International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol. 3, No.2, pp 1113-1117, April-June 2011

Plant Extracts with Antiamoebic Properties: A Theoretical Study with reference to *Entamoeba histolytica*

Dolly Rani*

Plant Biotechnology Lab, Department of Botany, Dayalbagh Educational Institute (Deemed University), Dayalbagh, Agra, 282110, India.

Corres.author: 12.dolly@gmail.com,Phone no.- 09457872407

Abstract: Indigenous plant remedies are widely used in the treatment of variety of disorders because these products are safe, widely available at low cost, and easy to access. Developing countries are severely threatened by amoebic diseases mainly caused by *Entamoeba histolytica*. Drugs available have been reported to be mutagenic so an alternate source of medicines is highly required. Plant extracts have found to possess antiamoebic properties which could effectively cure amoebic diseases. Active molecules from these potential plants can be widely used to cure amoebic diseases. These molecules might have more potential to treat amoebic diseases and have lesser chances to develop resistance and are less prone to develop mutagenic effects in host.
Key words: *Entamoeba histolytica*, plant extracts, IC_{50}, MIC.

Introduction and Study:

Developing countries are often victim of morbidity and mortality due to enteric protozoan infections and are an important health problem worldwide. Diarrhoea and dysentery are mostly common where living conditions are crowded and hygiene is poor. Dysentery is caused by the microbial infection in the gastrointestinal tract. Fever, vomiting, abdominal pain and diarrhea which often contain blood and pus are obvious symptoms. The onset of the disease usually occurs between 2-3 days after infection and lasts for up to several weeks. Dehydration occurs rapidly, especially in children and can cause death if treatment is not given. In developing countries, over five million children under the age of five die annually from severe diarrhoeal diseases (1). Rural people often lack access to formal and adequate health services and thus people depend on traditional healers who provide alternative health care services. Amoebiasis is predominant particularly in areas of Asia, Africa and South America. Protozoan parasites including *Entamoeba histolytica*, *Giardia lambia*, *Cryptosporidium parvum* and some other spore forming protozoa that can parasitize human intestine and affects approximately 25% of the world population (2). Of all the protozoan infections, amoebiasis is most severe human disease only next to malaria (3). According to current estimates almost 50 million people all over the world are affected which results in about 40,000-100,000 deaths annually mainly in tropical and subtropical countries (4). Of all the protozoans about 10 % of the world's population is infected with *Entamoeba histolytica* alone and most of the infections do not show any visible symptoms. Of these, 80-90 % exhibit symptoms of dysentery with amoebic infection of the intestinal mucosa (5). *Entamoeba histolytica* is a parasite with great invasive and evasive potential which allows it to penetrate and move in tissues and can also successfully paralyze immune system of host (6). Ingestion of cysts results in infection and excystation occurs in the small intestine, releasing trophozoites. They multiply in the colon by binary fission. Occasionally, the trophozoites can further migrate to the liver, lung, brain and skin by

hematogenous dissemination which develops abscess and potentially serious symptoms. There are currently no vaccines available to control the infection caused by *E. histolytica* (7). The knowledge about molecular biology of this parasite and the mechanisms which regulate its pathogenecity is restricted (8). In recent years there has been a rise in the number of reports of amoebic brain abscess. Metronidazole is considered as highly effective amoebicide and is often used for the treatment of amoebiasis. Metronidazole produces mutagenic effects in the microbiological system and is also seen to be carcinogenic to rodents (9). The use of metronidazole is also known to produce several side effects as nausea, vomiting, dry mouth, metallic taste, abdominal pain, headache, constipation and diarrhea. The use of drugs also exhibit lower immune response which can be both cell and humoral mediated in recipients (10). This can also quite possibly lead to drug resistance as demonstrated in other protozoan parasites (11). Even in some reports the *in vitro* generation of strains resistant to metronidazole has been reported (12). In spite of the tremendous use of metronidazole, currently used treatments for treating diseases caused by *Entamoeba* are insufficient to provide complete protection and hence continuous research for discovering and developing novel antiamoebic agents is required so that the limitations prevalent can be overcomed and more safer, effective antiamoebic drugs or vaccines can find applicability to minimize the treat posed by *E. histolytica* (9).

