Trop J Nat Prod Res, March 2021; 5(3):555-558 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

In Vitro Antiamoebic Activity of *Cnestis ferruginea* and *Dianthus basuticus* on *Entamoeba histolytica* Trophozoites

Teslim O. Ojuromi[1], Tom A. O. Ashafa[1], Jesus N. Garza-Gonzalez[2], Francisco Gonzalez-Salazar[2,3]*

[1]*University of the Free State Qwaqwa Campus, Faculty of Natural and Agricultural Sciences, Department of Plant Sciences, Phuthaditjhaba, 9866, South Africa*
[2]*Departamento de Ciencias Básicas, Universidad de Monterrey, Av Ignacio Morones Prieto 4500, San Pedro Garza García, 66238, México*
[3]*Centro de Investigaciones Biomédicas Del Noreste, Instituto Mexicano Del Seguro Social, Monterrey, 64720, México*

ARTICLE INFO

Article history:
Received 27 January 2021
Revised 23 february 2021
Accepted 12 March 2021
Published online 01 April 2021

Copyright: © 2021 Teslim *et al*. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

ABSTRACT

Amoebiasis is still a great disease burden in developing countries; this may cause around 100,000 deaths every year worldwide. The appearance of the strains of parasites resistant to conventional drugs used to treat this disease compels the search for new antiparasitic agents. As a result, a growing emphasis has been placed on plants used in traditional medicine to discover new agents with antiparasitic activity. The study determined the antiamoebic activity of *Cnestis ferruginea* and *Dianthus basuticus* found in Nigeria and South Africa. The assays were performed on cultures of *Entamoeba histolytica* growing in the logarithmic phase that were exposed to various concentrations of the ethanol extract of *D. basuticus* and *C. ferruginea* plants. The probit of the cultures were calculated, and the IC_{50} was determined for each extract for antiamoebic activity. The ethanol extract of both *C. ferruginea* and *D. basuticus* demonstrated a remarkable *in-vitro* inhibitory activity against *E. histolytica* trophozoites. The 50% inhibitory concentrations (IC_{50}) of *C. ferruginea* and *D. basuticus* on *E. histolytica* cultures were 15.07 µg/mL and 12.45 µg/mL, respectively. The results showed that *D. basuticus* and *C. ferruginea* have inhibitory activity against *Entamoeba histolytica.*

Keywords: *Dianthus basuticus*, *Cnestis ferruginea*, Connaraceae, Dianthus, *Entamoeba histolytica*, Amoebiasis.

Introduction

Gastrointestinal infections are considered a big burden in public health, impacting people mainly in developing countries.[1] Even though worldwide deaths due to diarrhoea have lessened in the last 25 years, this is still a great cause of death. Diarrhoea was reported as the leading cause of death throughout the world in 2015 among all ages.[2] Most of these cases of diarrhoea and correlated deaths occur in sub-Saharan Africa.[3] The most common agents correlated with gastrointestinal infections are the bacteria *Escherichia coli* and *Salmonella typhi*, parasites including *Giardia duodenalis* and *Entamoeba histolytica*, and viruses such as rotavirus and adenovirus.[4] Amoebiasis due to protozoan parasite *E. histolytica* is related to increased morbidity and mortality globally, and it has become a serious public health problem in developing countries.[5] It is known that, worldwide, approximately 50 million people are infected by *E. histolytica* and around 100,000 deaths occur yearly due to amoebiasis.[6] Following malaria, amoebiasis is the second cause of death due to parasitic diseases.[7] Traditionally, the drugs used to treat amoebiasis are 5-nitroimidazoles derivatives, including metronidazole or tinidazole.[8] Presently, there are reports that *E. histolytica* strains have an increased resistance to metronidazole and 5-nitroimidazoles, posing a serious problem.[9] This has resulted in a heightened interest in the discovery as well as development of new compounds to treat these diseases.[10] A feasible alternative to tackle this problem are medicinal plants which are considered important sources for the development of new chemotherapeutic agents.[11] In South Africa, one of the plants used in the management of diabetes and other sugar-related conditions is *Dianthus basuticus*. *Dianthus basuticus* Burtt Davy is known as Hlokoa-la-tsela and Lesotho Dianthus, in Sesotho and English, respectively. It is widely distributed in several regions of South Africa especially the Free State Province and Lesotho.[12] Among the Basotho people, the plant is used as an immune modulator, for flatulence and to increase the fertility of bulls. It is also widely used in the management of chest pains and mumps.[13] On the other hand, *Cnestis ferruginea* DC (Connaraceae), a shrub or a tree growing to approximately 6 m tall, is widely distributed throughout tropical Africa. The plant is widely used in traditional medicine in Africa. The scarlet fruit of the plant adds to its value as an ornamental plant (Burkill). This plant has been used traditionally to treat conjunctivitis, syphilis, gum pain, wounds, dysentery, and gonorrhoea. [14, 15] The fruit is used in Nigeria to treat gingivitis and *Streptococci* infections of the mouth. Its root is used as laxative, and the stem is rubbed on the skin as well as used to treat throat infections. The plant has been reported to have bioactive compounds which inhibit bacterial growth.[16] According to the several studies, *C. ferruginea* is a potent antioxidant, anti-inflammatory, analgesic, antimicrobial, laxative, anti-convulsant, aphrodisiac, hypoglycaemic, and hepatoprotective agent,[14-17] but an antiprotozoal activity has not been reported.

