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Antimicrobial activity and cytotoxicity of *Chromolaena odorata* (L. f.) King and Robinson and *Uncaria perrottetii* (A. Rich) Merr. extracts

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Ethanol extracts of leaves of *Chromolaena odorata* and ethyl acetate extracts of stem bark of *Uncaria perrottetii* were examined for their antimicrobial and cytotoxic properties. To determine these activities, *C. odorata* and *U. perrottetii* were tested against bacteria and fungus through disc diffusion and minimum inhibitory concentration (MIC) assays; protozoa through growth curve determination, antiprotozoal and cytotoxicity assays using *in situ* cell death detection kit (Roche Diagnostics). *C. odorata* extracts revealed antibacterial activities, inhibiting the growth of *Bacillus subtilis*, *Staphylococcus aureus*, and *Salmonella typhimurium*. Antibacterial and antifungal assays for *U. perrottetii* extracts showed that it inhibited *B. subtilis*, *S. aureus*, and *Candida albicans*. Both plant extracts presented low MIC (0.1%). The antiprotozoal and cytotoxicity assays against *Trichomonas vaginalis* and *Blastocystis hominis* illustrated that both plant extracts can reduce the number of parasites. With the *in situ* cell death detection kit, the parasites exposed to the plant extract concentrations were observed to fluoresce in yellow-green and red simultaneously signifying apoptotic-like changes. Preliminary phytochemical screening revealed the chemical composition of *C. odorata* extracts containing flavonoids, saponins, tannins and steroids, while *U. perrottetii* possessing alkaloids, tannins and leucoanthocyanin. Thus, these plant extracts can possibly be used to produce alternative forms of antimicrobials.

Key words: Plant extract, antibacterial, antifungal, antiprotozoal, cytotoxic, *Chromolaena odorata, Uncaria perrottetii*.

INTRODUCTION

Medicinal plants have been used for centuries as remedies for human diseases because they contain chemical components of therapeutic value (Nostro et al., 2000). According to the World Health Organization (WHO) in 2008, more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. In the Philippines, the importance of the country's diverse medicinal plants lies not only in their chemotherapeutic value in traditional healthcare but also in their potential as sources of new chemical entities for drug discovery. Although the country boasts of its remarkable biodiversity and rich cultural traditions of plant use, scientific understand-

ing of medicinal plants remains largely unexplored and pharmacological investigation of the Philippine flora gained momentum only recently. Two of these plants were consi-dered in this study.

Chromolaena odorata (L. *f.*) King and Robinson (synonym: *Eupatorium odoratum* L.) (Asteraceae) is a perennial scandent or semi-woody shrub. In traditional medicine, a decoction of the leaf is used as a cough remedy and as an ingredient with lemon grass and guava leaves for the treatment of malaria. Other medicinal uses include anti-diarrheal, astringent, antispasmodic, antihypertensive, anti-inflammatory and diuretic (Iwu et al., 1999). A decoction of flowers is used as tonic, antipyretic and heart tonic (Bunyapraphatsara and Chokechaijaroenporn, 2000). A study was done using another species of *Chromolaena* and it was found to possess antiprotozoal activity (Taleb-

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Contini et al., 2004).

Uncaria perrottetii (A. Rich) Merr. (Rubiaceae) is a slow-growing, high-climbing, woody vine. It is a medicinal plant used over the centuries by the indigenous civilization of the Peruvian rainforest, as an alternative treatment for different diseases. Studies performed on species of *Uncaria* in the last decades proved its anti-inflammatory, antineoplastic, anticonceptive, immunostimulant and antioxidant properties (Ccahuana-Vasquez et al., 2006).

There is a continuous and urgent need to discover plants with antimicrobial activities. The wide acceptance of traditional medicine as an alternative form of healthcare and the alarming increase in the incidence of new and re-emerging infectious diseases bring about the necessity to investigate these medicinal plants. Moreover, since many plants are unexamined, therapeutic results have been mixed resulting to poisoning (Nostro et al., 2000). Another concern is the development of resistance to the antibiotics in current clinical use (Ertürk et al., 2006). Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased.

In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents (Nascimento et al., 2000). Moreover, protozoal diseases, such as trichomoniasis and blastocystosis, are leading causes of parasitic infections worldwide (Matsuo, 2007; Sawangjaroen and Sawangjaroen, 2005; Stenzel and Boreham, 1996). The recommended treatment for these pathogenic protozoa is metronidazole, although this drug can cause undesirable side effects (Ertürk et al., 2006; Nostro et al., 2000). Therefore, there is a need to develop safe and alternative antimicrobial agents such as the use of medicinal plants.

