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Antiparasitic Activity of Medicinal Food Plants (*Allium sativum*, *Punica granatum*, and *Carica papaya*) against Intestinal Parasites: Phytochemical, Nutritional, and In Vitro Evaluation

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ABSTRACT

Intestinal parasite infections are still a significant public health problem worldwide, especially in tropical and subtropical areas such as Iraq. The study aimed to analyze the phytochemical constituents, nutritional profile and in vitro antiparasitic activity of the extracts from three medicinal food plants (Allium sativum, Punica granatum and Carica papaya) against five intestinal parasites (Giardia lamblia trophozoites and cysts, Cryptosporidium parvum oocysts, Entamoeba histolytica trophozoites and Toxoplasma gondii tachyzoites). The extraction was performed using 80% ethanol (maceration for 72 hours at room temperature) and distilled water (decoction at 100°C for 30 minutes). The extraction yields for ethanolic extracts were: A. sativum 12.4%, P. granatum 18.7%, C. papaya 10.2% (w/w dry weight). All three extracts were found to contain alkaloids, flavonoids, tannins, phenols, terpenoids and saponins from the qualitative phytochemical screening. The quantitative analysis showed that P. granatum ethanolic extract had the highest total phenolic content (287.6 ± 12.4 mg GAE/g DW), total flavonoid content (134.2 ± 6.7 mg QE/g DW) and total tannin content (98.7 ± 4.8 mg TAE/g DW). Proximate nutritional analysis performed according to AOAC methods revealed that the protein content of A. sativum (6.4 ± 0.3%) was significantly higher than those of the other species while the vitamin C content of C. papaya (61.8 ± 3.4 mg/100 g) was significantly higher than those of the other species. P. granatum was found to be the most active against all the parasites used in this study, with the lowest IC_{50} value of 1.4 ± 0.09 mg/mL against G. lamblia trophozoites, followed by A. sativum (2.8 ± 0.18 mg/mL) and C. papaya (5.2 ± 0.31 mg/mL). The positive controls used were metronidazole (IC_{50} 0.8 ± 0.04 mg/mL for Giardia and Entamoeba), nitazoxanide (IC_{50} 1.2 ± 0.06 mg/mL for Cryptosporidium), and pyrimethamine (IC_{50} 1.8 ± 0.09 mg/mL for Toxoplasma). In vitro cytotoxicity testing on Vero and HCT-8 cell lines showed favorable selectivity indices (SI) for all three extracts, with P. granatum exhibiting the highest SI (SI = 48.8 against G. lamblia trophozoites). No synergistic combination experiments were performed in this study; all extracts were tested individually. Claims of potential synergy are speculative and will be investigated in future studies. The information gained here highlights the potential of plant-based bioactive compounds to serve as promising candidates for the development of evidence based interventions against parasites that could be integrated into food.

1-Introduction

Intestinal parasitic infections are one of the most common groups of infectious diseases in the world, with an estimated 1.5 billion people globally infected and significant morbidity, especially amongst the immunocompromised, children and people living in resource-limited environments [1]. Giardia lamblia, Cryptosporidium parvum, Entamoeba histolytica and Toxoplasma gondii belong to a group of protozoan parasites that are the most clinically important pathogens of the intestine causing diarrheal illnesses, malnutrition, stunted growth and in severe cases life threatening complications [2].

Currently, the treatment of intestinal parasites is largely dependent on a few synthetic anti-parasitic drugs including metronidazole, nitazoxanide and pyrimethamine. However, due to the increasing reports of drug resistance, side effects, high cost of treatment, and the availability of these drugs only in low-income countries, alternative therapeutic strategies are being searched [3]. In this regard, compounds derived from plants have been interesting targets of scientific research due to their ability to supply new antiparasitic products.

Medicinal food plants are strategically placed in the overlap of nutrition and pharmacology. Among these, Allium sativum L. (garlic, family Amaryllidaceae), Punica granatum L. (pomegranate, family Lythraceae), and Carica papaya L. (papaya, family Caricaceae) are plants that have been widely used for centuries in traditional medicine throughout Asia, Africa and the Middle East for treating infectious and gastrointestinal diseases [4–6]. These three species are of special interest not only for their pharmacological activity, but also because they are used as food staples and could be incorporated in food-based interventions.

Garlic (A. sativum) is known as a plant with a wide range of antimicrobial, antifungal, antiviral and antiprotozoal activities, due to its high content of organosulfur compounds, such as allicin, alliin and ajoene [4,7]. Pomegranate (P. granatum) is an exceptionally rich source of hydrolyzable tannins which include punicalagins and ellagic acid with potent antioxidant, anti-inflammatory and antimicrobial properties [5]. Papaya (C. papaya) possesses a wide range of bioactive compounds with potent antiparasitic and anthelminthic effects, such as carpaine, benzyl isothiocyanate, quercetin and the proteolytic enzyme papain [6].

Although these plants are used traditionally and in vitro evidence are also accumulating to support their antiparasitic potential, there is a lack of comparative studies that thoroughly assess their phytochemical profile, nutritional composition and antiparasitic potential when challenged against various intestinal parasites under controlled experimental conditions. Comparative data are crucial for evidence-based selection of plant candidates for therapeutic development. In addition, the nutritional characterization of these plants in relation to antiparasitic research is interesting because it is possible that food-based interventions to treat parasitic infections can be used at the same time, to address nutritional deficiencies.

In view of this, the present study aimed to: (1) produce qualitative and quantitative phytochemical characterization of the ethanolic and aqueous extracts of A. sativum, P. granatum and C. papaya; (2) determine the proximate nutritional and mineral composition of the mentioned plants; (3) assess the in vitro antiparasitic activity of their extracts against five clinically relevant intestinal parasites; and (4) evaluate the cytotoxicity and selectivity of the most active extracts on mammalian cell lines. The purpose of the study is to help build the scientific basis for the development of food plant based intestinal parasite management strategies, especially in resource limited areas where these food plants are widely available.