Medicinal plants are an important health resource to many Indian communities and in many regions of the world. Indigenous plant remedies are widely used in the treatment of variety of disorders because these products are safe, widely available at low cost, and easy to access. However, only a few of them have been tested clinically or studied chemically to identify their active constituents. Medicinal plants are considered as an important source of potentially useful structures for the development of new chemotherapeutic agents. Crude drugs are usually the dried parts of medicinal plants (roots, stem wood, bark, leaves, flowers seeds, fruits, and whole plants etc.) that form the essential raw materials for the production of traditional remedies of Ayurveda, Siddha, Unani, Homeopathy, Tibetan and other systems of medicine including the folk, ethno or tribal medicines. The crude drugs are also used to obtain therapeutically active chemical constituents by specialized methods of extraction, isolation, fractionation and purification and are used as phytochemicals for the production of modern allopathic medicines or herbal/phytomedicines (13, 14). Successful strategies for investigating these preparations involve the selection of test crude extracts based on a combination of ethnopharmacology and daily healer's practices.

Screening of plant extracts for antiamoebic activity:

The antiamoebic effect of a crude drug formulation was explored against *E. histolytica*. This formulation has been earlier used in traditional system of medicine in India for effective use against intestinal disorders. The formulation comprised of five medicinal herbs, namely, *Boerhaavia diffusa*, *Berberis aristata*, *Tinospora cordifolia*, *Terminalia chebula* and *Zingiber officinale*. The dried plants were extracted in ethanol together and individually. Further *in vitro* amoebicidal activity was studied to determine minimum inhibitory concentration (MIC) values of all plant extracts as well as the whole formulation. The formulation had a MIC of 1000µg/ml as compared with 10µg/ml for metronidazole (14).

Active principle from root bark of *Adina cordifolia* was extracted in benzene and ethyl acetate and was seen to exhibit antiamoebic activity with IC_{50} values of 2.92 and 2.50µg/ml, respectively. In an attempt to identify a compound rendering antiamoebic activity bioassay-guided fractionation of benzene and ethyl acetate extracts was done which led to the isolation of 7-hydroxycoumarin (umbelliferone 1)and 7-b-D-glucosylcoumarin (skimmin 2),respectively. Further, Umbelliferone 1 was converted into 7-hydroxy-8-acetylcoumarin 2a in a two step conversion. A new series of thiosemicarbazones 3a-e of 7-hydroxy-8-acetylcoumarin with different thiosemicarbazides were synthesized. Umbelliferone was also converted into its methoxy derivative (7-methoxycoumarin 4). All the compounds synthesized were assessed for antiamoebic activity against HM1: IMMS strain of *E. histolytica*. Umbelliferone and skimmin were found to possess a very good activity with IC_{50} values of 6.38 and 4.35 µM/ml, respectively. These results indicate that umbelliferone and skimmin may be a potential compound for the development of new antiamoebic drugs (9).

Medicinal plants are very actively used in many regions of Americas and in India they serve a greater role. Ethanobotanical survery in Mexico led to identification of 29 medicinal plants which could possibly posses' antiamoebic activity. The significant results obtained were in case of *Annona muricata*, *Castela texana*, *Chenopodium graveolens*, *Gouania polygama* and *Quercus oleoides*. *Castela texana* and *Annona muricata* which showed activity at a comparatively lower concentration of 63 µg/ml and 31-63 µg/ml, respectively in crude and Ch_2Cl_2 fraction (15).

