**Research Article**
Volume 6:1, 2020
DOI: 10.37421/jpnp.2020.6.163

**Journal of Pharmacognosy & Natural Products**

**ISSN:2472-0992** **Open Access**

# Identification and Management of Vaginitis Prevalent Causal Agents using Herbal Aqueous Extracts

**Mai Abdalla Ali[1*], Intisar Kamaleldin Izzeldin[1], Awad Mohamed Abdel-Rahim[1], Yasir Mohamed Hasan[2] and Ivan Kahwa[3]**

*[1]Center of Bioscience and Biotechnology, University of Gezira, Wad Medani, Sudan*
*[2]The University of Gezira, Wad Medani, Sudan*
*[3]Department of Pharmacy, Pharm-Biotechnology and Traditional Medicine Centre of Excellence (ACEII), Mbarara University of Science & Technology, Mbarara, Uganda*

## Abstract

Vaginitis is defined as a spectrum of conditions that cause vaginal and sometimes vulvar symptoms, such as itching, burning, irritation, odor, and vaginal discharges. This study aims to identify and isolate the most prevalent causal agents associated with vaginitis at Gezira area – Sudan, evaluating the antimicrobial activity of the commonly used herbs like *Commiphora myrrha*, *Solenostemma argel*, *Azadirachta indica*, Salvia officinalis and *Eucalyptus camaldulensis* against the isolated causal agents and comparing the herbal effects at different concentrations with a known commercial vaginal washer. 50 subjects of high vaginal swabs were collected, to identify and isolate the target organisms during the period from January to May 2015, and then aseptically cultured on sterile and appropriate media. A total number of 31 bacterial isolates were represented by 4 different species of bacteria; 17 (34%) *Staphylococcus aureus*, 7 (14%) *E. coli*, 6(12%) *Streptococcus pyogenes* and only 1(2%) *Pseudomonas aeruginosa* whereas 14 (28%) yeast isolates were represented by Candida *albicans*. 11 aqueous extracts were prepared from each of the five herbs with concentrations of 5%, 15%, 25%, 35%, and 45%. The aqueous extracts of *C. myrrha*, *E. camaldulensis* and *A. indica* respectively achieved the highest means of inhibition zones (12.1, 11.5 and 10.4 mm) when compared to the others and showing non-significant differences when compared to each other (p value 0.08, 0.2 and 0.7). The combination of (*S. argel*, *A. indica*, and *E. camaldulensis*) showed relatively strong antimicrobial activity with inhibition zone range between (18.5–24 mm) in its different concentrations (25%, 35%, and 45%) when comparing the effect of this extract (mean=15.2) with the impact of the commercial washer (mean=9.9), it was found that the herbal extract has top influence, although, the difference was statistically non-significant (p value ± 0.07).

**Keywords:** Vaginitis • Commiphora myrrha • Solenostemma argel • Azadirachta indica • Salvia officinalis • Eucalyptus camaldulensis

## Introduction

The vagina has a squamous epithelium and is susceptible to bacterial vaginosis, *trichomoniasis*, and candidiasis. Vaginitis is defined as a spectrum of conditions that cause vaginal and sometimes vulvar symptoms, such as itching, burning, irritation, odor, and vaginal discharge. Vulvovaginal complaints are one of the most common reasons for women to seek medical advice. The most common infectious causes of vaginitis are bacterial vaginosis, vulvovaginal candidiasis, and *trichomoniasis*. Physicians traditionally diagnose vaginitis using the combination of symptoms, physical examination, and pH of vaginal fluid, microscopy, and the whiff test [1]. Among the causes of vaginal discharge, Bacterial Vaginosis (BV) is the commonest in most communities, with variation in the prevalence from one place to another and according to the method used and the group of patients studied. Evidence is available that, the disease is associated with preterm labor, premature rupture of the membranes, post-induced abortion pelvic inflammatory disease, post-hysterectomy vaginal cuff cellulitis, and plasma cell endometritis. Moreover, in pregnant women, bacterial vaginosis may be associated with amniotic fluid infection and post-partum endometritis [2]. In Sudan, the disease was first reported in 2000 by Kafi et al. who found bacterial vaginosis to be the commonest cause of vaginal discharge (17.2%) in a suburban Sudanese community [3].

Bacterial vaginosis is the most prevalent cause of vaginal discharge or malodor, occurring in up to 30 percent of women [4]. It occurs when the normal Lactobacillus species in the vagina are replaced with anaerobic bacteria, resulting in reduced levels of hydrogen peroxide and organic acids usually present in the vagina. The underlying cause of bacterial vaginosis is not fully understood. More than 50 percent of women with bacterial vaginosis are asymptomatic. The fishy odor caused by the production of amines from anaerobic bacteria found in many of these patients is predictive of bacterial vaginosis [5]. When vaginal alkalinity increases after sexual intercourse (with the presence of semen) and during menses (with the presence of blood), the odor becomes more prevalent. Vaginal discharge is more common but with a less specific symptom. Bacterial vaginosis is not associated with vaginal mucosal inflammation and rarely causes vulvar itch [6].

Trichomonas vaginalis infection is one of the major health problems in the world and one of the most commonly transmitted infections in many regions including developed countries such as the United States [7]. Prevalence estimates vary between populations studied falling in the range from 0.4 to 27.4% in women and 0.0 to 5.6% in men [8]. The annual worldwide incidence of *trichomoniasis* is more than 250 million cases [9]. In Sudan, *trichomoniasis* was reported among women by Omer [10] and more recently by Salih [11]. But the actual burden of the disease in Sudan remains unknown and no information on risk factors for *T. vaginalis* infection in women is available.

Vulvovaginal candidiasis (VVC) is a very common condition that affects up to 75% of women at least once in their lifetime [12]. Risk factors for VVC include sexual activity, recent antibiotic use, pregnancy and immunosuppression from such conditions as poorly controlled HIV infection or diabetes [13]. It is most often caused by *Candida albicans* [14].

Treatment depends on the type of vaginitis by using antibiotics but recently antibiotic resistance microorganisms has become a major clinical and public health problem within the lifetime of most people living today

***Address for Correspondence:** Mai Abdalla Ali, Center of Bioscience and Biotechnology, Faculty of Engineering and Technology, University of Gezira, Wad Medani, Sudan, Tel: +249923328080; E-mail: maiabdalla222@gmail.com*



**Received** 11 December 2019; **Accepted** 02 January 2020; **Published** 10 January 2020

that confronted by increasing amounts of antibiotics over the past 60 years, bacteria have responded to the deluge with the propagation of progeny no longer susceptible to them. While it is clear that antibiotics are pivotal in the selection of bacterial resistance, the spread of resistance genes and resistant bacteria also contributes to the problem [15].

