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RESEARCH TITLE

ANTIFUNGAL ACTIVITIES AND APPROXIMATE ANALYSIS OF THE EXTRACTS OF SPEARMINT (*MENTHASPICATA*), GUAVA (*PSIDIUM GUAJAVA*) AND SABAR (*ALOE VERA*)

Sahar M. A. ElSheikh[1], Awad M. Abdel-Rahim[2]and Abasher A. Abasher[2]

[1] University of Alsheikh Abdallah albdree, Sudan
Email: sahargado97@gmail.com

[2] Faculty of Sciences, University of Gezira, Sudan

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Abstract

There is an increasing demand for biologically active substances from plant origin. However, Sudan possesses an immense wealth of medicinal plants. The present was therefore conducted to to study the antifungal properties of the leaf and stem extracts of Spearmint, Guava and Sabar plants against both *Aspergillus niger* and *Penicillium digitatum*. The study was carried out by testing the fungal radial growth, wet and dry weight, as well as germination of the spores of both fungi. The study was also investigated the proximate analysis of the extracts. The leaf extracts of Spearmint were found very effective in reducing the radial of the fungus *A. niger*, giving 1.4 cm at the higher concentration (100%) c0mpared to 11.5 cm at the control treatment (0.0%). The stem extracts of the same plant were found less effective. The Guava leaf and stem extracts were both less effective. On the other hand the Sabar leaf extracts were found effective. The leaf and stem extracts of the Spearmint were found very effective against the radial growth of the fungus *P. digitatum* giving 1.4 cm at the higher concentration compared to 12.5 cm at the control treatment. All the extracts of Guava and Sabar were less effective. The test of the extracts on the fresh and dry weights of *A. niger* showed that the Spearmint leaf extracts are the best followed by the Sabar leaf extracts, while the Guava leaf extracts were less effective. The stem extracts of both Spearmint and Guava less effective . however, the extracts of the plants were found less effective on the fresh and dry weights of *P. digitatum*. The test of the extracts on the spore germination of *A. niger* indicated that both the leaf and the stem of Spearmint plant were the most effective. All the other extracts were less effective. However, similar effects were indicated against the spore germination of *P. digitatum*. The approximate analysis. The approximate analysis showed that the Spearmint leaf extracts contain more moisture, more fats and more fiber, but less ash, less protein and less carbohydrates than both the leaf extracts of Guava and Sabar. On the other hand, the stem extracts of the Spearmint were found to contain more moisture, more fats and more proteins but less ash, less fiber and less carbohydrates than the stem extracts of Guava .The study recommends that more studies and research should be conducted to investigate the effects of these extracts on bacteria as well as to study the photochemical composition.

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 fungal growth:

The method used was as described by Abdel-Rahim *et al*.(2002).The medium used for fungal radial growth was the PDA. The Potato Dextrose Broth (PDB) medium was used for mycelial weights. The five concentrations under test were each was mixed with each of the media. The media containing the different concentrations were then sterilized and kept at room temperature (28- 30 C^0). For radial growth each solidified Petri-dish containing the PDA with the extract was inoculated by a fungal growth disc cut by a sterile cork borer (5.0 mm diameter) from the edge of an actively growing culture of *A. niger* and *P. digtatum* grown on PDA. The inoculated Petri-dished were then inoculated at room temperature for 8 days. All treatment were done in triplicates. The diameter of growth was measured, every 48 hours by taking the average of two crossed dimensions for each disc in Petri-dish. The radial growth was calculated as percentage from the diameter of the discs. For mycelial weights each flask was inoculated by three discs (5.0 mm diameter), taken from an edge of an actively growing culture each fungus on a solidified PDA medium. Inoculated flasks were incubated at room temperature (28–30^{0}C).After incubation mycelia were collected by filtering the culture through a Whatman No. 1 filter paper and the fresh weight was recorded. The mycelia mats were then dried at 80^{0}C for 24 hours, before being reweighed for the dry weights. All treatments were done in triplicates. To study the effect of the extracts of the different plants on spore germination of *A. niger* and *P. digitatum*, the spore germination medium was used. Germination percentage was assessed by examining100 spores in a microscopic field every 6 hours for 24 hours.

Approximate Analysis .

