**Original Article**

# Effect of *Syzygium aromaticum* and *Rosmarinus officinalis* extracts on *Giardia lamblia* and *Streptococcus agalactiae* isolated from Nile tilapia (*Oreochromis niloticus*)

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**Abstract:**

Two herbs (*Syzygium aromaticum* and *Rosmarinus officinalis*) were assessed for their antimicrobial activity against *Giardia lamblia* causing giardiasis in humans and *Streptococcus agalactiae*, a major fish pathogen causing streptococcosis. Each herb was extracted with 3 solvents: water, 95% ethanol, and methanol. Methanol extract from *R. officinalis* was strongly active against *G. lamblia* ($IC_{50}$ = 0.689 mg/mL) and *Syzygium aromaticum* was good activity against *G. lamblia* ($IC_{50}$ = 0.755 mg/mL). On the other hand, ethanol extract from *Syzygium aromaticum* was strongly active against *G. lamblia* ($IC_{50}$ = 1.227 mg/mL) and *R. officinalis* was good active against *G. lamblia* ($IC_{50}$ = 2.874 mg/mL). None of the aqueous extracts of the two plants were active against tested protozoan. Using swab paper disc assays, methanol extracts of *Syzygium aromaticum* and *Rosmarinus officinalis* produced the largest (22.2±1.2 mm) and (25.4±0.9 mm) inhibition zones, respectively. Determination of minimal inhibitory concentration (MIC) of herb extracts against *S. agalactiae* showed that the methanol extract of *Rosmarinus officinalis* had the lowest MIC value (6.25 mg/L). methanol extract of *Syzygium aromaticum* was a moderate MIC (12.5 mg/L). Such results indicate *S. aromaticum* methanol extract and ethanol extract from *R. officinalis* as possible candidates for further investigations to isolate and characterize their active principles as possible new natural anti-bacterial and anti-protozoal agents.

*Key words: Syzygium aromaticum, Rosmarinus officinalis, Giardia lamblia, Streptococcus agalactiae*

## INTRODUCTION

*Giardia lamblia* is a binucleate flagellated protozoan parasite that infects the upper intestinal tract of human and many animals' species **(Davies and Hibler, 1979)**. *Giardia* is an intestinal flagellate that infects a wide range of vertebrate hosts. The genus currently comprises six species, namely: *G. agilis*, *G. ardeae*, *G. duodenalis*, *G. microti*, *G. muris*, and *G. psittaci*, which are distinguished on the basis of the morphology and ultrastructure of their trophozoites **(Adam, 2001)**. It was also a frequently encountered parasite of domestic animals, especially livestock, dogs and cats, and numerous species of wild mammals and birds, and even fish have been documented as hosts of *Giardia* **(Hugo and Staffan, 2011)**. Symptoms in mammals characterized by acute watery diarrhea, dehydration and weight loss and abdominal discomfort **(Adam, 2001)**. The infection spreads via the fecal oral route. It is generally caused by contaminated drinking water and only 10 cysts are enough to cause infection **(Hugo and Staffan, 2011)**. Recent study revealed a low prevalence of *Giardia*, 3.8% (27/709), in fish hosts. The zoonotic *Giardia* species, *Giardia duodenalis* assemblages A, B as well as *G. duodenalis* assemblage E and *Giardia*

*microti* were detected. The identification of zoonotic species of *Giardia* highlights the public health importance of investigating parasites within fish host species **(Rongchang et al., 2010)**. Another study investigated the occurrence of zoonotic *G. duodenalis* assemblages in fish (*Tilapia nilotica* and *Mugil cephalus*) collected from (fish farms and Nile River) at different governorates in Egypt to explore the possible role of fish in the epidemiology of human giardiasis and that contributes in the contamination of water with this pathogen **(Ghoneim et al., 2012).**