The study evaluated the *in vitro* inhibitory activity of the *D. basuticus* and *C. ferruginea* ethanol extracts and of some of its components against *E. histolytica* trophozoites.

*Corresponding author. E mail: fgonz75@hotmail.com
Tel: 52- 81- 82533190

Citation: Ojuromi OT, Ashafa AOT, Garza-Gonzalez JN, Gonzalez-Salazar F. *In Vitro* Antiamoebic Activity of *Cnestis ferruginea* and *Dianthus basuticus* on *Entamoeba histolytica* Trophozoites. Trop J Nat Prod Res. 2021; 5(3):555-558. doi.org/10.26538/tjnpr/v5i3.22

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Materials and Methods

Chemicals and stock solutions

All chemicals were of analytical grade. DMSO and the standard drug (Metronidazole) were acquired from Sigma Chemical Co. (St. Louis, MO, USA). The other chemicals used in this study were bought from J. T. Baker (Xalostoc, Edo. de Mexico, Mexico). Sterile bovine serum and peptone, liver, and pancreas extract and serum (PEHPS) medium were

© 2021 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

prepared in our laboratory as described elsewhere.[18] Metronidazole (Sigma Chemical Co.) was dissolved in DMSO to get 500 µg/mL concentration and was further diluted, adding 500 µL in 10 mL of dimethyl sulfoxide (DMSO); ultimately this solution was added to media 1:1 v/v to give a 12.5 µg/mL final concentration.

Plant materials

The plant material (whole plant, i.e., aerial parts and the roots) was collected from multiple populations in the field around Qwaqwa within the Golden Gate Mountains. Proper identification and authentication as *Dianthus basuticus* were carried out at the News Herbarium of the University of KwaZulu-Natal, Pietermaritzburg Campus by Dr C.J. Potgieter in January 2012. Then, a herbarium voucher with a reference number (LamMed/01/2013/Qhb) was already deposited at the UFS-Qwaqwa campus herbarium. *Cnestis ferruginea* was acquired from traditional healers in Nigeria, and the proper identification of the plant was validated at the UFS-Qwaqwa campus herbarium by Dr. AOT Ashafa by August 2016.

Preparation of crude extract

The ethanol extract was prepared by macerating 100 g of powdered dry plant material in stoppered flasks that contain 300 ml of absolute ethanol for 72h at room temperature on Labcon platform shaker (Labcon Supplies, Durban, South Africa). Following filtration, the solvent was evaporated to become dry in an IncoTherm incubator (Labotec, Johanessburg, South Africa) at 37°C to acquire the crude extract. The various extracts were stored in tightly sealed glass vials. In a 1.5 mL sterile Eppendorf vial, 15 mg of each crude extract was diluted in 250 µL of dimethyl sulfoxide (DMSO). Each vial that contains an extract was covered with aluminium foil and stored in 4°C until use.

