Phytochemical Analysis of Aqueous and Ethanolic Extracts of Seeds Powder of Sunut (*Acacia nilotica* L.), Bitter Apple (*Citrullus colocynthis*) L. (Schrad) and Senna (*Cassia acutifolia*) (Del.) and their Antibacterial Activity Against *Xanthomonas campesteris* pv *malvacearum*

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ABSTRACT

Cotton (Gossypiun spp.) is one of the most important fiber and oil crop worldwide. It is cultivated in many countries over the world. Bacterial blight disease is a devastated disease of cotton. Systemic pesticides are the most effective tool of controlling bacterial blight disease which affects cotton, however they possess a real threaten to human, animals and environment. This study aimed to determine the active constituents of some Sudanese indigenous plants such as sunt (Acacia nilotica L), Bitter Apple (Citrullus Colocynthis L.) Schrad and Senna (Cassia acutifolia Del.) Through phytochemical analysis of their seeds. In addition to reveal their anti-bacterial activity of their water and ethanolic extracts against causal agent of bacterial blight on cotton. Seeds of tested plants were collected from different parts of Gezira area. Tetracycline was used as a control. Phytochemical analysis was done to investigate active components of the previous mentioned plants. Disc filter paper and diffused technique were used to evaluate the bacterial inhibition zone. Three concentrations of each plant extracts were tested at 350,550 and 750 ppm. Inhibition of bacterial growth for each treatment were measured by using an ordinary ruler in millimeter (mm). The results obtained from the phytochemical analysis revealed that, flavonoid, steroids, triterpenes, and alkaloids were detected in all plants under the study. However, tannins have been detected in Sunut and Senna whereas glycosides were detected in Hanzal as well as in Senna. Additionally, Senna was the only plant that has no Nitrogen base. The interaction between those treatments revealed a very interesting result with highly significant (<.001) between the plant and concentrations. On the other hand, the interaction between plant and extracts was not significant with value equal to (0.941). Among the tested plants, Hanzal has no effect on the growth of bacterial blight. On the other hand, Sunt and Senna inhibited the bacterial growth to different degrees. Inhibition zone diameter produced by Snut and Senna ethanol extract reached to 22.22, 18.56 mm, respectively compared to Hanzal, while the inhibition zones of water extract of Snut and senna were 21.89, 26.78 mm, respectively. The ethanolic and water extracts of Sunut and Senna seeds showed high antibacterial activity than Hanzal, based on the measured zone of inhibition .The water extract reflected more antibacterial activity against the colonies grows of Xanthomonas campesteris pv malvacearum; it was 19.86 mm and that of ethanolic extract was 18.278mm, The inhibition zone increased with the increased concentrations (i.e. Senna in 350,550,750 ppm the inhibition zones were18.333, 23.833, 25.833mm, respectively). The study indicated that the potentiality of Sunut and Senna seeds extracts in the management of bacterial blight disease in cotton crop due to presence of bioactive compounds.

Key words: *Phytochemical analysis, Cotton, Bitter Apple, Sunut, Senna, Antibacterial activity.*