The most common strategy to fight aquaculture disease is the use of antibiotics; however, such usage has been reported to have adverse effects. *Streptococcus agalactiae* is a Gram-positive, non-spore-forming, non-acid fast, non-motile, oxidase-negative, catalase-negative coccus with a diameter around 2 μm. It usually occurs in pairs or short chains. This bacterium is often referred to as Group B Streptococcus because it has group B Lancefield antigen present **(Johri et al., 2006)**. This bacterium causes diseases in mammals, especially in human and cattle, and is a leading cause of streptococcosis in several fish species

(Johri et al., 2006). Streptococcosis is considered a destructive disease as it can result in massive kills of fish and is responsible for heavy economic losses in many species of freshwater, marine, and estuarine fish worldwide.

The first outbreak of streptococcosis was recorded in rainbow trout (*Oncorhynchus mykiss*) cultured in Japan. It was later reported in the US in freshwater fish, the golden shiner (*Notemigonus crysoleucas*), and in seawater fish, including striped mullet (*Mugil cephalus*), menhaden (*Brevoortia patronus*), sea catfish (*Arius felis*). Other outbreaks of streptococcosis were reported in rainbow trout cultured in South Africa and Spain **(Abutbul et al., 2004)**. Drug resistance in fish pathogens can occur and transfer to environmental and human pathogenic bacteria **(Smith et al., 1994; Abutbul et al., 2004)**. Thus, accumulation of antibiotics in fish can be harmful to the environment as well as consumers **(Smith et al., 1994)**. Such adverse effects of antibiotics lead to problems in aquaculture; therefore, only few have been approved. Many countries refuse to import cultured products in which antibiotics were used. Therefore, these problems have prompted scientists to search for an alternative to replace antibiotics in controlling diseases in aquaculture.

Herbs are an interesting alternative because they are cheaper than antibiotics. In addition, since herbs may be readily available in local markets or easily grown, user autonomy can increase. Moreover, herbs are often considered environmentally friendly, and therefore, can be used in aquaculture. Research on using herbs to control diseases in aquatic animals is increasing with the demand for environmentally friendly aquaculture processes. Many herbs have promising characteristics for use in controlling fish diseases **(Rajandra, 1990)**. For example, **Shangliang et al., (1990)** reported the antimicrobial activity of 5 herb extracts, *Stella aquatica*, *Impatiens biflora*, *Oenothera biennis*, *Artemisia vulgaris*, and *Lonicera japonica* against bacterial and viral infections in fish; **Adithepchaikarn et al., (2008)** reported that *Psidium guajava* could control fish disease caused by *Aeromonas hydrophila*; and **Abutbul et al. (2004)** used *Rosmarinus officinalis* to treat *Streptococcus* infection in tilapia (*Oreochromis sp.*).

In this study, two herbs, *Syzygium aromaticum* and *Rosmarinus officinalis* were investigated for their anti-bacterial activity against *S. agalactiae* and antiprotozoal activity against *G. lamblia*.

## MATERIALS AND METHODS

### Standardization of protozoal suspensions

*Giardia lamblia* isolated from infected tilapia (*O. niloticus*), and obtained from Parasitology Department, Faculty of medicine, University at Assiut. *G. lamblia* trophozoites were grown axenically in Diamond's modified culture medium TYI-S-33 broth medium was used for stock culture of *G. lamblia*; when the logarithmic phase of growth was reached, the trophozoites were detached from the substrate by cooling on water-ice for 20 min. They were then distributed into 1.5 mL volumes of fresh culture medium in polypropylene microcentrifuge tubes to achieve inocula ranging from $1.0 \times l0^3$ to $5.0 \times 10^5$ per mL as determined by counting in a Neubauer hemocytometer. After incubation for 48 h at 37°C, the number of trophozoites was again counted **(Diamond et al., 1978)**.

