can cause infections (known as tinea) of the skin, hair and nails because of their ability to use keratin. In this work, two plant extracts *Hibiscus sabdariffa* (*H. sabdariffa*) and *Cuminum cyminum* (*C. cyminum*) were tested for their possible biological activity against two dermatophytes *Trichophyton rubrum* (*T. rubrum*) and *Trichophyton mentagrophytes* (*T. mentagrophytes*). Boiled aqueous extracts of both plants at the following concentrations (5, 7.5, and 10%) were used after cooling. Agar dilution method was used to examine the biological activity of each extract and the results expressed as diameter of colonies in (mm). 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). The results of both plants showed that the inhibition of fungal growth is dependent on the concentration of plant extract. The *T. mentagrophytes* is more sensitive in growth than *T. rubrum* in response to both plant extracts. In general, there is no statistical difference ($p>0.05$) in the activity of both aqueous extracts against the two fungi. The pHs of 10% solution of *C. cyminum* and *H. sabdariffa* extract were 5.95 and 2.40, respectively. In *C. 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 *C. cyminum* have antifungal activity at concentrations $\geq 5\%$. There is no significant difference in the antifungal activity between the aqueous extract of *H. sabdariffa* and *C. cyminum* against both fungi. In general, both fungi showed same growth inhibition in response to both plant extracts. The results indicated that both of the plant extracts used in the present work are acidic ($\text{pH}<7$) and *H. sabdariffa* had higher acidity than cumin indicated the presence of different acidic compound in the extract. The acidity affects the growth of fungi and may decrease the rate of growth. This antifungal activity of the plant extract may be due, in part, to the presence of different active substances in the extracts. The results can be explained through the presence of different active substances, especially tannins and glycosides, of in the aqueous extract of both plants. More studies are required to isolate the specific antifungal compounds and to use the extract clinically.

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

الخلاصة :

إن تطوير مواد فعالة مثبتة لنمو الفطريات وقليلة السمية للمضيف لازالت دراسة الفطريات الجلدية المرضية. لازالت دراسة الفطريات الجلدية المهمة كون هذه المستخلصات متوفرة و رخيصة و أمينة إن الفطريات الجلدية الممرضة تتسبب الاتهابات للجلد والأظافر والشعر لقدرتها على استعمال الكيراتين الموجود في هذا الأعضاء في هذا البحث تمت دراسة الفعالية الباليلوجية للمستخلصات النباتية لنباتي الكرجات *Trichophyton* و *Trichophyton rubrum* (*T. rubrum*) ضد نمو الفطريات الجلدية الممرضة (*Hibiscus sabdariffa*) و الكمون (*Cuminum cyminum*) كما تم الكشف التوعي عن بعض المواد الفعلية فيها. استعملت المستخلصات المائية المغلية لكلا النباتتين ضد *Trichophyton mentagrophytes* (*T. mentagrophytes*) بتركيز 5% و 7.5% و 10% بعد تبريدها. واستعملت طريقة تخفيف الأكار (Agar dilution method) لاختبار الفعالية الباليلوجية لكل مستخلص و حسب الفعالية بقياس قطر المستمرة بوحدة الملميت. كما أجريت بعض الاختبارات لكشف عن وجود المجاميع الفعالة فيما حيث أجريت الكشوفات عن القلويدات و التانينات و الصابونينات والكلايكوسيدات بينما تم استنتاج وجود الزيوت من ملاحظة وجودها من عدمه على سطح المستخلصات المائية. أظهرت النتائج لكلا المستخلصين ان تثبيط النمو يزداد بزيادة تركيز المستخلص أي ان الفعالية معتمدة على التركيز. كما أن *T. mentagrophytes* هو أكثر تأثرا بالمستخلصات المائية لكلا النباتين من الفطر *T. rubrum* وبصورة عامة لا توجد فروقات معنوية ($p>0.05$) في فعالية كل المستخلصين ضد الفطرين المدروسين. إن قيم الدالة الحامضية للمستخلصين النباتيين *C. cyminum* و *H. sabdariffa* اللذان ترتكزهما 5.95 و 2.40 على التوالي. بالنسبة للمجاميع الفعالة فقد اظهرت البحث وجود كميات محسوبة من الكلايكوسيدات و الدهون و كميات كبيرة من التانينات بينما لم يلاحظ وجود القلويدات و الصابونينات في مستخلص الكمون. أما الكرجات فقد وجدت كميات من الصابونينات و كميات كبيرة من التانينات بينما لم يلاحظ وجود الدهون الأساسية و القلويدات. يسنتج من البحث أن المستخلصات المائية لكلا النباتين تمتلك فعالية ضد الفطريات الجلدية الممرضة عند ترتكز تساوي أو أعلى من 5%. كما أنه لا توجد فروق معنوية في الفعالية المضادة لنمو الفطريات الجلدية الممرضة لكلا المستخلصين، وبصورة عامة كل الفطرين أظهروا نفس التثبيط في النمو تجاه كل المستخلصين مثيرا إلى وجود مواد عالية الحامضية في كل من نباتي الكمون والكرجات. إن الحامضية العالمية تؤثر على سرعة نمو الفطريات وهذه الخاصية بالإضافة إلى وجود العديد من المواد الفعالة في المستخلصات المائية وخصوصاً التانينات والكلايكوسيدات هي التي سببت تثبيط نمو الفطريات. و من الممكن تفسير النتائج من خلال طبيعة و نوعية المواد الفعالة الموجودة في المستخلصات المائية لكلا النباتين والتي تحتاج إلى دراسة مستقبلية لغرض فصل و تشخيص المواد ذات القدرة على تثبيط نمو الفطريات لغرض استعمالها سريرا لمعالجة الإصابة بهذه الفطريات المرضية.