INTRODUCTION

Cotton (*Gossypiun* spp.) is the most economically important fiber and oil crop worldwide. It is cultivated in many countries over the world such as USA, India, Former USSR, China, Egypt, Mali, Sudan and Zimbabwe (Elbashir, 2000). In the Sudan ,cotton is one of the most economically important crops and its lint represent as one of the major source of foreign currency which represent 40-60% of the national income (Fadl Elseed, 2005). Bacterial blight of cotton (sometimes referred to as angular leaf spot or vein blight), black arm and bacterial boll rot are caused by *Xanthomonas campesteris* pv malvacearum (Smith) Dye (= *Xanthomonas malvacearum* (EFS) Dowson) the genus Xanthomonas is an economically important group of bacterial pathogens. Bacterial blight (BL) of cotton caused by *Xanthomonas axonopodis* pv. malvacearum (Smith) Dye affects the entire aerial parts of cotton plant i.e. Necrosis of parenchymatous tissue in the local phase and blockage of xylem vessels in its systemic phase (Cason *et al.,* 1977). The use of resistant varieties is an economical option for disease management but currently none of the high yielding commercial varieties has a durable resistance against the disease (Hussain *et al.,* 1985 and Khan and Rashid, 1996). In the absence of durable resistance in varieties, control the disease through chemicals, seed treatment or acid delinting is recommended but bactericide alone or in combination with fungicides dose not eradicate the pathogen completely (Hussain *et al.,* 1985 and Khan and Rashid, 1996). The use of chemicals for disease management is recommended as an alternative method but has very limited success (Hussain *et al.,* 1985 and Khan and Rashid, 1996) due to systemic nature of the bacterium. Disease management through plant extracts has been reported by different workers in different crops (Mukhtar *et al.,* 1994 and Mughal *et al.,* 1996) but very little is known about the antibacterial effects of plant extracts against bacterial blight of cotton. Biological control against foliar and soil borne disease in cotton has already achieved some success (Mondal and Verma, 2002). For bacterial blight, bacillus Sp- based plant spray has resulted in 40% control level .Biological seed treatment is cost effective for eradicating pathogens from seeds and protecting plants from infection (Cook, 1993).Nowadays, medicinal plants receive attention to research centers because their special importance in safety of communities. The curative properties of medicinal plants are mainly due to the presence of various complex chemical substances of different composition which occur as secondary metabolites (Karthikeyan *et al.,* 2009; Lozoya and Lozoya, 1989). They are grouped as alkaloids, glycosides, flavonoids, saponins, tannins, carbohydrates, and essential oils. Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds etc. (Gordon and David, 2001). Many plants possess antimicrobial activities and are used for the treatment of different disease (Arora and Kaur, 1999).

The present study aimed to determine the relationship between phytochemical components and antibacterial activity potential of (*Acacia nilotica* (L) Willd. ExDEL), (*Citrullus Colocynthis* (L.) and *Cassia acutifolia* (Del.) against *Xanthomonas campesteris* pv malvacearum on cotton.

MATERIALS AND METHODS

Site of experiments

The experiments were carried out in the Plant Pathology Center, Faculty of Agricultural Sciences and Food Science and Technology Laboratory University of Gezira, Wad Medani Sudan (14º24´N 33º 29´E, 408 m above sea level).

Collection of research materials

Seeds of three different plants species were collected from local market in Gezira State and University of Gezira campus. These plants were included *Citrullus colocynthis* L. commonly known as hanzel, *Acacia nilotica* commonly known assunnut and *Cassia acutifolia* Del. known as Senna. The seeds were dried under shade and subsequently ground to a fine powder using electric blender. The powder was kept inside brown bottles. Seeds were extracted by both water and ethanol solvents. The scientific names, Arabic names, and English names and parts used for preparing different extracts were as presented in Table (1).

Table (1): Scientific, local, and English names of plant materials.

Local name	Common name	Scientific name	Part used
Hanzel	Colocynth	*Citrullus colocynthis* L.	Seeds
Senna	Cassia	*Cassia acutifolia* Del.	Seeds
Seeds	*Acacia nilotica* (L.) Delile	Acacia acacia	Sunnut

Phytochemical screening

Test for glycosides

A known weight (30 g) the dried powder of Sunt, Senna, Hanzal seeds were boiled with an aliquot of distilled water (100 ml) and filtered aliquots (2 ml each) of the filtrate were tested for glycosides as described by (Harborne, 1998). The filtrate was dissolved in 2ml of glacial acetic acid. Two drops of ferric chloride solution were added to solution and mixed with. The mixture was transferred to a narrow test tube.

One to two ml of concentration sulpheric acid was added carefully on the side of the tube using pipette to form layer. Presence of glycosides is determined by formed of a reddish brown layer are the interface was formed and the upper layer gradually acquired a bluish – green color which darkened on standing.