The approximate analysis was done according to the standard method of the Association of Official Analysis Chemists(AOAC,2010).

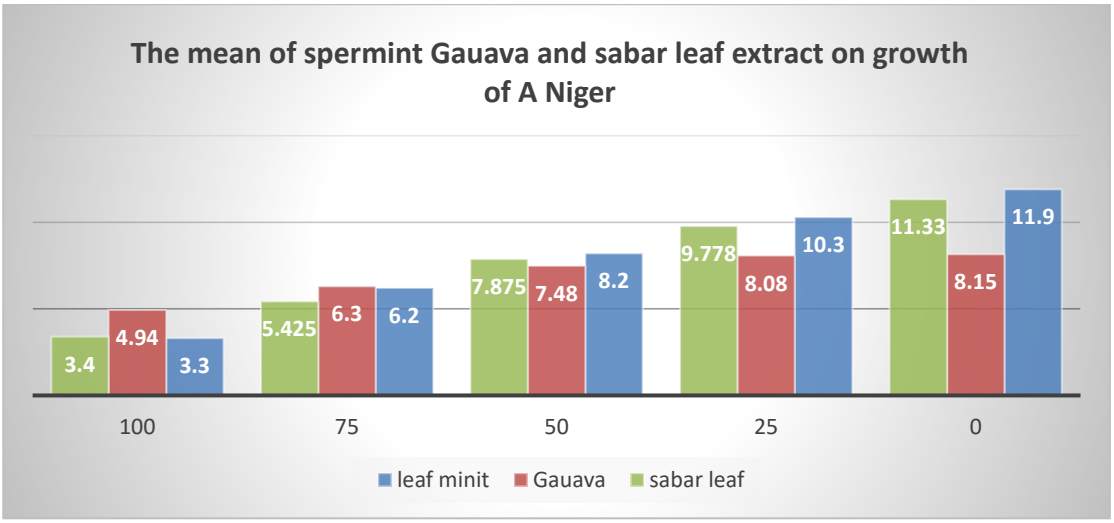
Experimental Results

From the results table (1) it is clear that the extracts of the leaves of the Spearmint are very effective in reducing the radial growth of A. niger. The growth was reduced from 11.5 cm at the control treatment to 1.4cm at the higher extract concentration (100%), at the 8th day of incubation. However, the stem extracts of the Spearmint were less effective. Table (1) also showed the effects the leaf and stem of Guava and leaf of Sabar. Both the leaf and the stem extracts of Guava were less effective while, the leaf extracts of Sabar were highly effective in reducing the radial growth of *A. niger*. Fig. (1) is showing the effects of leaf and the stem extracts of the three plants on radial growth of A. niger over a period of 8 days. The radial growth was reducing with increasing time and with increasing concentration.

Table (1): Effect of different concentrations of leaf' extracts of the plants on the radial growth of *A. niger* after 8 days

Concentrations %	Spearmint leaf extract	Spearmint stem extract	Guava leaf extract	Guava stem extract	Sabar leaf extract
0	11.5	7.4	8.4	9.9	9.9
25	9.5	10.0	9.0	8.0	8.0
50	6.9	10.5	8.4	8.5	8.5
75	3.6	8.5	7.2	7.0	7.0
100	1.4	4.5	4.6	4.6	4.6

Fig, (1). Effect of different concentrations of the leaf 'extracts of the plants on the radial growth of *A. niger* over a period of 8 days



Tests of Between-Subjects Effects- extract on A .niger radial growth

Dependent Variable: growth

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	245.792[a]	19	12.936	1.813	.035
Intercept	6587.433	1	6587.433	923.336	<.001
plants	123.944	4	30.986	4.343	.003
day	79.741	3	26.580	3.726	.015
plants * day	42.107	12	3.509	.492	.914
Error	570.751	80	7.134		
Total	7403.975	100			
Corrected Total	816.542	99			