Thus, the aim of this study was to evaluate the potential antimicrobial activities, namely, antibacterial, antifungal, antiprotozoal, and cytotoxic properties of a common herbaceous species, *C. odorata* and a woody forest species, *U. perrottetii* against different groups of microorganisms. Moreover, the plant extracts were subjected to preliminary phytochemical screening to analyze the possible antimicrobial compounds they contain. The study provides scientific evidence on the use of these plants which are being utilized traditionally as herbal medicines.

MATERIALS AND METHODS

Collection and identification of plant material

Leaves of *C. odorata* were collected from the University of the Philippines Diliman, Quezon City, Philippines. *C. odorata* was identified by the Dr. Jose Vera Santos Memorial Herbarium (Philippine University Herbarium) of the Institute of Biology, University of the Philippines. Bark of stems of *U. perrottetii* was collected from Lamau, Bataan, Philippines. *U. perrottetii* was identified by the Botany Division of the National Museum in Manila, Philippines. Voucher specimens of the herbs were prepared and deposited at the Institute of Biology and National Museum.

Preparation of extracts

Plant samples were air dried and ground to a coarse powder using a dry mill. For the extraction of *C. odorata*, 500 g of powdered leaf was soaked in 95% ethanol (1:5) for 72 h. On the other hand, a decoction method was used for the extraction of the stem bark (20 g/L) of *U. perrottetii* to yield a brown aqueous extract. Then, ethyl acetate was added to this extract. Following agitation and settling, the organic layer was separated from the aqueous layer. The solvents were removed under vacuum using a rotary evaporator. The yield (with respect to dry powdered material) of *C. odorata* extract was 2.0% and *U. perrottettii* aqueous and organic extracts was 5.5%.

Microorganisms and culture media

Microorganisms were obtained from the culture collection of the Natural Sciences Research Institute at the University of the Philippines. Organisms were as follows: bacteria: *Escherichia coli* UPCC 1195, *Salmonella typhimurium* UPCC 1368, *Pseudomonas aeruginosa* UPCC 1244, *Staphylococcus aureus* UPCC 1143, *Bacillus subtilis* UPCC 1295; fungus: *Candida albicans* UPCC 2168. Bacterial cultures were maintained on nutrient agar (NA). *C. albicans* was maintained on Sabouraud dextrose agar (SDA). Protozoans used in the study were *Trichomonas vaginalis* DSHC 2021 and *Blastocystis hominis* SVM ATCC 50613. *T. vaginalis* was grown in BI-S-33 medium (Diamond et al., 1978). *B. hominis* was grown in *Blastocystis* egg medium (ATCC medium 1671).

Antibacterial activity of the plant extracts

Disc diffusion assay on agar plates were used to determine the antibacterial and antifungal activities of *C. odorata* and *U. perrottetii* extracts. Bacteria were inoculated into nutrient broth (NB), while fungi were inoculated into Sabouraud dextrose broth (SDB) at 37°C for 6 h. The turbidity of the resulting suspensions was diluted with NB and SDB to obtain a transmittance of 74.3% (absorbance of 0.132) at 600 nm (Rojas et al., 2006). Then, these bacterial cultures were inoculated on the surface of Mueller-Hinton agar (MHA) plates for bacteria and SDA for fungi. Subsequently, filter paper discs (6 mm in diameter) saturated with extracts (25 µL) dissolved in water were placed on the surface of each inoculated plate. Antibiotics were used as positive control (ampicillin and gentamicin for bacteria, while nystatin and amphotericin B for fungi), while solvents (95% ethanol for *C. odorata* and ethyl acetate for *U. perrottetii*) of the plant extracts as negative control. The tests were carried out in triplicates. The plates were incubated at 37°C for 24 h. At the end of incubation, zones of inhibition were measured.

The plant extracts that showed antimicrobial activity were tested to determine the minimum inhibitory concentration (MIC) for each bacterial and fungal sample (Nascimento et al., 2000). MIC of each sample was determined by dilution in liquid culture medium then measuring the optical density after 24 h of incubation in the Beckman DU®-65 spectrophotometer (600 nm). MIC is defined as the lowest concentration of the crude plant extract that does not permit any visible growth. Assays were performed in triplicates.

