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RESEARCH TITLE

ANTIBACTERIAL ACTIVITIES AND PHOTOCHEMICAL SCREENING OF THE EXTRACTS OF SPEARMINT (*MENTHASPICATA*), GUAVA (*PSIDIUM GUAJAVA*) AND SABAR (*ALOE VERA*)

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Abstract

The Spearmint , Guava and Sabar are one of the plants found in the North African region. These plants have medicinal and pharmaceutical properties as well as antioxidants and food flavorings, and are used in skin care products and cosmetics. The aim of paper research is to study the antibacterial properties of Spearmint , Guava and Sabar leaves and stems. Against the following bacterial: *E. coli* and *staphylococcus . auras* .This study was conducted at the University of Gezira 2019/20 by selecting the bacterial number of colons .as well as the spore germination of the bacterial using the nutritional medium that nutritional medium that Nutrient Agar (NA) , of the leaves and stems Photochemical screening of Guava, Spearmint and Sabar leaves. Spearmint , Guava and Sabar were brought from the local market of wad Madani, sample of bacteria obtained from the laboratory of the University of Gezira. Four concentration (25 ,50 , 75 , 100%) were made from the aqueous extract of the leaves and stems of Spearmint and Guava . and only Sabar leaves. The result showed that the aqueous extracts of leaves and stems of Spearmint, Guava, and Sabar leaves showed the effected on *E. coli* the results showed that the higher concentration were highly effected. The Spearmint leaf extracts were found to decrease the number of colonies from 88 to 1and from 24 to 1 at the higher (75 and 100%), concentration respectively The effect of the different concentration of Guava and Sabar leaf extracts on *E. coli* are showing in Table (2, 3). The results indicated that the extracts are less effected compared to the Spearmint leaf extracts the Guava and the Sabar leaf extracts both gave 14 similar of control at the higher concentration (100%) respectively

However the Spearmint stem extracts are more effective allowing 11.7 colonies to grow compare to 12.9 colonies by stem extracts of Guava. the number of colonies of the bacteria *S. auras* are showing Table respectively the leaf extracts of Sabar were less effective allowing 12.7 colonies to grow, compared to that of Spearmint and Guava which allowed only 10.7 and 9.9 colonies to grow are showing the effects of the Guava leaf and stem extracts on the inhibition zone of *E. coli* both extracts were found highly effective. The inhibition zone was increased from 4.2 mm to 12.7 mm by the leaf extracts and from 7.2mm to 15.9 mm by the stem extracts .photochemical screening of the leaves the Spearmint , Guava and Sabar leaves were found to content all the compared except sterols .The Spearmint stems contain tannins , flavonids and alkaloid but not saponin are sterols. The Guava stem contains only Tannin and flavonoid but no saponin , alkaloid and sterol are detected

Introduction

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 (Odugbemi, 2008). However, 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). 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 (Abdel Daim, (2001; Abdel-Rahim, and Idris, 2010)

Spearmint is species of mint native to North Africa, Egypt and Morocco. It is an invasive species in Great Lakes region where it was first sighted in 1843. Spearmint has long tradition medicinal use. It was taken as a tea to treat general digestive problems. Spearmint is widely used in commercially manufactured product, cooking and medicine for its aromatic and flavorsome qualities. The objectives of this study were to determine the chemical composition of the spearmint, to determine the physicochemical properties Guava is a small tropical tree that grows up to 35feet tall; it is widely grown for its fruit in tropics. Itis a member of the Myrtaceae family, with about133 genera and more than 3,800 species. The leaves and bark of *P. guajava* tree have a long history of medicinal uses that are still employed today (Nwinyi *et al.,* 2008). Sabar is in sub-Saharan Africa, the Arabian Peninsula and a number of Indian Ocean islands. They occupy many different kinds of natural habitat, from forest to exposed rock surfaces, but they are absent from the moist lowland forests of (Kamath *et al.*,2008)