Test for flavonoids

A known weight (5 g) of the seeds dried powder was macerated in 1% of hydrochloric acid (100 ml) overnight, filtered and the filtrate was subjected to the following tests:

1) 5 ml from each filtrate was made alkaline with sodium hydroxide (10%, w/v), if yellow color was formed that indicate the presence of flavonoids.
2) Shinoda test: 5 ml of each filtrate was mixed with 1ml of concentrated hydrochloric acid and magnesium turning. The formations of red color indicate the presence of flavonoids, flavonones, and/ or flavonols (Harbone, 1998).

Test for saponins

Five gram of the dried seeds powder was extracted with 5ml ethanol (50%) and filtrate Aliquot of the alcoholic extracts then mixtures evaporate to dryness by using rotary evaporate under reduced pressure. The residue was dissolved in 4ml of distilled water and filtered. The filtrate was vigorously shaken, if voluminous forth was developed and persisted foam one hour this indicates the presence of saponins (Harbone, 1998).

Test for tannins

Five gram of dried seeds powder was extracted with ethanol (50%) and filtered then ferric chloride reagent (5%, w /v) was added; the appearance of green color which changes to a bluish black color or precipitate indicate the presence of tannins (Balbaa *et al.*, 1974).

Test of sterols and / or triterpenes

Five gram of dried seeds powder was extracted with ethanol and filtered. Aliquots from the ethanolic extract (5 ml each) were mixed with 10ml (10 %v/v) of hydrochloric acid and filtered. The filtered was rendered alkaline with ammonium hydroxide and extracted with successive portions of chloroform. the combined chlorform extract was evaporated to dryness , the residue was dissolved in hydrochloric acid (2 ml 10%v/v) and tested with Mayer's reagent and Dragendroffs reagents, respectively, if precipitate was formed, it indicate the presence of alkaloids and/ or nitrogen bases ((Balbaa *et al*., 1974).

Test of alkaloids

Five gram of the dried powder of each seeds were extracted with ethanol and filtered. 10ml of ethologic were mixed with hydrochloric acid (10 ml ;10%v/v) and filtered. The filtrate was rendered alkaline with ammonium hydroxide and extracted with successive portion of chloroform –extract was evaporated to dryness. The residue was dissolved in hydrochloric acid (2 ml ;10%v/v) and tested with Mayer's reagent, and Dragendorff's reagent, respectively. The formation of a precipitate was indicated the presence of alkaloids and or nitrogenous bases (Harbone, 1973).

Preparation of plant extracts

Preparation of water plant extracts

Twenty grams of seed powder of each tested plant were dissolving in 200 SDW, in a volumetric flask and kept at room temperature, mixing well by shaking overnight till it homogenized. Mixtures were filtered in two steps, firstly by sieving to remove large particles and secondly, by filtration using Whatman No.1 filter paper to remove the fine particles. The residues were discarded and later the final concentration of the extraction were obtained following evaporation to dryness using rotary evaporate (R165) at 95 ºC and the residue of the extract kept in a brown bottle till it was used (Alias, 2004).Concentration of water extract is calculated according to the following formula:

Dissolved weight = Total weight – Insoluble matter weight ……..…. (1)

The concentration % =

Total weight–Insoluble matter weight/ Volume of extract)*100…….. (2)

Preparation of ethanol seeds extracts

Ethanol extract was prepared according to the method adopted by (Alias, 2004). Using Soxhlet apparatus extraction was done as follows steps:

Fifty gram of each seed powder placed in the extract thimble and 400 ml of ethanol 98% as a solvent was added in a clean, dry Soxhlet flask. Then the solvent was boiled at 90ºC. Extraction procedure was taken over 6 hrs. Then the thimble with the sample was removed, and the Desiccator was cooling and the extracted volume was measured. The extracts were concentrated by evaporating the ethanol solvent at 70ºC using a rotary vacuum evaporator and kept in small glass ware . Following is the formula for estimating the ethanol extract concentration:

The concentration = (The volume of extract)/ (Total weight) ×100 …….. (3)

Collection of infected plants

Leaves sample of cotton that showed angular leaf spots were collected from Agricultural Research Corporation (ARC) farm, the collected leaves were dried in paper sheets under shade and kept at room temperature for bacterial isolation.