In an extensive study where 45 plants used in traditional medicine of some Congolese plants, as high as 35 plants were found to exhibit in vitro antiamoebic activity and only 10 showed no activity. The significant results were with extracts obtained from root bark of *Paropsia brazzeana*, *Cryptolepis sanguinolenta*, *Alchornea cordifolia*, *Hensia pulchella*, *Maprounea africana*, *Rauwolfia obscura* and *Voacanga africana*, leaves and stem bark of *Psidium guajava*, stem bark of *Dialum englerianum*, *Harungana madagascariensis* and *Mangifera indica*, mature seeds of *Carica papaya*, and leaves of *Morinda morindoides* and *Tithonia diversifolia*. The MIC value found was fairly low and was found to be 100 mg/ml. Metronidazole was used as a reference drug which showed highly significant activity than that of all plant extracts tested (16).

To use ethnobotanical knowledge in practical inquiries among traditional healers, community leaders, and native people of that particular area is important. In this particular study ethanobotanical survey in Lomela villages in Congo were done. Six medicinal plants namely *Epinetrum villosum*, *Roureopsis obliquifoliolata*, *Croton mubango*, *Cissus rubiginosa*, *Vernonia amygdalina* and *Quassia africana* that were used widely used in this region were identified ones having antidysenteric and antidiarrhoeal properties. All the six chosen plants were screened for its antiamoebic potential and also were tested for phytochemical compounds rendering them antiamoebic activity. Phytochemical screening of extracts revealed the presence of tannins, alkaloids, saponins, flavonoids, sterols and/or triterpenes and reducing sugars. Out of the six plants only two acted against *Entamoeba histolytica*. The prominent results were in case of those are where *Epinetrum villosum* and *Quassia africana* (17).

Hexane, ethanol and water extracts of plants used by South African traditional healers for treating stomach ailments were tested for having anti-amoebic activities. A microdilution technique was employed to investigate anti-amoebic activity against the enteropathogenic *Entamoeba histolytica*. These assays were suitable for the screening of a large number of extracts at one time. Several plants exhibited significant activity against these test organisms (18).

Forty-two extracts from 21 genera (18 families) were assayed in a similar experiment. *B. latifolia*, *C. inerme*, *C. pulchella*, *Ekebergia capensis*, *H. trifoliata*, *K. africana*, *Leonotis leonurus*, *L. japanica*, *Senna didymobotrya*, *Spirostachys africana*, *Tecomaria* and *T. orientalis* did not exhibit activity in both ethanol and water extracts. Reason may be attributed to the fact that the extracts of many plant species were inactive, attributing this to the selectivity of the anti-amoebic test (19). The greatest activity was found in methanol extracts and even water extracts of the same plants displayed less activity. The ethanolic extracts of *A. calamus* and *S.birrea* were more active than water extracts. In total, eight ethanol extracts showed anti-amoebic activity while five water extracts were active. The high activity of *A. calamus* may be attributed to the presence of the toxic phenylpropanoid b-asarone. Other plants having significant activities were *A. adianthifolia*, *D. oblongifolia* and *S. birrea*. Several compounds have been successfully isolated from the bark and roots of *A. adianthifolia* (20) but little work has been reported on the leaves. The bark of *S. birrea* contains tannin and traces of alkaloids (18).

Another experiment was performed at North Eastern Mexico with *Lepidium virginicum* L. (*Cruciferae*), *Acacia farnesiana* L. (*Leguminosae*), *Acacia rigidula* Benth(*Leguminosae*), *Parkinsonia aculeate* L., *and Carlowrightia cordifolia* were included in the study. All aqueous extracts tested inhibited the growth of *E. histolytica* trophozoites. The highest activity was detected in leaf extracts with the only exception of *L. virginicum*, in which stem extracts were more effective than leaves. *Acacia rigidula* showed pronounced activity than all plants included in this study. It is important to emphasize that activity was tested in aqueous extracts because teas or infusions from whole plants or their components are an extended practice to treat amoebiasis in folk medicine around the world. The potential plants could be used as infusions which would in turn cut down the cost of medication (21).

