Antifungal Activity of the Extracts of Garad (*Acacia nilotica* L.)

Abdel-Rahim, A. M[1].; Wafa Y. A.[1] and Idris, F. A.[2]

[1]Center of Biosc. and Biotech, Fac. of Eng.and Tech. [2] Fac of textile , University of Gezira

ABSTRACT

Plants may represent a potential source of antimicrobial compounds as evidenced by the huge studies dealing with antimicrobial activities of medicinal plants. However, Sudan possess an immense wealth of medicinal plants that is still unexploited. The present study was mainly conducted to investigate the antifungal of the most important and widely distributed Garad tree (*Acacia nilotica L.*) in the country. The extracts of the different parts of the tree were tested against the mycelial growth, mycelial weights and spore germination of two fungi (*Penicillium italicum* and *Aspergillus niger*). The inhibition zone and the radial growth methods were used for mycelial growth using PDA media. Extraction with different solvents confirmed that, Methanol, Diethyl ether, Acetone and the aqueous extracts were found highly effective in inhibiting growth of both fungi. The results also showed that the different tree parts had different effects with the extracts of both the tree bark and pods as the best effective. It is also clear that the extracts reduced both fresh and dry weights of mycelia of the two fungi. However, *A. niger* was found more sensitive to the extracts compared to *P. italicum*. The extracts reduced germination effectively, where only 23% and 37.7% of the spores of *A. niger* and *P. italicum* germinated, respectively, at the higher concentration of the extract (100mg/ml). It could be concluded that the extracts of Garad are highly effective as antifungal agents, specially the bark and pods of the tree. It could be recommended that the test should be carried on other microorganisms.

Key words: Antifungal activity, Garad tree, Aqueous extracts

INTRODUCTION

Since modern medicals have been beyond the financial reach of substantial proportion safe of the third world population, the use of plants as herbal medicine is emerging as and cheap alternative pharmaceuticals (Himmel *et al*., 1993; Legrand *et al*., 1993).

Factors such as easy availability of herbal remedies, accessibility to practioners at all times and places are encouraging.

Plants have formed the basis for traditional medicine systems in most societies and have been used for thousands of years. It was estimated that there are 250000 – 500000 species of plants on earth (Schultes, 1978; Borris, 1996).

Today, the WHO has estimated that about 80% of the world's inhabitants rely mainly on traditional medicines for their primary health care, where plant based systems still play a vital role in health care (Mullholand, 2000).

There is some literature dealing with the medical practices of Sudanese people. Most of the studies done by El-Ghazali (1987) were integrating ethno- botanical, agro-medical and pharmacological studies of Sudanese flora in different parts of the country. Due to the wide diversity of botanical and large number of species, Sudanese medical plants can be considered as very promising candidates as bioactive agents.

The Garad tree (*Acacia nilotica*), is one of most diverse of all the shrub species. It is a shrub that belongs to the family Fabaceae and the subfamily Mimosaceae and to the genus *Acacia*. The tree is called the Egyptian Mimosa in Egypt, the Garad in the Sudan and is referred to as Booni in Nigerian Yoruba language (Rahaman, 2009).

Acacia has multiple benefits to human beings. It is widely used as an ornamental tree as well as in the perfume industry. The roots of the acacia tree were boiled and used as a treatment for rabies, and the feathery shoots are used in fries, soups and gravies. The honey made from the forage is flavourful and has a very smooth texture. The seeds are either eaten raw, roasted or salted. Sauce and fritters are also made from the acacia tree and more popular in Mexico (Rahaman, 2009).

An infusion make from the bark of this tree or the gum is an effective medicine for diarrhea. The bark of *A. nilotica* tree is useful in the treatment of eczema. Chewing the fresh bark of the tree daily, helps strengthening loose teeth and arrest any bleeding from the gums (Ameh *et al*., 2010). The leaves of the tree are effective in the treatment of

conjunctivitis. The leaves of the *A. nilotica* were also found beneficial in treating epiphora that is the watering of the eyes (Internet, 2010).

