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# The Effect of *Tamarindus indica* Fruit Pulp Extract on *Blastocystis hominis*

**Suhad Kareem Nghaimesh[1], Nazar Shiaa Mohammad[2], Mohammad Abd Alaziz Kader[3]**

[1] *Kirkuk Teaching Hospital, Kirkuk Health Directorate, Ministry of Health, Kirkuk, Iraq*

[2] *College of Health and Medical Technology, Middle Technical University, Baghdad, Iraq*

[3] *College of Medicine, Kirkuk University, Kirkuk, Iraq*

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## ABSTRACT

*Blastocystis hominis* is a single-celled parasite found in the human gastrointestinal tract. Information about treatment options indicates that successful anti-parasitic eradication of this parasite is yet to be straightforward. Furthermore, the parasite has demonstrated some resistance to metronidazole. To evaluate the anti-*Blastocystis* effect of *Tamarindus indica* in vitro among patients who visit Kirkuk hospitals. Between September 2023 and March 2024, a total of (300) stool samples, including (224) patients who were referred to the Gastroenterology and Endoscopy Unit in Kirkuk Teaching Hospital and private laboratories complaining of abdominal pain and diarrhea and (76) healthy individual's control. The study included (134) males and (166) females; their ages ranged (from 5 to 70 years). Stool samples were examined by direct microscopic examination using double wet preparation, cultured on Modified Boeck and Drbohlav's medium, then tested by enzyme-linked immunosorbent assay (ELISA) using *Blastocystis* copro-antigen ELISA kit, and deoxyribonucleic acid (DNA) was extracted, followed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The susceptibility of (36) *Blastocystis hominis* (*B. hominis*) isolates was tested for *Tamarindus indica* (*T. indica*) using the broth macrodilution method. The *Tamarindus* was diluted two-fold dilution to obtain concentrations of (0.5-1042 µg/mL) and added to the broth. The detection rate of *Blastocystis* was (100 %) using PCR-RFLP, followed by culture (90 %). *Tamarindus indica's* aqueous extract demonstrated anti-*Blastocystis hominis* activity with the minimum inhibitory concentration (MIC) values ranging within (4-512 µg/mL). Following (72 hours) of incubation, the parasite counts were completely eliminated at a concentration of (4 µg/mL) of the *T. indica* aqueous extracts. Our study revealed the potential anti-parasitic effect of *T. indica* aqueous extract at a concentration of (4 µg /mL) in the growth inhibition of *B. hominis*, and it could be a source of new anti-parasitic agents.

***Keywords:*** *B. hominis, Tamarindus indica*, MIC, Modified Boeck and Drbohlav's .

**Name:** **Suhad Kareem Nghaimesh** **E-mail:** tofekkamel@yahoo.com

# تأثير مستخلص لب نبات التمر الهندي على طفيلي *Blastocystis hominis*

سهاد كريم نغيمش[1]، نزار شياع محمد[2]، محمد عبد العزيز قادر[3]