Growth curve analysis

A growth curve was constructed for *T. vaginalis* and *B. hominis*. *T. vaginalis* trophozoites (1.5 x 10^5/mL) were screened in tubes with BI-S-33 medium and incubated at 37°C for 170 h (Diamond et al., 1978). On the other hand, *B. hominis* were examined with egg medium and incubated at 37°C for 240 h. Every 24 h, the trophozoites were detached and counted to obtain the curve of growth for each time.

Table 1. Antimicrobial activity of *C. odorata*, *U. perrottetii* (organic and aqueous layers) extracts, positive and negative control determined by disc diffusion assay.

Test extracts and control	Zone of inhibition (mm)					
	E. coli	*P. aeruginosa*	*B. subtilis*	*S. aureus*	*S. typhimurium*	*C. albicans*
C. odorata	-	-	8.33 ± 0.58	10.00 ± 0.00	9.00 ± 0.00	
U. perrottetii (organic)	-	-	-	-	-	
U. perrottetii (aqueous)	-	-	11.00 ± 0.00	8.00 ± 0.00	-	9.33 ± 1.15
Ampicillin (10 µg)	-	-	-	40.00 ± 0.00	27.67 ± 4.04	nd
Gentamicin (10 µg)	18.00 ± .00	18.00 ± 0.00	20.67 ± 0.58	27.33 ± 2.89	20.00 ± 0.00	nd
Nystatin	nd	nd	nd	nd	nd	19.00 ± 1.00
Amphotericin B	nd	nd	nd	nd	nd	9.67 ± 1.15
Ethyl acetate	-	-	-	-	-	
Ethanol	-	-	-	-	-	

(-): No activity; nd: not determined; values: mean of 3 replicates and expressed as mean ± SD.

Antiprotozoal assay

In this assay, 1.5 x 10^5 parasites/mL were grown in the same culture media and simultaneously exposed to different concentrations (0.1, 0.5, 1.0% concentrations) of the plant extracts to study the viability of the parasites exposed to the plant extracts for three periods. Only three periods of time were considered for the experiment (24, 48 and 72 h for *T. vaginalis*, while 96, 120, and 144 h for *B. hominis*) of the parasite exposure to the plant extracts based on the growth curve constructed. Afterwards, the parasites were detached and counted in a Neubauer counting chamber and the counts were compared with those of the positive (metronidazole) and negative (95% ethanol for *C. odorata* and ethyl acetate for *U. perrottetii*) control (Fournet et al., 1994). Percent reduction was computed for all concentrations of the plant extracts (Perez-Arriaga et al., 2006). Each assay was performed in triplicate.

Detection of apoptosis (Cytotoxicity Assay)

T. vaginalis trophozoites and *B. hominis* were observed to determine the presence of apoptosis by a Tunel method. To observe apoptotic-like changes, the *In Situ* Cell Death Detection Kit, Fluorescein (Roche Diagnostics) was used. This method allows the recognition of apoptotic nuclei in *T. vaginalis* and *B. hominis* preparations by labeling the free 3'-OH termini with modified nucleotides in an enzymatic reaction (fragment end labeling). Fluorescein labels incorporated in the nucleotide polymers were detected by fluorescence microscopy. Viable cells were stained in yellow-green by a fluorescein derivative. The apoptotic cells exhibited reddish and yellow-green fluorescence and necrotic cells were stained only in red.

Data and statistical analysis

All values obtained were expressed as means ± standard deviation. The MIC data for each microorganism were analyzed using one-way analysis of variance (ANOVA). P value of < 0.05 was considered as significant.

Phytochemical screening

The plant extracts were submitted to the Chemical and Mineral Division of the Department of Science and Technology (DOST) for chemical analysis to identify and characterize some of their composition. The tests done followed the procedure in the Laboratory Manual for the UNESCO Sponsored Workshop on the Phytochemical, Microbiological, and Pharmacological Screening of Medicinal Plants (1986).

Ethics

Prior consent was obtained and authorized by the corresponding agencies of the government. The field work and data collection were conducted in accordance with the institutional, national, and international principles and guidelines of plant use and conservation of biodiversity.

RESULTS AND DISCUSSION

Antibacterial activity of the plant extracts

The ethanolic extract of *C. odorata* leaves obtained by rotary evaporation showed antimicrobial activity against *B. subtilis*, *S. aureus*, and *S. typhimurium* (Table 1). The solvents used as control exerted no effect against the microorganisms which suggest the effectiveness of the plant extract.

Ethyl acetate extractions of *U. perrottetii* stem bark yielded two components. Table 1 shows the antibacterial activity of the aqueous and organic extracts of *U. perrottetii*. From the two extracts, only the aqueous portion revealed variable degrees of antibacterial activity. The aqueous extract exhibited inhibitions on *B. subtilis* and *S. aureus*, whereas the organic extract showed no activity. The solvents used as control exerted no effect against the microorganisms that proves the efficiency of the plant extract.

