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Antiamoebic and phytochemical studies of two medicinal plants: traditional recipes used against amoebiasis

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ABSTRACT

Background: Amoebiasis, an intestinal infection caused by the parasitic amoeba *Entamoeba histolytica*, presents varying symptoms from none to severe. Traditional non-herbal treatments often carry adverse effects, prompting interest in herbal remedies. *Aim*: This study evaluates the effectiveness of *Tamarindus indica* (T. *indica*) and *Cynodon dactylon* (C. *dactylon*) extracts against *E. histolytica*. Using the Soxhlet extraction method, extracts were obtained in different solvents and analyzed for percentage yield, phytoconstituents (PCs), quality control parameters, fluorescence, high-performance thin layer chromatography (HPTLC), and Fourier transform infrared (FTIR) spectroscopy. The antiprotozoal study was conducted using the TY1-S33 inoculum of E. *histolytica*. *Results*: The methanolic extracts of *T.indica* and *C.dactylon* had higher yields (22.2% and 8.11%, respectively) compared to the chloroform extracts. Methanolic extracts revealed the presence of carbohydrates, flavonoids, tannins, and proteins. Quality control tests showed loss on drying (8.1% for T. *indica* and 6.38% for C. *dactylon*), total ash value (9.8% and 9.89%), and methanol soluble extractive (22.2% and 12.60%). Fluorescence analysis and HPTLC confirmed the presence of PCs. FTIR analysis identified O-H & N-H bonds at 3210.04, 2916.78, and 2830 cm^{-1} for T. *indica*, and N-H & C=O peaks at 3402.2 & 2215 cm^{-1} for C. *dactylon. Conclusion*: The antiprotozoal study demonstrated significant inhibition of trophozoites by *T. indica* (98.2%) and *C. dactylon* (97.7%). Thus, both plant extracts were found to be safe and effective for treating amoebiasis, suggesting improved patient compliance with herbal treatments.

KEYWORDS

amoebiasis, *Cynodon Dactylon*, *Tamarindus Indica*, *E. histolytica,* phytoconstituents

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1. INTRODUCTION

An anaerobic parasite *E. histolytica* is responsible for intestinal or extraintestinal amoebiasis [1,2]. According to Kantor et al., amoebiasis is anticipated to affect 50 million people worldwide annually and cause 55,000 deaths [3]. It has a biphasic life cycle and a latent cyst stage immune to the environment and can spread the infection [4,5]. It is mostly asymptomatic, but it can also cause mild to severe symptoms like diarrhea or bloody diarrhea,

and abdominal pain. On the other hand, peritonitis results from severe complications. The ingestion of cysts takes place via fecal-oral contamination of water, food, or transmitted person-to-person. It can be sexually transmitted by oral-rectal contact (especially among male homosexuals) or by vectors such as flies, cockroaches, and rodents [6].

Mature cysts are consumed and pass through the stomach's acidic environment unharmed before reaching the intestine. Cysts undergo excystation into trophozoites and infiltrate the large intestine when they are exposed to an alkaline environment. The trophozoites attach themselves to the colonic epithelial cells through lectins like galactose or N- acetylgalactosamine. Mucin in the stomach, however, inhibits these protozoa from adhering to the lectin; yet, the amoeba consumes mucus and lessens the protective function. The pleomorphic form of the *E. histolytica* trophozoites aids their mobility as they travel between the cells. The inclusion of a sizable, persistent ion channel (amoeba pore), which results in cytolysis, damages the host cell membrane during this migration. The trophozoites use binary fission to multiply in this area. The trophozoites also harm mammalian cells by causing apoptosis. The parasite invades and enters the intestine (mucosal and submucosal tissues) and extraintestinal (liver) systems, where it releases parasitic cysteine proteinase that can break host cell adhesion and reproduce asexually. When the trophozoites increase, necrotic components spread unevenly and cause cytotoxicity. Trophozoites that do not penetrate proceed through encystation and produce cysts, which are then expelled in faeces [7,8].

There are other non-herbal therapeutic alternatives available to treat amoebiasis, e.g., nitroimidazole agents, tinidazole, metronidazole, triazines, and bisphosphonates. However, these therapies have certain disadvantages and side effects that harm people's health including nausea, vomiting, headaches, a metallic or bitter taste in the mouth, and more severe effects such as anorexia, ataxia, and skin rashes/itching [9,10]. Thus, herbal remedies can be utilized to mitigate these effects. The goal of Ayurvedic medicine is to improve one's overall mental, bodily, and spiritual well-being. It is possible to stop the body's physical deterioration and disease processes by maintaining a correct balance of all essential energy [11,12]. *T. indica* and *C. dactylon* plants have tranditional uses for many years. Leaves of *T. indica is* used for amoebiasis, applied to reduce swellings and tumors, also useful in blood diseases. *C. dactylon* Pers. grows all over the state and has medicinal benefits for many diseases. The grass is presumed as a cure for epistaxis, haematuria, inflamed tumors, whitlows plump excrescences, cuts, wounds, draining piles, cystitis, nephritis, scabies, and other skin ailments [13,14]. Consequently, *T. indica* and *C. dactylon* plant extracts were used in this experiment were used to evaluate the preliminary tests and quality control parameters such as LOD, % yield, ash value, and extractive value. Fluorescence analysis, HPTLC, and FTIR were also performed for structural identification. And finally, an antiamoebic study wascarried out to check the potency of both plant extracts.