[1] مستشفى كركوك التعليمي، دائرة صحة كركوك، وزارة الصحة، كركوك، العراق

[2] كلية التقنيات الصحية والطبية، الجامعة التقنية الوسطى، بغداد، العراق

[3] كلية الطب، جامعة كركوك، كركوك، العراق

## الملخص

*Blastocystis hominis* هو طفيلي وحيد الخلية يوجد في القناة الهضمية للإنسان. تشير المعلومات المتعلقة بخيارات العلاج الى ان الاستئصال الناجح لهذا الطفيلي لايزال بعيدا عن المسار الصحيح. علاوة على ذلك فإن الطفيلي لديه بعض المقاومة للمترونيدازول. لغرض تقييم تأثير التمر الهندي ضد *Blastocystis hominis* بين المرضى الوافدين لمستشفيات كركوك. خلال الفترة ما بين شهر ايلول 2023 لغاية شهر اذار 2024 تم جمع (300) عينة براز بما في ذلك (224) مريضا تم احالتهم الى وحدة امراض الجهاز الهضمي والناظور في مستشفى كركوك التعليمي والمختبرات الاهلية يعانون من الم البطن والاسهال ومن ضمنهم (76) شخصا سليما كمجموعة سيطرة. شملت الدراسة (134) ذكرا و (166) انثى تراوحت اعمارهم ما بين (5–70) سنة. تم فحص عينات البراز عن طريق الفحص المجهري المباشر باستخدام مستحضر رطب مزدوج وتم زرع العينات على وسط Modified Boeck and Drbohlav's medium بعدها تم اختبار العينات باختبار ELISA باستعمال Blastocystis copro-antigen ELISA kit وتم استخلاص الـ DNA واجراء فحص PCR-RFLP. تم اختبار حساسية (36) عزلة من *Blastocystis hominis* للتمر الهندي باستخدام طريقة تخفيف المرق. تم تخفيف التمر الهندي بالتخفيف المضاعف للحصول على تراكيز (0.5-1024 µg/mL) واضيف الى المرق. كانت نسبة الكشف عن *Blastocystis hominis* (100 %) باستعمال PCR-RFLP ونسبة الزرع الموجب (90 %). اظهر المستخلص المائي للتمر الهندي تأثيرا ضد *Blastocystis hominis* حيث تراوح MIC ما بين (4-512 µg/mL). ادى تركيز (4 µg/mL) للمستخلص المائي للتمر الهندي الى تقليل اعداد الطفيلي الى الصفر بعد (72 hours) من فترة الحضانة. كشفت دراستنا عن التأثير المحتمل للمستخلص المائي للتمر الهندي في تثبيط نمو *Blastocystis hominis* بتركيز (4 µg/mL) وانها قد تكون مصدرا لعامل جديد ضد الطفيلي.

## INTRODUCTION

*Blastocystis* is a parasite previously considered a harmless yeast or a commensal organism but then reclassified as a pathogenic protozoon [(1)]. This protozoan parasite infects children and adults and has been associated with many symptoms, including diarrhea, constipation, flatulence, abdominal pain, and nausea [(2)]. *B. hominis* was shown by some reports as being related to the development of the nervous colon and to a pro-inflammatory response, in which the regulation of the expression of some cytokines is absent [(3)]. As it is present in patients with and without symptoms, its pathogenicity is disputed [(4)]. *Blastocystis species* are identified in many forms, including vacuolar, granular, multi-vacuolar, amoeboid, and cystic forms. Like other enteric parasites, this parasite is transmitted through fecal-oral contact with food and water; however, this has not been demonstrated experimentally [(5)]. The prevalence of this parasite has been shown to vary, varying between (1.5 and 10 %) in developed nations and (30 to 50 %) in developing nations [(6)]. Even with the widespread occurrence of *B. hominis*,

little is known about this parasite, including treatment options [7]. Despite the availability of various drugs for decades, Blastocystosis is indicated as "an infection that is difficult to get rid of" [8]. Finding another treatment option is essential because data about therapeutic options indicates that successful antiparasitic eradication of *Blastocystis* is yet to be straightforward [9]. Also, the resistance appeared due to Metronidazole, the patient's non-compliance, and taking it for an extended period. Several studies have tried to use other agents like medicinal plants for treating infections caused by *Blastocystis* due to their availability, low cost, and since they were used for hundreds of years [7,10]. Several of these herbal extracts have considerable effects on various parasites and may be regarded as a therapy for these parasites in the future, like *Tamarindus indica* [11]. This plant is used in herbal medicine to treat abdominal pain, diarrhea, inflammations, helminth infections, wounds, colds, and fever [12]. These effects may be because they contain polyphenols like n-hexacosane, pinitol, and phenolic antioxidants [13]. *Tamarindus indica* comprises many different biologically active compounds in its various parts, such as seeds, bark, pulp, leaves, and flowers, that are useful for human health and can be utilized in the production of drugs [14]. The aim of our study is the therapeutic investigation of *Tamarindus indica* pulp aqueous extract on *Blastocystis hominis* infection.