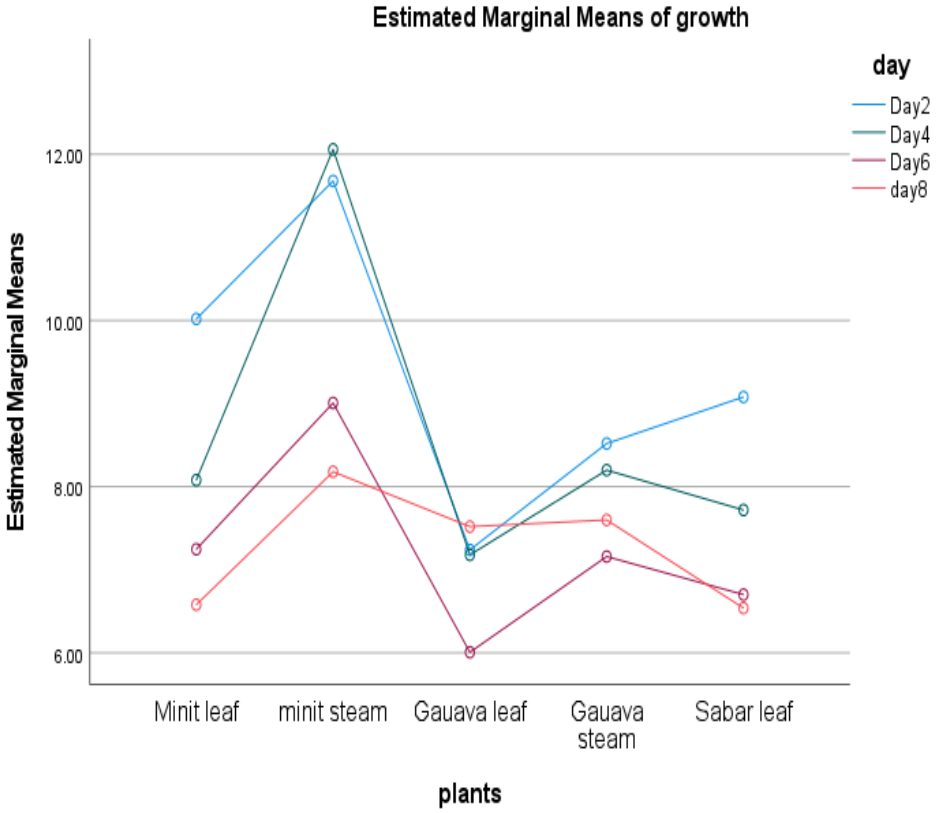
a. R Squared = .301 (Adjusted R Squared = .135)

Table (2) and Fig. (2) are showing the effects of the three plant part extracts on the radial growth of the fungus *P. digitatum* with increasing concentration and incubation time. The Spearmint leaf extracts were found the most effective, giving 1.4 cm at the higher concentration compared to 12.5 cm at the control treatment. All the other extracts were found less effective

Table (2) Effect of different concentrations of leaf' extracts of the plants on *P .digitatum* radial growth after 8 days

Concentrations %	Spearmint leaf extract	Spearmint stem extract	Guava leaf extract	Guava stem extract	Sabar leaf extract
0	12.5	9.4	9.4	10.4	8.4
25	11.4	9.0	9.6	9.9	8.0
50	7.4	8.5	8.5	8.3	7.9
75	4.6	7.5	7.4	7.2	6.5
100	1.4	4.6	4.2	7.6	3.6

.Fig, (2). Effect of different concentrations of leaf' extracts of the plants on P .digitatum radial growth over a period of 8 days.

