



In-vitro anti-giardial activity of ethanolic extract and fractions from *Phlebophyllum kunthianum*

D. N. Singh^{1*}, N. Verma¹, D. K. Kulshreshtha² and A. K. Agrawal³

1. K.S. Saket PG College, Dr. Ram Manohar Lohia Avadh University, Faizabad-224001, India,

2. Medicinal and Process Chemistry Division, Central Drug Research Institute, Lucknow-226001, India,

3. Division of Microbiology, Central Drug Research Institute, Lucknow-226001, India,

Abstract

The ethanolic extract (D002) of the *Phlebophyllum kunthianum* (whole plant) was tested *in vitro* against *Giardia lamblia* and has been found to exhibit 100% anti-giardial activity at the dose of 125 µg/ml. On bioassay guided fractionation of D002 by using the solvents of different polarity, n-butanol (F005) and aqueous fraction (F006) have been exhibited 100% anti-giardial activity at the dose of 500 µg/ml and 250 µg/ml respectively. Fraction F011 prepared from F006 was found to be most active comparable to the D002 and exhibited 100% anti-giardial activity at the dose of 125 µg/ml and number of live trophozoites of 13.48±0.97 at the MIC value of 62.5 µg/ml.

Keywords: *Phlebophyllum kunthianum*;, *Acanthaceae*; Anti-giardial activity; *Giardia lamblia*

1. Objective of the study

Giardia lamblia is a flagellated protozoan parasite most frequently the cause of intestinal protozoal infection in the world [1-3]. and is a common cause of waterborne diarrhea in North America [4]. In certain regions in India, the endemicity is reported to be as high as 87% with inadequate hygiene conditions [5]. Giardiasis is especially prevalent in infants and children in the developing country and can have devastating effects because of causes malabsorption and thus malnutrition [2]. The drugs which are currently used for the treatment

of giardiasis such as metronidazole, tinidazole, paramomycin, nitazoxanide, mepacrine and furazolidone [6-8]. However, many problems are associated with the currently used chemotherapeutic agents, including unpleasant side effects such as activity against normal intestinal flora leading to gastrointestinal disturbances, possible carcinogenicity, hemolytic anemia, disulfiram-like reactions to alcohol, hypersensitivity reactions as well as evidence of tumorigenicity in rodent. The treatment failures have occurred in up to 20%

* Corresponding author
Email: dnsinghsaket@yahoo.com

of patients and report of resistance have appeared and hence parasite resistance is a potential problem [2, 9-14]. Therefore, the development of effective and safer agents from natural sources for the treatment of giardiasis is urgently needed. Previously, we have reported potential antifungal agents from the aerial parts of *Saprosma fragrans* [15], and from the rhizomes of *Agapanthus africanus* [16]. In our continuous effort and programme for the development of new therapeutic agents from terrestrial plants including traditional remedies, The preliminary biological screening of the ethanolic extract of the *Phlebophyllum kunthianum* (whole plant) exhibited significant anti-giardial activity *in vitro*. This prompted us to undertake the detailed study on the plant so as to identify most active fraction by bioassay guided fractionation and leading to isolate the active constituent (s). *Phlebophyllum kunthianum* Nees wall (*Acanthaceae*) is a gregarious bushy shrub [17]. Literature survey revealed that no chemical and biological work have been carried out on this plant. The present communication deals with the detailed *in vitro* anti-giardial activity of the ethanolic extract and fractions of the *P. kunthianum*.

1.1 Plant material used

The ground powder of the *Phlebophyllum kunthianum* (whole plant) was employed for the study. The plants *Phlebophyllum kunthianum* were collected from Tamil Nadu, India and authenticated by Botany Division, Central Drug Research Institute, Lucknow, where a voucher specimen has been preserved.