Therefore, using medicinal herbs can sometimes be better than using antibiotics or medical drugs, due to some reasons in addition to the antibiotic resistance e.g.; In Sudan nowadays, the rising cost of prescription drugs has led the people to look for alternatives. While medicinal herbs may not be as strong or as fast-acting as conventional medicine. Much herbal medication has fewer side effects than conventional medicine. Medicinal herbs can be used in a variety of ways, depending on the kind of herb that to be used. Some herbs can be mixed with food. Some can be made as a tea and there are some of them available in capsule or tablet form, also most prescriptive drugs are designed for one specific health problem. By contrast, much herbal medicine act on several parts of the body at once, therefore this research aims to study the effect of different herbs with different concentrations on the causal agents of vaginal infections.

The objectives of this study were to identify and isolate the most prevalent causal agents associated with vaginitis at Wad Medani hospital, evaluate the antimicrobial activity of five plant extracts against the isolated causal agents and compare the herbal effects at different concentrations with a known commercial vaginal washer.

# Material and Methods

## Sampling methodology

**Microorganisms collection:** 50 subjects of high vaginal swabs (HVS) was collected and investigated at the Medical Laboratory, University of Gezira to identify and isolate the target organisms, during the period from January to May 2015. Ethical clearance was done by the Ethical Commitee of the Faculty of Science, University of Gezira in 2016.

**Herbs collection:** The plants of Commiphora myrrha, Solenostemma argel, Azadirachta indica, Salvia officinalis, and Eucalyptus camaldulensis was selected for this study. They were obtained from Wad Medani local market during May 2015. The herbs were approved by the Department of Botany and Microbiology, Faculty of Science, The University of Gezira, Sudan.

The completely dried leaves of the plant material were powdered and allowed for overnight extraction by distilled water, with a concentration of (5%, 15%, 25%, 35%, and 45%). Both negative controls with a concentration of 0% (distilled water) and positive control (common vaginal wash) with concentration 100% were used.

## Culture media

**Nutrient agar:** This was a general-purpose cultured medium for bacteria. It was obtained in a dehydrated form. The constituent of the medium was a beef extract, yeast extract, peptone, sodium chloride, and agar. It was prepared according to the manufactures instruction by suspending 28 g in one liter distilled water. The medium was allowed to boil until it has completely dissolved. The pH of the medium was adjusted to pH 7.4 ± 0.2 and then the medium was sterilized in an autoclave at 121°C (115 b\in$^2$) for 15 min [16].

**Sabouraud Dextrose Agar (SDA):** This was a suitable culture medium for cultivation and differentiation of fungi. It was obtained in a dehydrated form. The constituents of the medium were peptone, dextrose, and agar. It was prepared according to the manufactures instruction by suspending 65 g in one liter distilled water. The medium was allowed to boil until it has completely dissolved. The pH of the medium was adjusted to pH 5.6 and then the medium was sterilized in an autoclave at 121°C (115 b\in$^2$) for 15 min. Then 0.1 g chloramphenicol was added to one liter of the medium after autoclaving to inhibit bacterial growth [16].

## Effect of herbal extract on micro-organisms growth

**The disc diffusion (Inhibition zone) method:** This method was used, using Nutrient Agar (NA) for bacteria and Sabouraud dextrose agar for *C. albicans*. In this method different herbal aqueous suspensions of the herb powder (50 g/500 ml) was used to prepare different concentrations (5%, 15%, 25%, 35%, and 45% extract) and incubated overnight at room temperature, then the media were prepared, sterilized and distributed into sterile Petri-dishes and was left to solidify at room temperature for 24 hours. After that by using sterile cotton swabs each organism (*S. aureus, S. pyogenes, E. coli, P. aeruginosa* and *C. albicans*) was inoculated in 6 Petri-dishes by full streaking, then a sterile glass fiber discs (size 6 mm) were saturated with the extract of (*C. myrrha, S. argel, A. indica, S. officinalis, E. camaldulensis* and their combinations) allowed to dry and transferred on the surface of the solidified medium in each plate. The plates were then incubated at room temperature for 24 hours and the inhibition zones were measured by mm and the susceptibility is determined.

Two replicates were made for each solution. After the isolation of microorganisms, herbal solutions were prepared and tested their antimicrobial activities on these microorganisms. After that, the susceptibility of microorganisms and the effectivity of different concentrations of extracts were reported.

Combinations that consist of two or three herbs mixed by equal volumes according to concentration were also tested for their antimicrobial activities, they were 6 combinations of (*S. argel* and *A. indica*), (*S. argel* and *E. camaldulensis*), (*S. argel*, *A. indica*, and *E. camaldulensis*), (*S. officinalis* and *A. indica*), (*S. Officinalis* and *E. camaldulensis*) and (*S. Officinalis*, *A. indica* and *E. camaldulensis*).

At the end of the study, a common vaginal washer was used as a positive control and the results were compared with the effective concentrations of each herbal solution.

## Statistical analysis

Anova two factors without replication were used for data analysis, and the comparison between aqueous effects was done by T-test and F-test using M STAT-C.

# Results and Discussion

## Description of the samples

In the present study, 50 random subjects of High Vaginal Swab (HVS) were used, the appearance of microorganisms was as followed; 32% *S. aureus*, 28% *C. albicans*, 14% *E. coli*, 12% *S. pyogenes* and only 2% *P. aeruginosa*. Also, some objects were containing mixed organisms of 10% *S. aureus* with *C. albicans* and 2% *S. aureus* with *S. pyogenes* (Table 1 and Figure 1). The minimum age of the subjects was 13 years old whereas the maximum age was 90 years old but the range of age between (16–45)years old was the common range, frequency and percentage of age were showed in (Table 2 and Figure 2).