In the view of the immense medicinal importance of *P. guajava* plant evidenced in the various studies mentioned above and also corroborated in a recent review article by Kamath *et al.* (2008). The medicinal plants display antioxidant and antimicrobial properties which can protect the human body against both cellular oxidation reactions and pathogens. Thus it is important to characterize different types of medicinal plants for their antioxidant and antimicrobial potentials (Mothana and Lindequist , 2005., Bajpai et al ,. 2005, Wojdylo et al 2007). Herbal medicine is the oldest from of medicine known to mankind. It was the mainstay of many early practiced form of medicine in the world today according to world health organization figures. While 25 to 50 % of the current pharmaceuticals are derived from plants, none are used as antimicrobials. Traditional healers have long used plants to prevent or cure infectious conditions plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, and flavonoids, which have been found in vitro to have antimicrobial properties. The structure and antimicrobial properties of the phytochemicals are also addressed . Since many of these compounds are currently available as unregulated botanical preparations and their use by the public is increasing rapidly, clinicians need to consider the consequences of patients self-medicating with these preparations

Materials and Methods

Preparation of plant part extracts:

The plants under test (Spearmint, Guava and Sabar) were collected from Wad Medani City, Sudan. The leaves and stems were washed in tap water, dried for 10 days at room temperature and blended into a powder using a mortar and pestle. Five concentrations (0.0, 25.0, 50.0, 75.0 and 100.0 mg/ml) were made by serial dilution of the different extracts with the medium in the flasks. All solutions were sterilized in an autoclave at 121C0 (15/b/in2) for 15 minutes and then cooled to room temperature.

Effect of the extracts on bacterial growth

Twenty-eight grams of an already prepared medium (Himedia Ltd.),were used per 1000ml of distilled water . the medium was dispensed into flasks (each containing 250ml),and autoclaved at 121^0 C (151 b/in2) for 15 minutes , then poured into sterile petri dishes, which were allowed to solidity and kept inverted into a refrigerator before use. The Cup –plate agar diffusion (Inhibition zone) method

This method was used , using Nutrient Agar (NA). In this method 2ml of a standardized bacterial cell suspension (10×10^5) of *E. coli* or of *Staphylococcus aurous* were thoroughly mixed with 200ml of sterile molten nutrient agar, then the medium was distributed into sterile Petri-dishes and was left to solidity at room temperature for 24 hours. Sterile Whatsman glass fiber discs (No.5) were saturated with the extract then allowed to dry and transferred centrally on the surface of the solidified medium in each plate. The plates were then incubated at room temperature for 72hours and the inhibition zones were measured as described by Barry *et a*l (1970).

Photochemical screening

photochemical screening to identify classes of secondary metabolites. Solvent extract of the plant material with help of different solvent (choroform, ethanol and Hcl). Photochemical analysis of the extract revealed presence of Saponin , Tannin , Flavonoid, Alkaloid, steroids and phenolic compound.

Experimental Result

The effect of the different concentration of the leaf plants extracts on both the number of colonies of *E. coli* were receded on Table(1-3). Table (1) showed the effected on *E. coli* the results showed that the higher concentration were highly effected. The Spearmint leaf extracts were found to decrease the number of colonies from 88 to 1and from 24 to 1 at the higher 75 and 100%), concentration respectively (Table, 1).