2. METHODOLOGY

2.1. Materials and methods

The Soxhlet extraction method was used to get each extract in different solvents. Both extracts were then subjected to determine percentage (%) yield, phytoconstituents (PCs) screening, quality control parameters, fluorescence analysis, high-performance thin layer chromatography (HPTLC), and Fourier transform infrared (FTIR) spectroscopy. Finally, the antiprotozoal study was performed using the TY1-S33 inoculum of *E. histolytica.*

2.2. Methodology pharmacognostic evaluation

2.2.1. Preparation of extracts of selected plants

A hot extraction method, using the Soxhlet apparatus was used to extract the plants *T. indica* Linn (leaves) and *C. dactylon* Pers. (whole plants). Accurately weighed 20 gm of chosen plant parts were introduced one at a time to various solutions, such as petroleum (pet.) ether (60–80°C), chloroform, acetone, methanol, and water, following their polarity. The procedure continued until all solvents had been removed. After that, the product was gathered, cleaned, filtered, dried using a rotary evaporator, and placed for cooling. Extracts were measured, and the calculated percentage of yield [15] was given by equation 1.

$$Yield(\%) = \frac{oWeight\ of\ dry\ extractbtained}{Weightof\ dry\ part\ used} X\ 100 - - - -1$$

2.2.2. Preliminary phytochemical screening of various extracts

The extracts were investigated for phytoconstituents (PCs) /secondary metabolites, indicating the medicine's therapeutic actions of alkaloids, glycosides, carbohydrates, tannins-phenolic compounds, proteins and amino acids, flavonoids, saponins, steroids [16], etc. These qualitative phytochemical analyses were performed according to standard procedures as summarized below (Table 1).

2.2.3. Evaluation of quality control parameters of each plant extract

Loss on drying (LOD)

About 1.5 grams of the powdered plant extracts were transferred to the porcelain dish. Dried in the hot air oven at 100°C for a definite period. Placed it for cooling and measured [17,18]. The weight loss was determined by using equation 2.

$$LOD\ (\%) = \frac{Wt.of\ sample\ before\ drying\ -\ Wt.of\ sample\ afterdrying}{Wt.of\ sample\ before\ drying} X\ 100 - - - 2$$

Table 1. Preliminary phytochemical evaluation of plant extracts.

Secondary metabolites	Test	Indication for presence
Alkaloids	Dragendroff"s test	Reddish brown ppt.
Glycosides	Keller-Killiani test	A pale blue color layer
Proteins	Biuret test	The violet color appears
Carbohydrates and sugars	Fehling's test	brick-red ppt.
	Benedict's test	Red color ppt.
Phenolic compounds & Tannins	Extract + a few drops of 5% alcohol+ $FeCl_3$ solution	Bluish black (or) dark green color
Flavonoids	Shinoda's test	Orange or red color
Steroids	Salkowski test	Blue-green-coloured solution
Saponin	Foam test	The formation of a 1cm foam layer
Amino acids	Ninhydrin test	Purple or bluish colour

b) Total ash value

Two grams of each plant extract were measured and transferred into the crucible. Support the crucible with the tripod stand and then applied heat with a burner till the vapors almost ceased and the carbon was burnt off. The residue moistened with about 2 mL water, dried, and ignited. Cooled the ash and calculated its percentage [19] using equation 3.

$$Total\ ash\ (\%) = \frac{Weight\ ofash}{Weight\ of\ sample} X\ 100 - - - 3$$

c) Acid-insoluble ash value

To determine acid insoluble ash value, follow the same step as given in the above section by using 25 mL of dilute HCl. The residue was collected, weighed, and calculated using equation 4 [20].

$$Acid\ insoluble\ ash(\%) = \frac{Weight\ of\ acid\ treatedash}{Weight\ of\ sample} X\ 100 - - - 4$$

d) Water soluble ash value

The water-soluble ash of each plant extract was determined using 25 mL of H_2O. The solution boiled for 5 min. Filtered through filter paper, the residue was washed twice with hot water. Placed filter paper and residue for heating in the crucible till all carbon was removed. The residue was weighed and calculated as water-soluble ash value using equation 5 [20].

