

Novel antibacterial, antifungal and antiparasitic activities of *Quassia amara* wood extract

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ABSTRACT. *Quassia amara* is a plant of the family Simaroubaceae of Northern Brazilian origin. Its use in folk medicine is widespread, especially as an antiparasitic, antifungal and antibacterial agent. Our purpose was testing a *Quassia amara* ethanol wood extract (QWE) on various parasites, fungi and bacteria which had not been previously screened for this ingredient. QWE was found to have a strong antiparasitic effect on *Demodex* spp by counting the number of mites extracted from biopsies of pustules of patients with erythematotelangiectatic and papulopustular subtypes of rosacea along a topical treatment with 4% QWE, these numbers reaching their physiological value after a 42-day course. In vitro testing of this extract on cultures of *Trichomonas vaginalis* collected from symptomatic patients showed a rapid inhibition of the growth of the trophozoites after 48 hours of contact. QWE also showed a marked antifungal activity on *Candida* spp (namely *C. albicans*, *C. parapsilosis*, *C. glabrata* and *C. krusei*, the latest at a lesser extent, and *Malassezia furfur* isolated from samples of infected patients, inhibiting the growth of fungi in both a time- and dose-dependent manner. The antibacterial activity of QWE was demonstrated in cultures of *P. acnes* and coagulase- positive *Staphylococci* where the growth of the bacteria was reduced in a significant manner ($p < 0.05$) and at a lesser extent in cultures of coagulase-negative *Staphylococci* where the growth inhibition was not statistically significant. Contrarily, QWE had no effect on the growth of *Chlamydia trachomatis*, but uniquely altered the morphology and quantity of chlamydial inclusions. To the best of our knowledge, this is the first time that QWE is shown to have antiparasitic activity on *Trichomonas vaginalis* and *Demodex* spp, an antifungal activity on *Malassezia furfur* and *Candida* spp and an antibacterial activity on *P. acnes*.

1. INTRODUCTION

Quassia amara belongs to the family of Simaroubaceae. It features a shrub or small tree growing 4 to 6 meters in height. *Quassia amara* is indigenous to Northern Brazil and the Guyanas and it also grows in Venezuela, Columbia, Argentina, Panama and Mexico. Its popular use as a medicinal plant is widespread in many indications, especially in gastro-intestinal disorders but also as an antimalarial. In Europe, *Quassia amara* is registered in the British Pharmacopoeia, but also in the Pharmacopoeia of Belgium, Denmark, France, Germany, Norway, Spain, Switzerland and Sweden. *Quassia amara* wood contains a great number of active ingredients, mainly quassinoids (triterpenoid compounds); the most important are quassin, neoquassin, 18 hydroxyquassin and Simalikalactone D but isoquassin, parain, quassamarin, quassinol and quassol are also present. Quassinoids are the major components responsible for the biological and pharmacological activities in this family.

Main reported pharmacological activities are antimalarial [1,2], amoebicide [3,4], antiviral [5,6], antitumoral [7,8], antiulceric [9,10] and anti-inflammatory [11-13]. In dermatological applications, it is frequently used in lice infestation [14-16], rosacea [17] and seborrheic dermatitis [18].

In the present work we intend to describe the effects of *Quassia amara* wood extract on various parasites, bacteria and fungi that may be the causative agents of disorders affecting the skin and mucosae.

2. MATERIALS and METHODS

Preparation of extract

The wood of *Quassia amara*, featuring chips of bark of the tree, is ground in a rough powder which was macerated into a mixture consisting of ethanol 60/glycerine 20/water 20. The maceration process was for 48 hours. After decantation, the liquid phase was filtered through a mesh and then its content in quassin was assessed by HPLC as described by Bertani *et al* [19], using a Discovery® C18 column (15 cm×4.6 mm, 5µm, Supelco®) and a standard of quassin (Cod. ABS-00017010-010, ChromaDex Inc., Irving CA, USA).

Determination of in vitro antifungal activity on *Candida* spp

The strains under study were *C. albicans*, *C. parapsilosis*, *C. glabrata* and *C. krusei* isolated from vaginal exudates and *C. parapsilosis* ATC 22019.

The strains were inoculated on solid Sabouraud Chloramphenicol Agar medium (Sigma Aldrich Corp, St Louis, MO, USA) in doses of 100µl in 0,5 MacFarland standard (1-5 10⁶ UFC/ml).

Quassia wood extract in various concentrations was added to this culture and evaluation of activity was performed at 30 minutes, 60 minutes, 24 and 48 hours.