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

Introduction:

The search for antimicrobial agents in plant extracts still an attractive field of study because they are available, cheap, safe, and easy to use by unspecialized populations ^(1, 2). Recently, much attention

has been directed toward extracts and biologically active compounds isolated from popular plant species. The use of medicinal plants plays a vital role in covering the basic health needs in developing countries, and these plants may offer a new source of antibacterial, antifungal and antiviral agents with significant activity against infective microorganisms. A lot of antimicrobial screening evaluations have been published during this period based on the traditional use of different plants from different areas. The studies about the effect of plant extracts against different types of bacteria^(3, 4, 5) and fungi^(6, 7) are still one of the most important fields of researches. Dermatophytes are fungi that can cause infections (known as tinea) of the skin, hair and nails because of their ability to use keratin. Superficial mycoses are probably the most prevalent of infectious diseases worldwide^(8, 9, 10). Tinea pedis (athlete's foot) and onychomycosis (infection of the toenails) caused by the dermatophyte fungi, *Trichophytons*, are highly prevalent in adults⁽¹¹⁾ and it represent a serious problem when associated with other diseases^(12, 13). Since the fifties of last century *Trichophyton rubrum* (*T. rubrum*) is the most frequently isolated dermatophyte accounting for 80-90% of the infectious strains, followed by *Trichophyton mentagrophytes* (*T. mentagrophytes*). They are connected with the increase in the incidence of tinea pedis⁽¹⁴⁾. Hence, the study of different plant extracts as a source for antidermatophyte drugs and support its use in folk medicine for the treatment of fungal skin infections is an important issue. Two plants; *Hibiscus sabdariffa* (*H. sabdariffa*) and *Cuminum cyminum L.* (*C. cyminum L.*) were screened for their antidermatophytic efficiency against different human pathogenic fungi responsible for tinea and other skin infections. *Hibiscus sabdariffa L.* (English: roselle, red sorrel; Arabic: karkade) 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 was found to be above 5000 mg kg⁻¹⁽¹⁵⁾. *Hibiscus sabdariffa* exhibits antihypertensive and cardioprotective effects *in vivo*^(15, 16, 17). In one study⁽¹⁸⁾, the results suggested that *Hibiscus* extract blocks adipogenesis⁽¹⁸⁾, inhibits serum lipids, shows an antiatherosclerotic activity⁽¹⁹⁾ and protected erythrocytes against lipid peroxidation⁽²⁰⁾. *Hibiscus* protocatechuic acid (PCA), a phenolic acid isolated from *Hibiscus sabdariffa L.*, possesses potential as a cancer chemopreventive agent against tumor promotion⁽²¹⁾, and as apoptosis inducer⁽²²⁾.