According to Odugbemi (2008) *A. nilotica* can allay any irritation of the skin and smooth the inflamed membranes of the pharynx, alimentary canal and the genitor-urinary organs. The fresh pods of were found effective in treating sexual disorders such as spermatorrhea, loss of viscidity of semen, frequent night discharges and premature ejaculation. The African Zulu tribe, takes the bark of *A. nilotica*, for cough treatment, and also uses it for treating diarrhea, dysentery, leprosy and as an astringent. The bark or gum of the tree is used in West Africa to treat cancers and/or tumors of ear, eye or testicles. In Senegal, the bark, leaves and young pods are chewed as an antisorbutic (Ameh, *et al*., 2010). In Lebanon, *A. nilotica* is infused with orange flower to treat typhoid convalescence and use the roots to treat tuberculosis. The Egyptians believe that diabetics may eat carbohydrates as long as they consume powdered pods of *A. nilotica*. In Ethiopia, A. *nilotica* is used as a lactogogue (increase milk supply). In Australia, the bark is used for the treatment of hemorrhoids, skin eruption leg sores, mouth ulcers, sore throat and dental infections as well as discharge and excess mucus (Rahaman, 2009). However, Abdel-Rahim and Idris (2010), found that

The present study was mainly designated to investigate the antifungal properties of the extracts of the different garad tree parts against both fungi; *Penicillium italicum* and *Aspergillus niger*.

MATERIALS AND METHODS

Seeds of Garad (*Acacia nilotica*) were purchased from Wad Medani local market and then blended into a powder using a blender. The cultures of both *Aspergillus niger* and *Penicillium italicum* were obtained from the Food Science and Technology Laboratory, Faculty of Engineering and Technology, University of Gezira.

The effects of extracts of Garad on fungal radial growth were not completed since the garad tree extracts prevent solidification of the agar medium. The inhibition zone method

using was used instead. In This method a filter paper disc impregnated with each extract concentration was placed on the center of the surface of a solidifying PDA in a Petri dish containing either growth of *P. italicum* or *A. niger*. Inoculated dishes were then incubated at room temperature and the inhibition zones were measured as described by Barry *et al*. (1970) and Cruickshank *et al*. (1975). The potato dextrose broth medium (PDB), was used for studying the effect on mycelial weights of both fungi (*A. niger* and *P. italicum*).

To study the effect of the extracts of Garad on spore germination of both *A. niger* and *P. italicum*, the method described by Abdel-Rahim and Arbab (1984) was used.

EXPERIMENTAL RESULTS

The present study investigated the antifungal activity of the extracts of garad tree parts, using both fungi *Aspergillus niger* and *Penicillium italicum*. Results on Tables (1 and 2), show the effects of the extracts obtained by different solvents on the inhibition zone of growth of both *P. italicum* and *A. niger*, respectively. Methanol, Diethyl ether, Acetone and the aqueous extracts were found highly effective in inhibiting growth of the fungus *P. italicum* (Table 1). However, Petroleum ether gave an intermediate effect, while, all the other solvents were less effective. On the other hand, the effect of the extracts on the fungus *A. niger* are shown in Table (2). The results indicated that the aqueous extract was far better than the others, although, methanol was also highly effective. The results also confirmed that the different tree parts were showing different effects, with the extracts of both bark and pods as the best effective. However, the extracts of both seeds and leaves were less effective (Tables, 1 and 2).

Table 1 . Effect of different tree part extracts obtained by different solvents on inhibition zone (mm) of *P. italicum*.

Solvents	Tree parts			
	seeds	leaves	pods	bark
Methanol	5.0	5.5	10.0	11.5
Diethyl ether	5.0	8.0	9.0	10.0
Hexane	5.0	5.0	5.0	5.0
Chloroform	5.0	5.0	5.5	6.5
Ethanol	5.0	5.0	5.5	6.0

Acetone	6.0	6.0	8.5	10.0
Pet. ether	6.0	5.0	6.5	9.5
water	6.7	6.3	10.1	10.8

SE = 0.91 ST = 2.58

Table 2. Effect of different tree part extracts obtained by different solvents on inhibition zone (mm) of *A. niger*