Table (1): Effects of different concentrations of the Spearmint leaf on the number of colonies of *E. coli*

concentration	Time (Hours)			
	6	12	18	24
0.0%	62	80	88	90
25%	50	68	76	80
50%	30	60	62	54
75%	7	10	12	18
100%	1	1	1	1

The effect of the different concentration of Guava and Sabar leaf extracts on *E. coli* are showing in Table (2- 3). The results indicated that the extracts are less effected compared to the Spearmint leaf extracts the Guava and the Sabar leaf extracts both gave (4.5 and 2,7) from Guava leaf and (4.5 and 10.5) from sabar leaf at the higher concentration (75 -100%) respectively

Table (2):Effects of different concentrations of the Guava leaf on the number of colonies *E. coli*

concentration	Time Hours			
	6	12	18	24
0.0%	4.2	4.2	4.2	4.2
25%	8.4	7.7	8.0	8.1
50%	9.4	9.8	10.0	10.0
75%	10.5	11.5	11.6	11.6
100%	2	2.7	2.7	2.7

Table (3):Effects of different concentrations of the Sabar leaf on the number of colonies *E. coli*

Concentration	Time (Hour)			
	6	12	18	24
0.0	4.5	4.5	4.5	4.5
25%	7.4	7.7	8.0	8.01
50%	9.4	9.8	10.0	10.0
75%	10.5	11.5	11.4	11.4
100%	10	10	10.3	10.5

The effect of the stem extracts of both Spearmint and Guava are shown in tables (4 and 5, respectively). The results indicated that both extracts are effective. However the Spearmint extracts are more effective allowing 8.7 colonies to grow (table ,4) compare to 12.9 colonies by stem extracts of Guava (table, 5).

Table (4): Effects of different concentrations of the Spearmint stem on the number of colonies of *E. coli*

concentration	Time (Hours)			
	6	12	18	24
0%	6.5	6.5	6.5	6.5
25%	8.4	7.7	8.4	8. 1
50%	9.4	9.8	10.0	10.0
75%	9.5	10.5	11.2	11.2
100%	8	8.7	8.7	8.7

Table (5):Effects of different concentrations of the Guava stem on the number of colonies *E. coli*

concentration	Time hours			
	6	12	18	24
0%	7.2	7.2	7.2	7.2
25%	8.4	7.7	8.0	8. 1
50%	9.4	9.8	10.0	10.0
75%	10.5	10.5	11.6	11.6
100%	12	12.9	12.9	12.9

The effect of the leaf extracts of the three plant (spearmint , Guava and Sabar) on the number of colonies of the bacteria *Staphy. auras* are showing Table (6,7,and 8) respectively. The leaf extracts of the Spearmint ,Guava and Sabar found effective in reducing the number of colonies of *Staphy. auras*. However the leaf extracts of Sabar were less effective allowing 12.7 colonies to grow (Table,8), compared to that of Spearmint and Guava which allowed only 4.7 and 9.9 colonies to grow (Tables, 6 and7).