### Bacteria and culture conditions

The *S. agalactiae* used in this study was *S. agalactiae* isolated from infected tilapia (*O. niloticus*), and obtained from Department of Fish Disease, Research Institute for Animal Health, Egypt. The identity of each bacterial strain was confirmed using the API 20 Strep test kit (bioMérieux Industry, Hazelwood, MO, USA). The bacterial culture was grown in brain heart infusion (BHI) medium at 25°C, and a stock of the organism was stored at −80°C in BHI broth supplemented with 15% glycerol **(Gorman and Adley, 2004)**. .

### Plant materials and preparation of herb extracts

The herbs used were: *Syzygium aromaticum* (clove), *Rosmarinus officinalis* (rosemary). Herbs were purchased from herb shops in Cairo, Egypt. Three different solvents, distilled water, 95% ethanol, and 99.8% methanol, were used to prepare extracts from each herb. In each case, the herb was oven-dried at 80°C for 72 h, finely ground in a mortar, and extracted with solvent in a ratio of 1:10 (w/v) for 24 h at room temperature. The mixture was then centrifuged at 13,000 rpm for 10 min at room temperature and the supernatant collected for filtration. For aqueous extracts, the filtrates were frozen at -80°C for 48 h before being freeze-dried. For the other extracts, the filtrates were evaporated to dryness in a rotary evaporator before being frozen at −80 °C for 48 h prior to being freeze-dried. Two extracts were stored at −20 °C in glass vials until use. For antimicrobial activity testing and determination of minimal inhibitory concentration (MIC), the concentration of each herb extract was adjusted to 100 mg/mL **(Bauer et al., 1966)**.

**Column Chromatography and Gas Chromatography-Mass Spectrum Analysis (GC-MS)**

One gram of the crude methanol extract was subjected to column chromatography using silica gel (100-200 mesh) and eluted with ethyl acetate. This ethyl acetate fraction of the plant extracts was taken for GC-MS analysis, since ethyl acetate could extract most of the bioactive compounds **(Sangeetha and Vijayalakshmi, 2011)**.

**Antiprotozoal activity**

Susceptibility of *G. lamblia* to the antimicrobial agents was determined by the culture method of **Hill et al. (1986)** with some modifications: serial dilutions of drugs were prepared in 1.5 mL volumes of culture medium in microcentrifuge tubes. The tubes were then inoculated with *G. lamblia* to achieve an inoculum of $5x10^4$ trophozoites/mL. Controls, prepared by adding drug-free solvents to the culture media, were similarly inoculated. Each test included metronidazole as positive control, a negative control (culture medium plus trophozoites and DMSO), and a blank (culture medium). The metronidazole compound was dissolved in DMSO 67 µg/mL. After incubation for 48 h at 37°C, trophozoites were detached by chilling on iced water for 20 min and 50 µl of each culture tube was subcultured into 1.5 mL of fresh culture medium without drug. After incubation for a further 48 h, the final number of parasites was determined by counting in a Neubauer hemocytometer. The number of viable trophozoites remaining after exposure to drug for 48 h was deduced from the number of trophozoites attained in drug-free control cultures. The 50% inhibitory concentration ($IC_{50}$) was defined as the concentration of the drug that inhibited growth by 50%. The results of antiprotozoal activity of the different extracts used were calculated as the percentage of growth inhibition when compared with the controls grown without plant extracts, the best straight line was determined by regression analysis, and the concentration that caused 50% inhibition ($IC_{50}$) were calculated for the log of the concentration using Graph Pad Prism **(Katiyar et al., 1994)**. Each experiment was done in triplicate.

The mortality % of parasite for each extracts activity was carried out according to the following formula:

$$Mortality\ of\ parasite\ (\%) = \frac{Control\ Negative - Tested\ Sample}{Control\ Negative} \times 100$$