	Tree parts			
Solvents	seeds	leaves	pods	bark
Methanol	7.0	5.5	7.0	15.0
Diethyl ether	7.0	6.0	9.0	10.0
Hexane	5.0	5.5	6.0	7.0
Chloroform	4.5	4.5	4.5	4.5
Ethanol	5.0	5.0	5.5	7.0
Acetone	5.5	6.0	7.5	8.0
Petroleum ether	4.5	5.0	5.5	5.5
water	8.0	10.0	10.3	20.1

ST = 1.57 SE = 0.56

The effect of autoclaving and membrane filtration on the biological activity of the different tree part extracts was also investigated. Results presented in Tables (3 and 4) show the effects on the inhibition zone of both the autoclaved and the membrane filtered extracts of *P. italicum*, respectively. The results indicated that autoclaving reduced the activity of the extracts compared to the effect caused by the membrane filtered extracts.

Table. 3. Effect of autoclaved sterilized extracts of different garad tree parts on inhibition zone (mm) of *P. italicum*

	Incubation period (days)			
Tree parts	2	4	6	8
Leaves	5.0	5.1	5.5	5.6
bark	10.1	10.2	10.2	10.2
Pods	5.7	5.9	6.0	6.0
Seeds	5.1	5.2	5.2	5.2

SE = 1.21 ST = 2.42

Table. 4. Effect of membrane filtered extracts of different garad tree parts on inhibition zone (mm) of *P. italicum*

	Incubation period (days)			
Tree parts	2	4	6	8

leaves	5.3	6.0	6.3	6.3
bark	10.2	10.3	10.3	10.5
pods	9.1	10.1	10.3	10.3
seeds	5.5	6.0	6.7	6.7

SD = 2.34 SE = 1.17

On the other hand, the effect was pronounced with the fungus *A. niger* (Tables 5 and 6). The membrane filtered extracts were highly effective on this fungus. The bark extracts were more effective compared to the extracts of the other three parts. It was followed by the extracts of the pods. The autoclaved bark extracts gave an inhibition zone of about 10.5 mm in diameter (Table 5), compared to an inhibition zone of about 20.3 mm in diameter caused by the membrane filtered extracts (Table 6). The results also proved that the membrane filtered extracts were highly effective against the fungus *A. niger* compared to their effects on the fungus *P. italicum.*

Table 5. Effectof autoclaved sterilized extracts of different garad tree parts on inhibition zone (mm) of *A. niger.*

	Incubation period (days)			
Tree parts	2	4	6	8
leaves	5.6	6.0	6.0	6.0
bark	8.3	10.3	10.5	10.5
pods	10.1	10.1	10.1	10.2
seeds	5.0	5.1	5.1	5.1

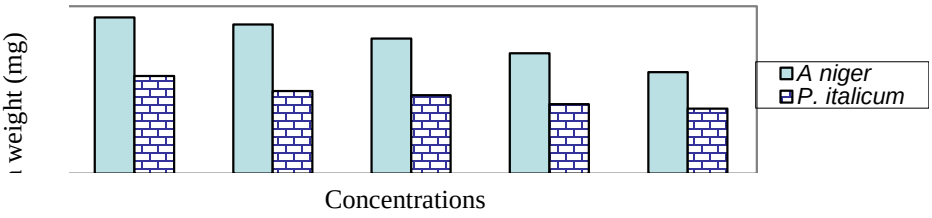
SE. = 2.37 SD = 4.74

Table 6. Effect of membrane sterilized extracts of different garad tree parts on inhibition zone (mm) of *A. niger*

Tree parts	Incubation period (days)			
	2	4	6	8
leaves	9.1	10.1	10.0	10.0
bark	11.0	20.0	20.3	20.3
pods	9.2	10.1	10.5	10.6
seeds	5.5	7.0	8.0	8.1

SE = 1.33 SD = 2.67

The effects of the membrane filtered bark extracts of the garad tree on mycelial growth of both fungi (*P. italicum* and *A. niger*) are shown on Fig. 1.and 2, respectively. Data on Fig. 1, compared the effect of the membrane filtered bark extracts on the fresh weights of mycelia of both P. italicum and A. niger, while, data on Fig. 2, compared the effects on the dry weights of both fungi.