Extract preparation and dilution

All dilutions were prepared immediately prior to use and remnants were discarded. Dilutions were realised by pipetting 50 µL of each DMSO diluted extract and adding 950 µL of sterile water (3mg/mL); from this dilution 50 µL were placed in microvial culture tubes, adding 950 µL of PEHPS media [19] to reach a final concentration of 150 µg/mL, which was named dilution 1. Serial dilutions to yield 75 µg/mL, 37.5 µg/mL, 18.75 µg/mL, 9.37 µg/ml were realised.

Parasite culture

For the parasite cultures, *E. histolytica* HM-1: IMSS strain was used in PEHPS medium, and *E histolytica* trophozoites were sub-cultured thrice a week. To determine the sensitivity of the parasites to both extracts and drugs, trophozoites of *E histolytica* were exposed to the extracts and drugs in the logarithmic growth phase. Metronidazole was used as positive inhibition control. The concentration of metronidazole was adjusted to a final concentration of 12.5 µg/mL by dissolving them in double distilled water. The solution was stored at −20°C until use. Before each assay, the stock solution had to be serially diluted. PEHPS medium was used with 500 µL of stock solution and 500 µL of medium; afterward 500 µL of the first dilution and 500 µL of the medium from the second dilution were taken and so on. Negative control was prepared, adding 50 µL of DMSO in 950 µL of sterile water.

Inhibition assays

A culture tube which contained HM1-IMSS growing in logarithmic phase (after 72 h incubation) was reviewed and observed under an inverted microscope, checking for contamination and excellent growth condition of the trophozoites. The culture tubes were cooled in ice for 10 min, and trophozoite density was determined by assessing a little sample with sterile Pasteur pipet in a hemocytometer. The trophozoite concentration was adjusted to 2 x 10^4 cells/mL, adding an inoculum from chilled culture tubes that contain 20,000 trophozoites in borosilicate Bellco brand vials plus culture media to complete 950 µL volumes. 50 µL of the ready-prepared dilutions of the extract were transferred, placed in each vial with the amount of trophozoites adjusted and incubated for 72 h at 36.5°C in triplicate [19]. The first step was the inhibition of growth of each extract with the use of 150µg/mL dilution, and only the extracts with greater than 65% of inhibition were chosen. To calculate IC_{50}, all vials were incubated at 36°C for 72 h. Next, the trophozoites were acquired by cooling the vials in ice for 20 minutes; the number of parasites per mL was calculated with the aid of a hemocytometer.

Statistical analysis

The proportion of live and dead cells was calculated by counting 100 cells directly in a hemocytometer. The percent growth inhibition was carried out by comparing the survival of the parasites in the treated tubes with respect to untreated controls. A table was created showing IC_{50} of each plant. Then, the concentration that inhibited 50% of the growth of the parasites was calculated using the probit analysis. These results were showed in graphic with linear formula calculation. All the experiments were carried out in triplicates, and 95% confidence intervals were calculated.

Results and Discussion

The results of the preliminary activities that led to the choice of *Cnestis ferruginea* and *Dianthus basuticus* are outlined in Table 1. The ethanol extract of both plants indicated solubility in the culture medium and exhibit a considerable inhibition of growth of *E. histolytica HM-1: IMSS* trophozoites. The antiprotozoal activity that was tested in the two plant extracts are shown in Table 1. *Cnestis ferruginea* have an IC_{50} against *E. histolytica* trophozoites of 15.07µg/mL, whereas *Dianthus basuticus* have an IC_{50} of 12.45µg/mL. The two extracts were soluble in the culture media, ensuring the interaction between the parasite's trophozoites. The lineal analysis of probit from each extract is shown in Figures 1 and 2, respectively. The extracts were considered active since they show an IC_{50} < 100 µg/mL. Protozoans including *E. histolytica* and other protozoans have been incriminated as serious causes of diarrhoea in Africa and other parts of the developing world.[20-22] Amoebiasis, the disease caused by *E. histolytica* parasites, is commonly treated with nitroimidazoles, [23] but recently various strains of *E. histolytica* have been reported to be resistant to imidazoles.[24,25]

