Anti-intestinal protozoan activities of 1-hydroxy-2-hydroxymethanlanthraquinone from Coptosapelta flavescens

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ABSTRACT

Objective: To investigate the antiprotozoal activity of medicinal plant extracts and isolated active compounds from the most active plant.

Methods: Twenty one medicinal plants with ethnobotanical use in Thailand, which were claimed to have anti-diarrhoeal or anti-parasitic activity, were screened for their anti-intestinal protozoan activity against Entamoeba histolytica (E. histolytica) and Giardia intestinalis (G. intestinalis). The most active compound was isolated and tested against E. histolytica and G. intestinalis.

Results: An acetone extract of Coptosapelta flavescens was the most active against both E. histolytica and G. intestinalis (minimal inhibitory concentration=125 and 15.63 μg/mL, respectively). Two anthraquinones and one naphthoquinone were isolated. The compound 1-hydroxy-2-hydroxymethanlanthraquinone was the most active chemical against E. histolytica and G. intestinalis with minimal inhibitory concentration values of 20 and 2.5 μg/mL, respectively. In time killing assay, the percentage of viable G. intestinalis, when compare to control, after exposure to compound 1-hydroxy-2-hydroxymethanlanthraquinone showed significantly (P<0.05) lower than when exposed to a standard drug, metronidazole, at 6 and 12 h of incubation time. While for E. histolytica, its activity was comparable to metronidazole.

Conclusions: These observations provide preliminary evidence that 1-hydroxy-2-hydroxymethanlanthraquinone from Coptosapelta flavescens can be considered to be a potential anti-parasitic agent against E. histolytica and G. intestinalis infections.

KEYWORDS

Antiprotozoal activity, Coptosapelta flavescens, Diarrhea, Entamoeba histolytica, Giardia intestinalis, Medicinal plant

1. Introduction

Intestinal infections caused by Entamoeba histolytica (E. histolytica) and Giardia intestinalis (G. intestinalis) are still major public health problems, particularly for children in most developing as well as in some of the more developed countries[1-3]. The major clinical symptoms caused by E. histolytica are colitis, dysentery and amoebic liver abscess whereas G. intestinalis may cause diarrhoea, malabsorption syndrome, cramp and weight loss[4,5]. It has been estimated that 50 million people around the world were infected by E. histolytica and resulted in 40–110 thousand deaths annually[6].

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Giardiasis is the most common intestinal protozoa to oCCur in humans and has been estimated at 280 million cases per year[7]. Metronidazole is the most commonly used drug to treat intestinal infections caused by E. histolytica or G. intestinalis[8]. However, unpleasant effects such as a metallic taste, headache, nausea, urticaria, pruritus and dark colored urine have been reported[9,10]. Furthermore, metronidazole is a mutagen when test in breast cancer cell lines and drug resistance varieties of the parasite have been detected[11,12]. The continuous search for new anti–protozoan compounds with high activity that are safe and produce little or no side effects is still a necessary and ongoing goal in medicine.

Medicinal plants are popular among people in developing countries because they are widely available at low cost and their products are mostly safe. Moreover, medicinal plants have a rich history of use for the treatment of diarrhoea and dysentery[13]. Several compounds from herbs have been proved to be effective against various organisms, i.e. curcumin from Curcuma longa (C. longa) is reported to have anti–bacterial, anti–oxidant, anti–HIV and anti–protozoan activities[14–16]; essential oils from Thymbra capitata, Origanum virens, Thymus zygis subsp. sylvestris, Lippia graveolens and Zygystium aromaticum are active against Giardia as well as providing some anti–bacterial activity[17–19]. We therefore plan to evaluate the in vitro activity of natural products obtained from selected Thai medicinal plants claimed to have ethnobotanical use as anti–diarrhoeal or anti–parasitic agents in order to detect alternative agents that are suitable for use in preventing and treating E. histolytica and G. intestinalis infections.

2. Materials and methods

2.1. Plant materials

Twenty one medicinal plants claimed to act as agents that cured diarrhoea or parasitic infection were evaluated. The plants were dried and then extracted with acetone or ethanol. The solvent was evaporated under reduced pressure. Each dried extract was then dissolved in dimethyl sulphoxide (DMSO) at a concentration of 100 mg/ml. The maximum concentration of DMSO in the test did not exceed 1%, and this concentration had no effect on the growth of E. histolytica or G. intestinalis.