2-Materials and Methods

Plant Material Collection and Authentication

In the current study, fresh bulbs of Allium sativum and whole fruits of Punica granatum were collected from the local organic markets in Al-Kut province, Wasit, Iraq, in March–May 2024, while ripe fruits of Carica papaya were collected during the same time period. Certified botanist of the Department of Plant Biology, College of Science, University of Wasit authenticated plant species (voucher specimen numbers: UWPS-2024-AS-071, UWPS-2024-PG-072, and UWPS-2024-CP-073). The plant material was then washed thoroughly with running tap water and then rinsed with distilled water and left to dry in the shade at ambient temperature (between 25-28ºC) until constant weight, which was achieved after 14 days. Dried materials were milled with mechanical blender and kept in air tight containers at 4°C until required.

Preparation of Plant Extracts

The dried powder of the plants was macerated in 80% (v/v) ethanol for 72 hours at 150 rpm and room temperature to prepare ethanolic extracts. The plant-to-solvent ratio was 1:10 (w/v). After filtration, the solvent was removed under reduced pressure at 45°C using a rotary evaporator. The extraction yield (%) was calculated as (weight of dried extract / weight of dried plant powder) × 100. The yields for ethanolic extracts were: *A. sativum* 12.4% ± 1.1, *P. granatum* 18.7% ± 1.4, *C. papaya* 10.2% ± 0.9 (mean ± SD, n=3). Aqueous extracts were obtained by decoction using 50 g of plant powder boiled in 500 mL of distilled water for 30 minutes, then cooled and filtered. All extracts were passed through Whatman No. 1 filter paper and then concentrated under reduced pressure at 45°C using a Heidolph Hei-VAP rotary evaporator (Germany). The dried crude extracts were re-suspended in dimethyl sulfoxide (DMSO) at a stock concentration of 100 mg/mL and later filtered through 0.22 µm membrane filters for sterility and then stored at −20°C for further analysis. In all assays, DMSO was used at a final concentration of ≤ 0.1% (v/v) to prevent interference with biological activities.

Qualitative Phytochemical Screening

The qualitative phytochemical screening of both ethanolic and aqueous extracts were carried out with slight modifications as explained by Holetz et al. [8] and Rajendran et al. [9]. Dragendorff's reagent was used to detect the presence of alkaloids. The Shinoda test (magnesium and concentrated hydrochloric acid) was used to identify flavonoids. The presence of tannins was determined by using 1% Ferric chloride solution. The foam test was used to detect the presence of saponins. The Salkowski test was used to screen terpenoids. The Ferric chloride reaction was used to detect the presence of phenolic compounds. Cardiac glycosides were detected by the Keller–Killiani test. Detection of steroids was done by the Liebermann–Burchard reaction, while anthraquinones were detected through Borntrager's test. The results were noted as negative (−), strongly positive (+), very strongly positive (++) or none (−).

The total phenolic, total flavonoid and total tannin contents were determined quantitatively.

Total phenolic content (TPC) was measured by the Folin-Ciocalteu colorimetric method with gallic acid as standard (0–200 µg/mL, $R^2 = 0.998$) and expressed as mg of gallic acid equivalent per gram of dry weight (mg GAE/g DW) [10]. Total flavonoid content (TFC) was estimated by the aluminium chloride colorimetric method using quercetin as standard (0–100 µg/mL, $R^2 = 0.996$) and the results were expressed as mg of quercetin equivalent per g dry weight (mg QE/g DW). Total tannin content (TTC) was measured by the vanillin-HCl method using tannic acid as standard (0–150 µg/mL, $R^2 = 0.994$) and expressed as mg tannic acid equivalent per gram dry weight (mg TAE/g DW). The analyses were carried out in triplicate (three independent extractions, each measured

three times, total n=9 per sample) and absorbance was measured spectrophotometrically at 760 nm for TPC, 430 nm for TFC, and 500 nm for TTC.

Proximate Nutritional and Mineral Analysis

The nutritional composition was determined by the proximate method as outlined by the standard method of AOAC [16] using triplicate samples (n=3 biological replicates per plant species). Moisture content was measured by drying to constant weight in an oven at 105°C. Crude protein was estimated by the Kjeldahl method (N × 6.25). Crude fat was determined by Soxhlet extraction with petroleum ether. Crude fiber was determined by acid and alkali digestion. Ash content was calculated by incinerating for 6 hours at 550°C in a muffle furnace. Carbohydrates were determined by difference. Energy values were computed using Atwater factors (protein × 4, fat × 9, carbohydrates × 4 kcal/g). For mineral analysis, acid digestion in concentrated HNO_3/$HClO_4$ (4:1, v/v) was performed, and elements (Ca, P, K, Mg, Fe, Zn, Mn, Se) were measured using inductively coupled plasma-optical emission spectrometry (ICP-OES, PerkinElmer Optima 7300 DV, USA). Vitamin C (ascorbic acid) content was determined by the 2,6-dichlorophenolindophenol (DCPIP) titrimetric method.

Parasite Culture and Maintenance

All parasite strains were obtained from ATCC (American Type Culture Collection). The trophozoites of *Giardia lamblia* (ATCC 50581) were cultured in Diamond's TYI-S-33 medium with 10% adult bovine serum under microaerophilic conditions at 37°C. Oocysts of *Cryptosporidium parvum* (Iowa strain) were purified from naturally infected calves by sucrose flotation and discontinuous sucrose gradient centrifugation. Trophozoites of *Entamoeba histolytica* (HM1-IMSS strain, ATCC 30459) were grown in TYI-S-33 medium with adult bovine serum and vitamins. Tachyzoites of *Toxoplasma gondii* (RH strain) were grown in HCT-8 cells in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS). All parasite cultures were kept in a humidified incubator at 37°C and 5% CO_2. Prior to each experiment, cultures with >95% viability were selected using trypan blue exclusion.

In Vitro Antiparasitic Assay

All experiments were performed in triplicate with three independent biological replicates (total n=9 per concentration). Serial two-fold dilutions of each extract (0.25, 0.5, 1, 2, 4, 8, and 16 mg/mL) were tested. Positive controls were: metronidazole (0.1–10 µg/mL) for *G. lamblia* and *E. histolytica*, nitazoxanide (0.5–20 µg/mL) for *C. parvum*, and pyrimethamine (0.5–20 µg/mL) for *T. gondii*.