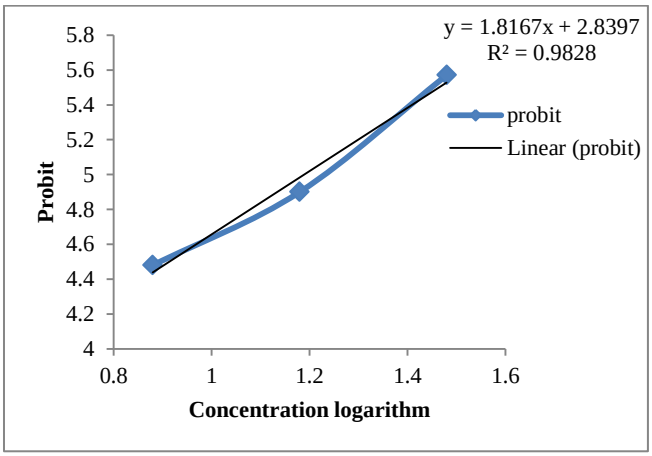

Figure 1: Probit analysis of *Cnestis ferruginea* extract.

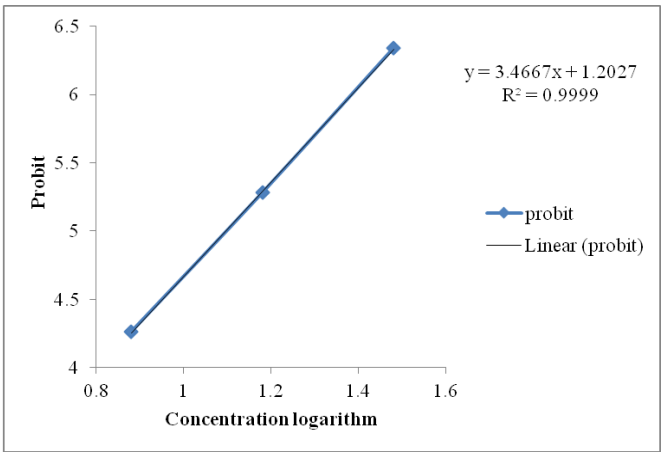

Figure 2: Probit analysis of *Dianthus basuticus* extract.

© 2021 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

Furthermore, there is documented evidence of the resistance with metronidazole treatment of protozoan parasites and other protozoan pathogens.[26-29] Several studies in the past have determined the phytochemical constituents of plants and their cytotoxic, antidiabetic, antioxidant, antimicrobial, hypoglycaemic and biochemical properties. [30-32] Furthermore, various compounds have been isolated from *C. ferruginea* including octacosanyl stearate and 1-myristo-2-stearo-3-palmitin [30] and a novel isoflavone glycoside, afrormosin-7-O-beta-D- galactoside with antimicrobial activity isolated from the testa fruit.[33] Other compounds including squalene, myricyl alcohol, ß-sitosterol, cyanidin, delphinidin and apigenidin have also been isolated from the plant. [34] Nonetheless, the antiprotozoal activity of some of these compounds on *E. histolytica* has not been evaluated. The study assessed the antiamoebic activity of *C. ferruginea* and *D. basuticus* and compared the inhibitory concentration (IC_{50}) of these extracts with metronidazole. The two plants showed good activity against *E. histolytica* trophozoites. The high activity of both plant extracts may be due to the presence of any of the previously isolated compounds. For instance, in a study, *Acorus calamus* and other medicinal plants from South Africa displayed high activity against *E. histolytica*, and this was due to the presence of phenyl propanoid β- asarone. [35] The activity of hexane extract from *Aristolochia elegans* was very active against *E. histolytica* and *Giardia lamblia* with an $IC_{50} < 0.624$ μg/mL^{-1},[36] and another similar study reported an antiprotozoal activity of dichloromethane extract and a pure compound from the roots of *Geranium mexicanum* on *E. histolytica* and *G. lamblia* trophozoites; the results validated the activity of the organic fraction and a pure flavonoid to be active against both protozoans with an IC_{50} of 1.9 to 79.2 μg/mL for *E. histolytica* and 1.6 to 100.4μg/mL for *G. lamblia*. The confirmed active compound was flavan-3-ol, (−) epicatechin. [37] Comparing the results of both studies with ours, the IC_{50} from this study was higher than that of 0.624μg/ml^{-1} but lower than 79.2μg/mL despite the use of various solvents for extraction.