2.2. Isolation of pure compounds from Coptosapelita flavescens

The most active plant extract against both E. histolytica and G. intestinalis in vitro was from Coptosapelita flavescens (C. flavescens) and this was further investigated. The dried and chopped whole plant (3.5 kg) was extracted three times with acetone (3 L) each for 5 d at room temperature. The combined acetone extract was evaporated to dryness under reduced pressure to afford a dark brown gum (23.7 g). The crude acetone extract was fractionated by column chromatography (CC) over silica gel with a gradient system of methanol (MeOH)–dichloromethane (CH2Cl2) to afford four fractions (A–D). Fraction A (0.8 g) was further separated by CC over silica gel to give six fractions (A1–A6). Fraction A2 (75.4 mg) upon CC over silica gel using gradient systems of CH2Cl2–Hexane and MeOH–CH2Cl2 afforded five subfractions (A2.1–A2.5). Subfraction A2.3 (12.7 mg) was subjected to CC over silica gel using a gradient systems of CH2Cl2–Hexane and MeOH–CH2Cl2 to yield compound 2 (3.5 mg).

Fraction A4 (92.3 mg) was purified using the same procedure as the crude acetone extract to give compound 3 (3.8 mg). Fraction B (2.2 g) was further separated using the same procedure as for fraction A to give six fractions (B1–B6). Fraction B2 (300.3 mg) was purified by CC over silica gel using a gradient elution system of CH2Cl2–Hexane and MeOH–CH2Cl2 as eluents to obtain compounds 2 (35.6 mg) and 3 (4.6 mg). Subfraction B3 (331.2 mg) was separated by CC over silica gel with the same procedure as for fraction B2 to afford compound 1 (16.8 mg). Their structures were assigned by spectroscopic methods and comparison of the nuclear magnetic resonance data with those reported in literatures.

2.3. Parasite cultures

E. histolytica strain HM1-IMSS purchased from the American Type Culture Collection and a Thai strain of G. intestinalis, originally as described previously were used in all experiments[20]. They were cultured axenically in screw capped tubes on Y1 medium supplemented with 10% heat inactivated bovine serum under anaerobic conditions at 37 °C[21]. Subculture was performed every 48 h. For the assays, cells were harvested by chilling the tube on ice for 20 min to detach the monolayer and then centrifuged at 3 000 r/min for 5 min. The supernatant was decanted, and cells were resuspended in fresh medium. The numbers of viable cells were calculated using a haemocytometer and 0.4% (w/v) trypan blue. The criteria for viability were motility and dye exclusion.

2.4. Screening of plant extracts and pure compounds for anti–protozoan activity

The anti–protozoan activity was performed according to standard methods as described elsewhere[22,23]. Briefly, E. histolytica and G. intestinalis trophozoites (2×105 cells/ml) were incubated in 96–well tissue culture plates (200 μl/well) in the presence of serial dilutions of each compound that ranged from 7.81 to 1 000.00 μg/ml. The percentage of viable trophozoites was calculated in a hemocytometer using a trypan blue exclusion assay. The percentage of viable trophozoite was calculated
against control well at the same time interval. IC₅₀ value was determined by probit analysis at 24 h of incubation. Each concentration was tested in duplicate, and at least two experiments were performed on separate occasions.

2.6. Statistic analysis

The percentage of viable trophozoite exposed to the most active pure compound at each time interval was compare with percentage of viable trophozoite after exposed to metronidazole using the student t−test. A P−value of <0.05 was considered as statistically significant.