The antiprotozoal activity of the dichloromethane–MeOH extract, fractions and pure compounds from the roots of *Geranium mexicanum* on *Entamoeba* was carried out. The result indicated that the extract, organic fraction and a pure flavonoid were active against *E. histolytica* with IC_{50} values ranging from 1.9 to79.2 µg/ml. The main active compound identified was the flavan-3-ol, (–)-epicatechin. In addition, the moderate active compounds (+)-catechin, tyramine and β-sitosterol 3-*O*- β -d glucopyranoside, also were isolated. These results support the traditional use of *Geranium mexicanum* roots in the treatment of diarrhoea and dysentery, illnesses caused by *E. histolytica* (22).

Twenty–six plants used in Mexican traditional medicine for the treatment of gastrointestinal disorders were selected and were extracted in methanol to test their efficacy against *E. histolytica* using *in vitro* tests. Significant antiprotozoal activity was observed in case of *Chiranthodendron pentadactylon*, *Annona*

cherimola and *Punica granatum* with IC_{50}<30 µg/ml. The potential of *Chiranthodendron pentadactylon* with IC_{50} value 2.5 µg/ml on *Entamoeba histolytica* was close that of to emetine, but far less than metronidazole, drugs used as control. The results of the antiprotozoal screening support the popular uses of the studied species for the treatment of diarrhoea and dysentery in Mexican traditional medicine (23).

Conclusion

Based on the above evidences and references it can be very well concluded that plant extracts possess a wide array of biological activities which can be easily used for the betterment of human beings. Many plants which were used in traditional medicine proved to be of immense importance and gave significant results in having antiamoebic activities. A detailed study of plants with antiamoebic properties should be done based on the ethnobotanical leads available. However these findings can only be of practical use when they would undergo stringent clinical trials.

Acknowledgement

I would like to thank Mr. Akash Singh Chauhan for helping out in the detailed literature survey.

References

1. WHO. WHO/PAHO/UNESCO report of a consultation of experts on amoebiasis. Week Epidem Rec. WHO 1997; 72, 97–99.
2. Petri, W. A.; Haque, R.; Mann, B. J. (2002). The bittersweet interface of parasite and host: Lectin-carbohydrate interactions during human invasion by the parasite *Entamoeba histolytica*. *Annu Rev Microbiol*. 56, 39–64.
3. Stanley, S. L. (2003). Amoebiasis. *The Lancet*. 361, 1025-1034.
4. Lotter, H.; Khajawa, F.; Stanley, Jr. S.L.; Tannich, E. (2000). Protection of gerbils from amebic liver abscess by vaccination with a 25-mer peptide derived from the cysteine-rich region of *Entamoeba histolytica* galactose-specific adherence lectin. *Infect Immun.* 68, 4416–4421.
5. Denis, M.; Chadee, K. (1988). Immunopathology of *Entamoeba histolytica* infections. *Parasitol. Today*. 4(9), 247-252.
6. Vega-Robledo, G. B.; Leandro, E.; Silva, R.; Olives, A.; Rico, G. (2005). Effect of Zinc Treated *Entamoeba histolytica* on the Human Polymorphonuclear Respiratory Burst. *Arch. Med. Res*. 36, 75–79.
7. Haque, R.; Ali, I. K. M.; Sack, R. B.; Farr, B.M.; Ramakrishnan, G.; Petri, Jr. W. A. (2001). Amebiasis and mucosal IgA antibody against the *Entamoeba histolytica* adherence lectin in Bangladeshi children. *J Infect Dis.* 183, 1787–1793.
8. Huber, M.; Garfinkel, L.; Gitler, C.; Mirelman, D.; Revel, M.; Rozenblatt, S. (1988). *Entamoeba histolytica:* cloning and characterization of actin cDNA. *Mole.Biochem. Parasitol.* 24, 227-235.
9. Iqbal, P. F.; Bhat, A.R.; Azam A. (2008). Antiamoebic coumarins from the root bark of *Adina cordifolia* and their new thiosemicarbazone derivatives. *European J. Medicinal Chemistry* xx, 1-8.
10. Saxena, A.; Chugh, S.; Vinayak, V. K. (1986). Antibody-dependent macrophage-mediated cytotoxicity against *Entamoeba histolytica. J. Med. Microbiol.* 22, 17-22.
11. Foley, J.E.; Harrus, S.; Polnad, A.; Chomel, B.; Pedersen, N.C. (1998). Molecular, clinical and pathologic comparison of two distinct strains of *Haemobartonella felis* in domestic cats. *Am. J. Vet. Res.* 59, 1581–1588.
12. Marchat, L. A.; Gomez, C.; Perez, D. G.; Paz, F.; Mendoza, L.; Orozco, E. (2002). The CCAAT/enhancer binding protein sites are cis-activator elements of the *Entamoeba histolytica* Ehpgp1 (mdr-like) gene expresion. *Cell. Microbiol.* 4, 725–737.
13. Alonso-Paz, E.; Cerdeiras, M. P.; Fernandez, J.; Ferreira, F., Moyna, P.; Soubes, M.; Vazquez, A.; Veros, S.; Zunno, L. (1995). Screening of Uruguayan medicinal plants for antimicrobial activity. *J. Ethanopharmcol.* 45, 67-70.
14. Sohni, Y. R.; Kaimal, P.; Bhatt, R. M. (1995). The antiamoebic effect of a crude drug formulation of herbal extracts against *Entamoeba histolytica* in vitro and in vivo. *J.Ethnopharmacol.* 45, 43-52.
15. Heinrich, M.; Kuhnta, M.; Wright, C.W.; Horst, R. J.; Phillipsonb D.; Schandelmaier, A.; Warhurst, D. C. (1991). Parasitological and microbiological evaluation of Mixe Indian medicinal plants (Mexico). *J. Ethnopharmacol.* 36, 81-85.
16. Tona, L.; Kambu, K.; Ngimbi, N., Cimanga, K.; Vlietinck, A.J.(1998). Antiamoebic and phyto - chemical screening of some Congolese medicinal plants. J. *Ethnopharmacol.* 61, 57–65.
17. Otshudi, A.; Vercruysse, A.; Foriers, A. (2000). Contribution to the ethnobotanical, phytochemical and pharmacological studies of traditionally used medicinal plants in the treatment of dysentery and diarrhoea in Lomela