## Antimicrobial activities of herbs aqueous extract on microorganisms

After the isolation of microorganisms, herbal solutions were prepared

**Table 1.** Frequency and percentage of the detected microorganisms.

| Variable | Subject | Frequency | Percentage |
|---|---|---|---|
| | *S. aureus* | 16 | 32 |
| | *C. albicans* | 14 | 28 |
| | *E. coli* | 7 | 14 |
| Microorganisms | *S. pyogenes* | 6 | 12 |
| | *P. aeruginosa* | 1 | 2 |
| | *S. aureus+C. albicans* | 5 | 10 |
| | *S. aureus+S. pyogenes* | 1 | 2 |
| Total | 6 | 50 | 100 |

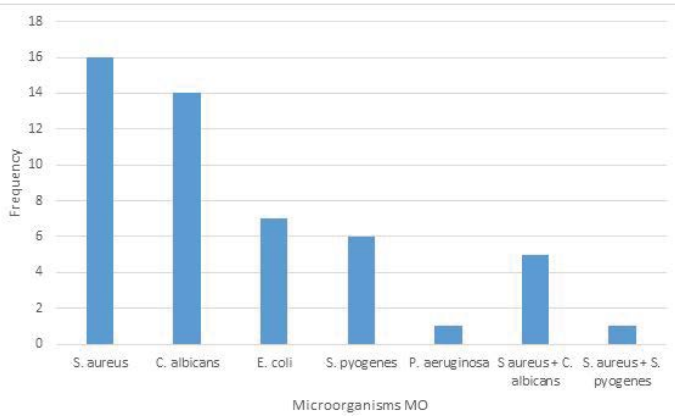


**Figure 1.** Plot of Frequency of microorganisms detected.

**Table 2.** Frequency and percentage according to the age class of the subjects.

| Variable | Subject | Frequency | Percentage |
|---|---|---|---|
| | 10 – 15 | 1 | 2 |
| | 16 – 30 | 18 | 36 |
| | 31 – 45 | 23 | 46 |
| Age classes | 46 – 60 | 7 | 14 |
| | 61 – 75 | 0 | 0 |
| | 76 – 90 | 1 | 2 |
| | Total | 50 | 100 |

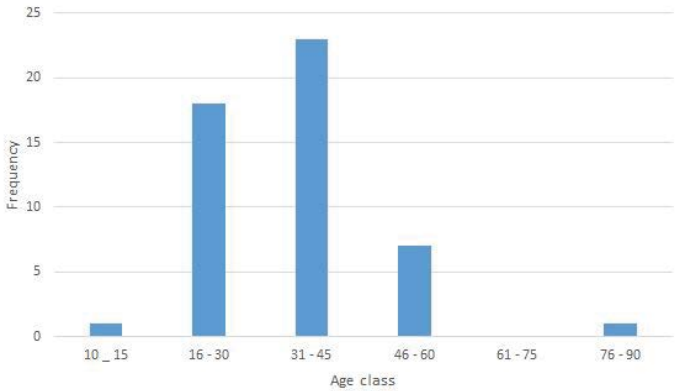


**Figure 2.** Frequency according to the age class of the subjects.

and tested their antimicrobial activities on these microorganisms. After that, the susceptibility of microorganisms and the effectivity of different concentrations of extracts were reported.

At the end of the study, a common vaginal wash was used as a positive control and the results were compared with the effective concentrations of each herbal solution.

## Antimicrobial activities of extracts on *S. Aureus*

In Table 3 and Figure 3, there are stated results of testing the antimicrobial activity of *Commiphora myrrha*, *Solenostemma argel*, *Azadirachta indica*, Salvia officinalis, Eucalyptus *camaldulensis*, and their combinations.

The combinations were consisting of two or three herbs mixed to gather by equal volumes according to concentration, they were 6 combinations of (*S. argel* and *A. indica*), (*S. argel* and *E. camaldulensis*), (*S. argel, A. indica*, and *E. camaldulensis*), (*S. officinalis* and *A. indica*), (*S. officinalis* and *E. camaldulensis*) and (*S. officinalis*, *A. indica* and *E. camaldulensis*).

The combination of (*S. argel, A. indica*, and *E. camaldulensis*) showed relatively strong antimicrobial activity with inhibition zone range between (18.5 – 24 mm) in its different concentrations (25%, 35%, and 45%). All the other extracts in concentration 45% except *S. argel* showed some antimicrobial activity as well with inhibition zone range (10 – 13.5 mm). Whereas *S. argel*, *S. officinalis* and *E. camaldulensis* with low concentrations of 25% and 35%. These three herbs did not show any significant antimicrobial activity.

## Antimicrobial activities of extracts on *C. albicans*

*C.albicans* was the only one yeast used in the present study, Table 4 and Figure 4 were showed results of antimicrobial activity of *Commiphora myrrha, Solenostemma argel, Azadirachta indica, Salvia officinalis*, *Eucalyptus camaldulensis*, and their combinations.

*C.albicans* was highly sensitive to the combination of (*S. argel, A. indica,* and *E. camaldulensis*) in the concentration of 45% with inhibition zone 17.50 mm, also it was recorded a moderate sensitivity to the combination of (*S. argel* and *A. indica*) in the concentration of 45% with an inhibition zone of 13.50 mm. Whereas it was have the same less sensitivity to the C. Myrrha in concentration of 45% with zone of 12.00 mm and combinations of (*S. argel, A. indica,* and *E. camaldulensis*) in concentration 35% with inhibition zone of 12.00, (*S. officinalis* and *A. indica*) in concentration of 45% with inhibition zone of 11.50 mm and combination of (*S. argel* and *E. camaldulensis*) in concentrations of 25%, 35% and 45% with inhibition zones 11.50, 12.00 and 11.50 mm.

*S. argel, S. officinalis, E. camaldulensis, A. indica* and combination of (*S. officinalis, A. indica* and *E. camaldulensis*) were showed few or no effects.

## Antimicrobial activities of extracts on *E. coli*

In Table 7, *E. coli* was showed low susceptibility to the herbal extracts in general, the aqueous of *A. indica* in concentration of 45% was made the largest inhibition zone 11.00 mm, the combinations of (*S. argel, A. indica,* and *E. camaldulensis*) and (*S. officinalis, A. indica* and *E. camaldulensis*) and the aqueous of *C. Myrrha* in concentration of 45% were achieved inhibition zones about 10.50 and 10.00 mm.

**Table 3.** Ranking of concentration and separation for herbs (*S. aureus*).

| | 0 | 0.25 | 0.35 | 0.45 | Means | |
|---|---|---|---|---|---|---|
| *C. myrrha* | 6 | 6 | 9 | 12 | 8.25 | cde |
| *S. argel* | 6 | 6.5 | 6.5 | 8.5 | 6.87 | G |
| *S. officinalis* | 6 | 6 | 7.5 | 10 | 7.37 | efg |
| *E. camaldulensis* | 6 | 6 | 7.5 | 9.5 | 7.25 | Fg |
| *A. indica* | 6 | 6 | 8 | 13.5 | 8.37 | bcd |
| *S. argel+E. camald* | 6 | 8 | 12 | 11 | 9.25 | B |
| *S. officin+A. indica* | 6 | 6 | 8.5 | 10.5 | 7.57 | defg |
| *S. argel+A. indica* | 6 | 6 | 9.5 | 11 | 8.12 | def |
| *S. officin+E. camald* | 6 | 7.5 | 10.5 | 12.5 | 9.12 | bc |
| *S. argel+E. camald+A. indica* | 6 | 18.5 | 22 | 24 | 17.62 | A |
| *S. officin+E. camald. +A. indica* | 6 | 9 | 10 | 11.5 | 9.12 | bc |
| Means | 6 | 7.77 | 10.09 | 12.18 | | |
| | d | c | b | a | | |