.

(1) Effect of the aqueous garad extracts of bark on mycelial fresh weight of *A. niger* and *P. italicum***.**

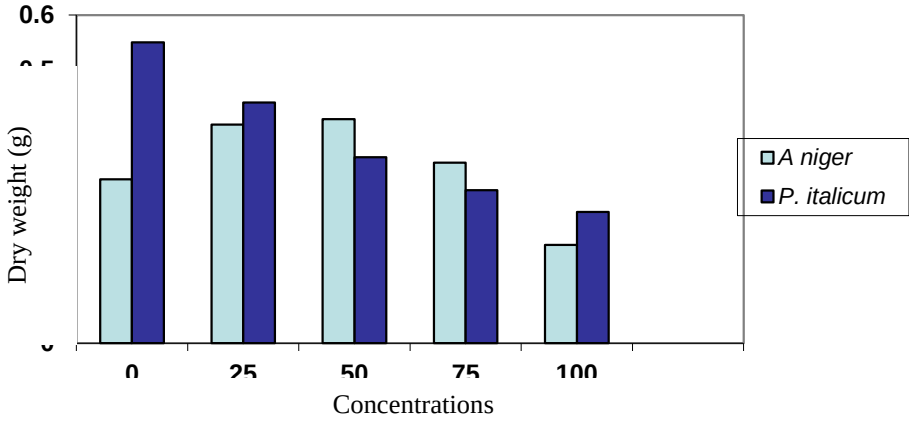
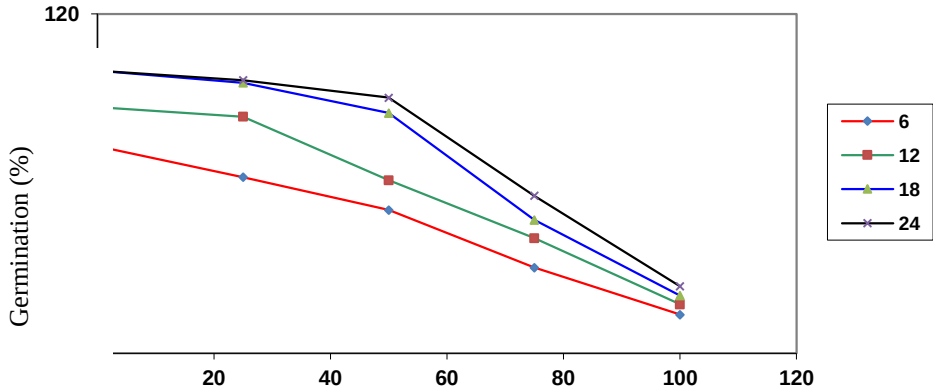


Fig. (2): Effect of the aqueous garad extracts of bark on mycelial dryweight of *A. niger* and *P. italicum.*

From the results it is clear that the extracts reduced both weights of mycelia of both fungi. However, *A. niger* was found more sensitive to the extracts compared to *P. italicum* (Fig. 1 and 2). Studies on the effects of the membrane filtered garad tree bark extracts on conidiospore germination of both *P. italicum* and *A. niger*, were made on clean sterilized glass slides. The numbers of conidiospores germinated were counted out of each 100 spores at different time intervals and germination was then calculated as percentage. Results displayed on Fig. (3) and Fig. (4), are showing the effects of the membrane filtered extracts of the bark of the garad tree on both conidiospore germination of *P. italicum* and *A. niger*, respectively.



Concentrations

Fig. (3). Effect of the aqueous garad bark extracts on conidiospore germination of *P. italicum*.