## MATERIAL AND METHODS

### Sample collection

During the period from September 2023 to March 2024, a total of (300) stool samples, including (224) samples from patients who were referred to the Gastroenterology and Endoscopy Unit in Kirkuk Teaching Hospital and private clinics with symptoms of diarrhea and abdominal pain and (76) samples from apparently healthy normal individuals as a control. The study included (134) males and (166) females; their age group was categorized as (5-10, 11-30, 31-50, and 51-70 years). During sampling, each patient was asked by a special questionnaire, including age, gender, and symptoms like diarrhea, abdominal pain, and flatulence. The stool samples were divided into two parts: one preserved in (2-3 ml) potassium dichromate (2.5 %) and kept in refrigerator for later examination by ELISA technique and the other without preservative for direct examination and culture. Upon arrival at the laboratory, stool samples were inspected microscopically using saline and iodine-stained wet mount preparations. The presence of 5 or more *B. hominis* in the microscopic field (40 X) was evaluated as positive, and 40 *Blastocystis*-positive specimens were selected for culture.

### Preparation of culture media

The Modified Boeck and Drbohlav's medium (Figure 1) was used to cultivate and harvest *Blastocystis hominis*. The components of the culture were dissolved in one liter of sterilized distilled water. The prepared culture was autoclaved at (121) atmospheric pressure for (30 minutes) to sterilize. Next, antibiotics—500 I.U. of streptomycin, (80 mg) of gentamycin, and (100 mg) of nystatin—were added to the medium in the safety cabinet. The upper part of the containers was rubbed with a tincture of iodine, folded with aluminum foil, and then kept in the refrigerator until use [15].

About (2 ml) of Boeck-Drbohlav's liquid medium was added to a test tube containing about (6 ml) of semi-solid media (1 g of agar-agar, NaCl, and peptone powder) to create a diphasic medium. Thus, the medium comprised two parts: Boeck-Drbohlav's liquid medium was found at the slant, and a semi-solid press was found at the bottom [15].

For culture, forty *Blastocystis*-positive specimens (about 50 mg of the formed stool/0.5 mL of a diarrheal stool) were cultivated immediately in a previously prepared diphasic medium. The inoculated media were incubated at (37 °C) for (72 hours). On the third day, each culture media was observed for turbidity and growth on the junction between the slant and bottom. Then, culture media were microscopically examined to demonstrate the

*Blastocystis* (vacuolar and granular) stages. The culture media were examined daily for seven days and, on the fifth day, were subcultured to harvest the parasite for DNA extraction. The culture that contains more than ($10^6$/mL) vacuolar forms of *Blastocystis* was used to assess the anti-parasitic activity of the plant.

**Fig. 1: Boeck-Drbohlav's liquid medium.**

**Detection of *Blastocystis* by ELISA Copro-Antigen Test**

The ELISA technique was performed for all (300) stool samples according to the leaflet of the manufactured company (Savyon Diagnostics Ltd.) The Netherlands. Specific polyclonal antibodies directed against *Blastocystis* antigens are coated on the surface of microwells Figure 2.

The diluted fecal sample is added to the wells, and *Blastocystis* antigens, if present, bind to the immobilized antibodies. All unbound non-specific antigens are washed away. Anti- *Blastocystis* polyclonal antibody conjugated to horseradish peroxidase (HRP) is added and incubated, which binds to the pre-bound antigen-antibody complex. The excess conjugate is washed off, and the blue color shows the complexed co-complex presence upon additional incubation with Tethe tetramethylbenzidine TMB) substrate reagent. The reaction is stopped with a stop solution, the blue color turns yellow, and absorbances are read at (450 / 620 nm) with an ELISA reader [16].