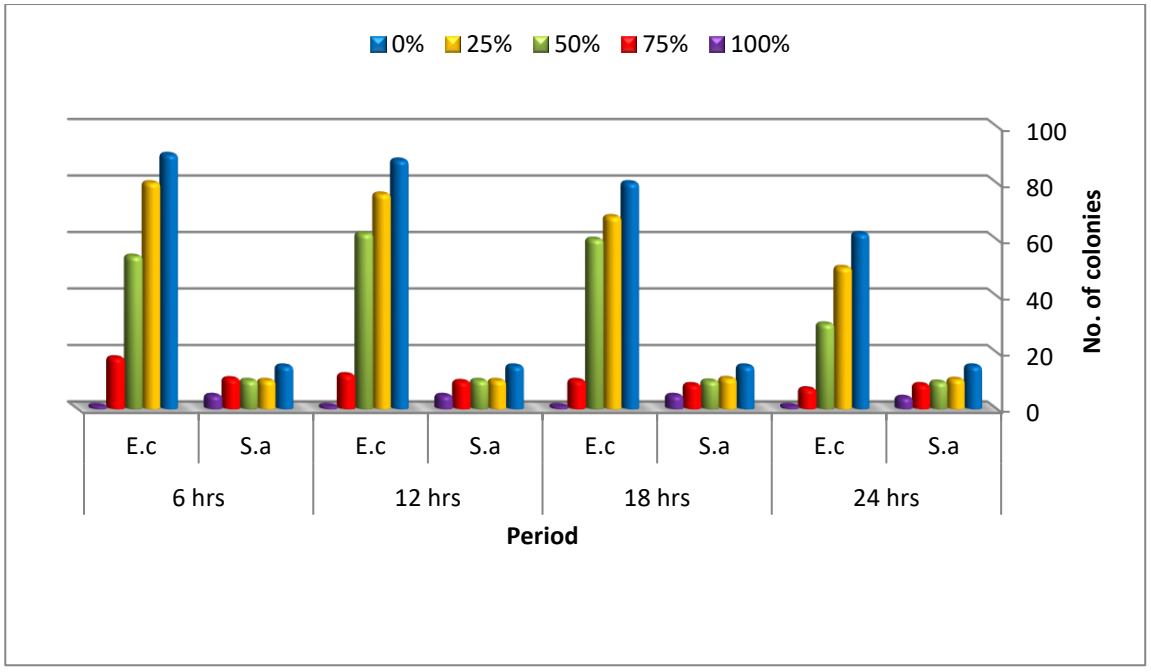


Fig (1) different of concentration to bacteria in plant extract steam

Table (6): Effects of different concentrations of the Spearmint leaf on the number of colonies *Staphylococcus. aurous*

concentration	Time Hours			
	6	12	18	24
0%	5.1	5.1	5.1	5.1
25%	7.4	7.7	8.0	8.01
50%	9.4	9.8	10.0	10.0
75%	8.5	8.5	9.6	10.6
100%	4	4.7	4.7	4.7

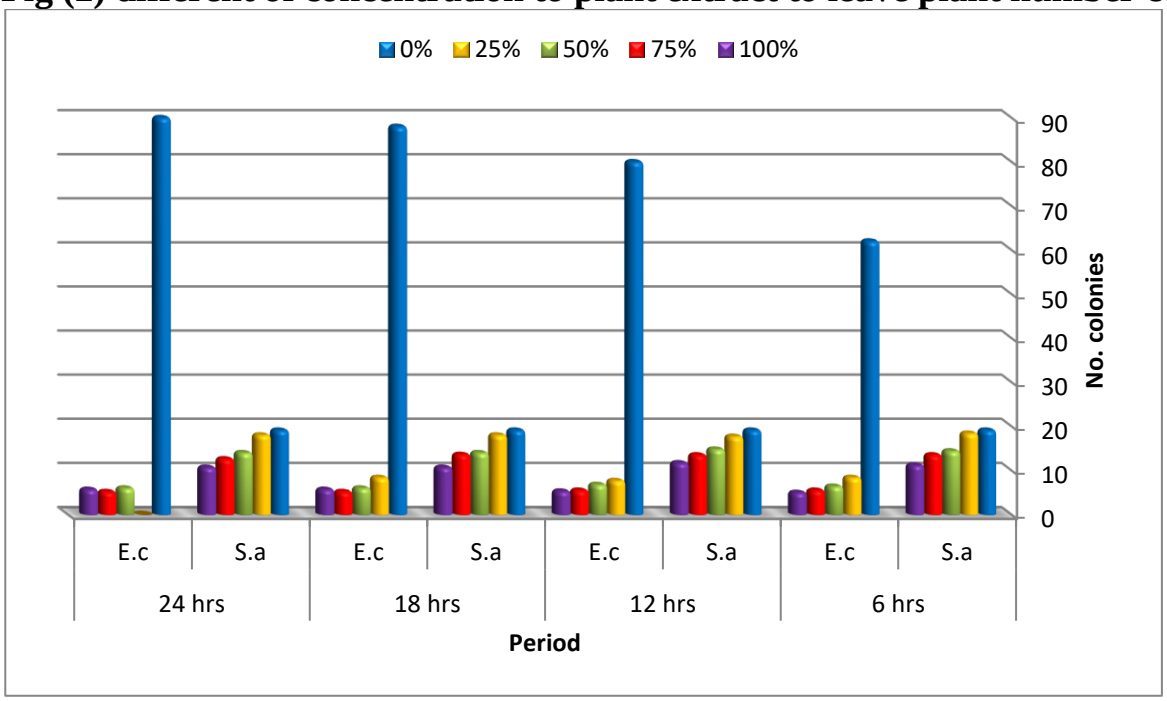
Table (7): Effects of different concentrations of the Guava leaf on the number of colonies of *Staphylococcus. aurous*