The inhibitory bacterial growth by this plant extract indicated by the MIC values obtained by broth dilution method is summarized in Table 2. Analysis of the MIC data revealed that values for *C. odorata* extracts against *B. subtilis* (P = 0.024) and *S. typhimurium* (P = 0.017) and for *U. perrottetii* extracts against *B. subtilis* (P = 0.017) were significantly different. This means that concentration or the amount of extract added to the medium will greatly affect the inhibition of microorganisms. On the other hand, leaf extracts of *C. odorata* against *S. aureus* and stem bark extracts of *U. perrottetii* against *S. aureus* were

Table 2. Minimum inhibitory concentration (MIC) of *C. odorata* and *U. perrottetii* plant extracts against susceptible microorganisms expressed in %v/v.

Source of plant extracts	Test organism			
	B. subtilis	*S. aureus*	*S. typhimurium*	*C. albicans*
C. odorata	0.5[a]	0.1	0.3[a]	nd
U. perrottetii	0.3[a]	0.1	nd	0.1[a]

[a] MIC value is significant at $P < 0.05$; nd – not determined (no zone of inhibition in the disc diffusion assay).

not significantly different. This implies that at any concentration, the plant extracts are effective. Thus, it is recommended to use lower concentration of the plant extracts. It is also noteworthy to say that *U. perrottetii* could possibly be a strong antimicrobial since it can inhibit the growth of microorganisms in small amounts.

Gram positive bacteria, such as *B. subtilis* and *S. aureus* were found to be more susceptible to all plant extracts than Gram negative bacteria. The reason for the difference in sensitivity between Gram positive and Gram negative bacteria could be ascribed to the morphological differences between these microorganisms. This could be due to the fact that the cell wall of Gram positive bacteria is less complex and lacks the natural sieve effect against large molecules due to the small pores in their cell envelope (El Astal et al., 2005). Gram-negative bacteria have an outer phospholipid membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to lipophilic solutes, while porins constitute a selective barrier to the hydrophilic solutes with an exclusion limit of about 600 Da (Nikaido and Vaara, 1985).

Gram positive bacteria should be more susceptible having only an outer peptidoglycan layer which is not an effective permeability barrier (Scherrer and Gerhardt, 1971). *E. coli* and *P. aeruginosa* showed no response to the extracts. Aside from cell membrane permeability, their observed resistance could also possibly be due to genetic factors (Ertürk et al., 2006). In spite of this permeability difference, *C. odorata* inhibited *S. typhimurium*. It could also be noted that the plant extracts can be more effective than ampicillin since the extracts inhibited microorganisms, such as *B. subtilis* and *S. typhimurium*, which were not inhibited by the antibiotic.

The possible antimicrobial mode of action of *C. odorata* is probably because of its ability to bind to the cell wall, thereby inhibiting its synthesis due to the presence of flavonoids and tannins found in the phytochemical screening. Moreover, *U. perrottetii* could probably target cell wall and cell membrane as it is found to contain alkaloids and tannins in the preliminary chemical analysis of the extracts. The use of ampicillin is no longer recommended due to the potency of widespread resistance to it (Ertürk et al., 2006).

Antifungal activity of the plant extracts

The antifungal activity of the plant extracts are shown in Table 1. The aqueous extract of *U. perrottetii* revealed pro-

mising antimicrobial activity against *C. albicans*. *C. albicans* is a diploid fungus and a causal agent of opportunistic oral and genital infections in humans. It can also be seen from the table that Amphotericin B has almost the same effectiveness with that of *U. perrottetii* aqueous extract. The solvents, on the other hand, used as negative control exerted no effect against the microorganisms in the broth medium. This implies the effectiveness of the plant extracts. The use of this plant may offer a new source of antifungal agent against the pathogenic *C. albicans* since this fungus is not easily inhibited by other drugs.

MIC value of *U. perrottetii* is found in Table 2. Unlike *C. odorata*, addition of *U. perrottetii* extracts even at low concentration (0.1%) kills the fungus ($P = 0.000$).

The activity of the ethyl acetate extracts of *U. perrottetii* was more pronounced against the fungal organism than against Gram negative bacteria (Table 1). The antifungal compounds of the plants assayed are not well known; however, the presence of alkaloids, flavonoids and a certain degree of lipophilicity might determine toxicity by the interactions with the membrane constituents and their arrangement (Tomas-Barberan et al., 1990). These components have been found present in the phytochemical screening of the plant extracts.