Table 1: The yields and outcomes of antiprotozoal activity from each extract versus *E. histolytica* trophozoites

Plant material	Soluble in culture	Activity	Yield	Part used	IC_{50} (μg/mL) (95% confidence intervals)
Cnestis ferruginea	Yes	Good	0.60g	Root	15.07 ± 2.35 μg/mL
Dianthus basuticus	Yes	Good	0.07g	Leaves	12.45 ± 2.32 μg/mL

Conclusion

The results show that *Dianthus basuticus* and *Cnestis ferruginea* have inhibitory activity against Entamoeba *histolytica.*

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors thank the Directorate of Research Development University of the Free State for Postdoctoral Fellowship of Dr. Ojuromi. The authors also acknowledge the support from the management of Lagos State University, Ojo, Lagos, Nigeria. Authors acknowledge the University of Monterrey, Mexico for the support to publish the manuscript.

References

1. Troeger C, Forouzanfar M, Rao PC, Khalil I, Brown A, Reiner Jr RC, Fullman N, Thompson RL, Abajobir A, Ahmed M, Alemayohu MA, Alvis-Guzman N, Amare AT, Antonio CA, Asayesh H, Avokpaho E, Awasthi A, Bacha U, Barac A, Betsue BD, Beyene AS, Boneya DJ, Malta DC, Dandona L, Dandona R, Dubey M, Eshrati B, Fitchett JRA, Gebrehiwot TT, Hailu GB, Horino M, Hotez PJ, Jibat T, Jonas JB, Kasaeian A, Kissoon N, Kotloff K, Koyanagi A, Kumar GA, Rai RK, Lal A, Abd El Razek HM, Mengistie MA, Moe C, Patton G, Platts-Mills JA, Qorbani M, Ram U, Roba HS, Sanabria J, Sartorius B, Sawhney M, Shigematsu M, Sreeramareddy C, Swaminathan S, Tedla BA, Jagiellonian RT, Ukwaja K, Werdecker A, Widdowson M, Yonemoto N, Zaki ME, Lim SS, Naghavi M, Vos T, Hay S, Murray CJL, Mokdad AH. "Estimates of global, regional, and national morbidity, mortality, and aetiologies of diarrhoeal diseases: a systematic analysis for the Global Burden of Disease Study 2015." Lancet Infect Dis. 2017; 17(9):909-948.
2. Troeger C, Blacker BF, Khalil IA, Rao PC, Cao S, Zimsen SR, Alvis-Guzman N. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of diarrhoea in 195 countries: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Infect Dis. 2018; 18(11):1211-1228.
3. Black RE, Morris SS, Bryce J. Where and why are 10 million children dying every year? Lancet. 2003; 361(9376):2226-2234.
4. Hawash YA, Ismail KA, Almehmadi M. High Frequency of Enteric Protozoan, Viral, and Bacterial Potential Pathogens in Community Acquired Acute Diarrheal Episodes: Evidence Based on Results of Luminex Gastrointestinal Pathogen Panel Assay. Korean J Parasitol. 2017; 55(5):513-521.
5. Carrero JC, Reyes-López M, Serrano-Luna J, Shibayama M, Unzueta J, León-Sicairos N, de la Garza M. Intestinal amoebiasis: 160 years of its first detection and still remains as a health problem in developing countries. Int J Med Microbiol. 2020; 310(1):151358.
6. Bercu TE, Petri WA, Behm JW. Amebic colitis: new insights into pathogenesis and treatment. Curr Gastroenterol Rep. 2007; 9(5):429-433.
7. Stanley SL Jr. Amoebiasis. Lancet. 200; 361(9362):1025-1034.
8. Petri WA Jr. Therapy of intestinal protozoa. Trends Parasitol. 2003; 19(11):523-6
9. Löfmark S, Edlund C, Nord CE. Metronidazole is still the drug of choice for treatment of anaerobic infections. Clin Infect Dis. 2010; 1:(50):S16-23.
10. Upcroft JA, Campbell RW, Benakli K, Upcroft P, Vanelle P. Efficacy of new 5-nitroimidazoles against metronidazole-susceptible and -resistant Giardia, Trichomonas, and Entamoeba spp. Antimicrob Agents Chemother. 1999; 43(1):73-76.