concentration	Time (Hours)			
	6	12	18	24
0%	4	4	4	4
25%	6.4	6.7	7.0	7.01
50%	9.4	9.8	10.0	10.0
75%	10.5	11.5	11.6	11.6
100%	9.9	9.9	9.9	9.9

Table (8) Effects of different concentrations of the Sabar leaf on the number of colonies Staphylococcus .aurous

concentration	Time Hours			
	6	12	18	24
0%	6	6.1	6.1	6.1
25%	7.3	7.6	8.0	8.02
50%	9.2	9.5	9.9	10.0
75%	10.5	11.3	11.6	11.6
100%	12	12.7	12.7	12.7

Fig (2) different of concentration to plant extract to leave plant number coloni



Tables (9 ,10) showed the effects of stem extracts of both Spearmint and Guava and number of colonies *Staphy. arouse* respectively both extracts were found effective given (10.7 and 13.9) respectively for concentration (100%)

Table (9): Effects of different concentrations of the Spearmint stem on the number of colonies of *Staphylococcus. aurous*

concentration	Time Hours			
	6	12	18	24
0%	6.1	6.1	5.1	5.1
25%	8.4	7.7	8.0	8.01
50%	9.4	9.8	10.0	10.0
75%	8.5	8.5	9.6	10.6
100%	11.2	12.7	10.7	10.7