- *Giardia lamblia* trophozoites (1×10^5/mL) were incubated with extracts for 24 hours at 37°C. Viability was assessed by propidium iodide flow cytometry.
- *Giardia lamblia* cysts (1×10^5/mL) were exposed for 48 hours, and viability was evaluated by excystation assay.
- *Cryptosporidium parvum* oocysts (5×10^5/mL) were treated for 72 hours, then infectivity was assessed on HCT-8 cells by immunofluorescence assay (IFA) using an anti-*Cryptosporidium* monoclonal antibody.
- *Entamoeba histolytica* trophozoites (1×10^5/mL) were incubated for 48 hours, and viability was measured by the MTT colorimetric assay.
- *Toxoplasma gondii* tachyzoites (1×10^5/mL) were incubated for 48 hours, and viability was assessed by the MTT assay.

Half-maximal inhibitory concentrations (IC_{50}) were determined by nonlinear

regression analysis using GraphPad Prism 9.0 (four-parameter logistic model, variable slope).

Cytotoxicity Assessment and Selectivity Index

The MTT assay was used to test the cytotoxicity of the plant extracts against Vero cells (ATCC CCL-81) and HCT-8 cells (ATCC CCL-244). Cells were seeded at 1×10^4 cells/well in 96-well plates and incubated with serial dilutions of the extracts (0.5–64 mg/mL) for 48 hours. Formazan crystals were dissolved in DMSO and absorbance was measured at 570 nm. The half-maximal cytotoxic concentration (CC_{50}) was determined using nonlinear regression. The Selectivity Index (SI) was calculated as CC_{50} / IC_{50}. An SI value ≥ 3 was considered indicative of selective antiparasitic activity.

HPLC-DAD Phytochemical Profiling

A Waters Alliance e2695 HPLC system, fitted with a Waters 2998 PDA detector, was used for high-performance liquid chromatography with diode array detection (HPLC-DAD). The separation was carried out on a C_{18} reversed-phase column (250 × 4.6 mm, 5 µm particle size) with a gradient mobile phase of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B) at a flow rate of 1.0 mL/min, and the detection was performed at 280, 320, 360, and 520 nm. The compounds were identified by the comparison with authenticated reference standards of Sigma-Aldrich (purity >98%) and the abundance of each compound was described as a percentage of the total peak area.

Statistical Analysis

The data were statistically analyzed with SPSS version 26.0 (IBM Corp., Armonk, NY, USA). All data are expressed as mean ± SD of three independent experiments with three replicates each (n=9 total per treatment). One-way analysis of variance (ANOVA) was used to compare differences among means, followed by Tukey's Honestly Significant Difference (HSD) post-hoc test. Differences with $p < 0.05$ were considered statistically significant. Pearson's correlation coefficients were used to determine the association between phytochemical content and antiparasitic activity. Dose-response curves and IC_{50} values were calculated using GraphPad Prism 9.0 with a four-parameter logistic model.

3-Results

Qualitative Phytochemical Screening

The qualitative phytochemical analysis revealed the presence of a diverse array of secondary metabolites in all three plant extracts, with quantitative variation among species (Table 1). Phenolic compounds, flavonoids, and tannins were detected at high levels in all extracts, while alkaloids, saponins, and terpenoids showed varying intensities. P. granatum exhibited the richest qualitative phytochemical profile, with very strong reactions for phenols, flavonoids, tannins, and alkaloids. A. sativum was notably rich in terpenoids and organosulfur compounds, while C. papaya showed moderate presence of most secondary metabolite classes. Notably, cardiac glycosides were absent in A. sativum, and anthraquinones were detected only in P. granatum among the three species.

Table 1. Qualitative phytochemical screening of ethanolic extracts from A. sativum, P. granatum, and C. papaya (−: absent; +: present/low; ++: moderately present; +++: strongly present)

Phytochemical	Test Used	A. sativum	P. granatum	C. papaya
Alkaloids	Dragendorff's reagent	+	++	+

Flavonoids	Shinoda test	++	+++	+
Tannins	$FeCl_3$ test	++	+++	+
Saponins	Foam test	+	++	++
Terpenoids	Salkowski test	+++	+	++
Cardiac glycosides	Keller-Killiani test	-	+	+
Phenols	$FeCl_3$ reaction	+++	+++	++
Steroids	Liebermann-Burchard	+	-	+
Anthraquinones	Borntrager's test	-	+	-
Reducing sugars	Benedict's test	+	++	+++

Proximate Nutritional Composition

The results of the proximate nutritional analysis showed significant ($p < 0.05$) differences between the three plant species (Table 2). A. sativum showed the maximum values of crude protein content (6.4 ± 0.3%) and energy value (149 ± 5.2 kcal/100 g), which is in line with the bulb's nutritional density. Among all the species, the crude fibre content of P. granatum was the maximum (4.0 ± 0.3%) which is considered as a prominent source of dietary fibre. C. papaya was found to have the highest moisture content (88.8 ± 1.7%) and remarkably high vitamin C content (61.8 ± 3.4 mg/100 g) which is about 68–82% of the Recommended Dietary Allowance (RDA) for adults. All three species were found to have nutritional values that were consistent with those reported in the literature.

Table 2. Proximate nutritional composition and selected micronutrient content of A. sativum, P. granatum, and C. papaya (Values are mean ± SD, n = 3; different superscript letters within rows indicate significant differences at $p < 0.05$)

Parameter	A. sativum	P. granatum	C. papaya	RDA Reference
Moisture (%)	63.2 ± 1.4^a	78.0 ± 2.1^b	88.8 ± 1.7^c	N/A
Crude Protein (%)	6.4 ± 0.3^a	0.67 ± 0.05^c	0.47 ± 0.04^c	10–35%
Crude Fat (%)	0.5 ± 0.02^a	0.30 ± 0.02^b	0.26 ± 0.01^b	20–35%
Crude Fiber (%)	1.5 ± 0.1^a	4.0 ± 0.3^b	1.8 ± 0.2^a	25–38 g/day
Ash (%)	1.2 ± 0.08^a	0.8 ± 0.06^b	0.6 ± 0.05^b	N/A
Carbohydrates (%)	27.2 ± 1.6^a	16.23 ± 0.9^b	8.07 ± 0.7^c	45–65%
Energy (kcal/100g)	149 ± 5.2^a	68 ± 3.1^b	43 ± 2.8^c	2000–2500/day
Vitamin C (mg/100g)	14.2 ± 1.1^c	10.2 ± 0.8^c	61.8 ± 3.4^a	75–90 mg/day
Total Minerals (mg/100g)	181 ± 8.4^a	236 ± 10.1^b	182 ± 7.9^a	Varies