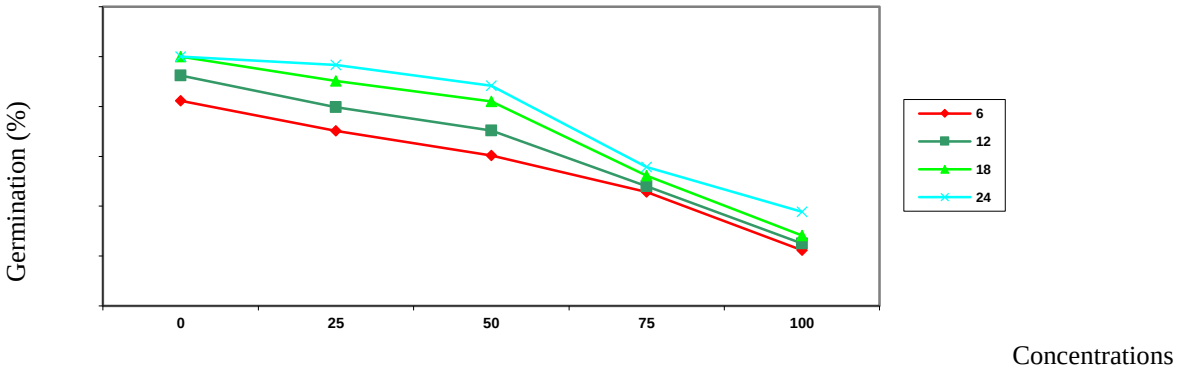


Fig.(4). Effect of the aqueous garad bark extracts on conidiospore germination of A. niger.

The results also showed that the extracts are highly effective in reducing conidiospore germination. Germination of the spores was decreasing steadily with increasing the extract concentrations, where only 37% of spores germinated in the case of *P. italicum*, while, only 23% germination was recorded for *A. niger* at the higher concentration 100mg/ ml. The effects of the different concentrations of each of the Garad tree parts (bark, pods, leaves and seeds) on the inhibition zone of both fungi (*P. italicum* and *A. niger),* were also investigated in the present study.

The effect of the different concentrations of the extracts on *A. niger* are shown on Tables (7). The results showed that the bark extracts were highly effective giving inhibition zones of about 20.4 and 15.1 mm in diameter at the concentrations of 100 and 75 mg/ml, respectively. It was followed by the extracts of the pods which gave about 20.1 mm inhibition zone at the higher concentration, then followed by the leaves and finally the seeds which are the less effective giving only 11.0 and 6.8 inhibition zones, respectively (Tables 7).

On the other hand, the extracts concentrations were comparatively less effective on growth of the fungus *P. italicum* as shown in Table (8). The results also indicated that the bark extracts were the highly effective, giving inhibition zones of about 19.3 and 15.8 mm in diameter at the concentrations of 100 and 75 mg/ml, respectively. It was followed by the extracts of the pods which gave about 10.8 mm inhibition zone at the higher concentration , then followed by the leaves and finally the seeds which were the less effective giving only 9.3 and 8.3 inhibition zones, respectively (Table, 8).

Table 7. Effect of different concentrations of membrane sterilized tree parts extracts on inhibition zone (mm) of *A. niger*.

	Tree parts)			
Concentrations	bark	pod	leaf	seed
0.0	4.0	4.0	4.0	40
25	7.5	7.2	6.5	6.6
50	10.3	10.0	8.2	6.8
75	15.1	14.8	10.7	6.8
100	20.4	20.1	11.2	6.8

ST = 6. 3 S. E. = 2.83

Table 8. Effect of different concentrations of membrane sterilized tree parts extracts on inhibition zone (mm) of P. italicum.

	Tree parts			
Concentrations	bark	pod	leaf	seed
0.0	4.0	4.0	4.0	4.0
25	12.0	7.0	7.0	7.0
50	13.5	10.3	7.5	7.5
75	15,8	10.5	7.7	7.8
100	19.3	10.8	9.3	8.3

SE = 2.22 ST = 4.96

DISCUSSIONS

Different Garad tree part extracts, were investigated against two fungi (*A. niger* and *P. italicum*). The results indicated that the extracts of the tree bark and the pods are highly effective against both fungi. Mycelial fresh and dry weights were reduced, however, the reduction was only statistically different compared to that of the leaves and the seeds as well as with the control. The use of plants and their extracts as remedies for curing many diseases have stimulated studies for investigating the presence of effective antimicrobial