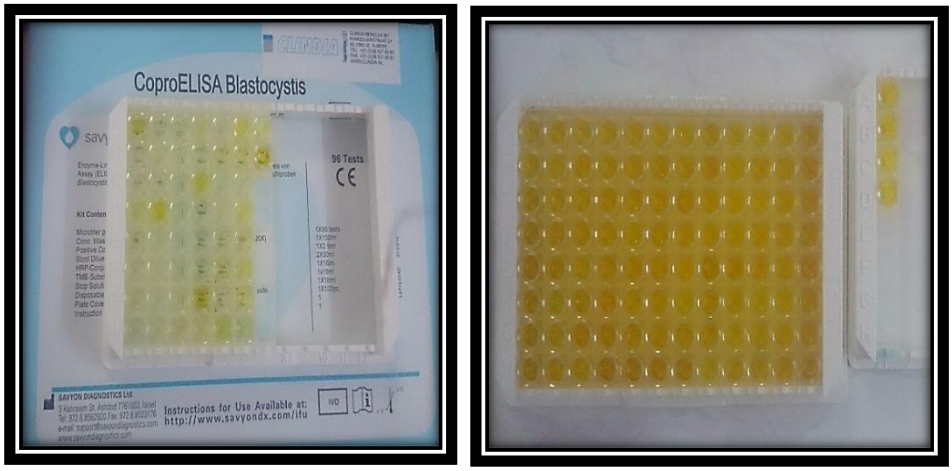


**Fig. 2: Copro ELISA *Blastocystis* Antigen Result (After adding stopping reagent).**

**Molecular characterization of *Blastocystis* Subtypes**

Only 20 *Blastocystis hominis* DNA extracts from (36 samples) on Boeck-Drbohlav's liquid medium were chosen according to the purity range (1.6 -1.8) for DNA amplification. Genomic DNA was extracted from *Blastocystis* positive subculture stool samples utilizing E.Z.N.A.® Stool DNA kit (OMEGA Bio-Tek, USA) following the manufacturer's instructions. DNA concentrations of the extracted DNA were measured and adjusted to (5 ng/µl) and were stored at (− 20 °C) until processed. The oligonucleotide primers were prepared according to the manufacturer's instructions. DNA of *Blastocystis species* was amplified using Restriction fragment length polymorphism (RFLP) analysis of PCR-amplified small subunit rDNA for identification of *B. hominis* genotypes [17].

Three pairs of sequences tagged Sequences Tagged Site (STS) primers (Table 1) were used for PCR amplification, including (SB 83, SB 227, and SB 155). The PCR products were digested with three restriction enzymes (HinfI, RsaI, and Sau3AI). Amplified DNA products were detected with (1.5%) agarose gel electrophoresis using ultraviolet trans-illumination after ethidium bromide staining[4].

**Table 1: The primers used for PCR amplification.**

| Subtypes | size (bp) | Forward and reverse Primer sequence (5′-3′) | GenBank Acc. No. |
|---|---|---|---|
| SB83 Sub1 | 351 | F: GAAGGACTCTCTGACGATGA<br>R: GTCCAAATGAAAGGCAGC | AF166086 |
| SB227 Sub3 | 526 | F: TAGGATTTGGTGTTTGGAGA<br>R: TTAGAAGTGAAGGAGATGGAAG | AF166088 |
| SB155 Sub7 | 650 | F: ATCAGCCTACAATCTCCTC<br>R: ATCGCCACTTCTCCAAT | AF166087 |

**Preparation of the Aqueous Extracts of *Tamarindus indica*:**

*Tamarindus indica* fruit pulp powder was purchased from the local markets in Kirkuk. The plant was extracted from Baghdad's College of Health and Medical Technology laboratories. The aqueous extract was made by mixing (50 g) of plant pulp powder with (500 mL) of distilled water, letting it sit for a day, and filtering it through several layers of gauze. With the evacuation, the filtrate was once more filtered via a Buchner funnel with filter paper Whatman NO.2. To dry; the filtrate was placed in an electric oven set to (40 °C). Until it is utilized, the dry powder is stored in a sterile glass bottle at (4 °C) in the refrigerator. To make the plant extract stock solution, (25 g) of plant powder were mixed with (100 mL) of (25 %) distilled water. The following aqueous extract concentrations were utilized in the study: (0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256, 512, and 1024 µg/mL), according to [18]. The effect of plant extract against *Blastocystis* was observed at (12, 24, 48, and 72 hours).

**Antimicrobial Susceptibility Test**

The study was conducted in vitro, and the susceptibility of (36) *Blastocystis hominis* isolates was tested for *Tamarindus indica* using the broth microdilution method. This test was carried out using twelve test tubes containing (1 mL) of Ringer solution, (10 %) horse serum, and (0.05 %) asparagine [19]. The *Tamarindus* was diluted two-fold dilution to obtain concentrations of (0.5-1042 µg/mL) and added to the broth. The tubes were cultured with (1 mL) of the parasite (106 cells /ml). The tubes were checked for growth after overnight incubation at (37 °C). The MIC is the tube containing the low inhibitory concentration [20]. The effect of plant extract against *Blastocystis* was observed at (12, 24, 48, and 72 hours). After (72 hours) incubation, the cultured Blastocystis cysts were assessed for viability using Lugol's iodine solution, which only stained viable cells [21]. The number of viable cysts in each culture tube was counted under the microscope (40 X), and the percentage of inhibition in their growth was calculated using a Neubauer's hemocytometer before and after treatment three times (24, 48, and 72 hours) from incubation [21]. The dead parasite was further confirmed by microscopic detection of cell wall disruption and destruction of internal structures. The total number of parasites was calculated by applying the following formula:

Total number of parasites = number of parasites in four large squares × 2500 × 2 [22].