Determination of in vitro antifungal activity on *Malassezia furfur*

The strain under study was *Malassezia furfur* isolated from skin scales of a patient with *Tinea versicolor*. It was inoculated on a Dixon's agar medium (Sigma Aldrich, St Louis, MO, USA) and the qualitative development of *Malassezia* yeasts was evaluated in function of time following the addition of a dilution of *Quassia* wood extract. The method used was screening, given the nutritional exigence featured by this lipophilic yeast of slow development. The concentration of inoculum was not adjusted.

Determination of in vitro antiparasitic activity on *Trichomonas vaginalis*

Patient-derived strains of *T. vaginalis* were obtained from female patients and isolated in culture for 48 hours in 7-ml Wassermann glass tubes with Trypticase-yeast extract-maltose (TYM) medium (Sigma Aldrich, St Louis, MO, USA). They were further cultured under aerobic conditions at 37°C in culture multiwell plates with Trypticase-yeast extract-maltose (TYM) medium (Sigma Aldrich, St Louis, MO, USA) and the concentration of motile trophozoites was adjusted to approximately 10⁵/ml in each well. The presence or absence of motility was observed at 24 and 48 hours with different concentrations of *Quassia* wood extract.

Determination of ex vivo antiparasitic activity on *Demodex* spp

In this experiment, the difficulty was that there is currently no in vitro experimental method permitting to culture *Demodex* spp. We have overcome it by counting with an optical microscope x50 the mites in measured skin surface biopsy specimens obtained with cyanoacrylate glue from six standard facial sites from 20 patients with erythematotelangiectasic-type rosacea and 37 patients with papulopustular rosacea at D0 and after a 14-day, 28-day and 42-day course of a topical cream containing 4% *Quassia* wood extract (QUASIX, Life Science Investments Ltd, London, UK) used twice per day.

Determination of in vitro antibacterial activity on Chlamydia trachomatis (CT)

Previous to the experiment itself, the cytotoxicity of Quassia wood extract was tested on cell cultures of CT. A suspension of the cellular line to-be-used was prepared and inoculated in sterile 96-well plates adjusting the number of cells to approximately 10^4 cells/ml.

0,1ml of this solution was inoculated in each well and the plates were incubated in an atmosphere with 5% CO₂ at 37°C during 24 hours. Then the quality of confluent cell cultures was checked by inverted optical microscopy in every one of the wells of all plates to-be-used in the experiment. Various concentrations of Quassia wood extract were tested after incubation at 37°C during 24, 48 and 72 hours. At these intervals the percentage of cell viability was evaluated. Non-cytotoxic concentrations will be used for determination of both Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). From 25ml-vials with confluent cultures, and using the processes to prepare cultures in Shell-type vials, suspensions of the cell lines to-be-used were prepared and inoculated in sterile 24-well plates. 1ml of cell suspension was inoculated in each well, each one containing a sterile circular coverslip where cells are sticking. The plates are incubated in an atmosphere with 5% CO₂ at 37°C during 24 hours. Afterwards, the quality of the confluent cell cultures is ascertained in each well of all plates by inverted optical microscopy. Vials containing 72h cultures of the standard strain of CT in infectious conditions between 90 and 100% are vigorously shaken in vortex. From them, and according to the technics used for sub-culturing CT cultures, a suspension containing 5000 inclusion-forming units (UFI) per ml of standard strain and cycloheximide 1µg/ml is prepared. In sterile conditions the culture medium is removed from each well, which are inoculated with 1ml of the standard strain of CT. The plates are centrifuged at 1200g at 32°C during 50 minutes. Then, again in sterile conditions, the infecting medium is removed from each well and 1ml of the diluted extract prepared in CT medium with addition of cycloheximide 1µg/ml. Each dilution of the extract is tested in quadruplicate. Each series of dilutions of the extract includes a control well where the CT medium was added with cycloheximide 1µg/ml but no extract. The plates are incubated during 72 hours in an oven with an atmosphere of 5% CO₂ at 37°C. During this period of incubation, the evolution of the infection is followed in each well of every plate with observation every 24 hours by inverted optical microscopy. At the end of the incubation period the medium is cautiously removed from all wells and treated with 1ml pure methanol during 10 minutes for its fixation. Methanol is then removed and each circular coverslip is lift up from the bottom of each well, dried by tepid air flow during 30 seconds and transferred to another clean, dry and non-sterile 24-well plate, the side containing the culture upside. It is then checked by immunofluorescence with specific monoclonal antibodies for CT. The results are observed in fluorescence optical microscopy and the percentage of chlamydial infection is determined by counting the number of typical inclusions compared with the total number of cultured cells. On the other hand the percentage of aberrant or atypical inclusions is also noted. To express the grade of inhibition of infection due to the extract, the percentage of infection in each well is compared with the percentage of chlamydial infection in the control well. The dilution of extract where no typical chlamydial infection is observed is considered as the MIC. In any case the MBC is determined as follows: at the moment of fixing the cultures, two of each of the quadruplicated wells are reserved and kept intact. Then in conditions of sterility the medium is extracted from each well and 1ml of fresh medium plus cycloheximide is added. One of the remaining wells is cultured in this manner and the other one is scrapped off with a pipette in order to put the medium in suspension again. With this suspension two vials with confluent cell cultures are inoculated and incubated at 37°C during 72 hours. At the end of this period the well and the vials are revealed with the aforementioned technic and the infective capacity is evaluated by observing the presence of typical inclusions. The concentration for which the suspension does not generate infection in the culture inoculated in the vial is considered as the MBC.