Growth curve analysis

Growth curve was constructed for *T. vaginalis* to determine the time for the addition of plant extracts in the antiprotozoal and cytotoxicity assays. The exponential growth of the parasite occurred well until 96 h. Since the maximum amount of microorganisms was achieved after 72 h of incubation (1.492×10^6 cells/mL), then, the optimal times for analysis are 24, 48 and 72 h after inoculation and initial exposure to the plant extracts. The parasites survived until 7th day, but drastically decreased in number. After 192 h, all the microorganisms were non-motile and presumed dead.

On the other hand, a growth curve of *B. hominis* was also constructed. The exponential growth of the parasite occurred well until 144 h. Since the maximum amount of microorganisms was achieved after 144 h of incubation (2.48×10^7 cells/mL), then, the optimal times for analysis are 96, 120 and 144 h after inoculation and initial exposure to the plant extracts. The parasites survived until 10th day, but drastically decreased in number. After 240 h all the parasites were granular in appearance and presumed dead.
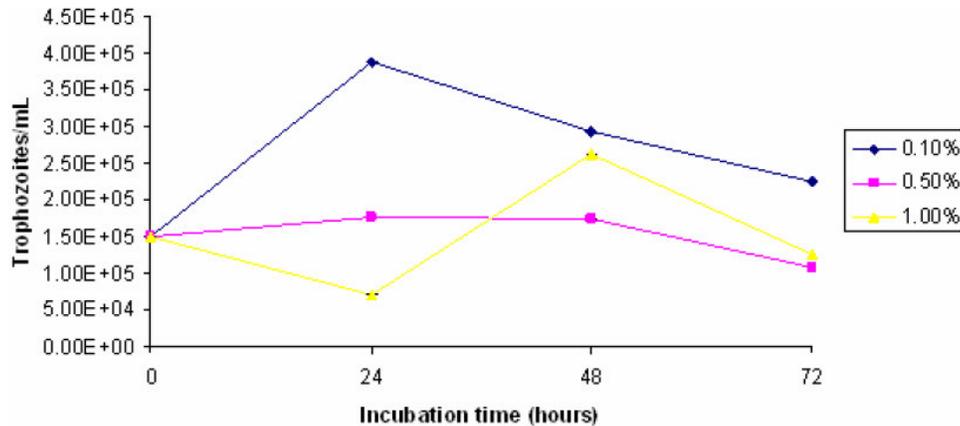
Figure 1. Effect of *Chromolaena odorata* plant extract on *T. vaginalis* trophozoites growth *in vitro*. Trophozoites were grown in BI-S-33 medium (Diamond et al. 1978) containing plant extracts and incubated at 37°C for 72 h. Every 24 h the trophozoites were counted in a Neubauer chamber. Each point represents the mean ± SD of triplicate determinations.
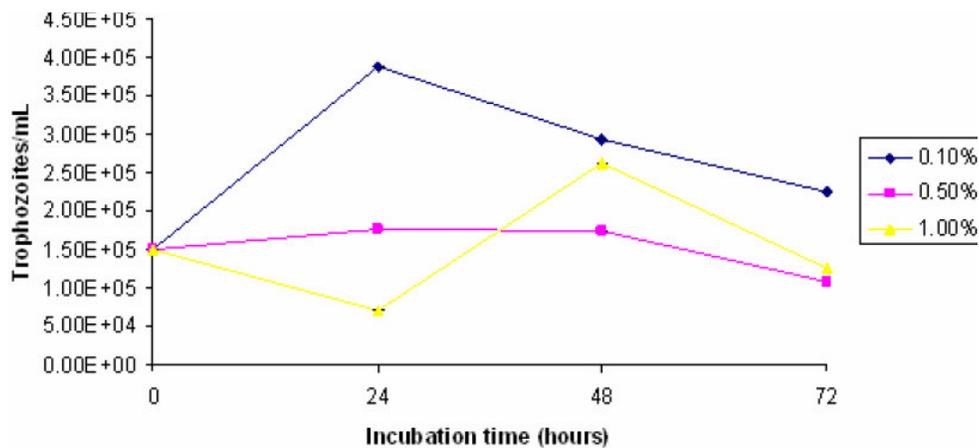
Figure 2. Effect of *U. perrottetii* plant extract on *T. vaginalis* trophozoites growth *in vitro*. Trophozoites were grown in BI-S-33 medium (Diamond et al., 1978) containing plant extracts and incubated at 37°C for 72 h. Every 24 h the trophozoites were counted in a Neubauer chamber. Each point represents the mean ± SD of triplicate determinations.