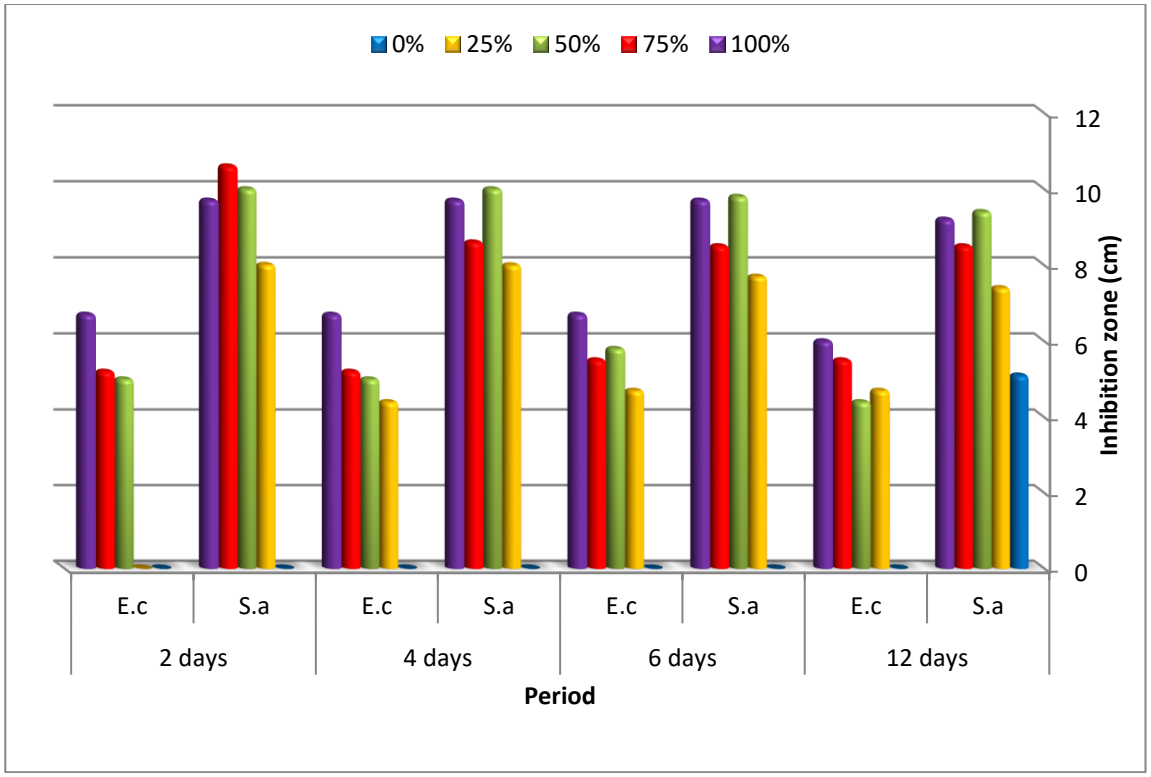


Fig (3) inhibition zone in plant extract leave

Table (10): Effects of different concentrations of the Guava stem on the number of colonies *Staphylococcus. aurous*

concentration	Time Hours			
	6	12	18	24
0%	5.5	5.5	5.5	5.5
25%	6.4	6.7	7.0	7.01
50%	9.4	9.8	10.0	10.0
75%	10.5	11.5	11.6	11.6
100%	13.9	13.9	13.9	13.9

The effect of the different concentration of Spearmint leaf and stem extract on the inhibition zone of *E. coli* are shown in table (11 and 12) the results indicated that both the leaf and the stem extracts are very effective increasing the inhibition from (5.5 to 6.5) and (6.7 to 10.7) at the 8^{th} day of incubation.

Table (11): Effects of different concentrations of the Spearmint leaf on the inhibition zone of *E. coli*

concentration	Days		
	2	4	6
0.0%	5.5	5.5	5.5
25%	7.4	7.7	8.4
50%	8.4	8.8	9.0
75%	9.5	10.5	11.2
100%	6	6.7	6.7

Table (12): Effects of different concentrations of the Spearmint stem on the inhibition zone of *E. coli*

concentration	Days			
	2	4	6	8
0%	6.5	6.5	6.5	6.5
25%	8.4	7.7	8.4	8. 1
50%	9.6	9.7	10.5	10.5
75%	9.9	10.4	11.9	11.9
100%	10	10.7	10.7	10.7

Tables (13 and 14) are showing the effects of the Guava leaf and stem extracts on the inhibition zone of *E. coli* both extracts were found highly effective. The inhibition zone was increased from 4.2 mm to 12.7 mm by the leaf extracts and from 7.2mm to 15.9 mm by the stem extracts

Table (13): Effects of different concentrations of the Guava leaf on the inhibition zone of *E. coli*

concentration	Days			
	2	4	6	8
0%	4.2	4.2	4.2	4.2
25%	8.4	7.7	8.0	8. 1
50%	9.4	9.8	10.0	10.0
75%	10.5	11.5	11.6	11.6
100%	12	12.7	12.7	12.7

Table (14):Effects of different concentrations of the Guava stem on the inhibition zone of *E. coli*

concentration	Days			
	2	4	6	8
0%	7.2	7.2	7.2	7.2
25%	8.1	8.7	9.0	8. 8
50%	9.4	9.8	10.0	10.0
75%	13.5	13.5	13.6	13.6
100%	15	15.9	15.9	15.9

The results on Table (15) are showing the effects of the Sabar leaf extracts of the inhibition zone of *E. coli* the results indicated that the extracts are also effective increasing the inhibition zone from 4.5 mm to 12.0 at the 8th days of incubation