3. Results

3.1. Anti–protozoan activity of plant extracts

The in vitro effect of each plant extract against E. histolytica and G. intestinalis has been summarized in Table 1. The extract from C. flavescens showed the best activity against both E. histolytica and G. intestinalis with MICs of 125 and 15.63 μg/mL, respectively. The ethanol extract of C. longa inhibited both E. histolytica and G. intestinalis at concentration of 250 μg/mL whereas Euphorbia thymifolia (E. thymifolia), Garcinia mangostana (G. mangostana), Panica granatum (P. granatum), Sandoricum koetjape (S. koetjape) and Terminalia bellirica (T. bellirica) inhibited both protozoa at 250–500 μg/mL. Derris scandens inhibited only E. histolytica (MIC 500 μg/mL). Piper betle, Psidium guajava, Rhizophora mucronata and Terminalia chebula (T. chebula) inhibited only G. intestinalis at 250–500 μg/mL. The remaining extracts showed no activity (MIC ≥1 000 μg/mL). The MIC of metronidazole against both E. histolytica and G. intestinalis was 2.5 μg/mL.

Table 1

<table>
<thead>
<tr>
<th>Plants</th>
<th>Part used</th>
<th>MICs (μg/mL)</th>
<th>E. histolytica</th>
<th>G. intestinalis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aegle marmelos (L.) Corr.</td>
<td>fruit</td>
<td>&gt;1 000</td>
<td>&gt;1 000</td>
<td></td>
</tr>
<tr>
<td>Ardisia colorata Roxb.</td>
<td>wood</td>
<td>&gt;1 000</td>
<td>&gt;1 000</td>
<td></td>
</tr>
<tr>
<td>Centella asiatica (L.) Urb.</td>
<td>whole</td>
<td>&gt;1 000</td>
<td>&gt;1 000</td>
<td></td>
</tr>
<tr>
<td>C. flavescens</td>
<td>whole</td>
<td>125</td>
<td>15.63</td>
<td></td>
</tr>
<tr>
<td>C. longa L.</td>
<td>rhizome</td>
<td>250</td>
<td>250</td>
<td></td>
</tr>
<tr>
<td>Derris scandens (Roxb.)</td>
<td>stem</td>
<td>500</td>
<td>1 000</td>
<td></td>
</tr>
<tr>
<td>E. thymifolia L.</td>
<td>whole</td>
<td>500</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>G. mangostana L.</td>
<td>skin</td>
<td>500</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>Holarrhena pubescens Wall. ex G. Don</td>
<td>bark</td>
<td>1000</td>
<td>1 000</td>
<td></td>
</tr>
<tr>
<td>Manilkara occlus (Mill.) Fosberg</td>
<td>fruit</td>
<td>1000</td>
<td>1 000</td>
<td></td>
</tr>
<tr>
<td>Morinda citrifolia L.</td>
<td>fruit</td>
<td>&gt;1 000</td>
<td>&gt;1 000</td>
<td></td>
</tr>
<tr>
<td>Peltophorum pterocarpum (DC.) Backer. ex K. Heyne</td>
<td>bark</td>
<td>1000</td>
<td>1 000</td>
<td></td>
</tr>
<tr>
<td>Piper aurantaceous</td>
<td>leaf</td>
<td>1000</td>
<td>1 000</td>
<td></td>
</tr>
<tr>
<td>Piper betle L.</td>
<td>leaf</td>
<td>1000</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>Piper chaba Vahl.</td>
<td>fruit</td>
<td>1000</td>
<td>1 000</td>
<td></td>
</tr>
<tr>
<td>Psidium guajava L.</td>
<td>leaf</td>
<td>1000</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>P. granatum L.</td>
<td>skin</td>
<td>500</td>
<td>250</td>
<td></td>
</tr>
<tr>
<td>Rhizophora mucronata Poir</td>
<td>bark</td>
<td>&gt;1 000</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>S. koetjape (Burm. f.) Merr.</td>
<td>root</td>
<td>500</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>T. bellirica (Gaertn.) Roxb.</td>
<td>fruit</td>
<td>500</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>Terminalia chebula Bate.</td>
<td>fruit</td>
<td>1000</td>
<td>250</td>
<td></td>
</tr>
</tbody>
</table>