Antiprotozoal assay

Figure 1 shows the effect of varying concentrations of *C. odorata* leaf extracts against *T. vaginalis*. Exposure to this plant extract in all concentrations, 0.1, 0.5 and 1.0% resulted in reductions of the trophozoites as compared to the control (without plant extract). *T. vaginalis* growth at 0.1% plant extract concentration had 49.5% reduction at 24 h, 65.8% at 48 h and 84.9% at 72 h. The extract concentration of 0.5% produced a decrease of 77.1% at 24 h, 79.8% at 48 h and 92.8% at 72 h. A 1.0% concentration of the plant extract produced a growth inhibition of *T. vaginalis* at 24 h 90.9%, at 48 h 69.5% and at 72 h 91.6%. It could also be noted that parasites are present in greater amount in the lower concentration of the extract than at the higher concentration. Furthermore, at 1.0% concentration, more parasites were stationary and presumed dead.

T. vaginalis trophozoites significantly decreased at all *U. perrottetii* extract concentrations (Figure 2). *T. vaginalis* growth at 0.1% plant extract concentration had 39.3% reduction at 24 h, 65.1% at 48 h and 94.0% at 72 h as compared with the growth of *T. vaginalis* without plant extract. The extract concentration of 0.5% produced a decrease of 63.5% at 24 h, 87.2% at 48 h and 89.4% at 72 h. A 1.0% concentration of the plant extract produced a growth inhibition of *T. vaginalis* at 24 h 70.3%, at 48 h 89.1%, and at 72 h 99.8%. Cell count of parasites in the lower concentration of the extract was greater than that of higher concentration. At 1%, there were generally a reduced number of cells signifying the probable effectiveness of the extract. Moreover, at this concentration, most of the parasites were either granular concentration, most
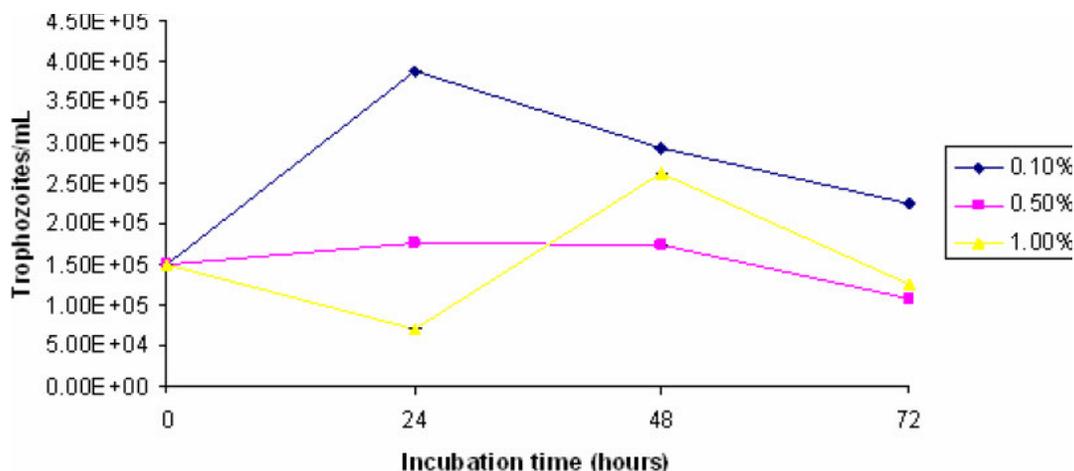
Figure 3. Effect of *Chromolaena odorata* plant extract on *B. hominis* growth *in vitro*. Parasites were grown in egg medium containing plant extracts and incubated at 37°C for 144 h. Every 24 h the trophozoites were counted in a Neubauer chamber. Each point represents the mean ± SD of triplicate determinations.
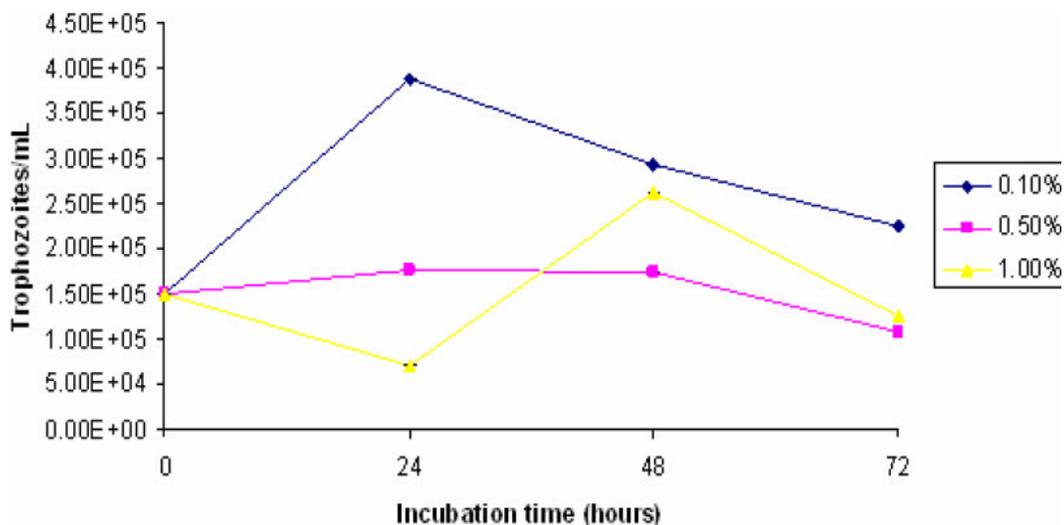
Figure 4. Effect of *U. perrottetii* plant extract on *B. hominis* growth *in vitro*. Parasites were grown in egg medium containing plant extracts and incubated at 37°C for 144 h. Every 24 h the trophozoites were counted in a Neubauer chamber. Each point represents the mean ± SD of triplicate determinations.