Determination of ex vivo antibacterial activity on P.acnes

Cultures were taken from pustules of 30 patients with acne by a swab and processed as previously described in the literature [20]. Samples were taken from these patients at D0 and at the end of a 42-

day course of application of a topical cream containing 4% Quassia wood extract (QUASIX, Life Science Investments Ltd, London, UK) used twice per day. Replica cultures of serial 10-fold dilutions in a phosphate buffer with triton x100 were prepared. The standard medium consisted of 3% trypticase soy, 2% agar, 1% yeast extract and 0,5% Tween-80 in plastic Petri dishes which were incubated 4 days at 37°C. Numbers of colony-forming units (CFU) were calculated directly on the samples.

Determination of in vitro antibacterial activity on Staphylococcus coagulase positive

Cultures were taken from the pustules of the same patients at D0 and D42 as aforementioned. The course of treatment was the same during this 42-day period. All specimen swabs were incubated overnight in 1.5 ml of tryptic soy broth with 6.5% NaCl, prior to subculture of 10 µl on BBL CHROMagar MRSA and CHROMagar SA (BD Diagnostics, Sparks, MD, USA). Presumptive positive colonies were subcultured to tryptic soy agar with 5% sheep red blood cells and incubated aerobically at 35°C for up to 48 h. Confirmation of coagulase positive Staphylococcus was performed using a tube coagulase test, and Gram staining.

Determination of in vitro antibacterial activity on Staphylococcus coagulase negative

The same methodology as aforementioned for Staphylococcus coagulase positive was applied. Isolates were sub-cultured twice on tryptic soy agar with 5% sheep blood to ensure purity and viability. The Rapid Gram-Positive Identification panels and the Conventional Gram-Positive Identification panels were tested concurrently according to the manufacturer's instructions. Bacterial suspensions for each panel were prepared from well-isolated colonies from a tryptic soy agar plate with 5% sheep blood incubated at 35°C for 18 to 24 h in a non-CO2 incubator.

3.RESULTS

Concentration in quassin of the extract

The extract prepared from Quassia amara wood featured a limpid, brownish-coloured liquid, with a characteristic odour. Concerning its physical properties its relative density was 1.028 and its refractive index 1.359. PH of the solution at 20°C was 3.9 and dry loss 3.56%. HPLC dosage of quassin gave a result of 0.797 g % w/v (equivalent to 0.764 g % w/w). As per microbiology, results were <1000 cfu for both count of total aerobic germs and count of total yeasts and moulds (Eur. Pharm. 5.0 2.6.12). Absence of Candida albicans, Staphylococcus aureus and Pseudomonas aeruginosa was assessed (Eur. Pharm. 5.0 2.6.13).

Antifungal activity on Candida spp

The results are reported in table 1.

Table 1. Development of Candida yeasts in contact with various concentrations of Quassia amara wood extract (QWE)

Time	Yeast	100	50	25	12,5	6,2	3,1	1,6	0,78	0,4	0,2	0,1
30 & 60'	C.albicans	D	D	D	D	D	D	D	D	D	D	D
	C.parapsilosis	D	D	D	D	D	D	D	D	D	D	D
	C.glabrata	D	D	D	D	D	D	D	D	D	D	D
	C.krusei	D	D	D	D	D	D	D	D	D	D	D
24 & 48h	C.albicans	ND	ND	D	D	D	D	D	D	D	D	D
	C.parapsilosis	ND	ND	D	D	D	D	D	D	D	D	D
	C.glabrata	ND	ND	D	D	D	D	D	D	D	D	D
	C.krusei	ND	D	D	D	D	D	D	D	D	D	D

D = Development

ND = No development

Concentrations feature % in grams of a 1/10 solution of the extract prepared.