© 2021 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

11. Garza-González J, Vargas-Villarreal J, Verde-Star M, Rivas-Morales C, Oranday-Cárdenas A, Hernandez-García M, Garza-Salinas L, González-Salazar F. Antiprotozoal Activity of a *Thymus vulgaris* Methanol Extract and Its Fractions. Health. 2017; 9:1081-1094.
12. Nafiu MO and Ashafa AOT. Antioxidant and Inhibitory Effects of Saponin Extracts from Dianthus basuticus Burtt Davy on Key Enzymes Implicated in Type 2 Diabetes *In vitro*. Pharmacogn Mag. 2017; 13(52):576-582.
13. Moteetee A and Van-Wyk BE. The medical ethnobotany of Lesotho: a review. Bothalia. 2011; 41(1):209-228.
14. Olayemi FO, Raji Y, Adegoke OA, Oyeyemi MO. Haematological and some biochemical profiles in male rats treated with *Cnestis ferruginea* (de Candolle) root extract and its pure fractions. Afr J Pharm Pharmacol. 2013; 7(20):1231-1235.
15. Ahmed HA. Therapeutic Potentials of *Cnestis ferruginea*: A Review. J Pharmacogn Phytochem. 2017; 6(6):1397-1401.
16. Akharaiyi C, Boboye B, Adetuyi FC. Antibacterial, phytochemical and antioxidant properties of *Cnestis ferruginea* DC (Connaraceae) extracts. J Microbiol Biotech and Food Sci. 2012; 2(2):592-609.
17. Yakubu MT and Nurudeen QO. Effects of aqueous extract of *Cnestis ferruginea* (Vahl ex De Cantolle) root on paroxetine-induced sexual dysfunction in male rats. Asian Pac J Reprod. 2012; 1(2):111-116.
18. Said-Fernández S, Vargas-Villarreal J, Castro-Garza J, Mata-Cárdenas BD, Navarro-Marmolejo L, Lozano-Garza G, Martínez-Rodríguez H. PEHPS medium: an alternative for axenic cultivation of Entamoeba histolytica and E. invadens. Trans R Soc Trop Med Hyg. 1988; 82(2):249-253.
19. Mata-Cárdenas BD, Vargas-Villarreal J, González-Salazar F, Palacios-Corona R, Said-Fernández S. A New Vial Microassay to Screen Antiprotozoal Drugs. Pharmacol Online. 2008; 1:529-537.
20. Okeke IN, Ojo O, Lamikanra A, Kaper JB. Etiology of acute diarrhea in adults in southwestern Nigeria. J Clin Microbiol. 2003; 41(10):4525-4530.
21. Mbae CK, Nokes DJ, Mulinge E, Nyambura J, Waruru A, Kariuki S. Intestinal parasitic infections in children presenting with diarrhoea in out-patient and in-patient settings in an informal settlement of Nairobi, Kenya. BMC Infect Dis. 2013; 13:243.
22. Sinniah B, Hassan AKR, Sabaridah I, Soe MM, Ibrahim Z, Ali O. Prevalence of intestinal parasitic infections among communities living in different habitats and its comparison with one hundred and one studies conducted over the past 42 years (1970 to 2013) in Malaysia. Trop Biomed. 2014; 31(2):190-206.
23. Gonzales MLM, Dans LF, Sio-Aguilar J. Antiamoebic drugs for treating amoebic colitis. Cochrane Database Syst Rev. 2019; 1(1):CD006085.