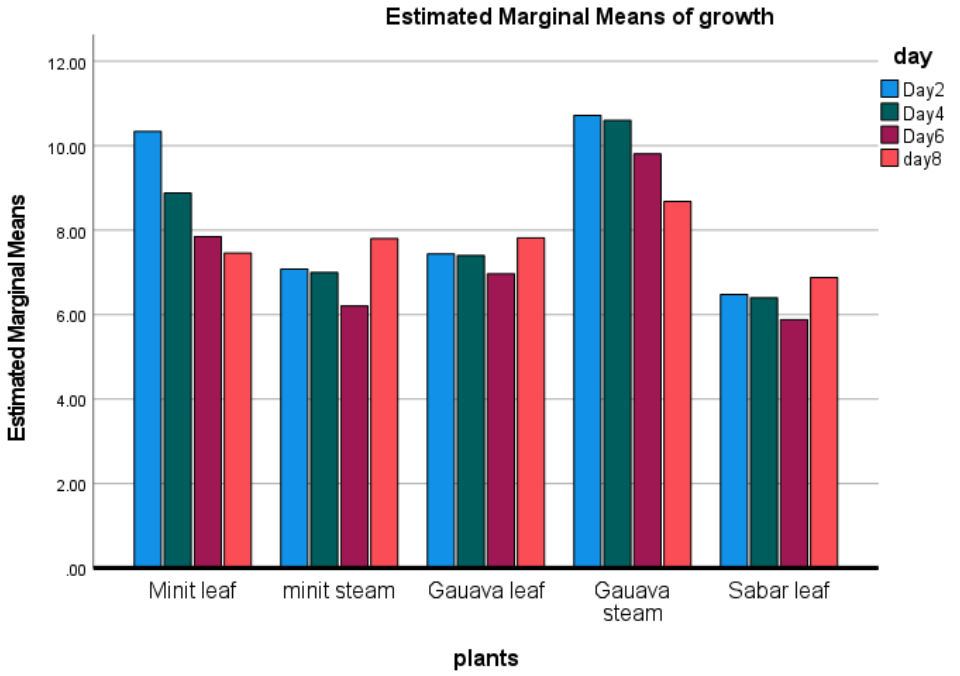


Tests of Between-Subjects Effects

Dependent Variable: growth

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	756.578[a]	33	22.927	25.234	<.001	.927
Intercept	1663.335	1	1663.335	1830.762	<.001	.965
concentration	28.902	4	7.225	7.953	<.001	.325
plants	79.277	4	19.819	21.814	<.001	.569
day	70.215	1	70.215	77.283	<.001	.539
concentration * plants * day	128.560	24	5.357	5.896	<.001	.682
Error	59.964	66	.909			
Total	7403.975	100				
Corrected Total	816.542	99				

a. R Squared = .927 (Adjusted R Squared = .890)



The effects of the of the extracts on the fresh and dry weights of the fungus *A. niger* are shown in table (3) and tale (4) respectively. The results indicated that the Spearmint leaf extracts are the best in reducing the mycelial weights followed by Sabar extracts, while the guava extracts are least effective (table, 3). However, the stem extracts of both Spearmint and Guava are effective giving 5.78 gm and 5.25 gm, respectively (table, 4). ..

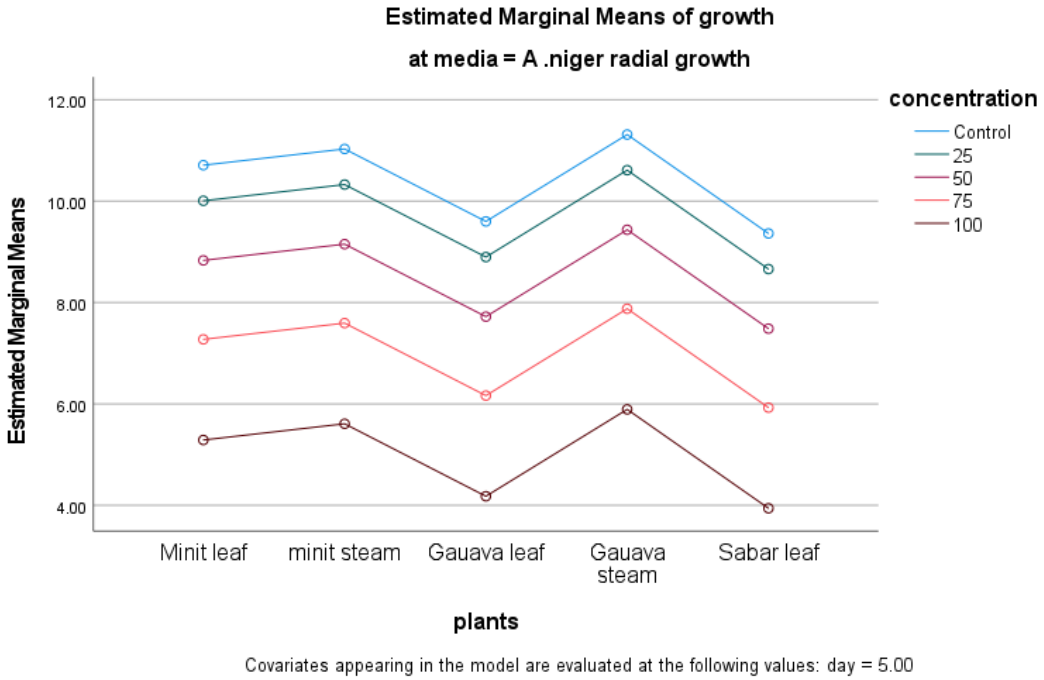


Table (4): comparison between the effect of the extracts of plants on *A .niger* mycelial dry weight