substances in them (Ahmed, 2004, Abdel Daim, 2001, Sulieman *et al.,* 2008 and Abdel-Rahim and Idris, 2010). Extracts and essential oils of both clove and cinnamon were found to inhibit growth and aflatoxin production by *Aspergillus flavus* and *A .parasiticus* (Alderman and Marth, 1976, Abdel-Rahim and Mohmmed Ali; 2002, Sulieman *et al.,* 2008, Abdel-Rahim *et al*., 2010). On the other hand, Zainal *et al.,* (1988) reported a pronounced effect of the leaf litter extracts of mesquite (*Prosopis juliflora)* on *A. niger* and *Condida albicans.* However, the extracts of *Cuppress, Juniper* and Rose marry were found more effective against *A. flavus* (Al-Jali *et al.*,1997).

The present study also investigated the effects of the different extracts on the spore germination of both fungi. From the results it is clear that the extracts have caused a highly significant reduction in the number of the spores germinated specially at the higher concentrations. Similar results were also obtained by different researchers (Abdel-Rahim *et al*. ,1989; Al-Jali *et al.*,1997 Abdel-Rahim and Mohmmed Ali; 2002, Sulieman *et al.,* 2008 and Abdel-Rahim *et al*., 2010).

In Sudan many studies were carried out for testing the antimicrobial activity of some medicinal plants. Ahmed (2004) tested the extracts of 10 plants against Gram positive and Gram negative bacteria as well as *Candida albicans*. He found a marked effect against the Gram positive *Staph. aureus* followed by the Gram negative *E. coli* and *Candida albicans*.

From the results, It could be concluded that Garad extracts which are traditionally used for curing many known diseases are useful for treating skin diseases orally. However, clinical trials should be performed under the supervision of a specialist.

REFERENCES

Abdel Daim, Z.J (2001). Phytochemical and Microbial Studies on some Senna Species. M. Sc., Faculty of Science, University of Khartom.

Abdel – Rahim, A. M., and Arbab, H. A. (1984). Factors affecting conidiophores germination in *Aspergillus niger*. ***Mycopathologia***, 89: 75 – 79.

Abdel-Rahim, A. M.; Osman, N. A. and Idris, M. O. (1989). Survey of some cereal grains and legume seeds for aflatoxin contamination in the Sudan. Zentralbl. Mikrobiol., 89: 75-79.

Abdel- Rahim, M. A. and Mohamed Ali, R. A. (2002). Effects of natural plant products on fungal growth and aflatoxin production by *A. flavus.* And *A.parasiticus.* African Journal of Mycology 15: 12-20.

Abdel-Rahim, A. M. and Idris, F. A. (2010). Survival of *Staphylococcus aureus* and *E. coli* on cotton fabrics treated with extracts of garad (*Acacia nilotica*). Gezira J. of Eng. & Applied Sci. 5(2): 127 – 134.

Abdel- Rahim, A. M., Mohammed Nour, H. A. and Sulieman, A. E. (2010) Effects of some essenetial oils on *Aspergillus flavus* growth and aflatoxin production. Journal of Science and Technology (In prss).

Ahmed, M. M. (2004). Phytochemical. Antimalarial, Molluscicigdal and Antimicrobial Activity of Selected Sudanese Medicinal Plants with Emphasis on: *Nigella sativa L.* seeds. Ph. D. Thesis, University of Gezira. Pp.75-78.

Al-Jali1, Z. I. and Al –Mismari, F. A. and Abdel-Rahim, A. M. (1997). Contamination of Seeds of Some Crops with Alfatoxins in the Jabel Al-Akhdar Region. Proceeding of the 6th . Arab Congress of plant protection. Beirut, Lebanon, p. 294.

Alderman G. G. and Marth, E. H. (1976). Inhibition of growth and aflatoxin production of *Aspergillus parasiticus* by citrus oils. Z. Lebensm. Unters Forsch 16o:353-358

Ameh, J. S. *et al*., (2010). Tropical Journal of Pharmaseutical Research. 9 (2): 119 – 125.

Barry, A. L.; Garacia, F. and Trupp, I. D. (1970). Interpretation of sensitivity Test results. Am. J. Clin. Path. 53: 149 – 155.