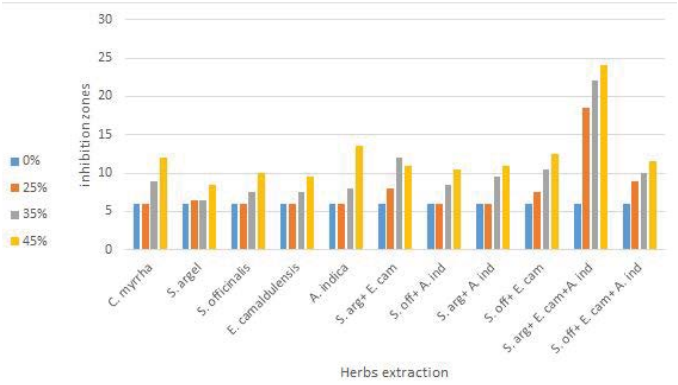


**Figure 3.** Antimicrobial activities of extracts on *S. Aureus*.

The inhibition zone of 9.00 mm was recorded by *S. argel* and combinations of (*S. argel* and *A. indica*) and (*S. argel* and *E. camaldulensis*) in the concentration of 45%, also by a combination of (*S. argel, A. indica,* and *E. camaldulensis*) in the concentration of 35%.

**Table 4.** The effective concentration of herbs and the mean separation for herbs.

| Source | Degrees of freedom | Sum of squares | Mean square | F value | Prob. |
|---|---|---|---|---|---|
| Herbs | 10 | 703.864 | 70.386 | 18.058 | 0 |
| Concentration | 3 | 480.034 | 160.011 | 41.052 | 0 |
| Herbs. Conc. | 30 | 277.591 | 9.253 | 2.374 | 0.0044 |
| Error | 44 | 171.5 | 3.898 | | |
| Total | 87 | 1632.989 | | | |

Analysis of variance:

Prob.: Probability: p value 0.05 significant ≤ p value>0.05 non-significant

Coefficient of variation 21.91%

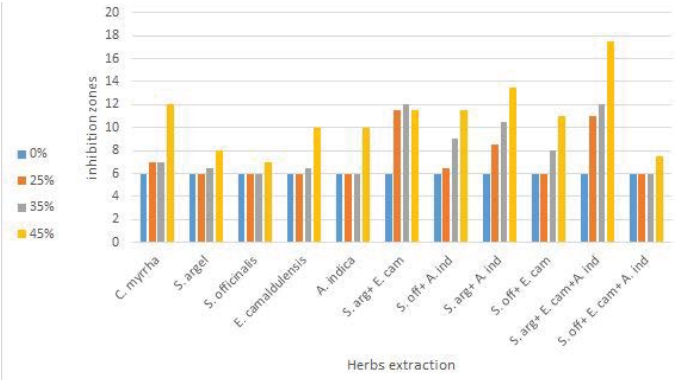


**Figure 4.** Antimicrobial activities of extracts on *C. Albicans*.

**Table 5.** Ranking of concentration and separation for herbs (*C. Albicans*).

| | 0 | 0.25 | 0.35 | 0.45 | Means | |
|---|---|---|---|---|---|---|
| *C. myrrha* | 6 | 7 | 7 | 12 | 8 | C |
| *S. argel* | 6 | 6 | 6.5 | 8 | 6.62 | ef |
| *S. officinalis* | 6 | 6 | 6 | 7 | 6.25 | F |
| *E. camaldulensis* | 6 | 6 | 6.5 | 10 | 7.12 | de |
| *A. Indica* | 6 | 6 | 6 | 10 | 7 | ef |
| *S. argel+E. camald.* | 6 | 11.5 | 12 | 11.5 | 10.25 | B |
| *S. officin.+A. indica* | 6 | 6.5 | 9 | 11.5 | 8.25 | C |
| *S. argel+A. indica* | 6 | 8.5 | 10.5 | 13.5 | 9.62 | B |
| *S. officin.+E. camald.* | 6 | 6 | 8 | 11 | 7.75 | cd |
| *S. argel+E. camald.+A. indica* | 6 | 11 | 12 | 17.5 | 11.62 | A |
| *S. officin.+E. camald. +A. indica* | 6 | 6 | 6 | 7.5 | 6.37 | ef |
| Means | 6 | 7.31 | 8.13 | 10.86 | | |
| | d | c | b | a | | |

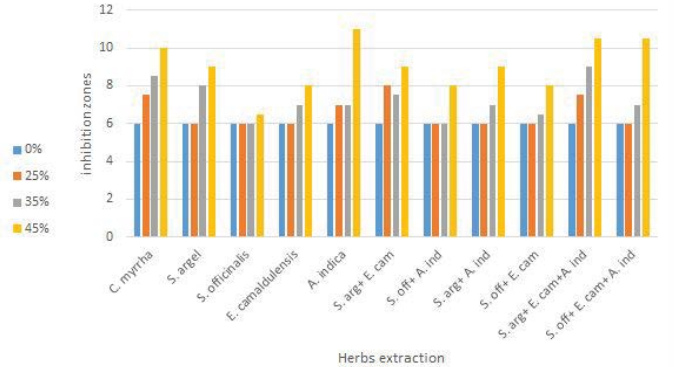


**Figure 5.** Antimicrobial activities of extracts on *E. coli*.

**Table 6.** Effective concentration of herbs and the mean separation for herbs.

| Source | Degrees of freedom | Sum of squares | Mean square | F value | Prob. |
|---|---|---|---|---|---|
| Herbs | 10 | 242.068 | 24.207 | 12.755 | 0 |
| Concentration | 3 | 278.489 | 92.83 | 48.916 | 0 |
| Herbs. Conc. | 30 | 136.386 | 4.546 | 2.395 | 0.0041 |
| Error | 44 | 83.5 | 1.898 | | |
| Total | 87 | 740.443 | | | |

Analysis of variance:

Prob.: Probability: p value 0.05 significant p value>0.05 non-significant

Coefficient of variation 17.05%

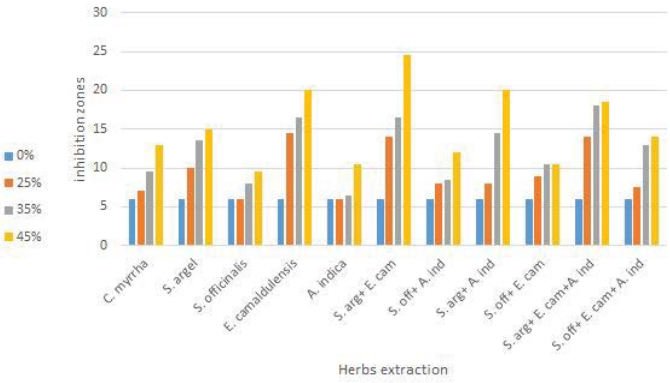


**Figure 6.** Antimicrobial activities of extracts on *S. Pyogenes*.

**Table 7.** Ranking of concentration and separation for herbs (*E. coli*).

| | 0 | 0.25 | 0.35 | 0.45 | Means | |
|---|---|---|---|---|---|---|
| *C. myrrha* | 6 | 7.5 | 8.5 | 10 | 8 | ab |
| *S. argel* | 6 | 6 | 8 | 9 | 7.25 | cdef |
| *S. officinalis* | 6 | 6 | 6 | 6.5 | 6.12 | h |
| *E. camaldulensis* | 6 | 6 | 7 | 8 | 6.75 | efgh |
| *A. indica* | 6 | 7 | 7 | 11 | 7.75 | abc |
| *S. argel+E. camald* | 6 | 8 | 7.5 | 9 | 7.62 | abcd |
| *S. officin+A. indica* | 6 | 6 | 6 | 8 | 6.5 | gh |
| *S. argel+A indica* | 6 | 6 | 7 | 9 | 7 | defg |
| *S. officin+E. camald* | 6 | 6 | 6.5 | 8 | 6.62 | fgh |
| *S. argel+E camald+A. Indica* | 6 | 7.5 | 9 | 10.5 | 8.25 | a |
| *S. officin+E. camald+A. indica* | 6 | 6 | 7 | 10.5 | 7.37 | bcde |
| Means | 6 | 6.54 | 7.27 | 9.04 | | |
| | d | c | b | a | | |

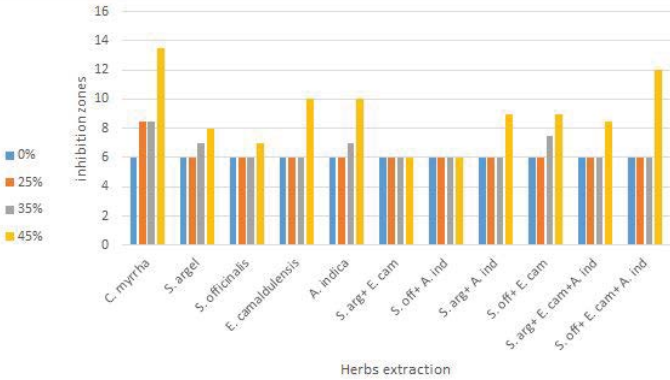


**Figure 7.** Antimicrobial activities of extracts on *P. Aeruginosa*.

The aqueous extract of S. officinalis does not affect *E. coli*, also *E. camaldulensis, the combinations of (S. officinalis and A. indica) and (S. officinalis* and *E. camaldulensis*) had non-significant effects.

## Antimicrobial activities of extracts on *S. Pyogenes*

Table 9 showed that 24.50 mm is the largest inhibition zone, was

achieved by 45% concentration of combination between (*S. argel* and *E. camaldulensis*), 20.00 mm inhibition zone achieved by *E. camaldulensis* and combination of (*S. argel* and *A. indica*).

There was a non-significant different between zones 18.00 and 18.50 mm of the two concentrations of combination (*S. argel*, *E. camaldulensis*, and *A. indica*) 34% and 35%. Also, the concentration of 35% of E. camaldulensis and combination of (*S. argel* and *E. camaldulensis*) were having the same inhibition zone of about 16.50 mm, 15.00 mm inhibition zone was achieved by *S. argel* in concentration 45%.

A combination of (*S. argel* and *A. indica*) with concentration of 35% and the extract of *E. camaldulensis* in concentration of 25% have inhibition zone about 14.50 mm, whereas a non-significant different were noted between extracts of *C. Myrrha* 45%, S. argel 35%, the combinations of (*S. argel* and *E. camaldulensis*) 25%, (*S. argel*, *E. camaldulensis*, and *A. indica*) 25% and (*S. officinalis*, *E. camaldulensis*, and *A. indica*) 35% and 45% with inhibition zones about 13.00 and 14.00 mm.

12.00 mm inhibition zone was in the concentration of 45% in the combination of (*S. officinalis* and *A. indica*), and 10.50 mm inhibition zone was found in *A. indica* 45% and combination of (*S. officinalis* and *E. camaldulensis*) 35% and a few or no effect was recorded with *S. officinalis* extract even in concentration of 45% and other extracts in low concentrations of 25%.

## Antimicrobial activities of extracts on *P. Aeruginosa*

In Table 11 the extract of *C. myrrha* showed a higher antimicrobial effect by inhibition zones of 13.50 mm in the concentration of 45% and 8.50 mm in the concentrations of 35% and 45%. The combination of (*S. officinalis*, *E. camaldulensis*, and *A. indica*) in a concentration of 45% 12.00 mm inhibition zone was found, with no effect in concentrations of 35% and 25%.

The aqueous extracts of *A. indica* and *E. camaldulensis* in concentration 45% provide an inhibition zone of about 10.00 mm without effect on the other concentrations of 35% and 25%.

**Table 8.** The effective concentration of herbs and the mean separation for herbs.

| Source | Degrees of freedom | Sum of squares | Mean square | F value | Prob. |
|---|---|---|---|---|---|
| Herbs | 10 | 35.818 | 3.582 | 2.541 | 0.0163 |
| Concentration | 3 | 116.045 | 38.682 | 27.451 | 0 |
| Herbs. Conc. | 30 | 32.455 | 1.082 | 0.767 | |
| Error | 44 | 62 | 1.409 | | |
| Total | 87 | | | | |

Analysis of variance:
Prob.: Probability: p value 0.05 significant ≤ p value>0.05 non-significant
Coefficient of variation 16.48%