Media preparation

The media was prepared by using ready-made nutrient agars (NA) media. It is consist of 5 g peptic digest of animal tissue, 1.50 g beef extract, 1.50 g yeast extract and 5g

sodium chloride, 15 g agar and the final pH 7.4±0.2. The 28 g of NA was dissolved in one liter of SDW in a flask closed carefully by cotton plug and aluminum foil. Media then autoclaved under standard condition i.e.121^{o}C for 20 minutes then cooled and poured into sterilized Petri – dishes. The Petri –dishes containing the media were kept at room temperature for 24 hours to ensure dishes free from microbial contamination.

Isolation of causal agent

Pieces of infected cotton leaf plant were cut off with sterilized blades. These pieces were washed in SDW, and then rinsed in 70% of ethanol for surface sterilization. Leaf pieces were washed three times in SDW to remove the ethanol residual. Then the infected plant tissues were mixed in a few drops of SDW to make bacterial suspension, under laminar air flow to ensure sterilize conditions, then a loop full of the bacterial suspension was spread on the surface of plates containing NA using sterilized loop. The inoculated plates were incubated 24 hours. Colonies growth show deep yellow, viscid and smooth that resembles to *X. malvacearum* was selected and sub – cultured to make pure culture of the bacteria using single colony.

Pathogenicity test

To confirm the virulence of the isolated bacteria colonies, pathogenicity test was carried out on disease free cotton hybrid Barakat seedlings.

Cotton plant preparation

Cotton seeds were planted in plastic pots containing 1: 2 mixture of sterilized sand –clay soil. Three to five seeds were sown in each pot. Plants are inoculated when the first true leaves were developed.

Inoculums preparation

Inoculums prepared by suspending of 24 hrs. Old bacterial culture in 10 ml of SDW shaking well and the bacterial suspension adjusted to 10^6 colony forming units (C.F.U) using dilution method.

Cotton inoculation procedure

Using sterilized hypodermic syringes, three labeled leaves on each seedling were inoculated by the bacterial inoculum in the lower surface of the leaves. Plants were infiltrated by SDW using sterilized hypodermic syringes for the check. Tested plants were kept till symptoms appearance. Observations were daily recorded till water – soaked lesions appeared after 4 - 5 days as indication of the compatible reaction and their pathogenicity to cotton plants.

Anti-bacterial activity assay of different plant extracts on growth of *Xanthomonas*

In vitro antibacterial activity of Aqueous ethanolic and Water extracts were determined by using filter paper disc diffusion method on nutrient agar medium .Full of bacterial suspension containing bacteria was spread on the NA solid plates with a sterile swab moistened with the bacterial suspension, square whatman filter paper 1cm^2 saturated with water and aqueous ethanolic extracts were placed in the bacteria inoculated petri dishes. Extraction was tested at 350,550 and 750 ppm for both the water and aqueous ethanol extracts .Tetracycline at same concentrations was used as a control. The plates were incubated for 24 hr. at room temperature. Inhibition zones were measured by using an ordinary ruler in millimeter (mm).All treatments (different concentrations) were replicated three times. MIC was determined based on the readings that have been taken using the ruler. Lowest concentration which inhibit bacterial growth for all tested plant extracts known as minimum inhibitory concentration (MIC), the concentration of extracts was prepared according to the equation 1mg/ ml= 1000 ppm.

Statistical analysis

Treatment and replication were arranged in completely randomized design (CRD). SAS software program was used to estimate the analysis of variance to determine the difference between treatments, Coefficient of variation (CV %), Standard Error (SE±) as well as the Mean separation, using LSD at level 5%.