Cruickshank, R. J., Dugide, J. P., and Swanin, R. H. (1975). Medicinal Microbiology. Vol. II Edinburgh, 12- Ehank-d.

El Ghazali, G. E. (1987). Medicinal Plants of the Sudan. Part II. Medicinal Plants of the Eastern Nuba mountains. Khartoum University Press, Sudan.

Himmel, W., Schulfe, M. and Kochen, M. (1993). Complementary medicine: are patients expectations met by their general practitioners?. Br. J. Gwen. Pract. 43: 232 – 235.

I**nternet** (2010). Benefits and uses of the Acacia Tree. http:/www. Buzzle. com/articles/acais-tree.html.

Legrand, A. Sri Ngernyang, L. and Streefland, P. (1993). Enhancing appropriate drug use: The contribution of herbal medicine Promotion. A-case study in rural Thialant Soc. Sci. Med. 36: 1023 – 1035.

Mullholand, A. D. (2000). Medicinal plants, A source for new drugs. African Conference on Medicinal Plant Research. Al-Fatih University, Faculty of p|harmcy, Tripoli, Libya.

Odugbemi, T. (2008). Outlines and Pictures of Medicinal Plants from Nigeria. University of Lagos, Nigeria, Press.

Rahaman, O. (2009). A review of uses of *Acacia nilotica* (Booni) in alternative medicine. Internet http://search.com/swa698858**.**

Schultes, R. E. (1978). The kingdom of plants. In: Thomson, W. A. R. ed). Medicines from the earth. McGrow- Hill Book Co. New York pp 208.

Sulieman, A. E.; Ahmed, H. E. and Abdel Rahim, A. M. (2008). The Chemical Composition of Fenugreek (*Trigonella foenum graceum L*) and the Antimicrobial Properties of its Seed Oil. Gezira J. of Eng. & App. Sci. 3(2): 1 – 21.

Zinal, A. S.,Abdel-Rahim, A. M.; Abu-Ali, R. M. and Radwan, S.S. (1988). Antimicrobial substances in the leaf litter of the *Xerophyte prospis Juliflora*. Zentralbl Mikrobial.143,375-381.

الملخص

تعتبر النباتات مصدر رئيسي للمواد المضادة للميكروبات. ويعتبر السودان من الدول التي لها ثروة من النباتات الطبية التي لم يتم استغلالها بعد. لقد تم في هذا البحث دراسة وجود المركبات المضادة للمكروبات في شجرة القرض والتي تنتشر بكثرة في ربوع القطر. وتم اختبار كفاءة مستخلصات الأجزاء المختلفة من الشجرة ضد النمو الميسليومي ووزن الميسليوم تم Penicillium و Aspergillus niger) . italicum) ونسبة انبات جراثيم الفطرين استخدام طريقة المنطقة المثبطة وطريقة النمو القطري لدراسة النمو اليسليومي وفي تم حين استخدام الوسط الغذائي السائل لمرق البطاطس لدراسة الوزن الميسليومي ووسط غذائي خاص لدراسة انبات الجراثيم. أظهرت النتائج أن استخدام مواد استخلاص مختافة قد اعطى تاثيرات مختلفة، حيث كانت مستخلصات الميثانول و الداي اثايل ايثر و الأستون بجانب المستخلص المائي هي الأكثر فاعلية. كما اوضحت النتائج أن مستخلصات الأجزاء المختلفة لشجرة القرض قد اختلفت في تاثيرها، حيث كانت مستخلصات اللحاء والحافظة أكثرها فعالية. وأظهرت النتائج كذلك أن الوزن الرطب و الجاف للفطرين كلاهما قد انخفض بدرجة كبيرة. هذا وكان التأثير على انبات الجراثيم أكثر وضوحاً، حيث لم تنبت غير 23% من جراثيم وحوالي 37% من جراثيم الفطر الأخر. ويستنتج من هذه الدراسة أن A. niger الفطر مستخلصات الأجزاء المختلفة لشجرة القرض لها تأثير فاعل ضد نمو الفطريات، خاصة اللحاء والحافظة. وتوصي الدراسة بالعمل على اجراء التجارب على أحياء اخرى.