**Table 9.** Ranking of concentration and separation for herbs (*S. pyogenes*).

| | 0 | 0.25 | 0.35 | 0.45 | Means | |
|---|---|---|---|---|---|---|
| *C. myrrha* | 6 | 7 | 9.5 | 13 | 8.87 | f |
| *S. argel* | 6 | 10 | 13.5 | 15 | 11.12 | d |
| *S. officinalis* | 6 | 6 | 8 | 9.5 | 7.37 | g |
| *E. camaldulensis* | 6 | 14.5 | 16.5 | 20 | 14.25 | b |
| *A. indica* | 6 | 6 | 6.5 | 10.5 | 7.25 | g |
| *S. argel+E. camald.* | 6 | 14 | 16.5 | 24.5 | 15.25 | a |
| *S. officin+A. indica* | 6 | 8 | 8.5 | 12 | 8.62 | f |
| *S. argel+A. indica* | 6 | 8 | 14.5 | 20 | 12.12 | c |
| *S. officin+E. camald.* | 6 | 9 | 10.5 | 10.5 | 9 | f |
| *S. argel+E. camald+A. indica* | 6 | 14 | 18 | 18.5 | 14.12 | b |
| *S. officin+E. camald* | 6 | 7.5 | 13 | 14 | 10.12 | e |
| *+A. indica* | | | | | | |
| Means | 6 | 9.45 | 12.27 | 15.22 | | |
| | d | c | b | A | | |

**Table 10.** Effective concentration of herbs and the mean separation for herbs.

| Source | Degrees of freedom | Sum of squares | Mean square | F value | Prob. |
|---|---|---|---|---|---|
| Herbs | 10 | 648.364 | 64.836 | 25.134 | 0 |
| Concentration | 3 | 1025.307 | 341.769 | 132.491 | 0 |
| Herbs. Conc. | 30 | 339.818 | 11.327 | 4391 | 0 |
| Error | 44 | 113.5 | 2.58 | | |
| Total | 87 | 2126.989 | | | |

Analysis of variance:
Prob.: Probability: p value 0.05 significant ≤ p value>0.05 non-significant
Coefficient of variation 14.96%

**Table 11.** Ranking of concentration and separation for herbs (*P. aeruginosa*).

| | 0 | 0.25 | 0.35 | 0.45 | Means | |
|---|---|---|---|---|---|---|
| *C. myrrha* | 6 | 8.5 | 8.5 | 13.5 | 9.12 | a |
| *S. argel* | 6 | 6 | 7 | 8 | 6.75 | bcde |
| *S. officinalis* | 6 | 6 | 6 | 7 | 6.25 | de |
| *E. camaldulensis* | 6 | 6 | 6 | 10 | 7 | bcd |
| *A. Indica* | 6 | 6 | 7 | 10 | 7.25 | bc |
| *S. argel+E. camald* | 6 | 6 | 6 | 6 | 6 | e |
| *S. officin+A. indica* | 6 | 6 | 6 | 6 | 6 | e |
| *S. argel+A. indica* | 6 | 6 | 6 | 9 | 6.75 | bcde |
| *S. officin.+E. camald* | 6 | 6 | 7.5 | 9 | 7.12 | bc |
| *S. argel+E. camald+A. indica* | 6 | 6 | 6 | 8.5 | 6.62 | cde |
| *S. officin+E. camald+A. indica* | 6 | 6 | 6 | 12 | 7.5 | b |
| Means | 6 | 6.22 | 6.54 | 9 | | |
| | c | bc | b | a | | |

An inhibition zone 9.00 mm was found in the combination of (*S. officinalis* and *E. camaldulensis*) with a concentration 45%, also inhibition zones of 8.50 mm and 8.00 mm were found in the concentration of 45% with aqueous extracts of combination (*S. argel, E. camaldulensis,* and *A. indica*) and *S. argel.* The extracts of (*S. argel* and *E. camaldulensis*), (*S. officinalis* and *A. indica)* and *S. officinalis* have no or very low antimicrobial effect.

## Susceptibility of Microorganisms to different extracts

Figure 8 shows the means of susceptibility of microorganisms *(S. aureus, S. pyogenes, C. albicans, E. coli* and *P. aeruginosa)* to different aqueous extracts, the combination of *(S. argel, A. indica,* and *E. camaldulensis)* was achieved the best effect with all most four organisms *S. aureus, S. pyogenes, C. albicans* and *E. coli* with means of inhibition zones (17.63), (14.13), (11.63) and (8.25) mm, whereas *P. aeruginosa* was all most resistant to all herbal extracts with few exceptions in *C. Myrrha,* which have a mean of inhibition zone about (9.13)mm.

Moreover, *S. pyogenes* was the most sensitive to more than one herbal extract as shown in Figure 8 with means of inhibition zones (15.25), (14.25), (12.13), (11.13) and (10.13)mm with aqueous of combinations *(S. argel and E. camaldulensis), E. camaldulensis, (S. argel and A. indica), S. argel* and *(S. officinalis, A. indica* and *E. camaldulensis).*

Although there were four extracts have very few or non-significant effects on the microorganisms like extracts of *S. officinalis, A. indica, (S. officinalis* and *A. indica),* and *(S. officinalis* and *E. camaldulensis).*

## Effectivity of different concentrations of extracts on microorganisms

Figure 9 shows the effectivity of different concentrations of herbal extracts on microorganisms *C. albicans*, *S. aureus*, *S. pyogenes, E.coli* and *P. aeruginosa*, the concentration of 45% was show the highest effect with means of inhibition zones about 15.23, 12.18, 10.86, 9.04 and 9 mm, although these zones were decreased when the concentrations decrease. The extracts of a concentration of at least 25% (15% and 5%) did not give results.