Metronidazole was used as drug control. It was purchased from a local pharmacy as Flagyl (500 mg) and dissolved in (500 mL) double distilled water to produce a stock solution of (1 mg/mL), kept in a dark bottle. Final concentrations of Metronidazole (MTZ) were added to the culture adjusted to (100 µg/mL) [21].

**Statistical Analysis**

Statistical Package for the Social Sciences (SPSS), the statistical analysis of the data was conducted using the Microsoft Office Excel program. The mean ± standard error of mean (SEM) was used to express the numerical data, and the Paired t-test was performed to compare the numerical data for the patient and healthy control groups [23].

## RESULTS AND DISCUSSION

To date, the emerging protozoal infection, blastocystosis, has no cure. Even though Metronidazole is the treatment of choice, doctors are still in doubt about an antibiotic prescription for *Blastocystis*. There is still some dispute about its pathogenicity and frequent reports of failure to respond to chemotherapy [2,10]. Due to its medicinal properties, being accessible, cost-effective, and naturally safe, herbal medicine gained particular interest as an alternative therapy for various human disorders and diseases. To find an alternative treatment for blastocystosis, the extract of *T. indica* was tested for its in vitro effect [9] . This study is focused on the antiprotozoal activity of *T. indica* pulp aqueous extract against *B. hominis*. The results showed that *T. indica* extract demonstrated antiparasitic activity against the pathogen.

In this study (Table 2, Figure 3), the PCR technique revealed the highest detection rate 100% (20/20), followed by culture 90% (36/40), ELISA 38.33% (115/300), while direct examination showed the lowest detection rate 34.66% (104/300). The variability of the detection rates can be explained by the variable sensitivity of different diagnostic methods and the technician's experience performing the diagnosis.

The current study's findings show that PCR is the most successful diagnostic technique, consistent with prior investigations that found molecular analysis to be the most successful technique for diagnosing *Blastocystis* [19,24]. This finding contrasts with another study's findings, showing that the in vitro culture outperformed the PCR [18]. While PCR-based molecular techniques are particular for diagnosing *Blastocystis* DNA, they are costly, time-consuming, and require specialized staff. Many recent studies found that because culture is simple and inexpensive, it is more reliable for diagnosing *Blastocystis* than microscopic examination [19, 25].

**Table 2: Comparison of different techniques for detection of *B. hominis.***

| Laboratory method | NO. Examined | NO. Positive | % | NO. Negative | % |
|---|---|---|---|---|---|
| **1**.Direct examination | 300 | 104 | 34.66 | 196 | 65.33 |
| **2**. ELISA | 300 | 115 | 38.33 | 185 | 61.66 |
| **3**. Stool culture | 40 | 36 | 90 | 4 | 10 |
| **4**. PCR | 20 | 20 | 100 | 0 | 0 |

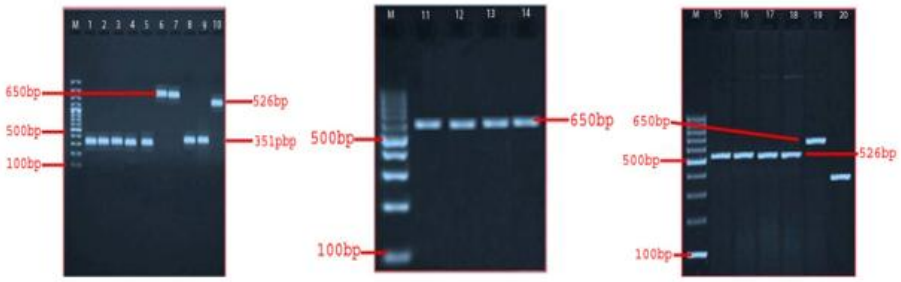


**Fig. 3: Agarose gel electrophoresis images show *Blastocystis* species suptypes (STs). M is the ladder at 100-1000bp. Isolates in lanes 1, 2, 3, 4, 5, 15, 16, 17 and 18 for SB83 subtype I. Isolates in lanes 6, 7, 11, 12, 13, 14 and 19 for SB155 subtype VII. Isolates in lane 10 for SB227 subtype III. DNA in lanes 1-10 was**