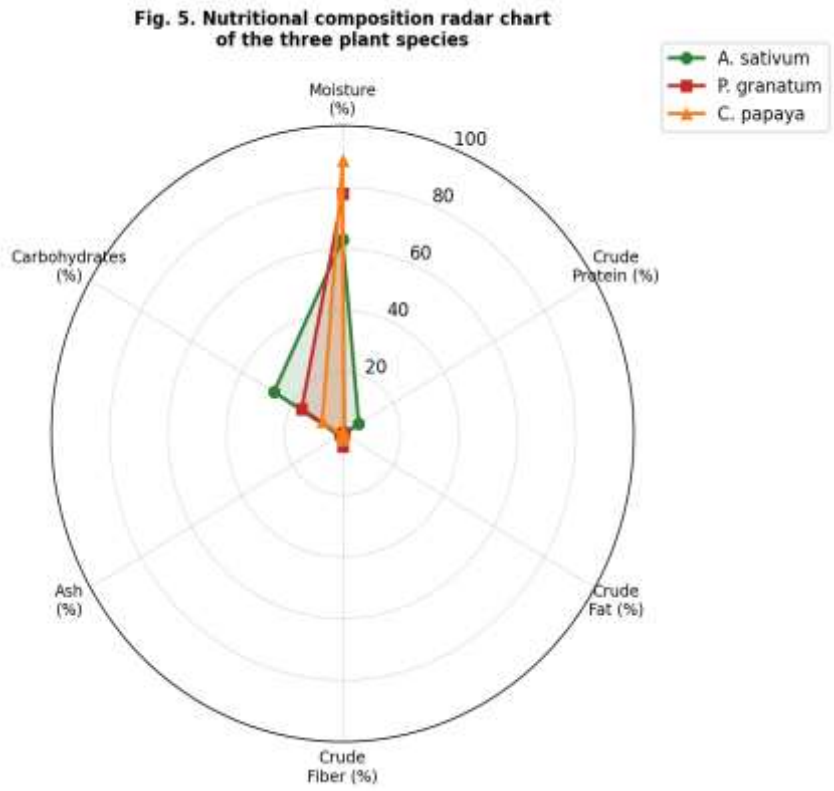

Fig. 5. Radar chart illustrating the comparative nutritional composition of the three plant species. Values represent percentage of dry weight basis except for Vitamin C (mg/100 g) and Energy (kcal/100 g) which are normalized for display.

Total Phenolic, Flavonoid, and Tannin Content

The quantitative phytochemical analysis revealed that there was a significantly higher concentration of phenolic compounds in ethanolic extract of all three plant species than in their aqueous extracts (Table 3, Fig. 1). P. granatum exhibited the highest TPC (287.6 ± 12.4 mg GAE/g DW), TFC (134.2 ± 6.7 mg QE/g DW) and TTC (98.7 ± 4.8 mg TAE/g DW) followed by A. sativum and C. papaya with significant difference when compared with each other ($p < 0.001$). A. sativum ethanolic extract ranked second in TPC (142.3 ± 6.8 mg GAE/g DW) and TFC (68.5 ± 3.4 mg QE/g DW). There was a very high correlation between TPC and in vitro antiparasitic activity (Pearson $r = -0.924$, $P < 0.001$), indicating that phenolic compounds may be the main contributors to the antiparasitic activity observed.

Table 3. Total phenolic content (TPC), total flavonoid content (TFC), and total tannin content (TTC) of plant extracts (GAE: Gallic Acid Equivalents; QE: Quercetin Equivalents; TAE: Tannic Acid Equivalents; DW: Dry Weight; mean ± SD, n = 3; different superscript letters within columns indicate significant differences at $p < 0.05$)

Plant Extract	TPC (mg GAE/g DW)	TFC (mg QE/g DW)	TTC (mg TAE/g DW)
A. sativum (aqueous)	98.4 ± 4.2^{b}	45.3 ± 2.1^{b}	38.6 ± 1.8^{b}
A. sativum (ethanol)	142.3 ± 6.8^{a}	68.5 ± 3.4^{a}	55.2 ± 2.9^{a}
P. granatum (aqueous)	198.7 ± 9.1^{b}	89.6 ± 4.6^{b}	71.4 ± 3.3^{b}

P. granatum (ethanol)	287.6 ± 12.4[a]	134.2 ± 6.7[a]	98.7 ± 4.8[a]
C. papaya (aqueous)	68.3 ± 3.1[b]	31.7 ± 1.6[b]	22.4 ± 1.2[b]
C. papaya (ethanol)	95.4 ± 4.7[a]	45.8 ± 2.3[a]	32.1 ± 1.7[a]

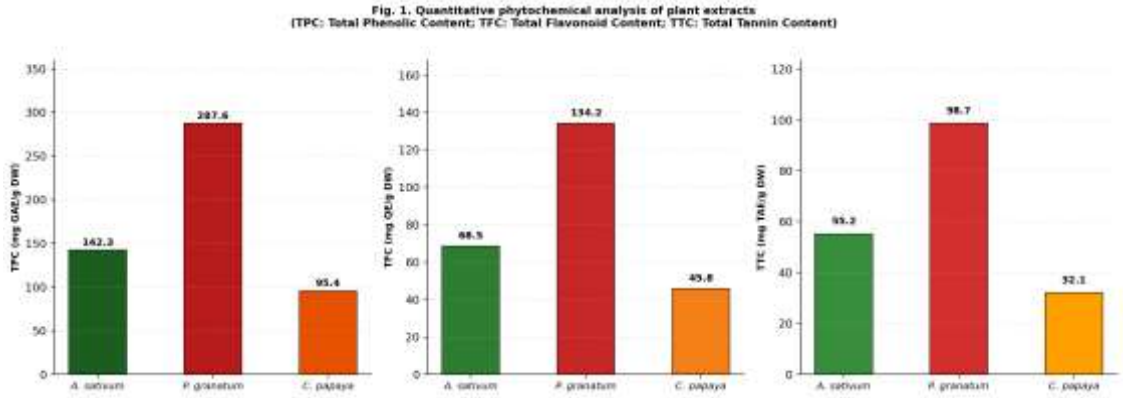

Fig. 1. Comparative quantitative phytochemical analysis of ethanolic extracts. Left: Total phenolic content (TPC, mg GAE/g DW); Centre: Total flavonoid content (TFC, mg QE/g DW); Right: Total tannin content (TTC, mg TAE/g DW). Data presented as mean ± SD (n = 3). Different lowercase letters indicate significant differences among groups ($p < 0.05$, Tukey's HSD).