We can observe from these results that QWE has no activity at any concentration after 30 and 60 minutes. Contrarily, highest concentrations of QWE (10% corresponding to a concentration in quassin of 0.0764% w/w) display antifungal properties after 24 and 48 hours on all four strains tested, and concentration of 5% (corresponding to a concentration in quassin of 0.0382% w/w) displays antifungal activity after 24 and 48 hours on *C.albicans*, *C.parapsilosis* and *C.glabrata* but not on *C.krusei*.

Antifungal activity on *Malassezia furfur*

The results are reported in Table 2.

Table 2. Growth of *Malassezia furfur* along time with various dilutions of QWE.

Days	1	2	3	4	5	6	7
Dilutions							
1/10	ND	ND	ND	ND	ND	ND	ND
1/20	ND	ND	ND	ND	ND	ND	ND
1/30	ND	ND	ND	ND	ND	ND	ND
1/40	ND	ND	ND	ND	ND	ND	ND
1/50	ND	ND	ND	ND	ND	ND	ND
1/60	ND	ND	ND	ND	ND	ND	ND
1/70	ND	ND	ND	ND	ND	ND	ND
1/80	ND	ND	ND	ND	ND	ND	ND
1/90	ND	ND	ND	ND	ND	ND	SD
1/100	ND	ND	ND	ND	ND	ND	SD
Control	ND	ND	ND	ND	ND	D	D

ND = No Development, SD = Slight Development, D = Development

From these results we can observe that *M.furfur* is growing in the medium only after 6 days (control data). QWE is showing a strong antifungal effect at dilutions up to 1/80 of the prepared solution (corresponding to a concentration in quassin of 0.01% w/w). This antifungal activity was decreased for higher dilutions.

Antiparasitic activity on *Trichomonas vaginalis*

The results are reported in Table 3.

Table 3. Growth of *Trichomonas vaginalis* in presence of different concentrations of QWE

Time	Sample A	Sample B	Sample A	Sample B	Control
Dilution	24 hours	24 hours	48 hours	48 hours	48 hours
1	-	-	-	-	
0,5	-	-	-	-	
0,25	-	-	-	-	
0,125	-	-	-	-	
0,075	-	-	-	-	
0,0375	-	-	-	-	
0,0187	+	+	-	-	
0,0093	+	+	-	-	
0,0046	+	+	-	-	
No QWE					+

+ = viability of TV - = viability of TV

As it can be seen, QWE has shown a strong toxic effect on *Trichomonas vaginalis* at concentrations > 0.0375 (corresponding to 0.029% w/w of quassin) at 24h and at concentrations > 0.0046 (corresponding to 0.0035% w/w of quassin) at 48 hours.

Antiparasitic effect on *Demodex* spp

Results are summarized in Table 4.

Table 4. Evolution of the number of *Demodex* spp units by cm² of skin during treatment with 4% QWE cream

Days	D0	D14	D28	D42
Number of <i>Demodex</i> spp per cm² Total sample	12.9 ± 0.6	7.7 ± 0.5	2.3 ± 0.3	0.9 ± 0.2
Number of <i>Demodex</i> spp per cm² Erythematotelangiectasic group	4.2 ± 0.5			0.2 ± 0.2
Number of <i>Demodex</i> spp per cm² Papulopustular group	20.5 ± 1.2			1.7 ± 0.3

As it can be seen from the table, there was a dramatic decrease in the population of *Demodex* spp along time in both groups of rosacea patients. The drug used in the course of the treatment was a cream formulated with 4% QWE corresponding to a concentration of roughly 0.03% in quassin.

Antibacterial activity on *Chlamydia trachomatis* (CT)

Previously to this experiment, the cytotoxicity of QWE was determined, resulting in that 80-90% of cell viability was corresponding to concentrations of 1.25 to 0.25% of QWE, i.e. concentrations of 0.01 to 0,002% in quassin. The study itself showed that QWE did not inhibit the cycle of CT, even when used at cytotoxic concentrations. Nevertheless, for concentrations ranging from 0.3% to 0.2% in QWE (i.e. respectively 0.0023 and 0.0015 in quassin) QWE was able to alter the morphology and quantity of chlamydial inclusions.