**digested with *Hin*fI, DNA in lanes 11-14, was digested with *Rsa*I and DNA in lanes 15-20 was digested *Sau3*AI.**

Among the examined cases, (104) were found to be positive for *Blastocystis hominis* based on microscopic examination, while (196) cases were negative results. The microscopic examination involved observing the samples under a microscope at both low and high power (10 X and 40 X). The observed forms of *Blastocystis hominis* exhibited a characteristic vacuolar morphology, characterized by a central body or vacuole surrounded by a thin cytoplasmic rim containing up to six nuclei (Figure 4: A and B).

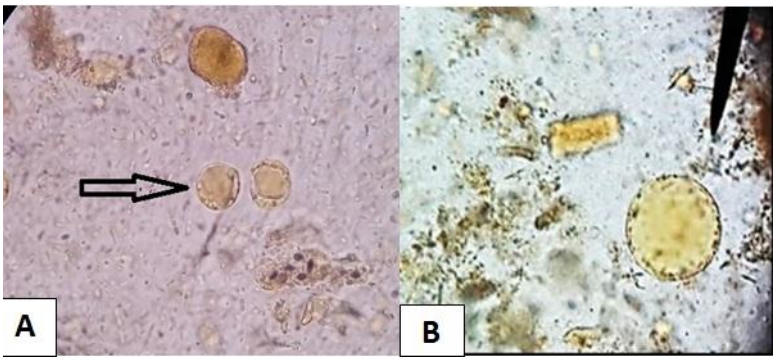


**Fig. 4: *Blastocystis hominis* stained with Lugol's iodine solution. Vacuolar form (A) 40 X, (B) 100 X.**

Most studies concerning detection of *Blastocystis* were based on direct microscopy [26]. Stained samples using Lugol's iodine solution was used to differentiate between viable cysts which stained yellow and dead cells (unstained) [27]. In this study, the obtained lower detection rate of microscopy is mainly due to the pleomorphic nature of the parasite, its resemblance to leukocytes, fat globules or other contaminants in the stool [28, 29]. Also, the current study demonstrated that ELISA had higher detection rate (38.33 %) than direct examination, which was in line with other studies [16,30]. This is because in ELISA test the parasite antigen present in stool samples will bound to specific immobilized antibody on the solid phase of ELISA microwells [16].

According to patients age Table (3), it was obvious that patients aging from (31-50 years) followed by (11-30 years) reveal high detection rate (35.6 %) and (33.9 %) respectively. While the lowest detection rate (10.4 %) was recorded among patients aging (5-10 years). These study findings are similar to previous [29] which reported that young adults have higher infection rate which can be explained by considerable outdoor activity and increased exposure to infectious diseases or because of the use of human feces as soil fertilizers which increase the chance of spreading infection. On the other hand, several studies showed that children have higher prevalence rate than adults [25, 28, 31].

**Table 3: Distribution of *B. hominis* positive cases according to age groups.**

| Age groups (Year) | No. examined (300) | *B. hominis* | |
|---|---|---|---|
| | | NO. positive (%) (n=115) | NO. Negative (%) (n= 185) |
| 5 -10 | 29 | 12 (10.4 %) | 17 (9.1%) |
| 11-30 | 81 | 39 (33.9 %) | 42 (22.7%) |
| 31 – 50 | 129 | 41 (35.6%) | 88 (47.5%) |
| 51 – 70 | 61 | 23 (20%) | 38 (20.5%) |

| $\chi^2$ test (P-value) | P= 0.076 > 0.05 (NS) |
|---|---|

*NS= non-significant, HS= highly significant

In this study Table (4), patients with abdominal pain have the highest detection rate (59.1 %) followed by flatulence (58.3 %) while patients with vomiting have the lowest detection rate (11.3 %). These findings align with previous studies conducted by [32], who also reported that *Blastocystis* is associated with abdominal pain.