RESULTS AND DISCUSSION

Isolation of *Xanthomonas campesteris* pv *malvacearum*

Isolated bacteria showed convex, smooth, domed, yellow and mucoid typical to *Xanthomonas* species colonies which suggested general morphological characteristic to *Xcm.* Moreover, pathogenicity tested on cotton plant produce the typical symptoms of cotton bacterial blight. This may confirm the isolation of *Xcm*

Phytochemical analysis of tested plant seeds

The results obtained from phytochemical analysis (for the main classes of phytochemicals) of *A. nilotica*, *C. colocynthis* and *Cassia acutifolia* seeds, were presented in (Table-1) Flavonoid, Saponins, Steroids, Triterpenes, and Alkaloids were detected in all plants under this study. However, Tanin has been detected in Sunt and Senna whereas Glycoside detected in Hanzel as well as in Senna. Additionally, Senna was the only plant that has no nitrogen base.

Table (2): phytochemical analysis of Sunt, Bitter apple and Senna in aqueous and ethanol extracts.

Active components	*Acacia nilotica*	*Citrullus colocynthis*	*Cassia acutifolia*
Glycoside	-	+	+
Flavonoid	+	+	+
Saponin	+	+	+
Steroid	+	+	+
Triterpenoid	+	+	+
Alkaloid	+	+	+
Nitrogen base	+	+	-
Tannin	+	-	+

+present, -not prescnt

The effect of aqueous ethanolic and water extract of tested plants on *Xanthomonas*

Among tested plants only Bitter apple any antibacterial activity whereas, Sunt and Senna inhibited the bacterial growth to different degree .Both water and aqueous ethanolic extracts of Senna and Sunt inhibited bacterial growth. Sunt aqueous ethanolic extract showed more antibacterial activity than Senna aqueous ethanolic extract, In contrast Senna in water extract showed better inhibition zone26.78 mm compared to Sunt water extract21.89 mm.Inhibition zone diameter produced by Sunt and Senna aqueous ethanolic extract reached 22.22 mm and 18.56 mm respectively, compared to the control 30.78 mm, (Table 3). Tetracycline showed high significantly bacterial inhibition zone compare to all plant extracts extracts .the results showed water seeds extract were better than seeds aqueous ethanolic extract against *Xanthomonas* growt.

Table (3): Interaction of plant and extracts against colony growth of Xam.

Plants	Extracts	
	Water (mm)	Aqueous ethanolic (mm)
Bitter apple	0.00 f	0.00 f
Sunt	21.89 d	22.22 c
Senna	26.78 b	18.56 e
Tetracycline	30.78 a	30.78 a

SE± Plants= 0.558 Extracts = 0.394 CV% = 12.5% LSD =5%

Table (4): Effect of different concentrations on the growth of Xanthomonas campestris pv. malvacearum.

Plants	350 ppm	550 ppm	750 ppm
	Inhibition Zone in (mm)		
Bitter apple	0.00 h	0.00 h	0.00 h
Sunt	20.17 f	22.17e	23.83 d
Senna	18.33 g	23.83 d	25.83 c
Tetracycline	27.67 b	28.67 b	36.00 a

SE± Plants= 0.558 concentrations = 0.483 CV% = 12.5% LSD =5%

The effect of different extract concentrations against colony growth of *Xanthomonas*

Generally inhibition zone diameter was increased according to increase in the concentration of active components, for tested plants extract and tetracycline as control. Among all tested plant except Bitter apple was showed significantly different effect on tested bacterial growth. Bitter apple in all concentration produce no effect on tested bacteria in contrast both Senna and Sunt were inhibited bacteria growth at different concentrations. The diameter of inhibition zone in Sunt increased at 350 ppm from20.17 to 23.83 mm in 750 ppm while in Senna increase from 18.33 at 350 ppm to 25.83 mm in 750 ppm (Table 4).Regarding the increase of concentrations followed by increase diameter of bacteria inhibition zone, Sunt produce systemic increase in inhibition zone, *i.e.* 20.17, 22.17 and 23.83 mm while in Senna, the increase in inhibition zone is more clear with concentration i.e. inhibition from 18.33 and 23.33 and 25.83, that mean there was significant different between concentration but there was no significant different between sunt and Senna as antibacterial against *Xcm*

Recently, plant extract have received much attention as source of antimicrobial substances. Although, The use of medicinal plant plays a vital role in covering the basic health needs in developing countries, is also plays role as new source of antibacterial and antiviral agents with significant activity against infective microorganisms .Therefore, there is a need to adopt cheap and environmental friendly control measures, which are available to the resource of small farmers that will enhance crop production as well as reducing pest and disease.