**Antibacterial activity testing**

Antibacterial activities of the herb extracts against *S. agalactiae* were examined using a swab paper disc method with some modifications. 40 µl of each herb extract were spotted on a sterile paper disc, placed onto a BHI agar plate, spread with a swab of overnight culture containing colony forming units (cfu) of *S. agalactiae* (approximately $10^8$/cfuL). Each extract was tested in triplicate. Negative control were prepared in the same way except that 40 µl of suitable solvent dimethyl-sulphoxide (DMSO) were used instead of the herb extracts. Oxytetracycline (5 µg on sterile paper disc), a common antibiotic used, served as the positive control. The prepared agar plates were then incubated at 25°C for 18 h, and their growth inhibition zones were same as the total diameter of the growth-free zones around the discs. The diameter of the growth inhibition zone in the negative control treatment was subtracted (in case of no growth inhibition in the negative control treatment, only the diameter of the disc was subtracted) giving the zones of growth inhibition beyond the paper disc **(Bauer et al., 1966)**.

**Determination of MIC**

To determine the MIC of the herb extracts against *S. agalactiae*, Serial concentrations of two plant extracts were prepared from this crude extract to contain, 0.049, 0.098, 0.195, 0.391, 0.781, 1.562, 3.125, 6.25, 12.5, 25 and 50 mg/mL. The series of concentrations were used to detect the minimum inhibitory concentration (MIC) of two plant extracts by disc diffusion method **(Bauer et al., 1966).** The series of concentrations were kept in refrigerator at 4°C until used. MIC was defined as the lowest concentration of extracts that inhibited visible growth. The herb extracts with the MIC values of 50 mg mL$^{-1}$ or more, 25 mg mL$^{-1}$ and 12.5 mg/mL or less were declared to have weak, moderate and strong antimicrobial activities, respectively.

**Phytochemical analysis**

Preliminary screening of phytochemical content of different extracts was carried out in order to discover the antimicrobial activities against *G. lamblia* that were used in this study. Tannins, alkaloids, saponins, steroids, terpeniods, flavonoids, volatile oils and cardiac glycosides were determined by standard technique. GC-MS analysis was carried out on a HP 5972 Mass Selective Detector and Agilent 6890 Series Autosampler (Agilent Technologies, Santa Clara, CA, USA) and gas chromatograph interfaced

to a mass spectrometer (GC-MS) **(Sangeetha and Vijayalakshmi, 2011)**.

### Identification of components

The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library by using the time retention (TR). The Name, Molecular weight and Structure of the components of the test materials were ascertained.

### Statistical analysis

Each parameter was tested in triplicate. The values were expressed as mean ± standard deviation (SD). Also determination of *p*-value (two tailed) of the results by using Graph Pad Prism **(Katiyar *et al.*, 1994)**.

### RESULTS

### Antiprotozoal activity of *Syzgium aromaticum* crude extracts

The 50% growth inhibition ($IC_{50}$) of methanol, ethanol, and aqueous extracts from *S. aromaticum* at different concentrations (0.098 - 50 mg/mL) against *G. lamblia* trophozoites cultures was displayed in (Table 1 and Figure 1). The aqueous showed no activity against trophozoites of *G. lamblia*. The methanol extract *S. aromaticum* showed strong activity against trophozoites of *G. lamblia* followed by ethanol extract with an $IC_{50}$ of 0.755 and 1.227 with the highest trophozoite mortality rate obtained (95.3±0.2%) and (92.3±0.1%) respectively. Trophozoites of *G. lamblia* were affected with increasing

concentrations of methanol extracts and ethanol of the plant *S. aromaticum*. Metronidazol (MET) at 67 µg/mL concentration were inhibited all growth culture of 100% trophozoites. The decline in the percentage of trophozoites number was significant for all concentration tested ($p < 0.05$).