Antibacterial activity on *P.acnes*

The results of bacterial counts showed that after a 42-day course with 4% QWE extract applied on the skin, the number of colony-forming units of *P.acnes* was significantly reduced by 58% (from 1.82 ± 0.20 to 0.76 ± 0.30 cfu – p<0.05)

Antibacterial activity on *Staphylococcus coagulase positive*

In the same manner, the number of colony-forming units of *Staphylococcus coagulase positive* was also reduced in a significant manner by 50.8% (from 2.4 ± 0.19 to 1.18 ± 0.36cfu p<0.05).

Antibacterial activity on *Staphylococcus coagulase negative*

The number of colony-forming units of *Staphylococcus coagulase negative* was also reduced by 27.8% (from 1.76 ± 0.18 to 1.27 ± 0.38) but this reduction was not statistically significant.

4.DISCUSSION

As mentioned in the introduction, *Quassia amara* is a plant widely used in its native areas as a folk medicine, mainly as an antimalarial, amoebicide, and topically in the treatment of lice infestations. More recent studies have corroborated the activity of *Quassia amara* on the inhibition of growth of *Plasmodium Berghei*¹ and *Plasmodium falciparum* [2,21]. The amoebicide activity of various quassinoids was also demonstrated in modern trials [3,4]. The efficacy of *Quassia* extract in pediculosis is also largely corroborated in various published studies [14-16].

These data permit to suggest that *Quassia amara* may be a first-line candidate in our armamentarium of antiparasitic vegetal active ingredients. For this reason it was tempting to check these antiparasitic properties in other parasites.

Trichomonas vaginalis (TV) is the causative agent of trichomoniasis, a common cause of vaginitis. The prevalence of trichomoniasis in inner city US STD clinics typically approaches 25% [22], with a peak of 38% among African American attendees at a public clinic in LA [23], and an annual incidence of 7.4 million new cases in the country [24]. Interestingly, earlier clinical studies conducted during the eighties [25] were reporting cure rates up to 97%; current data are estimating the prevalence of in-vitro resistance to be around 5% [26]. For this reason, a novel effective, non-expansive active ingredient is welcomed in the treatment of trichomoniasis. This was our initial idea when testing QWE on the growth of TV in vitro, and we have found that QWE was effectively capable of dramatically inhibiting this growth at concentrations > 0.0375 (corresponding to 0.029% w/w of quassin) at 24h and at concentrations > 0.0046 (corresponding to 0.0035% w/w of quassin) at 48 hours.

The efficacy of 4% QWE topical preparations in rosacea was once reported [17]. Rosacea is a common dermatological condition that predominantly affects the central regions of the face. Rosacea affects up to 3% of the world's population and a number of subtypes are recognized. The density of *Demodex* mites in the skin of rosacea patients is higher than in controls, suggesting a possible role for these mites in the induction of this condition [27]. Our purpose was checking if the reported efficacy of QWE in rosacea could be due to an antiparasitic activity of the same on *Demodex* mites. The gap was the inexistence of methods permitting the culture in vitro of this parasite. Hence we opted for an ex vivo trial, harvesting *Demodex* from the skin of rosacea patients by biopsy with cyanoacrylate glue at the beginning and at different stages of a treatment with 4% topical QWE. The microscope counts revealed that along this treatment, there was a dramatic decrease in the number of units, and the number of mites at the end of treatment reached values observed in normal skin. Hence we have demonstrated the antiparasitic efficacy of QWE on *Demodex* spp, which is probably one of the reasons explaining the efficacy of QWE in rosacea.

Further, we focused on the antifungal and antibacterial activities of QWE. A previous study in vitro [28] reported the inhibition of the growth of *Escherichia coli*, *Streptococcus faecalis*, *Staphylococcus aureus* and *Aspergillus niger* with QWE. It was tempting to test QWE on other pathogenic agents. The first candidate was *Candida albicans*, a diploid fungus that grows both as yeast and filamentous cells and a causal agent of opportunistic oral and genital infections in humans [29] and candidal onychomycosis, an infection of the nail plate. Systemic fungal infections including those by *C. albicans* have also emerged as important causes of morbidity and mortality in immunocompromised patients (e.g., AIDS, cancer chemotherapy, organ or bone marrow transplantation). Our in vitro results revealed that QWE (10% corresponding to a concentration in quassin of 0.0764% w/w) displayed antifungal properties after 24 and 48 hours on all four strains of *Candida* tested, and a concentration of 5% (corresponding to a concentration in quassin of 0.0382% w/w) displayed antifungal activity after 24 and 48 hours on *C. albicans*, *C. parapsilosis* and *C. glabrata* but not on *C. krusei*.