The analysis of phytochemical screening for *C. colocynthis*, *Acacia nilotica* and *Cassia acutifolia* proved the existing of phytochemicals namely, saponins, tannins, an

alkaloids, glycosides and flavonoids. The result obtained from this study showed that the seed extracts of selected plants were significantly affected the growth of *Xanthomonas campestris* pv *malvacearum*. Due to the presence of bioactive compounds they have mechanism action as good antimicrobial properties such as Tannins which have been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional proteins unavailable for them (Sadipo *et al*., 1991). The growth of many fungi, yeasts, bacteria and viruses was inhibited by tannins (Chung *et al*., 1998). Flavonoids are also reported to inhibit microbe's activity which are resistant to antibiotics by Linuma *et al.* (1994) the activity of flavonoids is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls. More lipophilic flavonoids may also disrupt microbial membranes. Saponins are a special class of glycosides which have soapy characteristics (Fluck, 1973).

The water and aqueous ethanol extracts *of A. nilotica, C. acutifolia* have good antimicrobial properties this could be attributed to the presence of most constituents of polar (ethanol and water extracts) which of these compounds are soluble in water or ethanol extract. This may explain the inhibition of tested bacterial growth produce by plant extracts.Sunt aqueous ethanol extract exhibited highest antibacterial activity than aqueous extract that result agree with (Mahesh and Satish, 2008) who observed high antibacterial activity of methanolic extracts of *Acacia nilotica* against *B.subtilis* and *Staphylococcus aureus*. Also, Mahesh and Satish, (2008) reported high antimicrobial activity of *Acacia nilotica* against three bacterial *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi*. Cos *et al.* (2006) found that compound easily extracted in polar solvent such as ethanol, methanol and ethyl acetate. Moreover, Saini, (2008) reported all extracts (water and acetone)of *Cassia acutifolia* demonstrated highest antimicrobial activity against *Xanthomonas* in acetone extract while the water extract demonstrated the least activity this result disagree with our finding in this study. Although, Gnanavel *et al* (2012) and Hatano *et al.* (1999) they found *Cassia acutifolia* act against positive and negative gram bacteria both in aqueous and ethanol extract these result agree with our finding. Bitter apple in phytochemical analysis has same active components however don't show any antimicrobial activity against *Xcm* this could be due to the lack of concentration of the active substance and the absence of tannin. These finding disagrees with (Ayana *et al*., 2008) who screened antibacterial activity of *Citrullus colocynthis* against *B. subtilis* and *Staphylococcus aureus*, *Escherichia coli*, *Psedomonas aeruginosa*, *Proteus vigorous* and *Klebsiella pneumoni.*The relation between plants extracts concentrations and effectiveness was observed, results were more significant at 550 ppm and at 750ppm concentrations as compared to 350 ppm. In this study, the results of MIC suggested the inhibition zone increases accordingly with the increase in concentration. This could be due to the highly active nature that can be regarded as a promising resource for antibacterial drug or seed dresser.

CONCLUSION

The results obtained from this study showed that both water and aqueous ethanolic extract of Senna (*Cassia acutifolia*), Sunt (*Acacia nilotica*) and Bitter apple (*Cittirllus colocynthis)* have the following effects in inhibition the growth of *Xanthomonas campesteris* pv *malvacearum*: Sunt and Senna have antibacterial activities against *X. c. m*

whereas Bitter apple did not show any antibacterial activity. Water extracts reflected good bacterial growth inhibition compared to ethanolic extracts. Also this study revealed that the antibacterial properties of tested plant extracts possess potentiality to reduce the bacterial disease incidence due to the active compounds existed in the various plants.