### Antiprotozoal activity of *Rosmarinus officinalis* crude extracts

The 50% growth inhibition ($IC_{50}$) of pet. ether, ethyl acetate, methanol and aqueous extracts from *R. officinalis* at different concentrations (0.098 - 50 mg mL$^{-1}$) against *G. lamblia* trophozoites cultures was displayed in (Table 2 and Figure 2). The aqueous extract showed no activity
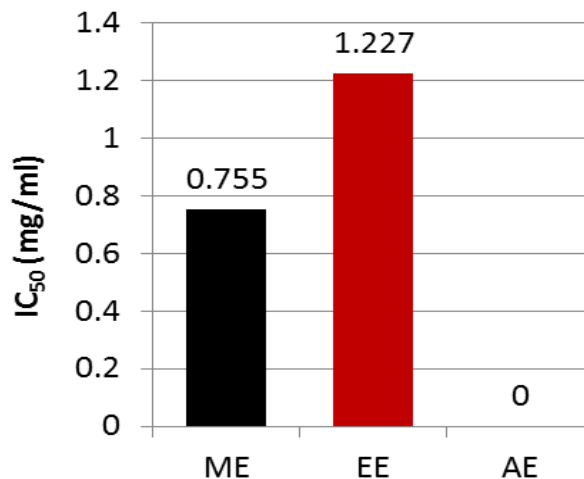


Figure 1: Inhibitory concentration 50% (IC50) of *S. aromaticum* crude extracts against tested *G. lamblia*

Table 1: *G. lamblia* trophozoites mortality rate and $IC_{50}$ after 48 h exposure to *S. aromaticum* extracts

| Concentration of plant extract mg/mL | ME | | EE | | AE | |
|---|---|---|---|---|---|---|
| | TM% | $IC_{50}$ | TM% | $IC_{50}$ | TM% | $IC_{50}$ |
| 50 | 95.3±0.2 | | 92.3±0.1 | | 0 | |
| 25 | 89.3±0.1 | | 86.3±0.1 | | ˙ | |
| 12.5 | 81.3±0.1 | | 80.3±0.1 | | ˙ | |
| 6.25 | 73.7±0.6 | | 74.7±0.6 | | ˙ | |
| 3.125 | 70.7±0.8 | | 68.7±0.8 | | ˙ | |
| 1.562 | 64.7±0.5 | 0.755* | 64.7±0.5 | 1.227* | ˙ | ˙ |
| 0.781 | 53.7±0.6 | | 52.7±0.6 | | ˙ | |
| 0.391 | 44.3±0.2 | | 42.3±0.2 | | ˙ | |
| 0.195 | 23.7±0.5 | | 23.7±0.5 | | ˙ | |
| 0.098 | 13.3±0.1 | | 13.3±0.1 | | ˙ | |
| DMSO (-ve C) | 0 | | 0 | | ˙ | |
| Metronidazole (+ve C) | 100±0.0 | | 100±0.0 | | ˙ | |

Values are means of inhibition effect (%) ± SD (n =3) *Correlation is significant at the *p* < 0.05 level (2-tailed).
TM% = Trophozoite mortality, -ve C = negative contole +ve C = positive control
ME = Methanol extract, EE = Ethanol extract, AE = Aqueous extract,
$IC_{50}$ = Inhibitory concentration of 50%, 0 : no sensitivity

Table 2: *G. lamblia* trophozoites mortality rate and $IC_{50}$ after 48 h exposure to *R. officinalis* extracts

| Concentration of plant extract mg/mL | ME | | EE | | AE | |
|---|---|---|---|---|---|---|
| | TM% | $IC_{50}$ | TM% | $IC_{50}$ | TM% | $IC_{50}$ |
| 50 | 96.7±0.8 | | 92.0±0.6 | | 0 | |
| 25 | 90.0±0.5 | | 86.0±0.5 | | . | |
| 12.5 | 84.0±0.7 | | 80.0±0.5 | | . | |
| 6.25 | 78.0±0.3 | | 74.0±0.5 | | . | |
| 3.125 | 72.0±0.5 | | 68.0±0.4 | | . | |
| 1.562 | 62.3±0.1 | 0.689* | 58.3±0.1 | 2.874* | . | . |
| 0.781 | 52.0±0.3 | | 47.3±0.2 | | . | |
| 0.391 | 41.0±0.3 | | 36.0±0.6 | | . | |
| 0.195 | 31.0±0.5 | | 26.0±0.3 | | . | |
| 0.098 | 19.0±0.3 | | 16.0±0.5 | | . | |
| DMSO (-ve C) | 0 | | 0 | | . | |
| Metronidazole (+ve C) | 100±0.0 | | 100±0.0 | | . | |