$$Weight\ soluble\ ash\ (\%) = \frac{Initial\ weight\ of\ ash\ -\ Mass\ of water\ insoluble\ residue\ in\ total\ ash}{Weight\ of\ sample} X\ 100 - - - 5$$

e) Extractive value

i. Water soluble: Weighed accurately 4 g of dry plant extracts with 100 mL water and transferred them to a conical flask. Allow the mixture to stand for one hour. The reflux condenser was connected to the container and boiled for one hour. Placed it for cooling and weighed it immediately. Calculated the percentage w/w of extractive using equation 6 [21].

$$Water\ soluble\ extractive\ = \frac{Initial\ mass\ -\ Mass\ of\ water\ solubleextractionresidue}{Initial\ mass} X\ 100 - - - 6$$

ii. Methanol soluble: Followed the same procedure previously performed in the above section (water-soluble extractive) using 100 mL methanol. The residues were collected and weighed immediately. Calculated the percentage w/w of extractive using equation 7 [22].

$$Methanol\ soluble\ extractive\ (\%) = \frac{Initial\ mass\ -\ Mass\ of\ alcoholsoluble\ extractionresidue}{Initial\ mass} X\ 100 - - - 7$$

f) Fluorescence analysis

It was performed on powdered plant extracts under UV light to analyse the fluorescence characteristics. A small quantity of dry plant extract powder was placed on a grease-free clean microscopic slide, and 1-2 drops of freshly prepared reagent solution were added, and mixed by gently tilting the slide and waiting for a few minutes. Then the slide was placed inside the UV chamber and observed the colour in visible light, short (254 nm) and long (365 nm) UV radiations [23–25].

g) High-performance thin-layer chromatography

The HPTLC is a general method to analyze plant drugs and had been used for varied applications. It is an essential tool, not only for the quality control of medicinal plants but also for the analysis of herbal drugs. A Silica gel 60 GF254 pre-coated aluminium plate was coated with each plant's alcoholic extract and standard solution using an applicator (Linomat-IV). The plates were generated in a glass tank employing a toluene: ethyl acetate (93:7) mixture that contained chromatographic solvents for specific plant components at room temperature (28°C). To accomplish efficient separation, the mobile solvents' composition was optimized. Each plate was kept in the horizontal direction and carefully dried first in the air and later in an oven at a temperature of 105ºC ± 5ºC for 5 min. Further, each plate was observed visually under UV light in the UV chamber at short wave (254 nm) and long wave (366 nm). The separation pattern and Rf value (Equation 8) were observed and carefully noted [26,27].

$$RF\ value = \frac{Distance\ travelled\ by\ solute\ from\ the\ origin}{Distance\ travelled\ by\ sovent\ from\ the\ origin} - - - -8$$

h) Fourier transform infrared (FTIR) spectroscopy
Every single extract of a plant was run through an individual FTIR for qualitative component identification. 10 mg of KBr (potassium bromide) pellets were ground up with 1 mg of the dry extract. The mixture was fed onto an FTIR spectroscope (Agilent Technologies Cary 650 FTIR), which scanned the sample with a resolution of 4 cm^{-1} over a range of 500 to 4000 cm^{-1} [28–30]. Pharmacological evaluation Anti-amoebic activity Parasite culture *E. histolytica* HM-1: IMSS strain was mixed with DMSO to prepare a 5% v/v culture medium, and the trophozoites were subcultured for a week thrice. To determine the sensitivity of the parasites to *T. indica* and *C. dactylon* extract, trophozoites were exposed to both extracts in the logarithmic growth phase [31].

2.3. Statistical analysis

The statistical analysis was performed using independent sample *t*-test. The proportion of live and dead cells was determined by counting 100 cells directly in a hemocytometer. The % growth inhibition was carried out by comparing the survival of the parasites in the treated tubes concerning untreated controls. The linear probit model developed the probit analysis from the log concentration-response relationships [31].

2.4. Pharmacological evaluation

2.4.1. Anti-amoebic activity parasite culture

E. histolytica HM-1: IMSS strain was mixed with DMSO to prepare a 5% v/v culture medium, and the trophozoites were subcultured for a week thrice. To determine the sensitivity of the parasites to *T. indica* and *C. dactylon* extract, trophozoites were exposed to both extracts in the logarithmic growth phase [31].