In contrast, our results disagreed with other reports from [29], describing a significant association between fatigue and *Blastocystis*.

**Table 4: Distribution of B. *hominis* according to clinical symptoms.**

| **Clinical symptoms** | | ***Blastocystis hominis*** | |
|---|---|---|---|
| | | **No. Positive (%) (n=115)** | **No. Negative (%) (n=185)** |
| Diarrhea | No. (%) | 33 (28.7 %) | 49 (26.5 %) NS |
| Abdominal Pain | No. (%) | 68 (59.1 %) | 68 (36.8 %) ** |
| Flatulence | No. (%) | 67 (58.3 %) | 84 (45.4 %) * |
| Nausea | No. (%) | 39 (33.9 %) | 43 (23.2 %) * |
| Vomiting | No. (%) | 13 (11.3 %) | 17 (9.2 %) NS |
| Weight loss | No. (%) | 18 (15.7 %) | 28 (15.1 %) NS |
| Anemia | No. (%) | 16 (14.0 %) | 22 (11.9 %) NS |
| Total | | 300 | |

NS= non-significant *<0.05= Significant **<0.01= highly significant

The therapeutic use of medicinal herb extracts for parasitic diseases has been reported since ancient times. It has more valuable effects than synthetic drugs for being cheap, having fewer side effects, acceptable and suitable for chronic treatments. The present study's findings (Table 5, Figure 5) showed that the aqueous extract of *Tamarindus* exhibited inhibitory activity against *B. hominis*, with a minimum inhibitory concentration (MIC) ranging from (4-512 µg/mL). After (72 hours) of incubation, the concentration of 4 µg/ml of the *T. indica* aqueous extracts demonstrates that the quantity of *Blastocystis hominis* has dropped to zero (Table 6). In the present study, *Tamarindus* and metronidazole demonstrated a statistically valuable ($p < 0.05$) reduction of *Blastocystis* development according to the used concentration. The phytochemical components in the extract and their direct impact on the parasite cell membrane may be the cause of *T. indica's* action. Entering the cell destroys the protozoa or interferes with the parasite body's protein synthesis process [33].

Several studies have demonstrated that extracts of certain plant species belonging to various families may have anti-*Blastocystis* growth activity (in vitro). Abd Allah [7] found that garlic suppressed *Blastocystis* growth at (0.1 and 0.01 mg/ mL). Also, [34] revealed that the ethyl acetate fraction of *Eurycoma longifolia* had a potent anti-protozoal activity against *B. hominis*.

Consistent with these findings [35]. demonstrated that the *T. indica* aqueous extract reduced *E. histolytica* count to zero after (72 hours). Also, [36] showed that *T. indica's* chloroform extract had the most vigorous anti-*Plasmodium falciparum* action, which could be because 5-hydroxymethyl furfural, the extract's primary constituent, is present. Furthermore, [11] demonstrated that, in comparison to standard medication, the hydroethanolic seed coat extract of *T. indica* exhibited an intense microbicidal and anti-helminthic action against both Gram-positive and Gram-negative strains, as well as against earthworms (*Eisonia fatida*) and tapeworms (*Taenia solium*). By creating 44 distinct extracts, [37] assessed the anti-*trypanosoma* activity of three

medicinal plants in vivo. All these extracts, including the aqueous leaf extract of *T. indica*, had anti-trypanosomal activity. Additionally, methanol, chloroform, and water extracts of the barks and fruits of *Acacia seyal*, *Acacia sengal*, and *T. indica* were tested for their anti-*Trichomonas vaginalis* activity [38]. The results showed that *T. indica* extracts were less active than the other two species. Also, extracts of *T. indica* revealed antibacterial activities against gram-negative due to its lupeol content [13] and antifungal activities against *Aspergillus niger* and *Candida albicans* [39].