Values are means of inhibition effect (%) ± S.D (n =3) *Correlation is significant at the *p* < 0.05 level (2-tailed).
TM% = Trophozoite mortality, -ve C = negative control +veC = positive control
ME = Methanol extract, EE = Ethanol extract, AE = Aqueous extract,
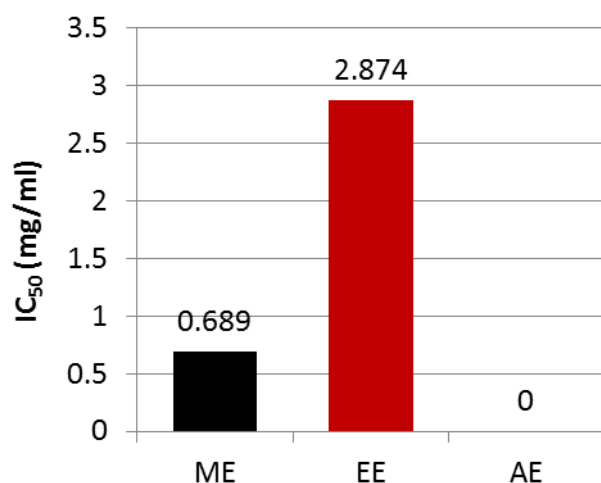$IC_{50}$ = Inhibitory concentration of 50%, 0: no sensitivity



Figure 2: Inhibitory concentration 50% (IC50) of *R. offisinalis* crude extracts against tested *G. lamblia*

Table 3: Antibacterial activity of herbs extracted in different solvents as determined by swab paper disc assay against *S. agalactiae*

| Herbs | Inhibition zone size (mm) | | |
|---|---|---|---|
| Solvent | methanol | ethanol | water |
| *S. aromaticum* | 22.2±1.2 | 20.8±1.2 | 10.3±0.6 |
| *R. officinalis* | 25.4±0.9 | 20.2±0.4 | 18.1±1.0 |
| DMSO (-ve control) | (-) | | |
| Oxytetracycline (+ve control) | 28.7±0.9 | | |

Values are means of Inhibition zone size (mm) ± SD (n=3).
*Correlation is significant at the *p* < 0.05 level (2-tailed), (-): No sensitivity.

activity against trophozoites of *G. lamblia*. The methanol extract *R. officinalis* showed strong activity against trophozoites of *G. lamblia* followed by ethanol extract with an $IC_{50}$ of 0.689 and 2.874 mg/mL with the highest trophozoite mortality rate obtained (96.7±0.8%) and (92.0±0.6%) respectively. Trophozoites of *G. lamblia* were affected with increasing concentrations of methanol and ethanol extracts of the plant *R. officinalis*. Metronidazole (MET) at 67 μg/mL concentration were inhibited all growth culture of 100% trophozoites. The decline in the percentage of trophozoites number was significant for all concentration tested (*p* < 0.05).

**Antibacterial activity testing**

The swab paper disc assay results are presented in Table 3. Two herb extracts had antimicrobial activity against *S. agalactiae*; however, different degrees of inhibition were observed. Of the herb extract types used in this study, the 98.8% methanol extracts exhibited the strongest antimicrobial activity, followed by 95% ethanol and aqueous extracts. Based on inhibition zone sizes, methanol extract of *R. officinalis* produced the largest inhibition zone, whereas aqueous extract of *S. aromaticum* produced the smallest.