2.4.2. Inhibition assays

The antiprotozoal activity of each extract was examined by micro-assay technique. As a positive control, methanolic extract (MeE) (1 mg/mL) was added to dimethyl sulfoxide (DMSO) to create a 5% v/v medium with inoculum. The solution was sterilized by running it via a nylon membrane filter with a pore size of 0.22 µm (Hawach Scientific, China), and it was then kept at 4°C until use, protected from light. By using 500 mL of the stock solution and an equal amount of sterile water, successive dilutions were performed. 0.031, 0.062, 0.125, 0.25, and 0.5 mg/mL were the final concentrations made. 50 µL of stock solution was then transferred to each culture tube, which was then incubated at 36.5°C for 72 h with 1 mL of *E. histolytica* trophozoites deposited in the log phase. The culture tubes were made by Fisher Scientific International, Inc. A positive control was each extract, while a negative control was 5% DMSO. The bioassays were carried out three times. After the period of incubation, the tubes were chilled in ice water for 15 min, and a hemocytometer was used to count the total quantity of trophozoites per mL for each tube. (Ajanta Export Industries, India) [32].

3. RESULTS

3.1. Pharmacognostic evaluation

3.1.1. Preparation of extracts of selected plants

The extraction of *T. indica* (leaves) and *C. dactylon* (whole plants) showed maximum percent yield with methanol, whereas lowest in chloroform. The summary of colour, % yield, and consistency of each successive solvent is given **in** Table 2. The results suggested that methanol was an effective solvent as it produced a higher percentage yield than other solvents due to its high polarity.

3.1.2. Preliminary phytochemical screening of various extracts

Chemical assays were used to identify the phytoconstituents (PCs), as stated in Table 3. The preliminary qualitative phytochemical investigation of various extracts of *T. indica* (leaves) indicated the presence of all PCs except steroids and amino acids in MeE. In addition, flavonoids were also absent in aq. Extract. While MeE of the whole plant of *C. dactylon* showed carbohydrates, flavonoids, tri-terpenoids, phenol, and tannins were present, and in aqueous extract (AqE) carbohydrates, flavonoids, proteins, phenols, and tannins were present. These PCs' presence has physiological and therapeutic effects.

3.1.3. Evaluation of each plant extract's quality control criteria

Table 4 provides a summary of the findings from the quality evaluations of several plant extracts. The above-mentioned findings confirmed that given extractive and ash values, which revealed quality and purity, and low LOD, which suggested less microbial growth. The quality control test therefore passed and was taken into consideration for the development of the final formulation.

Table 2. Yield of extracts in a successive solvent.

	Petroleum ether (40-60º C)	**Chloroform**	**Methanol**	**Aqueous extract**
Parameters	***T. indica***			
Color Consistency Yield (%W/W)	Yellow	Green	Green	Green
	Greasy	Sticky	Sticky	Sticky
	11.6	3.8	22.2	15.8
	C. dactylon			
	Yellow	Green	Brown	Brown
	Greasy	Sticky	Sticky	Sticky
	3.61	1.83	8.11	7.85

Table 3. Phytochemical analysis of particular plants.

Phytochemicals	**Solvents**			
	Methanol		**Aqueous**	
	T. indica	***C. dactylon***	***T. indica***	***C. dactylon***
Alkaloids	+	-	-	-
Carbohydrates	+	+	+	+
Glycosides	-	-	-	-
Flavonoids	+	+	-	+
Tannins & phenols	+	+	+	+
Steroids	-	-	-	-
Triterpenoids	+	+	-	-
Saponins	-	-	-	-
Proteins	-	-	+	+
Amino acids	-	-	-	-

(+) = Present; (-) = Absent

Table 4. Evaluation of each plant's extract for quality.

S. No.	Study parameters	*T. indica* (leaves)	*C. dactylon* (whole)
	LOD & Ash value		
1.	LOD (%)	8.1	6.38
2.	Total ash (%)	9.8	9.89
3.	Acid insoluble ash (%)	3.6	3.02
4.	Water soluble ash (%)	5.5	7.46
	Extractive value		
5.	Water soluble	15.8	18.54
6.	Methanol soluble	22.2	12.60

LOD: Loss on drying

3.1.4. Fluorescence analysis

The fluorescence analysis was performed at long and short wavelengths of UV light. A summary of the results is given in Table 5. The fluorescence analysis confirmed the various chemical constituents present in the plant extracts based on fluorescent properties.

3.1.5. High-performance thin-layer chromatography

The HPTLC was performed using a suitable mobile phase to optimize better separation and was visualized under UV light at 254 and 366 nm. The results obtained from this evaluation are depicted in Figure 1 for *T. indica* and *C. dactylon.* Plant extracts' HPTLC profiles revealed that they contained a variety of chemically active components. The intense peaks on the graph indicated the presence of a flavonoid functional group in herbal plant extracts which has potent ant-amoebic activities. Furthermore, it demonstrated that the extract came from a real plant or from plant components that contained secondary metabolites.