24. Samarawickrema NA, Brown DM, Upcroft JA, Thammapalerd N, Upcroft P. Involvement of superoxide dismutase and pyruvate:ferredoxin oxidoreductase in mechanisms of metronidazole resistance in Entamoeba histolytica. J Antimicrob Chemother. 1997; 40(6):833-40.
25. Wassmann C, Hellberg A, Tannich E, Bruchhaus I. Metronidazole resistance in the protozoan parasite *Entamoeba histolytica* is associated with increased expression of iron-containing superoxide dismutase and peroxiredoxin and decreased expression of ferredoxin 1 and flavin reductase. J Biol Chem. 1999; 274(37):26051-26056.
26. Leung AKC, Leung AAM, Wong AHC, Sergi CM, Kam JKM. Giardiasis: An Overview. Recent Pat Inflamm Allergy Drug Discov. 2019; 13(2):134-143.
27. Kissinger P. Trichomonas vaginalis: a review of epidemiologic, clinical and treatment issues. BMC Infect Dis. 2015; 15:307.
28. Upcroft P and Upcroft JA. Drug targets and mechanisms of resistance in the anaerobic protozoa. Clin Microbiol Rev. 2001; 14(1):150-164.
29. Batista L, Pérez Jove J, Rosinach M, Gonzalo V, Sainz E, Loras C, Forné M, Esteve M, Fernández-Bañares F. Low efficacy of metronidazole in the eradication of *Blastocystis hominis* in symptomatic patients: Case series and systematic literature review. Gastroenterol Hepatol. 2017; 40(6):381-387.
30. Adisa RA, Choudhary MI, Adewoye EO, Olorunsogo OO. Hypoglycaemic and biochemical properties of *Cnestis ferruginea*. Afr J Trad Compl Altern Med. 2010; 7(3):185-194.
31. Kazeem MI and Ashafa AOT. *In-vitro* antioxidant and antidiabetic potentials of *Dianthus basuticus* Burtt Davy whole plant extract. J Herbal Med. 2015; 5(3):158-164.
32. Lamula SQN and Ashafa AOT. Antimicrobial and cytotoxic potential of *Dianthus basuticus* used in Basotho traditional practice Bangladesh. J Pharmacol, 2014; 9:105-111.
33. Kouakou K, Panda SK, Yang MR, Lu JG, Jiang ZH, Van Puyvelde L, Luyten W. Isolation of Antimicrobial Compounds from *Cnestis ferruginea* Vahl ex. DC (Connaraceae) Leaves Through Bioassay-Guided Fractionation. Front Microbiol. 2019; 11(10):705.
34. Ogbede ON, Eguavoen OI, Parvez M. Chemical studies in the anthocyanins of the local plants. J Chem Soc Pak. 1986; 8:545-547.
35. Rajput SB, Tonge MB, Karuppayil SM. An overview on traditional uses and pharmacological profile of *Acorus calamus* Linn. (Sweet flag) and other Acorus species. Phytomed. 2014; 21(3):268-276.
36. Jiménez-Arellanes A, León-Díaz R, Meckes M, Tapia A, Molina-Salinas GM, Luna-Herrera J, Yepez-Mulia M. Antiprotozoal and Antimycobacterial Activities of Pure Compounds from *Aristolochia elegans* Rhizomes. Evid-Based Compl Altern Med. 2012; 2012:1-7.
37. Calzada F, Cervantes-Martínez JA, Yépez-Mulia L. *In vitro* antiprotozoal activity from the roots of *Geranium mexicanum* and its constituents on *Entamoeba histolytica* and *Giardia lamblia*. J Ethnopharmacol. 2005; 98(1-2):191-193.

© 2021 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License