**Determination of MIC**

The MIC values of herb extracts are shown in Table 4. In general, there was a significant correlation between the findings for the swab paper disc method and the MIC determination assay. Based on MIC values, Clove and Rosemary extracts were declared to have strong (MIC values 6.5 mg/mL), moderate (MIC values of 12.5 mg/mL), and weak (MIC values 50 mg/mL) inhibitory activity with methanol, ethanol and water extracts, respectively of clove and rosemary. None of the 95% ethanol extracts exhibited moderate activity. Among the herb extracts, showing strong antimicrobial activity, the methanol extract of *R. officinalis* had the lowest MIC (6.5 mg/mL). Within the group of extracts with weak activity, only one extract (aqueous extracts of *S. aromaticum*) had an MIC value of >50 mg/mL.

**Phytochemical analysis**

Some secondary products of *S. aromaticum* and *Rosmarinus officinalis* extracts contained flavonoid, volatile oil, sterols & triterpins, saponins, tannins and cardiac glycosides. GC-MS analysis for volatile compounds methanol extract of *S. aromaticum* contained sixteen compounds were identified. The major component was phenol, 2-methoxy-3-(2-propenyl)-[eugenol] (71.04%), phenol, 2-methoxy-4-(2-propenyl)-, acetate (12.03%) and caryophyllene (8.41%). Also, GC-MS analysis for volatile compounds methanol extract of *Rosmarinus officinalis* contained twenty six volatile compounds were identified. The major component were α-pinene (19.32%), camphor (6.12%), 1.8-cineole (5.44%), camphene (6.03%), β-pinene (5.60%), bornylacetate (4.55%), limonene (3.66%), borneol (3.20%), terpinen-4-ol (1.99%) and carvone (1.14%).

## DISCUSSION

Although antibiotics have been used to control bacterial infections in fish for many years, several antibiotics have been banned from use in aquaculture due to their adverse effects. In addition, products from aquaculture facilities using antibiotics are not accepted by many countries. Therefore, finding an alternative antimicrobial substance to replace antibiotics has become an issue of interest of many research groups. In this study, two herbs *Syzgium aromaticum* (clove) and *Rosmarinus officinalis* (rosemary) were evaluated for their antiprtozoal activity against *G. lamblia* and antibacterial activity against *S. agalactiae*. The essential oil inhibited trophozoites adherence since the first hour of incubation and was able to kill almost 50% of the parasites population in a time dependent manner.

Table 4: Minimal inhibitory concentrations (MIC) of herbs extracted in different solvents against *S. agalactiae*

| Herbs | MIC (mg/L) | | |
|---|---|---|---|
| Solvent | methanol | ethanol | water |
| *S. aromaticum* | 12.5 | 25 | (-) |
| *R. officinalis* | 6.25 | 12.5 | 50 |

(-): No sensitivity

The eugenol inhibited *G. lamblia* trophozoites adherence since the third hour and not induce cell lyses **(Machado et al., 2003)**. The main morphological alterations were modifications on the cell shape, presence of precipitates in the cytoplasm, autophagic vesicles, internalization of flagella and ventral disc, membrane blebs, and intracellular and nuclear clearing. Taken together, our findings lead us to propose that eugenol was responsible for the anti-giardial activity of the *S. aromaticum* essential oil and both have potential for use as therapeutic agents against giardiasis **(Machado et al., 2003)**. Plants and their extracts and essential oils have been used for many years in the treatment of several diseases, including parasite infections **(Jones, 1996)**. The essential oils can be extracted from different parts of the plant, obtained by crushing or by distillation. Others performed a literature review of the essential oils that have been shown to possess activity against *E. histolytica*, *G. lamblia*, *T. vaginalis*, *T. cruzi*, and/or *Leishmania* sp **(Perez et al., 2012)**.