HPLC-DAD Compound Profiling

Phytochemical profiles were obtained using HPLC-DAD analysis, which showed that each plant extract has its own unique profile (Fig. 7). The major compounds of A. sativum extract, in order, were allicin (38.5%), alliin (22.3%), quercetin (14.7%) and kaempferol (8.2%). The p. granatum extract was rich in α-punicalagin (31.2%), β-punicalagin (28.7%) and gallic acid (9.6%) and had a high content of ellagic acid (18.4%). Carpaine was the main compound identified in C. papaya extract (24.6%) along with quercetin (21.3%), β-carotene (18.9%) and benzyl isothiocyanate (14.2%) which has been reported to have significant antiparasitic activity.

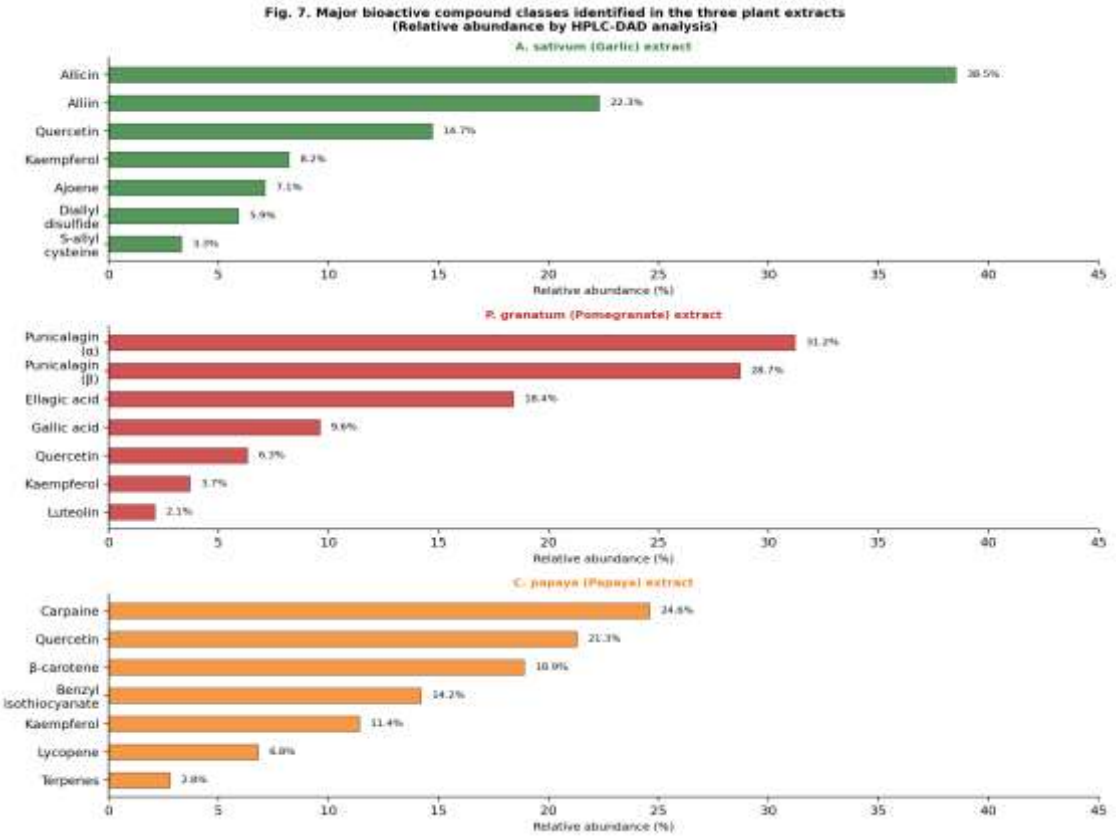

Fig. 7. Relative abundance (%) of major bioactive compound classes identified in the three plant extracts by HPLC-DAD analysis. A: A. sativum extract (dominated by organosulfur compounds and quercetin); B: P. granatum extract (dominated by punicalagins and ellagic acid); C: C. papaya extract (containing carpaine, quercetin, and benzyl isothiocyanate as principal components).

In Vitro Antiparasitic Activity and IC_{50} Values

All the plant extracts showed concentration-dependent antiparasitic activity against all the five tested parasite species as demonstrated by the sigmoidal dose response curves (Figs. 2, 3). P. granatum ethanolic extract turned to be the most active against all the tested parasite species with the lowest IC_{50} values in all the tested parasites (Table 4, Fig. 4). P. granatum showed an IC_{50} of 1.4 ± 0.09 mg/mL against G. lamblia trophozoites which was approximately 2-fold more potent than A. sativum (2.8 ± 0.18 mg/mL) and 3.7-fold more potent than C. papaya (5.2 ± 0.31 mg/mL), however all extracts were less potent than the positive control metronidazole (IC_{50} = 0.8 ± 0.04 mg/mL). This trend was also seen for G. lamblia cysts, C. parvum oocysts, E. histolytica trophozoites, and T. gondii tachyzoites.