of the parasites were either granular in appearance, lost their motility, or shriveled.

Figure 3 shows the effect of *C. odorata* leaf extracts against *B. hominis*. Exposure to this plant extract at 0.5 and 1.0% concentrations resulted in reductions of the parasites as compared to the control (without plant extract). The extract concentration of 0.5% produced a decrease of 90.1% at 96 h, 95.2% at 120 h, and 98.8% at 144 h. A 1.0% concentration of the plant extract produced a growth inhibition of *B. hominis* at 96 h 93.6%, at 120 h 92.0%, and at 144 h 97.2%. It could also be noted that parasites are present in greater amount in the lower concentration of the extract than at the higher concentration.

Moreover, the parasites appeared granular at higher concentration of the extract.

B. hominis significantly decreased at 0.5% and 1.0% *U. perrottetii* extract concentrations (Figure 4). *B. hominis* growth at 0.5% plant extract concentration had 90.0% reduction at 96 h, 94.2% at 120 h and 98.4% at 144 h as compared with the growth of *B. hominis* without plant extract. The extract concentration of 1.0% produced a decrease of 91.6% at 96 h, 94.2% at 120 h and 98.8% at144 h. Cell count of parasites in the lower concentration of the extracts was greater than that of higher concentration. At 1%, there were generally a reduced number of cells signifying the probable effectiveness of the extract.
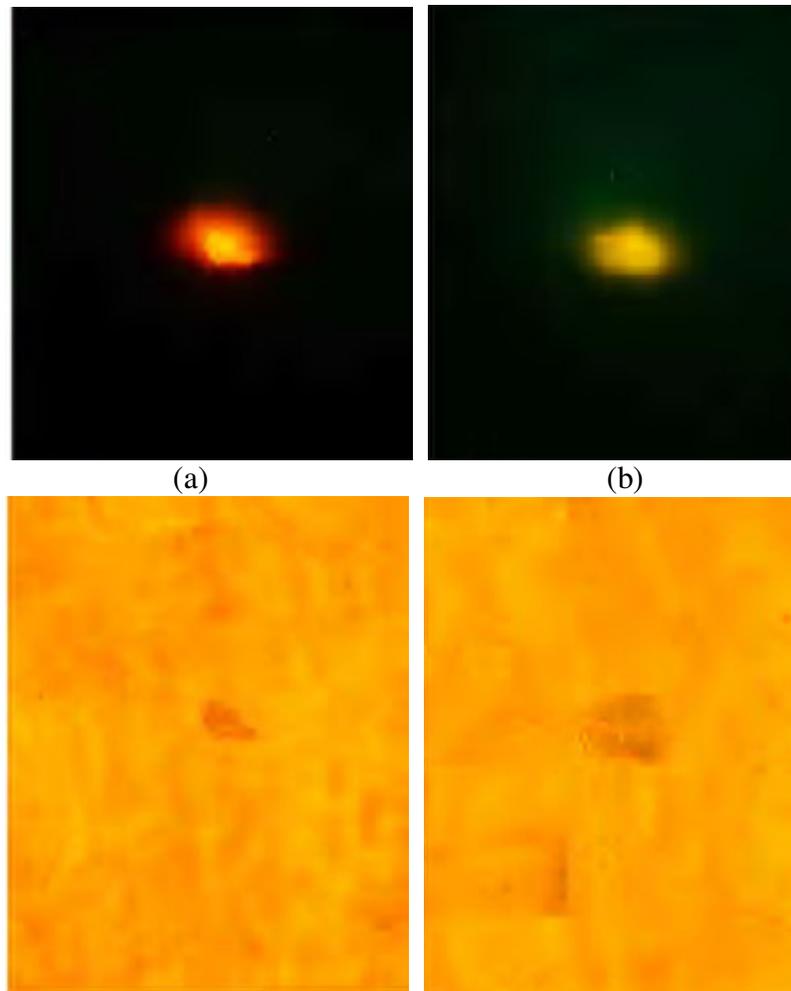
Figure 5. Apoptosis detected by Tunel method exposed to plant extracts: 0.1% concentration of *U. perrottetii* against *T. vaginalis* (a); 1.0% concentration of *U. perrottetii* against *B. hominis* (b) showing yellow green and red fluorescence. Photographs on the left panel are under fluorescence microscope (400x), while photographs on the right are under light microscope (400x).