C. cyminum L. (Cumin) is a small annual herb native to the Mediterranean region. 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 (The dried seeds of cumin have 2.5 to 5 percent essential oil on a dry weight), which contains cuminaldehyde,⁽²³⁾ p-mentha-1, 4-dien-7-al, cumin aldehyde, gamma-terpinene, and beta-pinene⁽²⁴⁾ as the main constituent. Other ingredients of the oil are dihydrocuminaldehyde, d, 1-pinene, d--pinene, Para-cymene, -pinene, dipentene, and cumanyl alcohol^(24, 25). As a medicinal plant, cumin has been utilized as an antispasmodic, carminative, and stimulant. Cumin is generally recognized as safe for human consumption as a spice/flavoring and plant extract⁽²³⁾. The essential oils extracted from the seeds of *C. cyminum*, have been studied for antibacterial activity against different pathogenic bacteria, causing infections in the human body⁽²⁶⁾. However, up to date, little research⁽²⁷⁾ has been done to investigate these traditionally used plants *Hibiscus sabdariffa* and dried seeds of *C. cyminum L.* as antifungal. Although numerous studies have been carried out using natural extract products for screening antifungal activity^(28, 29, 30) little attention was given to these plants antifungal activity. The present study has been designed to identify antifungal activity of aqueous extracts obtained from flower of *Hibiscus sabdariffa* and dried seeds of *C. cyminum L.* against two pathogenic fungi cultures that causes skin diseases; *T. mentagrophytes* and *T. rubrum* that were isolated form patients. Some tests for active groups in these aqueous plant extracts were also studied.

Materials and Methods

1. Collection and Extraction of Plant Material:

Dried seeds of *C. cyminum L.* and whole dried calyces of the flower of *Hibiscus sabdariffa* and 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 (5, 7.5, and 10 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 of both plants. Hence, tests about flavones, coumarins, and terpenes were not done while oils noticed as upper layer (if present). Alkaloids measured using Dragendorff's reagent according to Harborn (1973) method ⁽³¹⁾. 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 ⁽³²⁾. Tannins screened by ferric chloride method as described by Harborn (1984) ⁽³³⁾. Glycosides screened by Fehling's reagent as described by Adeday *et al* (2001) ⁽³⁴⁾.

3. Measurement of pH:

The acidity of the most concentrated solution of each plant extract (10%) was estimated by pH-meter (Hanna[®]).

4. Antifungal Activity:

Antifungal activity of the aqueous extracts from *C. cyminum L.* and *H. sabdariffa* was determined by the agar dilution method ⁽³⁵⁾. The aqueous plant extracts (5, 7.5, and 10%) of each plant were mixed with Sabouraud dextrose agar (SDA) which is dissolved and cooled at 50°C. Each experiment was repeated three times. After solidification of the media, a disk of 6millimeter diameter of fungi colony, which previously cultured for 7-10 days in SDA media, was put in the center of the disk. The Petri dishes were incubated at 25-28°C for a period of 14 days. The control group were made in the same way accept the addition of plant extract. The results were recorded in centimeters as diameter of the grown colonies.

5. Statistical Analysis:

The results were analyzed statistically, and values were expressed as (mean ± standard deviation). The level of significance was determined by employing (t) test. When the p-value was less than 0.05, the difference between two groups considered statistically significant. All the statistical analysis processes were done using Microsoft Excell[®] program.

Results:

Antifungal activity of *H. sabdariffa* and *C. cyminum* against *T. mentagrophytes* and *T. rubum* was expressed as diameter of colonies zone (mm) as shown in Tables (1 and 2), respectively. The results of both plants showed that the inhibitions of fungal growth are dependent on the concentration of plant extract.

The *T. mentagrophytes* is more sensitive in growth than *T. rubum* for the both plant extract. In general, there is no statistical difference ($p>0.05$) in the activity of both aqueous extracts against the two fungi.

The active components were studied in 10% of aqueous plant extract to detect the active components in both plants (Tables (3)). In *C. cyminum* there are detectable amounts of essential oils, glycosides and high amounts of tannins. While alkaloids and saponines are not detectable at these concentrations 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.

The pHs of 10% solution of *C. cyminum* and *H. sabdariffa* extract were 5.95 and 2.40, respectively.

Discussion:

The development of more effective and less toxic antifungal agents is required for the treatment of dermatophytosis. Different active drugs are present in the pharmacies against these dermatophytes including terbinafine, itraconazole, ketoconazole, and fluconazole but they are expensive and exported⁽³⁶⁾. Plants and their preparations have been used as medicines against different fungi. Many plant extracts were tested and found to have antifungal activity including *T. mentagrophytes*, and *T. rubrum*^(37, 38, 39, 40, 41, 42, 43). Some of plant extracts are active *in vitro* against human pathogenic dermatophytes^(44, 45). The finding of the present work are in accordance with the previously mentioned studies as shown in Table (1 and 2) and the *cumin* and *Sabdariffa* extracts have good anti growth activity on vitro against human pathogenic dermatophytes *T. mentagrophytes* and *T. rubrum*. The results indicated that both of the plant extracts used in the present work are acidic (pH<7) and *Hibiscus sabdariffa* had higher acidity than cumin indicated the presence of different acidic compound in the extract. The acidity affect the growth of fungi and may decrease the rate of growth. *Hibiscus* protocatechuic acid (PCA), a phenolic compound found in the dried flowers, was demonstrated to have an antioxidant effect *in vitro* and *in vivo*⁽⁴⁶⁾, and an antitumor property in a previous study⁽⁴⁷⁾. *Hibiscus sabdariffa* L. extract was also found to have an inhibitory activity against different enzymes⁽⁴⁸⁾. *T. mentagrophytes* has an exocellular keratinase enzyme⁽⁴⁹⁾. This ability to inhibit enzymes activity may be the reason about the antifungal activity (i.e. may inhibit some enzymes necessary for fungal biological activity. Furthermore, it is suggested that tannins are plausible candidates for the anti-dermatophytic activity especially, *T. mentagrophytes* and *T. rubrum*⁽⁵⁰⁾. Hence, tannins that present in cumin and sabdariffa as shown in Table (3) may be the most important factor for the antifungal activity of both plant extracts. Oil extracted from the *Hibiscus sabdariffa* L. has been shown to have an inhibitory effect on some bacteria and fungi *in vitro*⁽⁵¹⁾. The inhibition of growth of fungi that noticed in Table (1 and 2) is dose dependent and the growth decrease as the concentration of the two plant extracts increase. This fact was shown in other research where the growth of both *T. mentagrophytes* and *T. rubrum* activity against *T.* is dose- and contact time-dependent manner⁽⁶⁾. Anthocyanins are natural plant dyes present as glycosides and water soluble. The heat aided the extraction of the anthocyanins from calyces of *H. sabdariffa* and the aqueous extracts are concentrated with anthocyanins⁽⁵²⁾. *H. sabdariffa* contain different anthocyanins in addition to their colourful characteristics possess antioxidant properties^(53, 54). Antifungal agents affect fungal cells by different mechanisms, but most of the drugs act by binding to ergosterol in the cell membrane and inhibit ergosterol synthesis. Some antifungal act by inhibiting mitosis and nucleic acids synthesis of fungal cells⁽⁵⁵⁾ or lysis of cell membrane⁽⁵⁶⁾. The effect of the plant extracts on fungal growth may due to one of these mechanisms. The mechanism of inhibition of *T. mentagrophytes* and *T. rubrum* by plant extract can be related to the cell leakage as observed by irregular, wrinkle shape and loss in rigidity of the macroconidia⁽⁵⁷⁾. Therefore, microscopic investigation should be taken into consideration in addition to the chemistry of plant extract for interpretation of antifungal activity. Different aqueous extracts⁽⁵⁸⁾ and agent⁽⁵⁶⁾ targets the cell membrane of the two important dermatophytes, *T. rubrum* and *T. mentagrophytes*, as breaking down of both inner and outer membranes with consequent extrusion of materials into the surrounding medium. Cytoplasmic membranes and other membranous structures of organelles, such as nuclei and mitochondria, were also disrupted. Plasmolysis accompanied by an almost complete depletion and disorganization of cytoplasmic structures were found to be the final event which led to cell death⁽⁵⁸⁾. *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⁽⁵⁹⁾.

Raw polysaccharides isolated from the flowers of *Hibiscus sabdariffa* L. caused a strong induction of proliferation of human keratinocytes of up to 40 %. Raw polysaccharides induced early differentiation of primary natural human keratinocytes⁽⁶⁰⁾. The neutral polysaccharides are composed of arabinans and arabinogalactans of low relative molecular mass⁽⁶¹⁾. This is another important useful mechanism for aiding the treatment of skin diseases in addition to the active components found in the extract. Some plant extract have activity against both *T. mentagrophytes*