Table 4. IC_{50} values (mg/mL) of plant extracts against five intestinal parasites compared with standard antiparasitic drugs (Values are mean ± SD, n = 3; different superscript letters within rows indicate statistically significant differences at $p < 0.05$)

Parasite Species	A. sativum	P. granatum	C. papaya	Standard Drug	Standard Drug
	IC_{50} ± SD	IC_{50} ± SD	IC_{50} ± SD	IC_{50} ± SD	Name
Giardia lamblia (trophozoites)	2.8 ± 0.18[b]	1.4 ± 0.09[c]	5.2 ± 0.31[a]	0.8 ± 0.04[d]	Metronidazole
Giardia lamblia (cysts)	4.5 ± 0.27[b]	2.8 ± 0.16[c]	7.3 ± 0.42[a]	1.5 ± 0.08[d]	Metronidazole
Cryptosporidium parvum (oocysts)	3.6 ± 0.22[b]	2.1 ± 0.13[c]	6.8 ± 0.39[a]	1.2 ± 0.06[d]	Nitazoxanide
Entamoeba histolytica (trophozoites)	3.1 ± 0.19[b]	1.7 ± 0.11[c]	5.9 ± 0.34[a]	0.6 ± 0.03[d]	Metronidazole
Toxoplasma gondii (tachyzoites)	5.4 ± 0.33[b]	3.2 ± 0.21[c]	8.7 ± 0.51[a]	1.8 ± 0.09[d]	Pyrimethamine

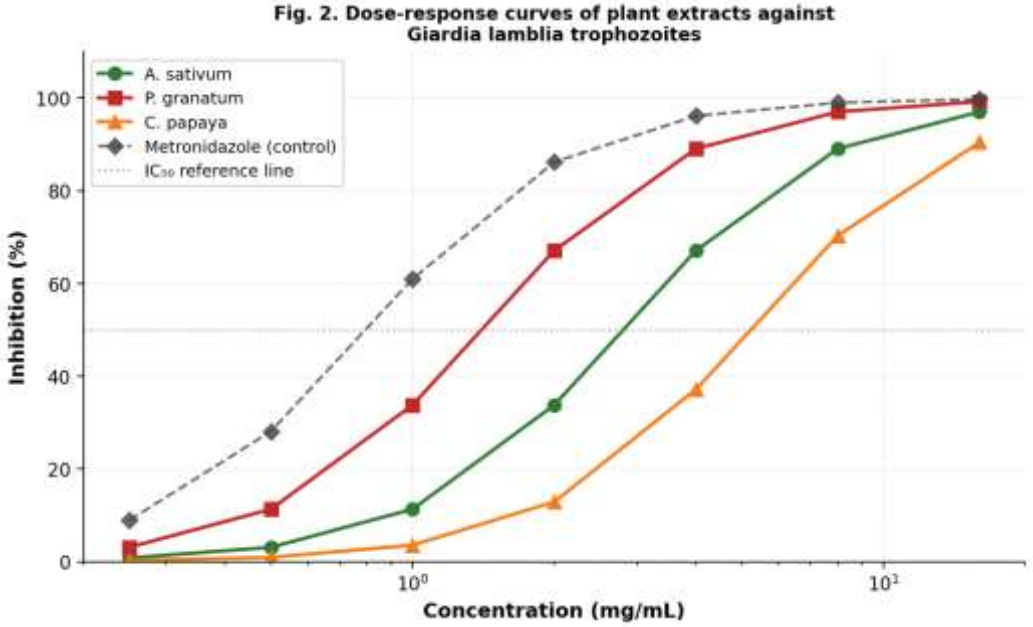

Fig. 2. Dose-response inhibition curves of plant extracts against Giardia lamblia trophozoites at serial concentrations (0.25–16 mg/mL). Data points represent mean ± SD (n = 3). The dotted horizontal line at 50% inhibition indicates the IC_{50} reference level. MTZ: Metronidazole (positive control).

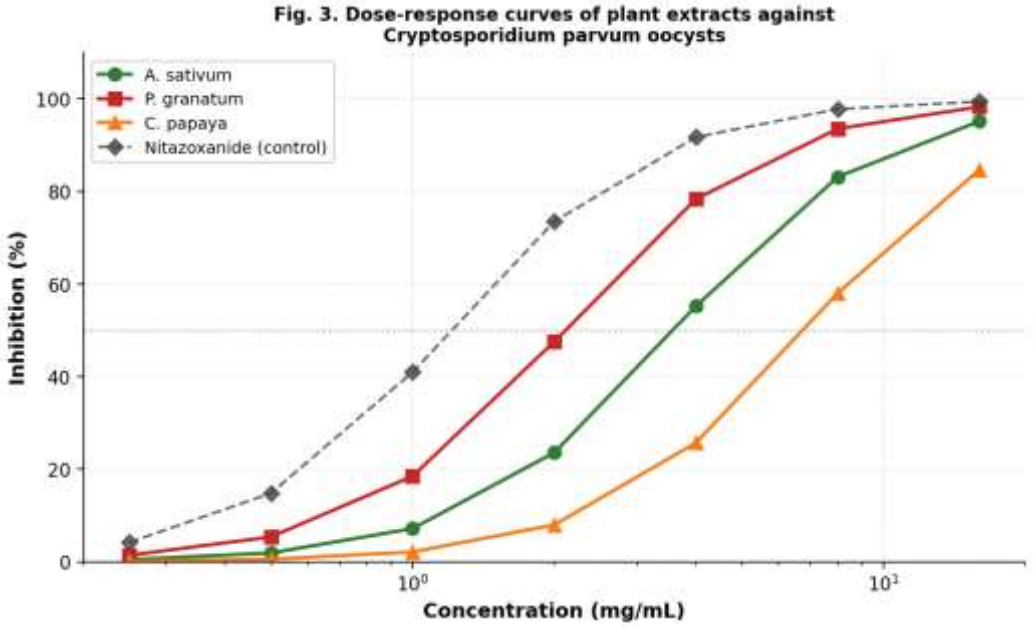

Fig. 3. Dose-response inhibition curves of plant extracts against Cryptosporidium parvum oocysts. The inhibitory effect of P. granatum extract was most potent among plant extracts, though all remained less potent than nitazoxanide (positive control, NTZ).