3.2. Purification of C. flavescens

Purification using chromatographic techniques afforded two anthraquinones and one naphthoquinone. Compound 2 was identified as 1-hydroxy-2-hydroxymethylanthraquinone which was previously isolated from Galium verum by Banthorpe and White.[25] 1-Hydroxy-2-methoxycarbonylanthraquinone (compound 3) was previously obtained from Rubia wallichiana whereas 2-amino-3-methoxycarbonyl-1,4-naphthoquinone (compound 1), as synthesized previously was isolated for the first time as a natural product.[26,27]
3.3. In vitro anti-amoebic and anti-giardial activity of pure compounds isolated from C. flavescens

The MICs of compound 2 against *E. histolytica* and *G. intestinalis* were 20.0 and 2.5 μg/ml, respectively. The morphology and approximate number of *E. histolytica* and *G. intestinalis* treated with compound 2 and metronidazole at the MIC concentration for 24 h under inverted microscope were shown in Figures 1A, 1B and 2A, 2B, respectively while the trophozoites from the control well were more than 90% confluent (Figures 1C and 2C). Compound 1 and compound 3 showed MIC values of >80 μg/ml which was considered to be not suitable for further study.

3.4. Time killing assay

The percentages of viable *E. histolytica* and *G. intestinalis* after exposed to metronidazole and compound 2 at 1/2 MIC, MIC and 2 MIC concentrations for 6, 12, 24 and 48 h, compare to the control at the same time interval, are shown in Figures 3 and 4, respectively. Metronidazole and compound 2 at MIC concentration decrease the number of viable *E. histolytica* and *G. intestinalis* trophozoites to <20% within 24 h. However, it is interesting to note that at 6 and 12 h the percentage of viable *G. intestinalis* exposed to compound 2 significantly (*P*<0.05) lower than when exposed to metronidazole.

![Figure 4](image_url)

*Figure 4.* Percentage of viable *G. intestinalis*, compared to control, after incubation with different concentrations of (A) metronidazole and (B) compound 2 for 6, 12, 24 and 48 h. Values are expressed as means and standard error of the mean, at *P*<0.05.

4. Discussion

In the present work, we have investigated the anti-protozoan activities of 21 medicinal plants (21 extracts) that have been used in Thai traditional medicine for the treatment of diarrhoea or parasitic infection. We found seven plant extracts, *C. flavescens*, *C. longa*, *E. thymifolia*, *G. mangostana*, *P. granatum*, *S. koetjape* and *T. bellirica*, exhibited both anti-amoebic and anti-giardial activities in vitro. These findings were in agreement with other reports such as compounds from *C. longa* and *P. granatum* were found to inhibit both *E. histolytica* and *G. intestinalis*.

*E. thymifolia*, is commonly used in folk medicine in Bangladesh for treatment of helminthisis[28], and was found to also possess various activities such as: an anti-viral, anti-oxidant, etc. activities[29]. This is the first report of an extract from *E. thymifolia* that inhibited *E. histolytica* and *G. intestinalis* in vitro.

*G. mangostana* is a tropical plant that grows well in tropical areas including Thailand, Indonesia and Malaysia. The pericarps of *G. mangostana* have been widely used as a traditional medicine for the treatment of diarrhoea, skin infections and chronic wounds in South East Asia for many years. Medicinal properties of *G. mangostana* extract such as: an anti-oxidant, anti-tumor, anti-inflammatory, anti-allergy, anti-malarial, and anti-bacterial/viral, anti-helmintic properties have been reported[30,31]. We further added more value to a *G. mangostana* extract as it also had a low anti-*E. histolytica* and anti-giardial activities.

An aqueous extract of *S. koetjape* bark is used traditionally in Malaysia as a tonic after giving birth[32]. The biological and phamacological properties of pure compounds isolated from different parts of *S. koetjape* have been reported to have an anti-viral[33], anti-inflammatory[32] and anti-cancer activities[34]. The roots of *S. koetjape* are used to treat intestinal disorders throughout Southeast Asia and we are the first to report its anti-*E. histolytica* and anti-*G. intestinalis* activities in vitro[35].

*T. chebula* is used as a remedy against a sore throat and cough, against diarrhoea connected to a prolapsed rectum and against ulcers and dysentery in China and Tibet[35]. The powder of *T. chebula* fruits has been used to treat chronic diarrhoea[36]. An anti-bacterial[37], anti-ciliate protozoa in ruminent[38] and anti-amoebic activities of *T. chebula* extracts have been reported[39]. We reported here its anti-giardial activity.

