

Evaluation of the *in vitro* Antiprotozoal Activity of Various Dry Plant Extracts against *Dientamoeba fragilis*

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Plants represent an important source of diverse biomolecules with unique properties, some of which make them attractive candidates for the development of novel antimicrobials. Plant extracts have been evaluated *in vitro* for their activity against human-infecting gastrointestinal protozoa, such as *Giardia intestinalis* [1] and *Entamoeba histolytica* [2], with some success. Some plant extracts are also active against the sexually transmitted parasite *Trichomonas vaginalis* [2].

For the treatment of infections with microaerophilic protozoa, such as *Dientamoeba fragilis* and *G. intestinalis*, metronidazole (Flagyl[®]) has been the drug of choice for decades. However, it is well established that the widespread use of monotherapeutic treatment regimens encourages drug resistance, leading to treatment failures and relapses of illness. Recently, resistance to Flagyl[®] has been observed in *T. vaginalis* [3] and *G. intestinalis* [4], and has been suggested in the bowel parasite; *D. fragilis*, due to the increasing frequency of treatment failures [5]. With the emergence of drug resistance, there is increasing pressure to identify new drugs. In response to reports of possible Flagyl[®] resistance in *D. fragilis* [5], a simple study was designed to determine whether non-prescription dry plant extracts commercially available from local health stores exhibit direct *anti-Dientamoeba* activity.

Dry plant extracts were selected based on reports of their antibacterial, antiprotozoal, and/or anthelmintic activity. The agents were also selected, as they are prescribed by many alternate health practitioners as effective treatment for dientamoebiasis. The products evaluated included pomegranate (*Punica granatum*) peel extract, Garlic (*Allium sativa*) extract (crushed tablets), Goldenseal (*Hydrastis canadensis*) extract (crushed tablets), Black Walnut (*Juglans nigra*) extract (crushed tablets), Wormwood (products from two species; *Artemisia annua* and *Artemisia absinthium*) each in crushed tablet form, Wormseed (*Chenopodium ambrosioides*) extract (crushed tablets), Pumpkin seeds (*Cucurbita* sp.) ground to fine powder, Grapefruit seed extract (powdered) and Ginger root extract (powdered).

Four clinical isolates of *D. fragilis* were used in this study. *Dientamoeba* trophozoites were cultured in a biphasic Loeffler's medium overlaid with PBS and supplemented with 2 mg of rice starch [6]. The plant extracts were dissolved or suspended in the PBS overlay, at a concentration of 500 µg/ml, resulting in 10 different media formulations (one for each of the plant extracts). Media vials were inoculated with trophozoites of *D. fragilis* and incubated at 37°C under microaerophilic conditions. Cultures were examined microscopically on a daily basis for several days, for the presence or absence of motile *D. fragilis* trophozoites. Each experiment was accompanied by a negative control consisting of trophozoites inoculated into the standard Loeffler's slope medium. This experiment was repeated for each of the four *D. fragilis* isolates. Upon completion of this experiment, it was found that even at 500 µg/ml, none of these plant extracts had an inhibitory effect on *D. fragilis* growth. Motile trophozoites were still present after several days (as per negative controls).

While plant derived biomolecules show promise for the development of novel antiprotozoals, it must be remembered that the beneficial properties ascribed to plants are often attributable to

a few select molecules. Usually, these molecules must be properly extracted and sufficiently concentrated to see any beneficial effects when administered. In the case of dry extracts, it can be difficult (or impossible) to administer a sufficient quantity to see any benefits at all. The plant extracts used in this study have no activity against cultured *D. fragilis* trophozoites at a concentration of 500 µg/ml, which exceeds that which could be achieved in the human body. However, based on the results of this study, it cannot be concluded that the plants evaluated do not possess *anti-Dientamoeba* activity. Rather, it is suggested that these dry plant extracts are ineffective. This finding is important for those considering natural, non-prescription therapies for treating gastrointestinal ailments in the place of conventional medicines.

Given that *T. vaginalis* and *D. fragilis* are both trichomonads, and that berberine salts from Goldenseal [7] and Pomegranate juice [8] are active against *T. vaginalis*, it was hypothesised that dry extracts from these plants would also possess *anti-Dientamoeba* activity. Similarly, *Histomonas meleagridis* is the closest known relative of *D. fragilis* [9], and essential oils extracted from garlic leaves possess *anti-Histomonas* activity [10], while powdered garlic extracts did not eradicate *D. fragilis*. The inability of the products used in this study to eradicate *D. fragilis* may be due to differences between the extracts used. For example, in this study dry plant extracts were used, while essential oil extracts [10], and purified berberine sulphate derived from Goldenseal [7], were used in studies involving other trichomonads.

To conclude, we demonstrate that various dry plant extracts available from local health stores are ineffective at eradicating cultured *D. fragilis*, for the first time. This does not confirm that these plants do not possess molecules capable of eradicating *D. fragilis*. Rather, it is concluded that dry extracts from commercial health product suppliers are ineffective. It is suggested that different types of extracts from the plants used in this study (such as lipid or organic solvent extracts) be evaluated for *anti-Dientamoeba* activity in future studies.

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