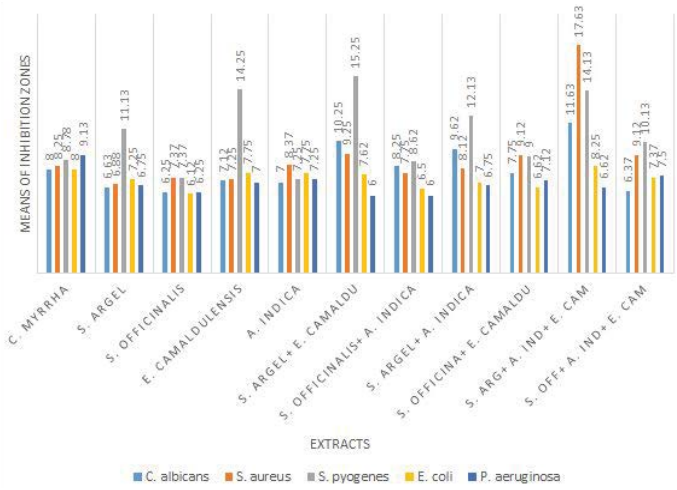


**Figure 8.** Susceptibility of Microorganisms to different extracts.

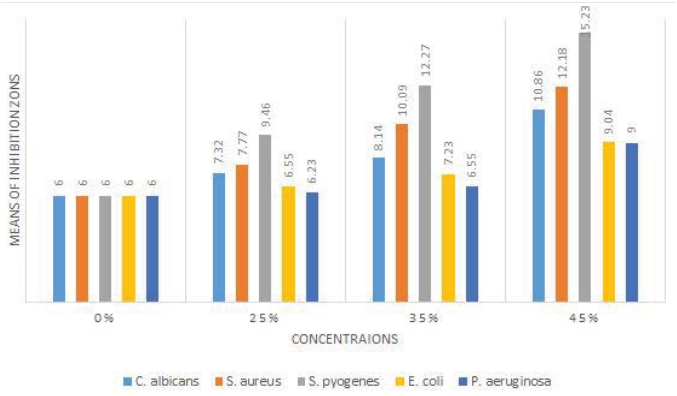


**Figure 9.** Effectivity of different concentrations on Microorganisms

**Table 12.** Effective concentration of herbs and the mean separation for herbs.

| Source | Degrees of freedom | Sum of squares | Mean square | F value | Prob. |
|---|---|---|---|---|---|
| Herbs | 10 | 61.091 | 6.109 | 2.307 | 0.027 |
| Concentration | 3 | 127.398 | 42.466 | 16.038 | 0 |
| Herbs. Conc | 30 | 73.727 | 2.458 | 0.928 | |
| Error | 44 | 116.5 | 2.648 | | |
| Total | 87 | 378.716 | | | |

Analysis of variance:
Prob.: Probability: p value 0.05 significant ≤ p value>0.05 non-significant
Coefficient of variation 23.44%

**Table 13.** Antimicrobial effect of a common vaginal wash against tested microorganism.

| | 0.5 | 1 | Mean |
|---|---|---|---|
| *C. albicans* | 8.5 | 9 | 8.75 |
| *S. aureus* | 8 | 8.5 | 8.25 |
| *S. pyogenes* | 7 | 12 | 9.5 |
| *E. coli* | 8 | 10 | 9 |
| *P. aeruginosa* | 7.5 | 10 | 8.75 |
| Mean | 7.8 | 9.9 | |

## Antimicrobial effect of a common vaginal wash against tested microorganism

In Table 13 a common vaginal wash was used as an antimicrobial solution in different concentrations 50% and 100%, in the concentration of 100%, the microorganisms showed different size of inhibition zones as followed *S. pyogenes* 12.00 mm, *E. coli* and *P. aeruginosa* 10.00 mm, *C. albicans* 9.00 mm and *S. aureus* was 8.5 mm in diameter.

# Discussion

The results obtained in this study revealed that the majority of vaginitis causal agents appeared to be bacteria in a percentage of 70%, however, only 30% or less was caused by *Candida albicans*, this finding was similar to the global statistic which done by Rein et al. [17] who reported that bacterial vaginosis is the most common causal of the lower genital tract disorder among women of reproductive ages. Never the less, the most common cause of vaginitis in both pregnant and non-pregnant women, and the most prevalent cause of vaginal discharge and odor. This also supports the study finding regarding the susceptible ages, with the range of age (16 – 45) years old to be subjected to a higher incidence of microbial growth. Some similarities also noticed in the presence of microorganisms in this study and previous studies, the study found that *S. aureus*, *C. albicans*, *E. coli*, *S. pyogenes* and rarely *P. aeruginosa* respectively also found that microorganisms such as *Staphylococcus epidermidis*, *Streptococcus spp*, *Corynebacterium spp*, *E. coli*, *peptostreptococci*, *Bacteroides melaninogenica*, *Gardnerella vaginalis*, *Ureaplasma*, *Mycoplasma*, *C. albicans* and some of the obligate and facultative anaerobic bacteria are associated with BV. In contrast, Hillier [18] said that there is no single organism whose presence confirms the diagnosis of bacterial vaginosis, but rather many different bacteria may be present, including *Gardnerella vaginalis*, *Mobiluncus species*, *Bacteroides* and *Prevotella species*, and *Mycoplasma species*.

Furthermore, Pabich et al. [19] reported that lactobacilli, yeasts and BV-associated bacteria are a less common component of the vaginal microbiota in postmenopausal women than in women of reproductive age, while *E. coli* is recovered at a higher frequency. This support the study finding which was note that *E. coli* was found in samples of subjects who their age between (50–90) years old.

The study data revealed that strains of Gram-positive bacteria were more sensitive than Gram-negative ones towards the plant extracts studied. This data is also supported by previous workers [20]. It has been proposed that the mechanism of the antimicrobial effects involves the inhibition of various cellular processes, followed by an increase in plasma membrane permeability and finally ion leakage from the cells. Amongst the tested Gram-negative bacteria, *E. coli* was found to be the most sensitive, while *P. aeruginosa* was the most resistant bacteria. In the case of Gram-positive bacteria, *S. pyogenes* was the most sensitive, while *S. aureus* was recorded less sensitivity. *C. albicans* was found to be highly sensitive to the action of a combination of (*S. argel, A. indica,* and *E. camaldulensis)*. The variation of the susceptibility of the tested microorganisms could be attributed to their intrinsic properties that are related to the permeability of their cell surface to the extracts. Due to the emergence of antibiotic-resistant pathogens in hospitals and homes, plants are being looked upon as an excellent alternate to combat the further spread of multidrug-resistant microorganisms.

The study found that the highest effects among the herbal and its combination were achieved by two herbs *S. argel* and *E. camaldulensis* and their combinations; this result was supported by many global studies. The previous study done by Abd [21] about antimicrobial activity of *S. argel* against eight bacteria: *Staphylococcus aureus; Micrococcus; Streptococcus spp; Bacillus anthracis; E. coli; Klebsiella pneumonia; Pseudomonas aeruginosa;* and Proteus Vulgaris and 14 fungi: Fusarium; Aspergillus parasiticus; *A. flavus; A. niger; A. candidus; A. glaucus; Penicillium; Chrisosporium; Cr. neoformans; Candida spp; C. albicans; Can. spp 20; Mucor* and *Rhodotorula*. The most powerful effect was observed in the case of *Streptococcus spp*; moderate action against *E. coli, B. anthracis; S. aureus; Klebsiella pneumonia* and Proteus Vulgaris. Also, another study by Akin-Osanaiye et al. [22] found, there was no significant difference in the antimicrobial activity of the extracts on Gram-negative and Gram-positive bacteria despite the differences in their cell wall components with reported the antibacterial activity of *E. camaldulensis* extracts against *Staphylococcus aureus*, while Sherry et al. [23] revealed that topical applications of eucalyptus oil clear methicillin-resistant *Staphylococcus*

*aureus* infections Lenka and Libor [24] reported that in some cases, the less concentrated extract was more effective. On the contrary, this study showed that the effect of different extracts increases as well as its concentrations.