3.1.5. Fourier transform infrared (FTIR) spectroscopy

Each plant extract's FTIR spectrum was recorded between 4000-500 cm^{-1} (Figure 2a, b). The prominent peaks showed that specific plant extracts contained the functional groups C-H, O-H, N-H, C=C, C=O, and CH_3. As a result, the FTIR analysis verified a trustworthy and accurate technique for identifying biomolecular composition. The bands of the FTIR analysis were recorded (in cm^{-1}) as following:

T. indica: 3210.04 (OH st.), 2916.78, 2830 (symm. and asymm. CH St.), 2173.79, ($C\equiv C$ st.), 1750 (C=O st.), 1498.11 (Ar. St.).

C. dactylon: 3402.2 (NH st.), 2850 (CH st.), 2215 ($C\equiv C$ st.), 1419 (Ar. St.).

3.2. Pharmacological evaluation

3.2.1. Anti-amoebic activity

The methanol extracts from *T. indica* and *C. dactylon* were able to inhibit trophozoite growth *in vitro* cultures under axenic conditions, according to the activity assays against *E. histolytica* (Figure 3 and Figure 4) demonstrate that when extract concentrations rise, the inhibition percentage also rises. Additionally, a positive control comprised of culture medium, inoculum, and each plant extract at 1 mg/mL as well as a blank control including the culture medium and inoculum were examined. The negative control had a culture medium with 5% DMSO infected with *E. histolytica*. The results

demonstrated that viability reduced as extract concentration increased. The inhibition for *T. indica* and *C. dactylon* was 93.5% and 92.4%, respectively, at a 500 µg/mL concentration. The extract nevertheless exhibited 18.8% and 14.4% inhibition for *T. indica* and *C. dactylon*, respectively, even at the lowest test dose of 31.3 µg/mL. The positive control reported 98.2% and 97.7% inhibition for *T. indica* and *C. dactylon*, respectively, whereas the negative control and inoculum control did not affect the parasite.

The probit analysis (Figure 5) revealed that *T. indica* and *C. dactylon's* methanolic extract had IC50 of 48.982±0.88 µg/mL and 49.71± 0.383 µg/mL respectively. Based on the above study, the results indicated that both extracts showed good activity against *E. histolytica* trophozoites. The potent activity of both plant extracts was the due presence of active chemical constituents.

Table 5. Fluorescence analysis of different extracts.

Type of extract	Light passed	***T. indica*** (leaves)	***C. dactylon*** (whole)
Methanol	Daylight	Green	Brown
	UV short light	Deep green	Deep brown
	UV long light	Brown	Black
Aqueous	Daylight	Green	Brown
	UV short light	Deep green	Deep brown
	UV long light	Brown	Black