Concentrations %	Spearmint Leaf extracts	Spearmint stem extract	Guava leaf extract	Guava stem extract	Sabar leaf extract
0.0	0.50	0,90	0.49	0,90	0,48
25.0	0.42	0,80	0,38	0,67	0,38
50.0	0.25	0,40	0,33	0,60	0,33
75.0	0.23	0,30	0,22	0,53	0,25
100.0	0.20	0,10	0,19	0,1	0,20

Table (5): comparison between the effect of the extracts of plants on *P. digitatum* mycelial fresh weight

Concentrations %	Spearmint Leaf extracts	Spearmint stem extract	Guava leaf extract	Guava stem extract	Sabar leaf extract
0.0	7.98	6.44	7.78	6.55	7.78
25.0	7.05	6.45	7.01	6.43	7.01
50.0	6.76	5.13	5.56	3.17	5.56
75.0	5.98	4.22	4.11	2.67	4.11
100.0	3.13	2025	3.00	2.54	3.00

ANOVA

growth

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	433.859	4	108.465	26.926	<.001
Within Groups	382.683	95	4.028		
Total	816.542	99			

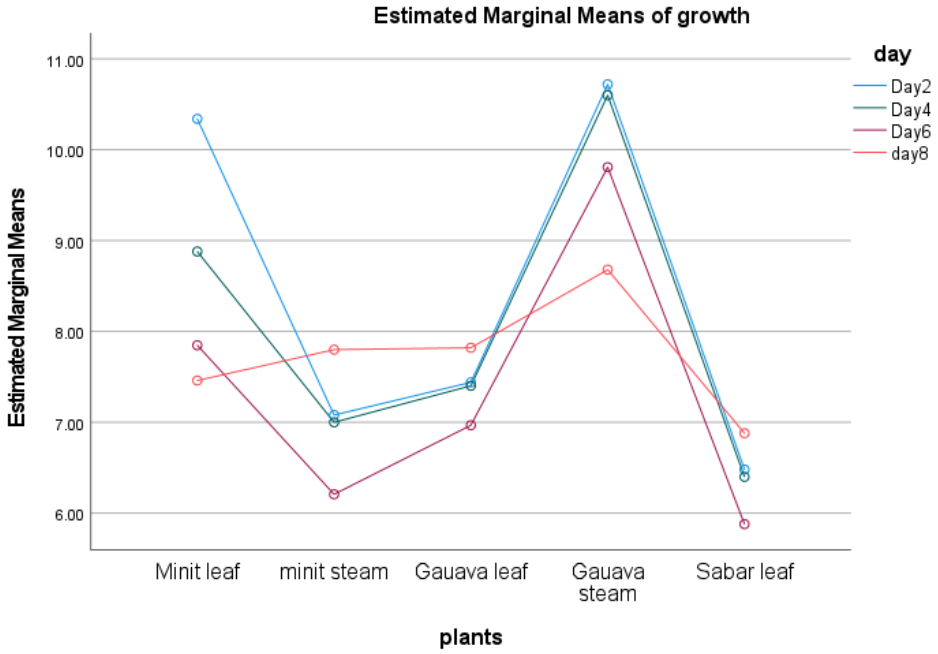
Table (6): comparison between the effect of the extracts of plants on *P. digitatum* mycelial dry weight

Concentrations %	Spearmint Leaf extracts	Spearmint stem extract	Guava leaf extract	Guava stem extract	Sabar leaf extract
0.0	0.80	0.45	0.80	0.45	0.80
25.0	0.70	0.33	0.63	0.35	0.60
50.0	0.50	0.30	0.55	0.30	0.55
75.0	0.02	0.25	0.10	0.23	0.50
100.0	0.01	0.22	0.0	0.20	0.0