Table (15). Effects of different concentrations of the Sabar on the inhibition zone of *E. coli*

concentration	Days			
	2	4	6	8
0%	4.5	4.5	4.5	4.5
25%	7.4	7.7	8.0	8.01
50%	9.4	9.8	10.0	10.0
75%	10.5	11.5	11.4	11.4
100%	12	12	12	12

The effect of the leaf and stem extracts of spearmint on the inhibition zone of *Staphy. arouse* are shown in Table (16, 17) respectively. The result showed an effective results increasing the zone from (5.1 to 9.7) and (5.1 to 10.7) extract at 8th days of incubation

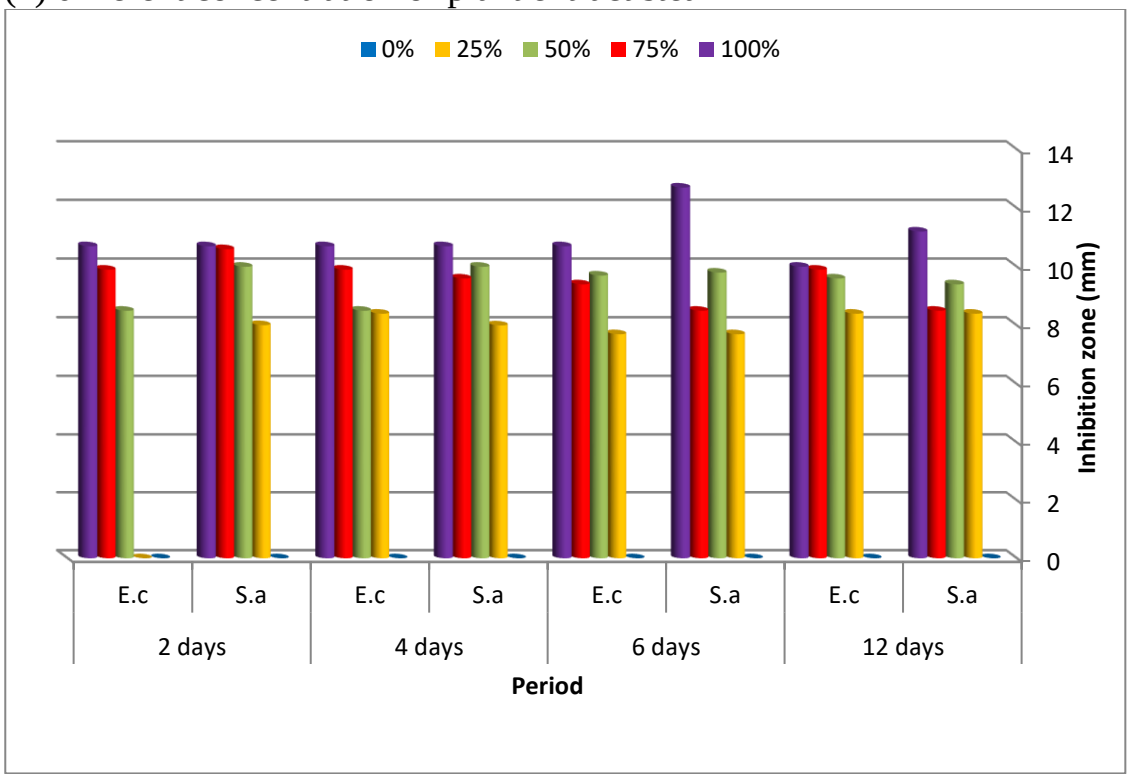
Table (16): Effects of different concentrations of the Spearmint leaf on the inhibition zone of *Staphylococcus. aurous*

concentration	Days			
	2	4	6	8
0%	5.1	5.1	5.1	5.1
25%	7.4	7.7	8.0	8.01
50%	9.4	9.8	10.0	10.0
75%	8.5	8.5	8.6	10.6
100%	9.2	9.7	9.7	9.7