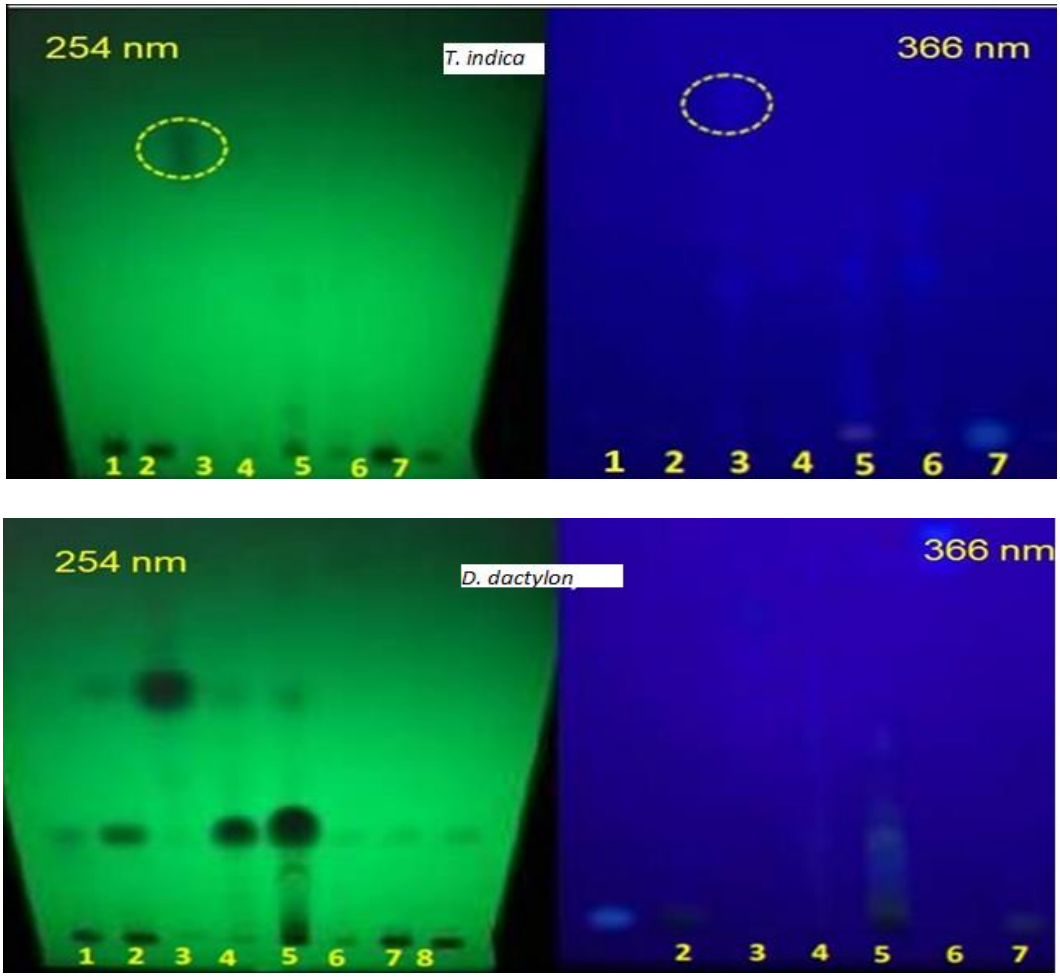

Figure 1. Photographs of HPTLC plates spotted with MeE of *T. indica* and *C. dactylon* under UV light at 254 and 366 nm respectively.

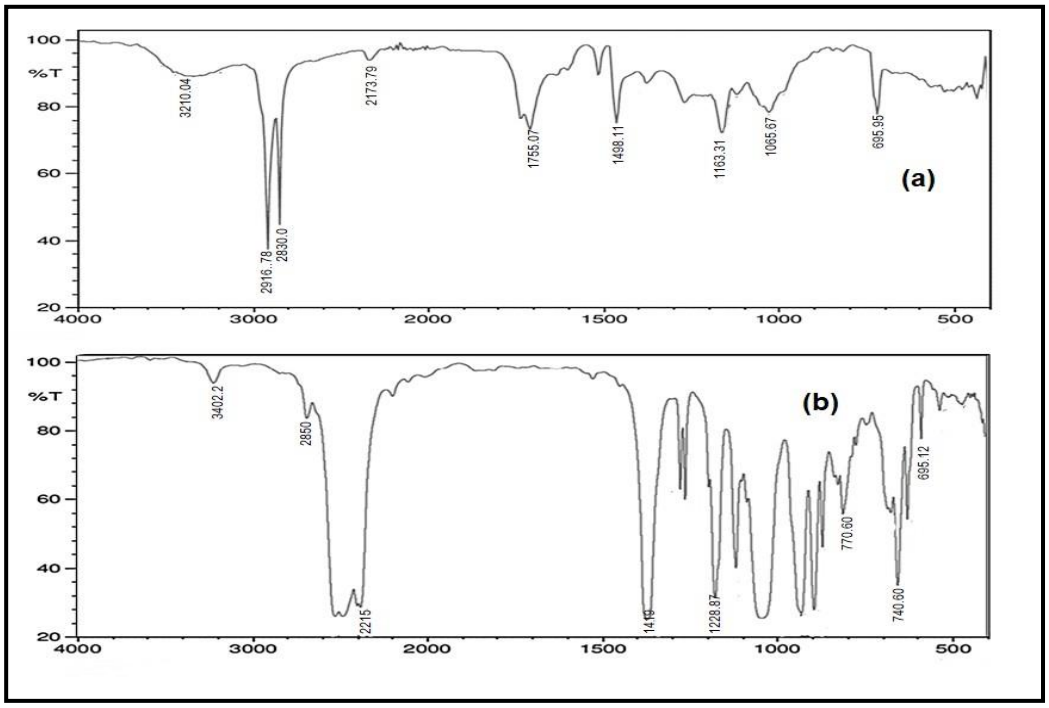

Figure 2. FTIR spectra of plant extracts a) *T. indica* and b) *C. dactylon*.