The results of the effect of different leaf extracts on the spore germination showed that the leaf extracts of Spearmint were highly effect in reducing the number of the spore germination of *A, niger*. The spore germination was only 13% at the higher concentration (100%) compared to 95% germination of the control treatment. However, the germination of that fungus was only 58% at the 25% of the extracts(Table, 7). The results also indicated that the guava leaf extracts were also effect in reducing spore germination of the fungus. However only 14% of the spores were germinated at the higher leaf concentration of that plant compared to 96% of the control after 24 hours. The effect of the Sabar leaf extracts on the spore germination of the fungus were almost similar to that of the leaf extracts of both Spearmint and Guava. Only 12% germination after 24 hours (Table, 7).

Table (7): comparison between the effect of the extracts of the plants on The spore germination *A .niger* .

Concentrations %	Spearmint Leaf extracts	Spearmint stem extract	Guava leaf extract	Guava stem extract	Sabar leaf extract
0.0	100%	100%	100%	100%	100%
25.0	95	97	96	96	95
50.0	58	58	59	59	55
75.0	34	34	36	36	30
100.0	11	13	14	14	12



The effect of the leaf extracts of Spearmint, Guava and Sabar on the spore germination of the fungus *P. digitatum* are shown in Tables (8,).The results showed that the spore germination of the fungus was highly reduced with the three different leaf extracts. However only 16.0% were germinated with the spearmint leaf extracts The effect of the stem extracts of Spearmint and Guava on the spore germination of the fungus *P. digitatum* are showing on Tables (5).The results showed that the spore germination of the fungus was highly reduced with the two different stem extracts.

The proximate analysis of the leaves of the three plants (Spearmint , Guava and Sabar) is shown in the Table (9). Guava and Sabar have more proteins 16.73% compared to only 3.47 in Spearmint similar effects are also found by the carbohydrates the Guava and Sabar leaves were found to contain 64.89 and 64.4% carbohydrates, respectively compared to only 10.38% in the Spearmint extracts. The Spearmint leaves on the other hand contain more fats 2.2% compared to 0.46 and 0.46 by the Guava and Sabar. The Spearmint extracts were also found to contain high moisture content 70.01% compared to only 6.39 and 6.30 in Guava and Sabar respectively. Table (7) is showing the proximate analysis of the stem of both Spearmint and Guava. The Spearmint stem have also very high moisture contain 30.02% compared to only 1.33 of the Guava. On the other hand the Guava stem contain high contend of ash (66.20), compared to only 4.49 of the Spearmint.

Table (9) proximate analysis of Spearmint, Guava and Sabar leaf and stem extracts

Chemical analysis	Spearmint leaf extracts	Guava leaf extracts	Sabar leaf extracts	Spearmint stem extract	Guava stem extracts
Moisture	76.01%	6.39%	6.3%	30.02	1.33
Ash	3.47	16.73	16.73	4.49	66.20
Fat	2.21	0,46	0.45	0.70	0.03
Fiber	6.3	5.08	5.00	13.3	15.40
Protein	1.74	6.64	6.64	1.45	0.52
Carbohydrate	10.38	64.89	64.4	2.13	3.11

Discussion and Conclusions

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 radial growth , mycelial weights and the spore germination of both fungi *A. niger* and *P. digitatum* 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 radial growth of both fungi while ,the extracts of the leaf and the stem of Guava were less effective. The effects of the of the extracts on the fresh and dry weights of the fungus *A. niger* indicated that the Spearmint leaf extracts are the best in reducing the mycelial weights giving followed by Sabar extracts, while the guava extracts are least effective. Extracts of many plant species were reported to have antifungal activities (Abdel-Rahim *et al.*, 1989, Aljsli *et al.*, 1997). In Sudan many studies were carried out for testing the antimicrobial activities of some medicinal plants. The extracts and essential oils of both clove and cinamon were found to inhibit growth and aflatoxin production of both *A. flavus* and *A. prasiticus* (Abdel-Rahim *et al.*, 2002 and Abdel-Rahim *et al.*, 2009).Ahmed (2004) tested the extracts of ten plants for antimicrobial activities.

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