Still among yeasts, we focused on *Malassezia furfur*. Formerly known as *Pityrosporum*, *Malassezia* is naturally found on the skin surfaces of many animals, including humans. In occasional opportunistic infections, some species can manifest hypopigmentation or hyperpigmentation on the trunk and other locations in humans in a disorder named *Tinea versicolor* or *Pityriasis versicolor*. *Malassezia* are also a causative agent for dandruff and seborrheic dermatitis (SD). One published double-blind study reported the efficacy of 4% QWE topical ointment in SD [18] with results significantly better than ketoconazole or cyclopiroxolamine, the standard treatments of this disease. Our findings are that QWE is showing a strong in vitro antifungal effect on *Malassezia furfur* at dilutions up to 1/80 of the prepared solution (corresponding to a concentration in quassin of 0.01% w/w), which could be the reason of its efficacy in the treatment of SD.

Regarding bacteria, we have tested QWE on *Propionibacterium acnes*, which is the relatively slow-growing, typically aerotolerant anaerobic, Gram-positive bacterium linked to the skin condition of acne. In previous unpublished work, we had found that topical applications of 4% QWE on acne

lesions resulted in a clear improvement of the same. Our in vitro results of bacterial counts on sub-cultures of *P.acnes* extracted from pustules of patients with rosacea showed that after a 42-day course with 4% QWE extract applied on the skin, the number of colony-forming units of *P.acnes* was significantly reduced by 58% (from 1.82 ± 0.20 to 0.76 ± 0.30 cfu – $p<0.05$) and hence that QWE possessed an in vitro inhibitory effect on the growth of this bacteria.

In this study we have also focused on *Staphylococcus* spp. One of the most important phenotypical features used in the classification of staphylococci is their ability to produce coagulase, an enzyme that causes blood clot formation. Six species are currently recognised as being coagulase-positive: *S. aureus*, *S. delphini*, *S. hyicus*, *S. intermedius*, *S. lutrae*, *S. pseudintermedius*, and *S. schleiferi* subsp. *coagulans*. Among coagulase-negative species, *S. epidermidis* is a commensal of the skin, but can cause severe infections in immunosuppressed patients and those with central venous catheters. *S. saprophyticus*, another coagulase-negative species that is part of the normal vaginal flora, is predominantly implicated in genitourinary tract infections in sexually active young women. In recent years, several other *Staphylococcus* species have been implicated in human infections, notably *S. lugdunensis*, *S. schleiferi*, and *S. caprae*.

Our in vitro testing showed that the number of colony-forming units of *Staphylococcus* coagulase positive reduced in a significant manner by 50.8% (from 2.4 ± 0.19 to 1.18 ± 0.36 cfu $p<0.05$) upon contact with QWE. These data are corroborating the antibacterial activity of QWE on *S. aureus* previously published²⁸. Under the same conditions, the number of colony-forming units of *Staphylococcus* coagulase-negative was also reduced by 27.8% (from 1.76 ± 0.18 to 1.27 ± 0.38) but this reduction was not statistically significant.

Finally, we tested QWE on *Chlamydia trachomatis* (CT). *Chlamydia* is a genus of pathogenic bacteria that are obligate intracellular parasites. *Chlamydia* infections are the most common bacterial sexually transmitted diseases in humans and are the leading cause of infectious blindness worldwide. As observed in our in vitro testing, QWE did not inhibit the cycle of CT, even when used at cytotoxic concentrations. Nevertheless, for concentrations ranging from 0.3% to 0.2% in QWE (i.e. respectively 0.0023% and 0.0015% in quassin) QWE was able to alter the morphology and quantity of chlamydial inclusions.

5.CONCLUSION

In this paper we described in vitro and ex vivo tests performed with QWE on various parasites, yeasts and bacteria. To the best of our knowledge, this is the first report of antiparasitic activity of QWE on both *Trichomonas vaginalis* and *Demodex* spp, antifungal activity on *Candida* spp and *Malassezia furfur* and antibacterial activity on *P.acnes*, coagulase-positive *Staphylococci* and at a lesser level coagulase-negative *Staphylococci*.

These promising results should motivate researchers for further studies, which could corroborate them and provide the industry with a novel wide-spectrum antiparasitic, antifungal and antibacterial ingredient.

6.REFERENCES

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