**Table 5: Minimum inhibitory concentration of *T. indica* for the 36 samples.**

| | The concentrations (µg /mL) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MIC | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | 256 | 512 | 1024 |
| No. of affected cases (36) | 0 | 0 | 0 | 7 | 6 | 6 | 5 | 4 | 4 | 3 | 1 | 0 |

**Fig. 5: *Tamarindus indica* concentrations active against *Blastocystis* parasite (4-512 µg /ml).**

**Table 6: Effects of aqueous extract of *T. indica* against *B. hominis* in vitro.**

| **Concentrations** | **Times (h) (Number of *B. hominis* x$10^6$ cells /ml)** | | | | **Means** |
|---|---|---|---|---|---|
| **MIC (µg /ml)** | **12** | **24** | **48** | **72** | |
| Control | 100.67 | 90.25 | 53.75 | 0.09 | 61.19 |
| 0.5 | 25.13 | 17.65 | 1.53 | 1.01 | 11.33 |
| 1 | 25.17 | 17.68 | 1.54 | 0.11 | 11.12 |
| 2 | 25.19 | 17.77 | 1.56 | 0.10 | 11.15 |
| 4 | 25.10 | 17.80 | 1.66 | 0.00 | 11.14 |
| 8 | 28.30 | 19.67 | 1.20 | 4.17 | 13.34 |
| 16 | 30.45 | 25.36 | 18.47 | 10.79 | 21.27 |
| 32 | 59.02 | 39.11 | 20.67 | 18.10 | 34.23 |
| 64 | 75.64 | 70.78 | 67.48 | 39.27 | 63.29 |
| 128 | 85.80 | 70.50 | 59. 64 | 11.93 | 56.97 |
| 256 | 89.50 | 76.20 | 61.77 | 45.25 | 68.18 |
| 512 | 90.69 | 82.44 | 63.89 | 49.75 | 71.69 |
| 1024 | 96.25 | 85.70 | 66.12 | 50.99 | 74.77 |
| **Means** | 58.22 | 48.53 | 32.25 | 17.81 | 39.20 |

This study used metronidazole as the positive control, which is active against various protozoa and bacteria. The drug diffuses into the organism, inhibits protein synthesis by interacting with DNA, and causes a loss of helical DNA structure and strand breakage. Therefore, it causes cell death in susceptible organisms [40]. *Blastocystis hominis* cells treated with *T. indica* and metronidazole showed deformity in withered shapes compared to the control.

When the aqueous extract was present, the average number of *B. hominis* cells per milliliter was (39.20×106) (Table 6). When compared to the control, the aqueous extract significantly ($p \leq 0.05$) increased the concentration and duration of *B. hominis* reduction (Table 6). After (72 hours) of

incubation, the *T. indica* aqueous extract concentration of (4 µg/mL) reduced the *B. hominis* populations to zero. This is because the extract contains harmful substances that directly affect the parasite cell membrane. If the parasite enters the cell, it will either be killed or its internal protein synthesis will be impacted [(33)]. The anti-*Blastocystis* inhibitory activity obtained from *T. indica* in this study may be due to its active constituents, such as saponins, alkaloids, and glycosides[(33)]. Also, Tannin was one of the constituents present in the phytochemical analysis of *T. indica* extracts, which binds with the free proteins in the parasite's gastrointestinal tract or glycoproteins on its outside, thereby eliciting antiparasitic activity [(41)].

In the present study, it has been shown that the interaction between time and concentration (Table 6) has a significant effect at ($p \leq 0.05$) on decreasing *B. hominis* number in culture. The (4 µg/ml) concentrations decreased *B. hominis* number to zero at (72 hours) of incubation. It was observed that the extract's severity scores were higher at the higher concentration than at the lower concentration. The effects become more noticeable the longer the incubation period. This could be because longer times allow the active ingredients in the parasite membranes to penetrate more easily, which either destroys the membranes or weakens the parasite.

Additionally, compared to the lesser concentration, the high concentration offers more room to affect the parasite. This could be accomplished by the concentration interpenetrating the parasite's outer membrane. This may have an impact on the membrane's tubulins. The parasite's contents exoded and eventually died as a result of this concentration creating a hole in the membrane [(28)].

## CONCLUSIONS

Our study revealed the potential anti-parasitic effect of *T. indica* aqueous extract in the growth inhibition of *B. hominis* at a concentration of (4 µg/mL) and it could be a source of new anti-parasitic agents after analyzing its phytochemical constituents.

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