Moreover, at this concentration, most of the parasites were either granular in appearance or shriveled.

There was no reduction in cell numbers observed in the control samples. The negative control used was ethanol and ethyl acetate. On the other hand, for the positive control, metronidazole lysed and killed the cells after 24 h of incubation. Metronidazole is a drug of choice recommended for the treatment of human trichomoniasis. However, potential carcinogenic, teratogenic, embryogenic effects of this drug and clinical and laboratory-based drug-resistant protozoan isolates have been reported (Calzada et al., 2007).

The results of counting and microscopic analysis suggest that the plant extracts may exert profound effects in parasite growth and morphology. The mode of action of the plant extract is probably via lysis of the cell. However, further methods are required to define the exact mechanism and to ensure death of the cells. Thus, the cytotoxicity assay

Detection of apoptosis (Cytotoxicity Assay)

Apoptosis or programmed cell death is the most common form of eukaryotic cell death. With the kit that was used, necrotic cells fluoresce in red color, living cells fluoresce in yellow green and apoptotic cells fluoresce in yellow green and red simultaneously (Perez-Arriaga et al., 2006). *C. odorata* extract induced apoptotic-like changes to *T. vaginalis* trophozoites at 0.1% and 1.0% concentrations after 72 h exposure. Moreover, yellow-green and red signal were also observed in 0.1% and 1.0% concentrations of *U. perrottetii* extract (Figure 5). Cells in all cases showed a clear loss of normal morphology.

C. odorata extract induced apoptotic-like changes to *B. hominis* 1.0% concentration after 120 h of exposure. Moreover, yellow-green and red signal were also observed in 0.5% and 1% concentrations of *U. perrottetii* extract (Figure 5). Cells in all cases showed a clear loss of nor-

mal morphology.

T. vaginalis and *B. hominis* exposed to metronidazole fluoresced in red showing cytoplasmic debris dispersed around the cells. On the other hand, the negative control (with ethanol and ethyl acetate) and the control without any extract or solvent gave a green color. This signified the presence of viable cells.

Apoptosis induced by anti-parasitic drugs has been barely studied in protozoan parasites (Perez-Arriaga et al., 2006). In the method that was used, the effects are attributable to the plant extracts since the kit preferentially and specifically labels DNA strand breaks generated during apoptosis. It allows the discrimination of apoptosis from necrosis and from primary DNA strand breaks induced by cytostatic drugs or irradiation. These effects might be consequences of the activation of apoptotic mechanisms that may be exclusive for microorganisms lacking mitochondria (Perez-Arriaga et al., 2006).

Phytochemical screening

Chemical tests showed the presence of flavonoids, saponins, tannins, and steroids in *C. odorata* leaf extracts. On the other hand, chemical analysis of the aqueous extracts of *U. perrottetii* stem bark revealed the presence of alkaloids, tannins, and leucoanthocyanin.

Thus, extracts from *C. odorata* can possibly be used as antibacterial and antiprotozoal sources; while those from *U. perrottetii* can be antibacterial and antifungal sources. It is of high probability that this potential can be developed into antimicrobial drugs if specific compounds can be isolated and purified.

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