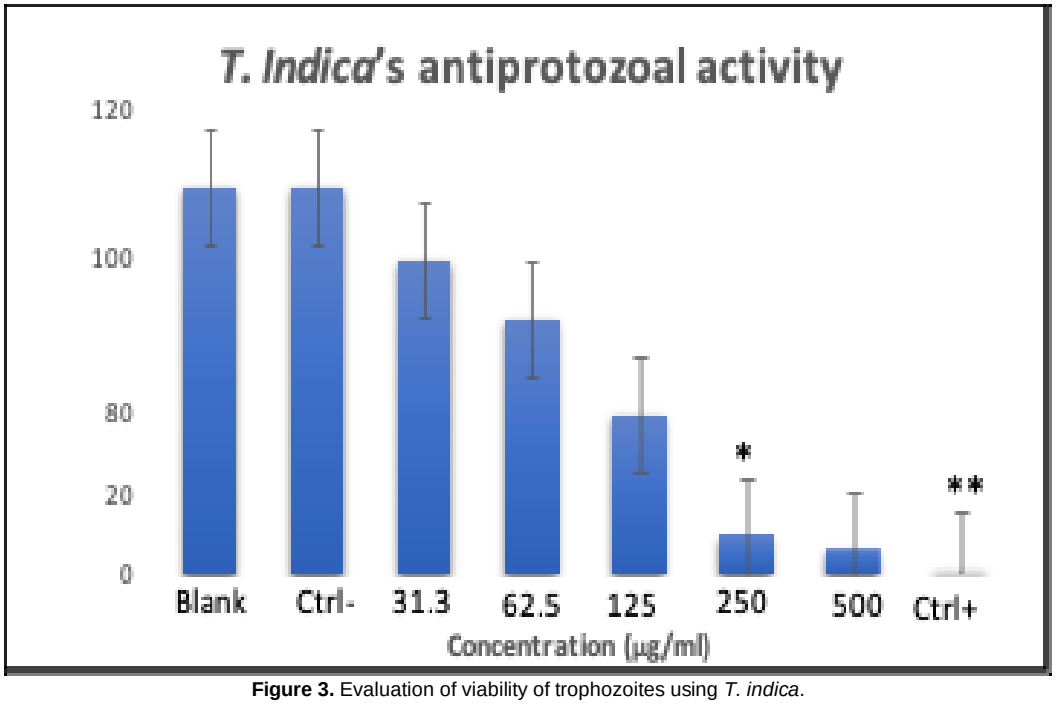

Figure 3. Evaluation of viability of trophozoites using *T. indica*.