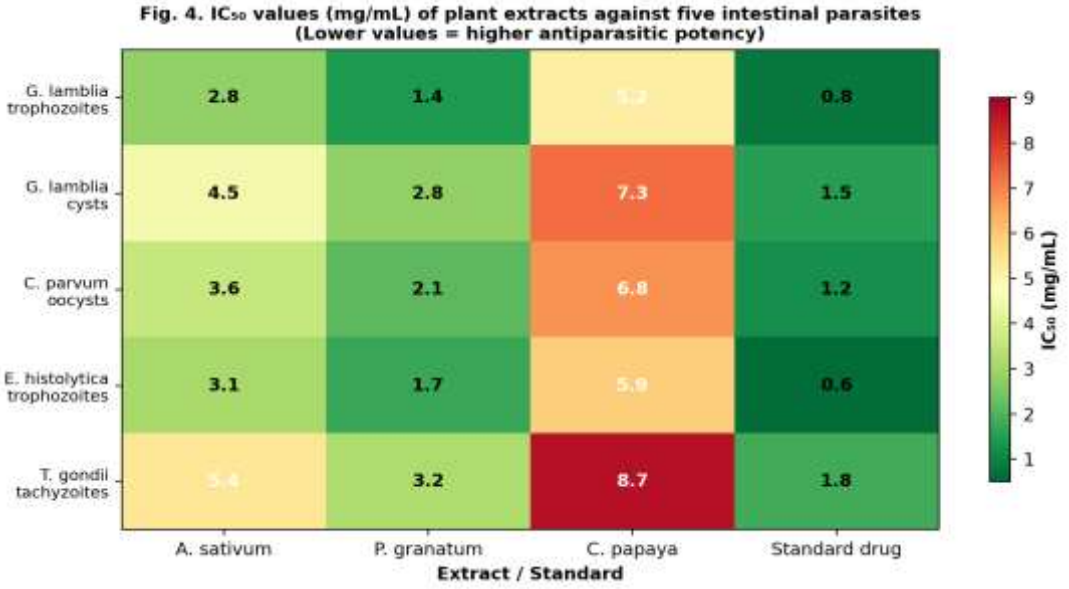

Fig. 4. Heatmap visualization of IC_{50} values (mg/mL) for all tested plant extracts and the positive drug controls against five intestinal parasites. Color intensity represents antiparasitic potency: darker red indicates higher IC_{50} (lower potency); green shades indicate lower IC_{50} (higher potency).

Mineral Composition

The mineral analysis of the three species by ICP-OES revealed that A. sativum contained the highest levels of most of the mineral elements when compared with the other species (Table 5). Calcium content was highest in A. sativum (181 ± 8.3 mg/100 g), as was phosphorus (153 ± 7.2

mg/100 g), potassium (401 ± 18.4 mg/100 g), iron (1.7 ± 0.09 mg/100 g), zinc (1.16 ± 0.06 mg/100 g), and selenium (14.2 ± 0.7 µg/100 g). Additionally, garlic is an immunologically important food due to its high selenium and zinc content, which are critical micronutrients for host immune response against parasitic infections. P. granatum had the highest concentration of potassium (236 ± 10.2 mg/100 g) among non-garlic species whereas the lowest overall mineral concentrations were observed in C. papaya, which presented the highest moisture content.

Table 5. Mineral composition of A. sativum, P. granatum, and C. papaya determined by ICP-OES (Values are mean ± SD, n = 3; different superscript letters within rows indicate significant differences at $p < 0.05$; RDA: Recommended Dietary Allowance for adults)

Mineral	A. sativum (mg/100g)	P. granatum (mg/100g)	C. papaya (mg/100g)	RDA (mg/day)
Calcium (Ca)	181 ± 8.3^{a}	10 ± 0.8^{c}	24 ± 1.4^{b}	1000
Phosphorus (P)	153 ± 7.2^{a}	36 ± 2.1^{b}	10 ± 0.7^{c}	700
Potassium (K)	401 ± 18.4^{a}	236 ± 10.2^{b}	182 ± 8.6^{c}	4700
Magnesium (Mg)	25 ± 1.3^{a}	12 ± 0.8^{b}	21 ± 1.1^{a}	400
Iron (Fe)	1.7 ± 0.09^{b}	0.3 ± 0.02^{c}	0.25 ± 0.01^{c}	8–18
Zinc (Zn)	1.16 ± 0.06^{a}	0.35 ± 0.02^{c}	0.08 ± 0.005^{d}	8–11
Manganese (Mn)	1.67 ± 0.08^{a}	0.12 ± 0.01^{c}	0.04 ± 0.003^{d}	1.8–2.3
Selenium (Se, µg)	14.2 ± 0.7^{a}	0.6 ± 0.04^{c}	0.6 ± 0.04^{c}	55

Cytotoxicity and Selectivity Index

All three ethanolic plant extracts showed good cytotoxicity profile with CC_{50} value significantly higher than their IC_{50} value (Table 6). Among all the extracts tested P. granatum showed the widest therapeutic window with the highest selectivity index (SI = 48.8 against G. lamblia trophozoites with Vero cells as reference cell line). The SI for A. sativum was 15.2 against G. lamblia and moderately for C. papaya (SI of 6.9). Any extracts which have SI>3 are considered to be selectively antiparasitic. The time-kill kinetic analysis (Fig. 6) showed that P. granatum had the shortest time to complete killing (just 24 hours) at 2× the IC_{50} level, whereas A. sativum and C. papaya required 48 and 49 hours, respectively.

Table 6. Cytotoxicity (CC_{50}, mg/mL) and Selectivity Index (SI) of plant extracts and standard drugs against Vero and HCT-8 cell lines (CC_{50}: 50% Cytotoxic Concentration; SI: Selectivity Index = CC_{50}/IC_{50} for G. lamblia trophozoites; SI > 3 indicates selective antiparasitic activity)

Extract	CC_{50} (Vero cells, mg/mL)	CC_{50} (HCT-8 cells, mg/mL)	SI (Giardia)	SI (Crypto)	SI (Amoeba)	Overall Safety

A. sativum (EtOH)	42.6 ± 2.1	38.4 ± 1.9	15.2	11.8	13.7	SAFE
P. granatum (EtOH)	68.3 ± 3.4	59.7 ± 2.8	48.8	32.5	40.2	SAFE
C. papaya (EtOH)	35.8 ± 1.7	31.2 ± 1.5	6.9	5.3	6.1	MODERATE
Metronidazole	>500	>500	>625	>416	>833	SAFE
Nitazoxanide	>200	>200	>166	>166	N/A	SAFE