In this study antigiardial activity of *R. officinalis* extracts may be attributed to their phytoconstituents which contained it. Plants and their extracts and essential oils have been used for many years in the treatment of several diseases, including parasite infections **(Jones, 1996)**. The essential oils can be extracted from different parts of the plant, obtained by crushing or by distillation. Others performed a literature review of the essential oils that have been shown to possess activity against *E. histolytica*, *G. lamblia*, *T. vaginalis*, *T. cruzi*, and/or *Leishmania* sp **(Perez *et al.*, 2012)**. Interestingly, many herbs used to treat diarrheal diseases contain considerable quantities of some of these flavonoids (e.g., *Quercus robur, Croton lechleri, and Hamamelis virginiana*) **(Cai *et al.*, 1991; Ubillas *et al.*, 1994; Blumenthal *et al.*, 2000)**. This study found that aqueous, 95% ethanol, and methanol extracts of two herbs used in this study were able to inhibit the growth of *S. agalactiae*. This may be because the herbs have more than one extractable active compound that provides

antimicrobial activity. Clove buds and their essential oils have been known to possess various antimicrobial and antioxidant properties **(Fu et al., 2007)**. **Shelef (1983)** reported that the essential oil substance isolated from clove, inhibited bacteria in culture media and also discovered that most of the antimicrobial substances were phenol compounds such as eugenol, thymol and carvacol. **Burt (2004)** proposed that different modes of action are involved in the antimicrobial activity of EOs. The activity may, in part, be due to their hydrophobicity, responsible for their partition into the lipid bilayer of the cell membrane, leading to an alteration of permeability and a consequent leakage of cell contents **(Burt, 2004).** Recent investigations on the antimicrobial action of some EOs showed disruption of the bacterial and fungal membrane **(Cox et al., 2000, 2001; Hammer et al., 2004; Di Pasqua et al., 2007; Bakkali et al., 2008).** Eugenol is the main volatile compound of extracted oil from bud *S. aromaticum* that is used in traditional medicine, as a bactericide, fungicide **(Arras and Usai, 2001; Ayoola et al., 2008)**, anesthetic, and others **(Santos et al., 2009)**. Tannin is a compound, which has antimicrobial activity, and can be isolated from plants by water **(Scalbert, 1991)**, ethanol **(Silva et al., 1997),** and methanol extraction **(Taylor et al., 1997)**. **Moreno et al. (2006)** reported that rosemary plants are rich sources of phenolic compounds with high antimicrobial activity against both Gram positive and Gram negative bacteria. Antimicrobial activities of plant essential oils have been known for centuries, but their strong flavor limited their use in food **(Del Campo et al., 2000).** Rosemary plants are rich sources of phenolic compounds with high antioxidative and antimicrobial properties, but their antimicrobial activities have not been deeply characterized **(Moreno et al., 2006)**. There is also some evidence that minor components have a critical part in antibacterial activity, possibly by producing a synergistic effect between other components **(Burt, 2004).** The absence of inhibition zone does not necessarily mean that compounds are inactive. For example, non-polar compounds may not diffuse into the culture medium **(Moreno et al., 2006)**. The antimicrobial activity of essential oils fractions from rosemary has been reported several times **(Tepe et al., 2005 ; Sacchetti et al., 2005)** although this activity was scarcely investigated in other fractions. In another report an inhibitory activity of a commercial rosemary extract against *S. aureus* was described, but in this case the composition and the active compound were not given **(Plouzek et al., 1999)**. It was reported that an antimicrobial action of phenolic compounds was related to the inactivation of cellular enzymes, which depended on the rate of penetration of the substance into the cell or caused by membrane permeability changes **(Shahidi and Naczk, 2004)**. The mechanism of action of terpenes is not fully understood but it is in general speculated to involve membrane disruption by the lipophilic compounds **(Cowan, 1999)**. Besides, another research done by **Cervenka et al. (2006)** on methanol and chloroform extracts of 17 plants such as rosemary, sage, chamomile and cinnamon, showed that methanol extract of these plants had higher antimicrobial effects than their chloroform extracts. The difference in obtained results is due to using different kinds and different concentrations of extracts in our study and previous researches. The antibacterial activity of clove is attributed to eugenol (2 methoxy-4 allyl-phenol) **(Gupta *et al.,* 2008)**. High tannin content (10-19%) in clove also provides additional antimicrobial activity **(Nanasombat and Lohasupthawee, 2005)**.

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