and *T. rubrum*. A qualitative chemical analysis of the extract and fractions showed the presence of biologically active constituents such as some coumarins, flavonoids, phenols, tannins and sterols^(62, 63, 64, 65). The essential oil of *Eucalyptus pauciflora* showed strong antifungal activity against human pathogenic fungi, including, *T. mentagrophytes* and *T. rubrum* *in vivo* and *in vitro*. Moreover, it did not exhibit any adverse effects on mammalian skin up to 5% concentrations⁽⁶⁶⁾. Anise fruits extract inhibited the growth of dermatophyte species (*T. rubrum*, *T. mentagrophytes*, due to the fact that essential oil exhibited stronger antifungal activities against yeasts and dermatophytes⁽⁶⁷⁾. Some essential oil and aqueous extract of others exhibited fungistatic activities against *T. mentagrophytes*^(68, 69). Different essential oils showed strong activity and were assessed for their fungi toxicity against different dermatophytes⁽⁷⁰⁾. Sterols and triterpenes, parts of essential oils, were found to inhibit the growth of the fungi *T. mentagrophytes*⁽⁷¹⁾. Table (2) showed the antifungal activity of cumin expressed as diameter of colonies. The chemical components of cumin plant is important for understanding its antifungal activity. There are about 16 hydrocarbons and 32 oxygenated volatile oils extracted from *C. cuminum* L.. The main components were cuminal and safranal (accounting for 32.26% and 24.46% respectively in the components identified). The other compounds were monoterpenes, sesquiterpenes, aromatic aldehydes, aromatic oxides, terpenes, terpenols, terpenals, terpenones, terpene esters, aromatic compounds⁽⁷²⁾, monoterpenoid and glucosides⁽⁷³⁾. 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. The effect of these compounds of cumin are not completely studied against fungi growth. Cumin, have potent antimicrobial activities against bacteria and yeasts. The results from different sources establish the traditional use of spices as food preservatives, disinfectants and antiseptics⁽⁷⁴⁾. The antifungal effects of different plant extracts including cumin, were investigated against fungi⁽⁷⁵⁾. Cumin inhibit growth and aflatoxin formation of *Aspergillus flavus*⁽⁷⁶⁾ and able to stop mycelial growth at only 0.1% in the medium⁽⁷⁷⁾. Many research results suggested the use of different spice oils, including Cumin may offer some advantage in the prevention of mycotoxin production⁽⁷⁸⁾. No aflatoxin was produced when Cumin and mint levels of 5% and 10% were used⁽⁷⁶⁾. The results in Table (1 and 2) showed that the *T. mentagrophytes* is more sensitive for the plant extract than *T. rubrum* for the both plant extract. These results are agreed with the researches that showed that *T. mentagrophytes* were the most susceptible fungal strains to inhibit by different herbal extracts⁽⁷⁹⁾. While in other research, the results are differ from our results that showed that the *T. rubrum*, was the most susceptible dermatophytes⁽⁴⁴⁾ for some plant extract indicating the specificity of each plant extract and fungus toward each other. Some plants extract were completely inhibited the growth of *T. mentagrophytes* by only 14% of the extracts, respectively⁽⁸⁰⁾.

While in other studies, *T. rubrum* was the most susceptible species to inhibition by different plant extracts⁽⁸¹⁾. Table (3) indicated the presence of high amounts of tannins in the aqueous extract of both plants. Tannins, in addition to the acidity, may play a major role in the inhibition of dermatophytes growth as noticed in many previous works^(50, 51, 65). In conclusion, the noticed biological activity in the present work showed that the activity depends on the chemical constituents, especially tannins, of each plant extract and the activity is dependent on the concentration of the water soluble chemicals from each plant. From this study we can conclude that the traditional use of this plant for the treatment of infectious diseases is promising, mainly against fungi. Purification of the bioactive components from the extracts is underway and further investigations may improve our understanding of possible and antifungal activities.

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Table (1): Effect of aqueous solution of *H. Sabdariffa* on the diameter of colonies (mm) of *Trichophyton mentagrophytes* and *Trichophyton rubum* in comparing with control group.

Conc.% of plant extract	<i>Trichophyton mentagrophytes</i>	<i>Trichophyton rubum</i>
0	72.5±2.5	62.5±2.5
5	15±0	25±0
7.5	10±0	20±0
10	5±0	11±1

Table (2): Effect of aqueous solution of *C. cyminum* on the diameter of colonies (mm) of *Trichophyton mentagrophytes* and *Trichophyton rubum* in comparing with control group.

Conc.% of plant extract	<i>Trichophyton mentagrophytes</i>	<i>Trichophyton rubum</i>
0	72.5±2.5	62.5±2.5
5	15±0	22.5±2.5
7.5	11±1	17±3
10	7±1	11.5±1.5

Table (3): The chemical components of the aqueous extract of *C. cyminum* L. and *Hibiscus sabdariffa* L..

Plant Extract	Presence of detectable active components in 10% of Plant Extract				
	Essential Oils	Alkaloids	Tannins	Glycosides	Saponines
<i>C. cyminum</i> L.	+	-	++	+	-
<i>H. sabdariffa</i> L.	-	-	++	+	+