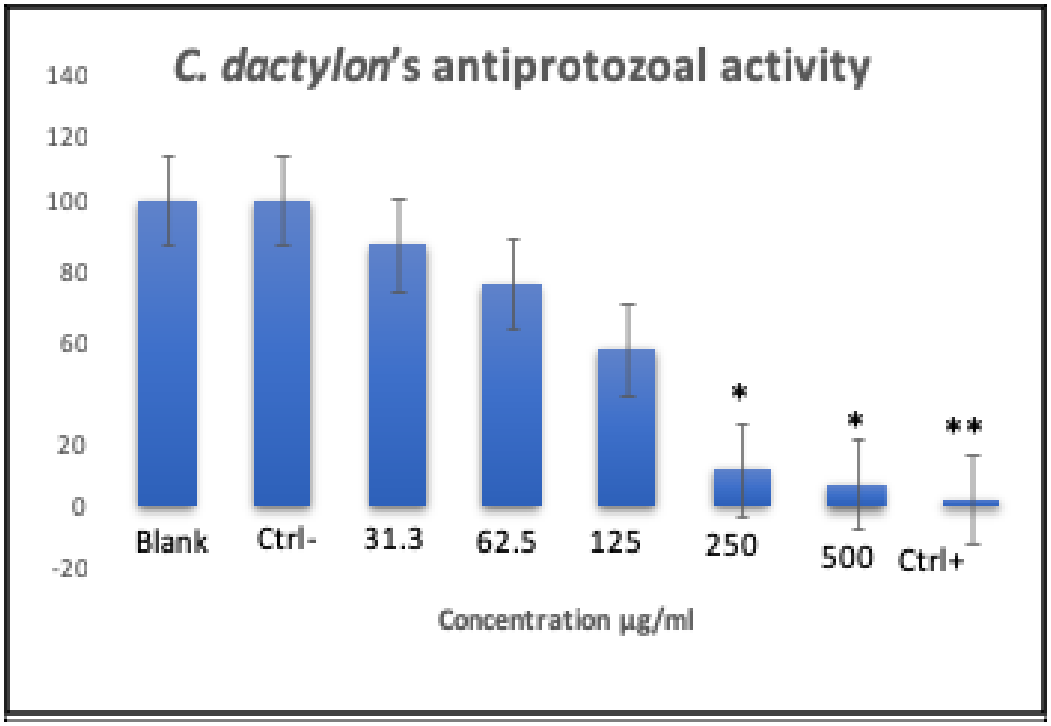

Figure 4. Evaluation of viability of trophozoites *C. dactylon.*

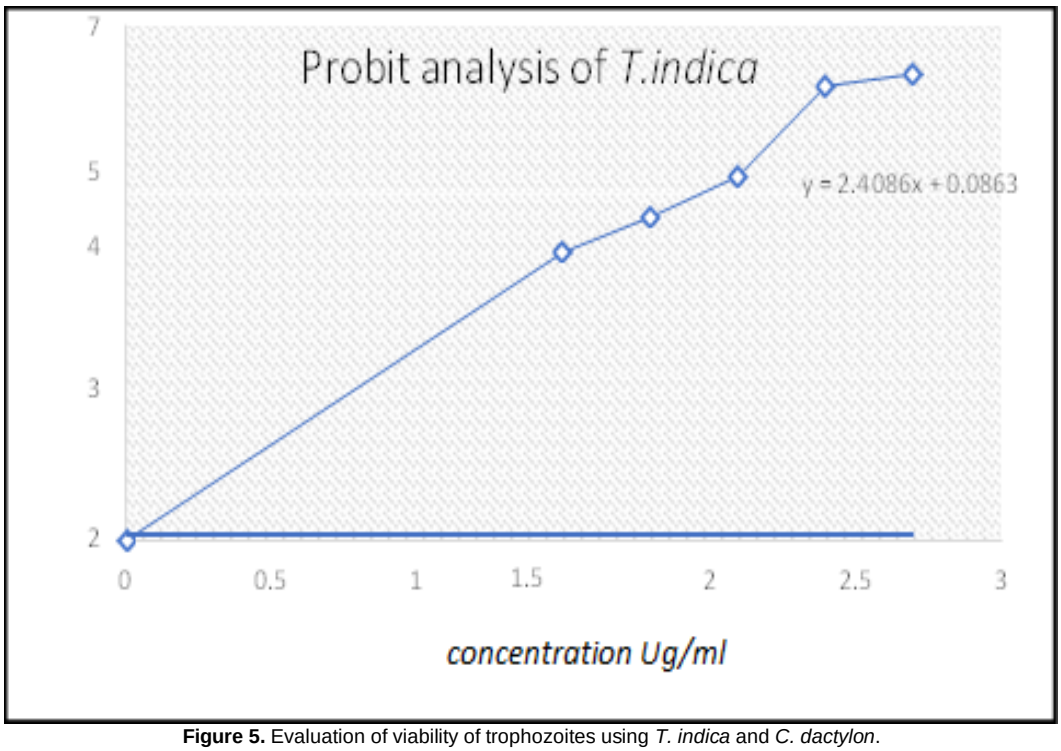

Figure 5. Evaluation of viability of trophozoites using *T. indica* and *C. dactylon*.

4. DISCUSSION

Numerous health issues have been brought about by the widespread spread of bacterial and fungal infections, particularly in nations that did not adhere to infection control recommendations. The development of antimicrobial medicines and their discovery are often carried out in extremely slow steps, which is inconsistent with the rate of evolution of pathways of antibiotic resistance to widely used drugs. In the same situation, the therapeutic substitution of previously used medications and the resurgence of interest in Plants could make up for the disparity in approaches to this problem. Thus, the utilization of complementary and alternative therapies, particularly those involving natural substances and vital Oils are the best option, when used with discretion, to avoid the aggravating of this disaster. It is necessary to employ medicinal plants [33].

The presence of phytoconstituents (such as flavonoids, carbohydrates, phenols, and tannins) in the extracts of *T. indica* and *C. dactylon* suggests potential physiological and therapeutic effects, which can contribute to the medicinal value of these plant extracts. HPTLC profiles showed the presence of several active ingredients, including flavonoids, which are well-known for their strong anti-amoebic properties. The chemical complexity and diversity of the plant extracts, as well as their possible medicinal uses, are confirmed by the distinct separation and strong peaks seen in the HPTLC profiles. Key functional groups (such as C-H, O-H, N-H, C=C, C=O, and CH_3) in the plant extracts were identified by FTIR spectroscopy, with spectra recorded between 4000-500 cm^-1. The same results were obtained in study the effect of extract on parasitic haemnochus [34].

Significant anti-amoebic action was demonstrated by the methanol extracts of *T. indica* and *C. dactylon* against trophozoites of *E. histolytica*. The results of the investigation showed that trophozoite growth was inhibited in a dose-dependent manner; for both plants, the maximum concentration (500 µg/mL) achieved over 93% inhibition. Strong anti-amoebic effects were identified even at the lowest concentration (31.3 µg/mL), indicating substantial inhibition. The effectiveness of the extracts was demonstrated by the negative control, which had no discernible impact, while the positive control verified high inhibition rates. According to Probit analysis, *T. indica* and *C. dactylon*. These results indicate that the active chemical ingredients in the products had strong anti-amoebic activity, the same results observed with the study of ethanol extract of *T. indica* [35].

5. CONCLUSION

According to above results we can concluded that serial extraction using Soxhlet equipment revealed that methanol, due to its high polarity, had a superior yield compared to chloroform. Furthermore, phytochemical screening, along with fluorescence analysis, HPTLC, and FTIR, confirmed the chemical composition and therapeutic potential of the extracts. Finally, the methanolic extracts of T. *indica* and C. *dactylon* showed significant inhibitory activity against *E. histolytica*, suggesting their potential as traditional treatments for amebiasis.

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CONFLICT OF INTEREST STATEMENT

The author declares no conflicts of interest.

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