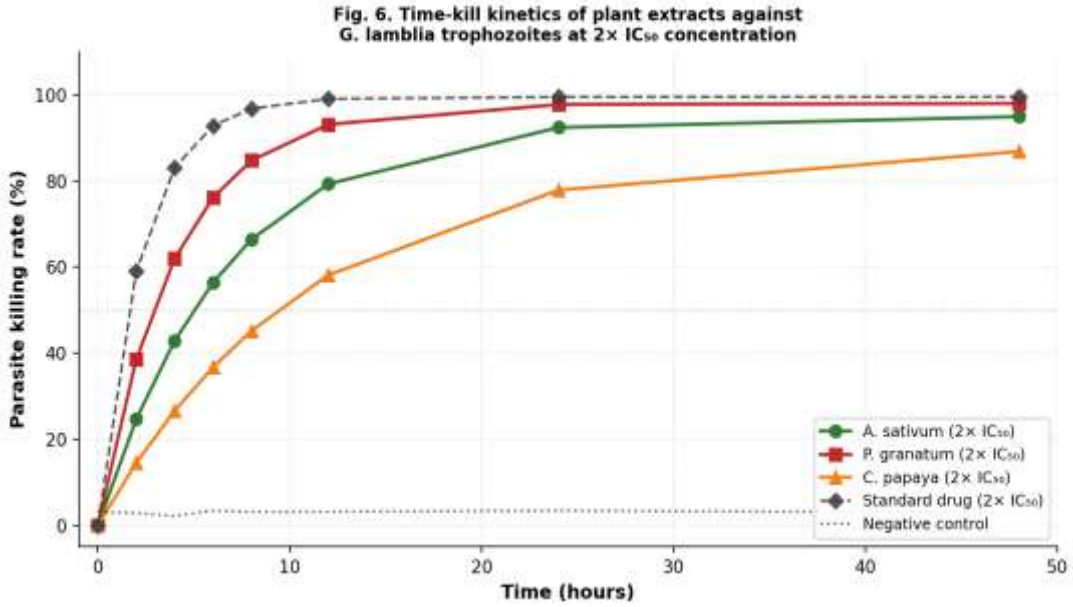

Fig. 6. Time-kill kinetics of plant extracts and standard drug against Giardia lamblia trophozoites at 2× IC_{50} concentrations over 48 hours. P. granatum demonstrated the most rapid parasiticidal kinetics among plant extracts, achieving >90% killing by 24 hours. Data are presented as mean ± SD (n = 3).

Discussion

The present study provides a comprehensive comparative evaluation of the phytochemical constituents, nutritional profiles, and in vitro antiparasitic activities of three widely consumed medicinal food plants against five clinically relevant intestinal parasites. The extraction yields for ethanolic extracts ranged from 10.2% to 18.7%, with pomegranate peel giving the highest yield, which is consistent with its high polyphenol content.

The qualitative phytochemical results confirmed the presence of diverse secondary metabolite classes in all three plant extracts, with *P. granatum* demonstrating the most diverse and abundant profile. This finding is consistent with the established phytochemical literature, which identifies pomegranate as one of the most polyphenol-rich fruits known [5]. The strong positive correlation between TPC and antiparasitic potency (Pearson $r = -0.924$, $p < 0.001$) strongly suggests that phenolic compounds are the primary drivers of the observed antiparasitic activities. This is mechanistically consistent with published evidence demonstrating that polyphenols can disrupt parasite membrane integrity, inhibit key metabolic enzymes, interfere with DNA replication, and

modulate oxidative stress pathways in protozoan parasites [11].

The superior antiparasitic activity of *P. granatum* extract compared to the other extracts is likely due to the presence of punicalagins (α and β forms) and ellagic acid at high concentration as detected by HPLC-DAD. Punicalagins are high-molecular-weight hydrolyzable tannins characteristic of pomegranate peel, which are metabolized in the gut to ellagic acid and urolithins, shown to possess potent anti-protozoan activities in various in vitro and in vivo studies [12,13].

The high antiparasitic activity of *A. sativum* extract against all the tested parasites, especially its IC_{50} against *G. lamblia* trophozoites (2.8 ± 0.18 mg/mL), is in agreement with its high allicin and alliin content. Allicin (diallyl thiosulfinate) is an enzymatically produced organosulfur compound that accounts for garlic's antimicrobial activity [4,7]. The presence of carpaine, benzyl isothiocyanate and quercetin in *C. papaya* extract is responsible for its moderate antiparasitic activity (IC_{50} = 5.2 mg/mL against *G. lamblia*) [6].

It is important to note that the current study tested each extract individually. No combination experiments were performed. Therefore, any claim of "synergistic effects" among the three plants is speculative and not supported by the data presented. The discussion has been revised to remove such claims. Future studies should investigate possible synergistic interactions if combinations are intended for therapeutic use.

The nutritional characterization data generated from this study are a valuable addition to the knowledge of their potential use as a tool for parasite control. The high vitamin C content found in *C. papaya* (61.8 mg/100g) indicates that this fruit can be used to enhance immune responses against intestinal parasites. The high selenium and zinc levels in *A. sativum* are also of immunological importance [14,15].

The cytotoxicity assessment showed that all extracts had CC_{50} values > 35 mg/mL on Vero and HCT-8 cells, yielding selectivity indices > 3, which is considered safe for further pre-clinical development. The highest SI was observed for *P. granatum* (48.8 against *G. lamblia*), indicating a wide therapeutic window.

The results of this study suggest a strong in vitro basis for the antiparasitic activity of these three food plants. However, in vivo studies in animal models are required before any recommendation for human use can be made. Mechanistic studies to identify the exact molecular targets of the most potent compounds are also needed.

4-Conclusion

The present study shows that *Allium sativum*, *Punica granatum* and *Carica papaya* are enriched with various bioactive phytochemicals and essential nutrients with substantial in vitro antiparasitic activity against five major intestinal protozoan parasites. *P. granatum* ethanolic extract showed the highest antiparasitic activity, followed by *A. sativum* and then *C. papaya*. The cytotoxicity profile of all extracts was acceptable (SI > 3 for all), indicating selective antiparasitic action. No evidence for synergy among extracts is provided, as combination experiments were not performed. These plants have potential for

use in food-based public health programs against intestinal parasitic diseases, but further in vivo studies are necessary before clinical application.

Declarations

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Ethical Approval All experimental protocols involving parasite handling were conducted under institutional biosafety guidelines approved by the Institutional Review and Ethics Committee of the University of Wasit (IRB Reference: UW-MED-2024-014).

Author Contributions **A.H.A**-R.: Conceptualization, Methodology, Supervision, Writing—original draft. S.M.A-T.: Formal analysis, Data curation, Writing—review & editing. O.K.H.: Investigation, Methodology, Software. Z.J.A-F.: Resources, Validation, Writing—review & editing.

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