Table (17): Effects of different concentrations of the Spearmint stem on the inhibition zone of *Staphylococcus. arouse*

concentration	Days			
	2	4	6	8
0%	6.1	6.1	5.1	5.1
25%	8.4	7.7	8.0	8.01
50%	9.4	9.8	10.0	10.0
75%	8.5	8.5	9.6	10.6
100%	11.2	12.7	10.7	10.7

Fig (4) different concentration of plant extract steam



Tables (18 ,19). Are showing the effect of both the leaf and stem extracts of Guava on the inhibition zone of the bacterium *Staphy. arouse* the extracts were less effective compared to that for Spearmint , although increased the zones from(4.0 mm to 9.9) only and (5.5 to 14.9) at the 8th day of incubation

Table (18)Effects of different concentrations of the Guava leaf on the inhibition zone of *Staphylococcus*

concentration	Days			
	2	4	6	8
0%	4	4	4	4
25%	6.4	6.7	7.0	7.01
50%	9.4	9.8	10.0	10.0
75%	10.5	11.5	11.6	11.6
100%	9.9	9.9	9.9	9.9

Table (19): Effects of different concentrations of the Guava stem on the inhibition zone of *Staphylococcus. aurous*

concentration	Days			
	2	4	6	8
0%	5.5	5.5	5.5	5.5
25%	6.4	6.7	7.0	7.01
50%	9.4	9.8	10.0	10.0
75%	10.5	11.5	11.6	11.6
100%	14.9	14.9	14.9	14.9

The effect of the leaf extracts of Sabar are showing in Table (20). From the result can be seen that they extracts are highly effective , increasing the zone from 6.1 mm to 12.7 mm at the 8th day of incubation

Table (20): Effects of different concentrations of the Sabar leaf on the inhibition zone of *Staphylococcus. aurous*

concentration	Days			
	2	4	6	8
0%	6	6.1	6.1	6.1
25%	7.3	7.6	8.0	8.02
50%	9.2	9.5	9.9	10.0
75%	10.5	11.3	11.6	11.6
100%	12	12.7	12.7	12.7

The photochemical screening of the leaves and stem of the Spearmint and Guava is stem on Table (21). However the table also contend photochemical screening of the Sabar leaves the Spearmint , Guava and Sabar leaves were found to content all the compared except sterols .The Spearmint stems contain tannins , flavonids and alkaloid but not saponin are sterols. The Guava stem contains only Tannin and flavonoid but no saponin , alkaloid and sterol are detected

Table(21): photochemical screening of the leaves and stem of the spearmint

Class chemical	Spearmint leaves	Spearmint stem	Guava leaves	Guava stem	Sabar leaves
Saponins	++	+	+	-	++
Tannins	+	+	++	+	+
Flavonoids	++	+	+++	+	+
Alkaloids	++	+	-	-	+
Sterols	-	-	-	-	-

\+ indicated the presences of the class
_ indicated that, the class was not tested

Discussions

The use of plants and their extracts as remedies for curing many diseases have stimulated interest for investigating the presence of antimicrobial substances (Suliman *et al.*, 2008 and Abdel-Rahim *et al.*, 2010). However, the antimicrobial of many plant extracts have already well documented The effect of the leaf extracts of Spearmint , Guava and Sabar on number of colony and spore germination for tow bacteria *E. coli* and *staphylococcus auras* are tested in the present study. The results of the study indicated that all the extracts were effective. The leaves extracts of the Spearmint are very effective in reducing the number of coloni of both bacteria while ,the extracts of the leaf and the stem of Guava were less effective. The effects of the of the extracts on the spore germination of the bacteria *E.coli* indicated that the Spearmint leaf extracts are the best in reducing the number of coloni giving followed by Sabar extracts, while the guava extracts are least effective. Extracts of many plant species were reported to have antibacterial activities. In Sudan many studies were carried out for testing the antimicrobial activities of some medicinal plants. The extracts tested of 3 plants for antimicrobial activities

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