area, Democratic Republic of Congo (DRC). *J. Ethnopharmacol.* 71, 411–423.
18. McGaw, L.J.; Jager, A.K.; Staden, J.V. (2000). Antibacterial, anthelmintic and anti-amoebic activity in South African medicinal plants. *J. Ethnopharmacol.* 72, 247–263.
19. Wright, C.W.; O'Neill, M.J.; Phillipson, J.D.; Warhurst, D.C. (1988). Use of microdilution to assess *in* v*itro* antiamoebic activities of *Brucea javanica* fruits, *Simarouba amara* stem, and a number of quassinoids. *Antimicrob. Agents and Chemother.* 32, 1725–1729.S
20. Hutchings, A.; Scott, A.H.; Lewis, G.; Cunningham, A.B. (1996). Zulu Medicinal Plants: An Inventory. University of Natal Press, Pietermaritzburg.
21. Cruz-Vega, D.E.; Aguilar, A.; Castro-Garza, J.; Gonzalez-Garza, M.A. (2000). Antiamebic Activity of Aqueous Extracts from Five Medicinal Plants from Northeastern Mexico. *Arch. Med. Res.* 31, S17–S18.
22. Calzada, F.; Cervantes-Martinez, J.A.; Yepez-Mulia, L. (2005). *In vitro* antiprotozoal activity from the roots of *Geranium mexicanum* and its constituents on *Entamoeba histolytica* and *Giardia lamblia*. *J. Ethnopharmacol.* 98, 191–193.
23. Calzada, F.; Yepez-Mulia, L.; Aguilar, A. (2006). *In vitro* susceptibility of *Entamoeba histolytica* and *Giardia lamblia* to plants used in Mexican traditional medicine for the treatment of gastrointestinal disorders, *J. Ethnopharmacol.* 108, 367-370.

*****