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الملخص العربي

التحليل الكيمائي النباتي لمسحوق بذور السنط (القرض) والسنمكة والحنظل وفاعليتها ضد المسبب المرضي للفحة البكتيرية في القطن

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يعتبرمحصول القطن واحد من أهم محاصيل الالياف والزيوت وتمتد زراعته على نطاق واسع من العالم. مرض اللفحه البكتيرية يعتبرمن الامراض المدمره لمحصول القطن.استخدام المبيدات الجهازية يعد من الطرق الاكثر فاعليه لإدارة المرض بالرغم من انها تسبب اضرارا على الانسان والبيئة والحيوان.هدفت هذه الدراسة للتحليل الكيمائي النباتي لمسحوق بذورالسنط والحنظل والسنمكه ودراسة فاعلية المستخلص المائي والكحولي لبذورهذه النباتات في تثبيط نمو المسبب المرضي للفحه البكتيريه في نبات القطن. تم جمع البذورمن مناطق مختلفة من ولاية الجزيرة. استخدم الماء والايثانول كمذيبات للاستخلاص .تم التحليل الكيمائي النباتي للكشف عن الموا د الفعاله. استخدمت تقنية قرص ورقة الترشيح وتقنية الانتشار لتقييم منطقة تثبيط نمو البكتيريا.تم اختبارثلاثة تراكيز للمستخلصات النباتية وهي ٣٥٠ و ٥٥٠ و ٧٥٠لجزءاً من المليون. استخدمت المسطرة المدرجة لقياس منطقة تثبيط نموالبكتيريا بالملم . كشفت النتائج المتحصل عليها من التحليل الكيمائي النباتي عن وجود الفلافنويدات والاسترويدات والالكلويدات والتربينات في جميع النباتات تحت الدراسه ووجود التانينات في السنط والسنمكة فقط والجلايكوسيدات في الحنظل والسنمكه بالاضافه لوجود مادة السابونين في بذورالسنمكة كما انها لاتحتوي على مركبات نتروجينية . اظهرت النتائج ان هناك فروقات معنوية لكل معامله على حده باحتمال معنوية (<٠.٠٠١) ومع ذلك اظهرت النتائج ان التفاعل بين تلك المعاملات نتيجة مهمة للغاية وذلك لان التركيز يلعب دورا هاما في تثبيط نموالبكتيريا ويظهرذلك ايضا في نوع النبات من الناحية الاخرى. ومن جانب اخرلم تكن هنالك فروقات معنوية (٠.٩٤١) عند تفاعل كل من انواع النبات والتركيز ونوع المستخلص . مستخلصات الماء والكحول لكل من نباتات القرض والسنمكه كانت ذات فاعليه في تثبيط نموالبكتيريا بحيث كانت منطقة التثبيط للمستخلص المائي لكل منهما هي٢١.٨٩ و٢٦.٧٨ ملم على التوالي ومنطقة التثبيط للمستخلص الايثانولي ١٨.٥٦ و ٢٢.٢٢ ملم على التوالي مقارنة مع نبات الحنظل. ومن جانب اخراظهرت النتائج ان المستخلص المائي اكثر فاعلية في تثبيط نمو البكتيريا (١٩.٨٦ ملم) مقارنة مع المستخلص الايثانولي (١٧.٨٩ملم) منطقة التثبيط تزداد بزيادة التركيز مثلا نبات السنمكة في التراكيز ٣٥٠ ، ٥٥٠،٧٥٠كانت منطقه التثبيط المقاسة هي ١٨.٨٣٣، ٢٣.٨٣٣ و٢٥.٨٣٣ ملم على التوالي . توصي هذه الدراسه الي استخدام مسحوق بذور القرض والسنمكه كمضاد لنموالمسبب المرضي للفحه البكتيريه بمختلف التراكيز و كما انها تحتاج للمزيد من الدراسات المستقبليه لتحديد فاعليه هذه المستخلصات واختبارتاثيرها على شدة حدوث المرض.