Antimicrobial activity of the herbal extracts were compared to chemical agent (common vaginal washer), it has been found that extracts of combination of (*S. argel*, *A. indica*, and *E. camaldulensis*) obtained better activity than the chemical agent against most of the tested microorganisms with a slightly lower result in the case of *E. coli* but this difference was statistically non-significant. In contrast, Leelapornpisid et al. [25] also found that when the antimicrobial activity of the plant extracts was compared to chemical agents, it has been found that extracts of *E. cochinchinensis* and *S. officinalis* revealed good activity equal to azelaic acid and benzoyl peroxide but lower than clindamycin. Although these findings supported by Patel et al. [26] when they found that, herbal formulations have reached widespread acceptability as therapeutic agents for diabetics, arthritics, liver diseases, cough remedies, memory enhancers, and adaptogens [27].

## Conclusion

The present study indicated that aqueous extracts of *S. argel* and *E. camaldulensis* have antimicrobial activity against the tested microorganisms. The inhibitory effect against the tested organisms was more effective when using the concentrated extract. From the present work, it could be recommended that these two herbs extracts can be used as antibacterial and antifungal agents.

It is important to research the antimicrobial effects of herbs and other plants in many various forms, like different extracts or essential oils, as a natural source of antimicrobial compounds. Natural antimicrobial appears to be the most promising solution for many problems.

## Competing Interests

Authors have declared that no competing interests exist.

## References


1. Lowe, Nancy, Jeremy Neal and Nancy Ryan-Wenger."Accuracy of the clinical diagnosis of vaginitis compared with a DNA probe laboratory standard." *Obstet Gynecology* 113 (2009): 89-95.
2. Barbara,Majeroni. "Bacterial vaginosis: An update." *Am Fam physician* 57 (1998):1285 – 1289.
3. Kafi, shak, Mohamed and Musa. "Prevalence of sexually transmitted diseases (STD) among women in a suburban Sudanese community." *Upsala J Med Sci* 105 (2000): 249 -254.
4. Jenifer, Allsworth and Jeffrey Peipert. "Prevalence of bacterial vaginosis: 2001-2004 National Health and Nutrition Examination Survey data." *Obstet Gynecol* 109 (2007): 114-120.
5. Anderson, Matthew, Kathleen Klink and Andreas Cohrssen. "Evaluation of vaginal complaints." *JAMA* 291 (2004):1368-1379.
6. Livengood, Charles, Jessica Thomason and Gale Hill."Bacterial vaginosis: Diagnostic and pathogenetic findings during topical clindamycin therapy." Am J Obstet Gynecology 163 (1990):515-520.
7. Barbara, Van. "Prevalence, incidence, natural history and response to treatment of Trichomonas vaginalis infection among adolescent women." *J Infect Dis* 192 (2005): 2039-2043.
8. Swygard, Sena Hobbs and Cohn. "Trichomoniasis: Clinical manifestations, diagnosis, and management." *Sex Trans Infect* 80 (2004): 91-95.
9. Seema, Sood and Arti Kapil. "An update on Trichomonas vaginalis." *Indian J Sex Transm Dis Rev* (2008) 29(1): 7-14.
10. Fadil, Omer. "Trichomoniasis in Sudanese women presenting with vaginal discharge." *Sudan Med J* 16 (1978): 1-5.
11. Salih. "Vaginalis Detection of Trichomonas individuals." At Bashair Hospital. M. Sc. Thesis, Faculty of Veterinary Sciences, University of Khartoum, Sudan (2005).
12. Sobel, Jack. "Management of patients with recurrent vulvovaginal" *candidiasis Drugs* 63 (2003):1059–66.
13. De, Leon, Jacober, Sobel and Foxman. "Prevalence and risk factors for vaginal Candida colonization in women with type 1 and type 2 diabetes." *BMC Infect Dis* 2002 2:1.
14. Odds. "Candidosis of the fenitalia. In: Odds FC. Candida and candidiasis: A review and bibliography (2nd edn)London." *Bailliere Tindal* (1988) p.124.
15. Levy. "The challenge of antibiotic resistance." *Sci Am* 278(1998): 46–53.
16. Harrigan. "Laboratory methods in food microbiology." Academic press limited, California (1998), USA.
17. Rein and Holmes. "Non-specific vaginitis, vulvovaginal candidiasis, and trichomoniasis: Clinical features, diagnosis, and management." *Curr Clin Top Infect Dis* 4 (1983):281–315.
18. Sharon,Hillier. "Diagnostic microbiology of bacterial vaginosis." *Am J Obstet Gynecology* 169 (1993): 455–9.
19. Wendy,Pabich, Stephan Fihn, Walter Stamm, Delia Scholes, Edward Boyko and et al. "Prevalence and determinants of vaginal flora alterations in postmenopausal women." *J Infect Dis* 188(7): 1054-1058.
20. Walsh, Maillard, Russel, Catrenich, Charbonneau and et al. "Activity and Mechanism of Action of Selected Biocidal Agents on Gram-positive and –negative bacteria." *J Appl Microbiol* 94 (2003): 240-247.
21. Abd,hady and Hegazi. "Studies for determining the antimicrobial activity of Solenostemma argel (Del) Hayne. 1-extraction with methanol/water in different proportions." *Qatar University Science Journal* 14 (1994):138- 142.
22. Osanaiye, Akin, Agbaji and Dakare. "Antimicrobial activity of oils and extracts of Cymbopogon citrates, Eucalyptus citriodora, and Eucalyptus camaldulensis." *J Med Sci* 7 (2007): 694- 697.
23. Eugene,Sherry, Harry Boeck and Patrick Warnke. "Topical application of a new formulation of eucalyptus oil phytochemical clears methicillin-resistant *Staphylococcus aureus* infection." *Am Infect Control* (2001) 29(5): 346.
24. Lenka, dostalova and Libor kalhotka. " Antimicrobial Activity of Aqueous Herbal Extracts." *MENDELNET* (2014): 403-406.
25. Leelapornpisid, Chansakao, Ittiwittayawat and Pruksakorn. "Antimicrobial Activity of Herbal Extracts on *Staphylococcus aureus*." *Acta Hort* (2005) 679 ISHS: 97-104.
26. Patel and Goyal "Quality control of herbal products." *The Indian Pharmacist* (2006) 5(45): 26-30.
27. 27. Bryan, Larsen and Gilles Monif. " Understanding the bacterial flora of the female genital tract." Clin Infect Dis 32 (2001): e69-77.


**How to cite this article:** Ali Abdalla, Intisar Kamaleldin Izzeldin, Awad Mohamed Abdel-Rahim and Yasir Mohamed Hasan et al. "Identification and Management of Vaginitis Prevalent Causal Agents using Herbal Aqueous Extracts." *J Pharmacogn Nat Prod* 6 (2020) doi: 10.37421/jpnp.2020.6.163