Results from the present study have demonstrated that an acetone extract of *C. flavescens* is the most effective against both *E. histolytica* and *G. intestinalis* growth. We therefore further isolated pure compounds from this plant and evaluated them for their anti-protozoan activities. *C. flavescens* is used in folk Thai medicine for treatment of helminthic infections. Three pure compounds were isolated, but only compound 2 exhibited both anti-amoebic and anti-giardial activities. For *E. histolytica*, its MIC concentration (2.5 μg/ml for metronidazole and 20.0 μg/ml for compound 2), the percentage of viability trophozoite were comparable. On the other hand, at a similar MIC concentration (2.5 μg/ml for both metronidazole and compound 2), the percentage of viable *G. intestinalis* trophozoite exposed to compound 2 significantly (*P*<0.05) lower than when exposed to...
metronidazole within 12 h of incubation. These results have indicated that compound 2 from \textit{C. flavescens} can inhibit \textit{G. intestinalis} quicker than metronidazole. This would be one advantage of its future therapeutic use. Compound 2 is an anthraquinone derivative and has not been studied for its bioactive activity. Anthraquinones from \textit{Cassia} sp. are known to possess anti-fungal, anti-plasmodial, anti-inflammatory and anti-diarrhoeal activities\cite{40}.

In conclusion, several medicinal plants that have been commonly prescribed as an anti-diarrhoeal or anti-parasitic remedy in Thailand showed anti-intestinal protozoan activity. Among them, 1-hydroxy-2-hydroxymethylanthraquinone isolated from \textit{C. flavescens} seems to be a good candidate for use against amoeba and giardial infections. Further studies on the mechanism of this compound and some \textit{in vivo} studies with regard to its effects on animals and human should be investigated.

**Conflict of interest statement**

We declare that we have no conflict of interest.

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**Comments**

**Background**

Intestinal infections caused by \textit{E. histolytica} and \textit{G. intestinalis} are major public health problems, particularly for children in most developing countries. So it is necessary to find low-cost and safe medicine. Medicinal plants are popular among people in developing countries because they are widely available at low cost and their products are mostly safe as expected. Moreover, medicinal plants have a rich history of use for the treatment of diarrhoea and dysentery.

**Research frontiers**

The manuscript evaluated the \textit{in vitro} activity of natural products obtained from selected Thai medicinal plants already claimed to have ethnobotanical use as potential anti-diarrhoeal or anti-parasitic agents and detected alternative agents that are suitable for use in preventing and treating \textit{E. histolytica} and \textit{G. intestinalis} infections.

**Related reports**

Several compounds from herbs have been proved to be effective against various organisms, \textit{i.e.} curcumin from \textit{C. longa} is reported to have anti-bacterial, anti-oxidant, anti-HIV and anti-protozoan activities; essential oils from \textit{Thymbra capitata}, \textit{Origaronirens}, \textit{Thymus zygis} subsp. \textit{sylvestris}, \textit{Lippia graveolens} and \textit{Zyzygium aromaticum} are active against \textit{Giardia} as well as providing some anti-bacterial activity.

**Innovations & breakthroughs**

Data regarding anti-intestinal protozoan activities of compounds from \textit{C. flavescens} are scarce. This study has showed that 1-hydroxy-2-hydroxymethylanthraquinone from \textit{C. flavescens} can be considered to be a potential anti-parasitic agent against \textit{E. histolytica} and \textit{G. intestinalis} infections.

**Applications**

1-hydroxy-2-hydroxymethylanthraquinone isolated from \textit{C. flavescens} may be a hopeful candidate for use against amoeba and giardial infections. After further studies on this compound \textit{in vitro} and \textit{in vivo}, it is possible for the compound to be used in clinical medicine.

**Peer review**

This is a good study in which the authors evaluated anti-amoeba and anti-giardial activities of several medicinal plants. Among them, 1-hydroxy-2-hydroxymethylanthraquinone isolated from \textit{C. flavescens} seems to be a good candidate for use against amoeba and giardial infections. In general, writing is fine, results are interesting, the topic of MS is covered by APJTD.

**References**