1.2 Preparation of extract and fractions

Air dried and powdered (10 kg) of the whole plants were pulverized and extracted with 95% ethanol (4 x 25 litre) for 24 h at room temperature. The ethanolic extract was

concentrated under reduced pressure by using rotatory evaporator at 50° C and finally dried under *vacuo* to give solid mass (D002, 300g). The crude ethanolic extract (D002, 125g) was macerated with n-hexane to give n-hexane fraction (F003). The insoluble residue was then suspended in water and partitioned with chloroform and n-butanol in succession to give chloroform (F004), n-butanol (F005) and water soluble (F006) fractions,. All these fractions were evaporated in rotatory evaporator and finally dried under *vacuo* to furnish solid mass F003 (10g), F004 (85g) and F005 (0.5g) and F006 (29g) respectively. Aqueous fraction (F006, 15g) was chromatographed over silica gel (60-120 mesh) column eluting with polar gradient of chloroform and 5% aqueous methanol and collected eluants having same TLC pattern were pooled together, evaporated in rotatory evaporator and dried under *vacuo* to give fractions F007-F0011.

1.3 Tested activity

In vitro anti-giardial activity of the extract and fractions were tested using a method previously reported [18,19]. Briefly, a 0.1 ml inoculum of exponentially growing (24h old) cultures of *Giardia lamblia* containing approximately 2000 trophozoites was dispensed into a cavity slide. The test sample in the desired concentration of 0.1 ml TYI-S-33 was added in the cavity mounted with glass coverslip and the edge of the cavity were sealed with the paraffin wax. The slides were kept in the moist chamber at 37°C and incubated for 24 h. the number of dead and live trophozoites was recorded to determine the anti-giardial activity of the test sample. Metronidazole a standard drug was served as the positive control. The details of the anti-giardial activity profile of the tested ethanolic extract and fractions of *Phlebophyllum kunthianum* are given in Table-1

Table 1: In vitro anti-giardial activity of the ethanolic extract and fractions of the of *P. kunthianum* (whole plant) against *Giardia lamblia*

Code	Trophozoite survival vs dose ($\mu\text{g/ml}$)						
No	1000	500	250	125	62.5	31.0	Control
D002	0.00 ^a	0.00	0.00	0.00	32.74 ^b ± 0.63	51.09 ^d ± 0.39	57.06 ±0.97
F003	55.76 ^b ±0.37	Nt	Nt	Nt	Nt	Nt	
F004	54.09 ^b ±0.71	Nt	Nt	Nt	Nt	Nt	
F005	0.00	0.00	19.30 ^b ±1.07	54.50 ^c ±0.87	Nt	Nt	
F006	0.00	0.00	0.00	15.30 ^b ±0.37	51.35 ^b ± 0.63	Nt	
F007	0.00	32.76 ^b ±0.63	44.7 ^b ±0.31	Nt	Nt	Nt	
F008	0.00	37.72 ^c ±0.30	49.15 ^b ±0.27	Nt	Nt	Nt	
F009	0.00	0.00	39.16 ^c ±0.62	55.54 ^b ±0.29	Nt	Nt	
F010	0.00	0.00	0.00	15.36 ^c ±0.82	48.67 ^b ± 0.51	Nt	
F011	0.00	0.00	0.00	0.00	13.48 ^b ± 0.97	44.69 ^b ± 0.43	
Metro-nidazole	0.00	0.00	0.00	0.00	0.00	25.11 ^b ± 0.93	

Values expressed as mean $\pm$ SE of five observations of three experiments, ^a(0.00) No live trophozoites recorded (100% mortality), Nt Not tested since negligible effect was recorded at higher doses, ^b $p > 0.001$, ^c $p > 0.1$, ^d $p > 0.002$, ^e $p > 0.01$.

2. Results and discussion

The ethanolic extract (D002) was displayed prominent anti-giardial activity as exhibited 100% anti-giardial activity up to the dose of 125 $\mu\text{g/ml}$, weak activity at 62.5 $\mu\text{g/ml}$ with number of live trophozoites of 32.74 $\pm$ 0.63 and possessed no anti-giardial activity as the number of live trophozoites was recorded 51.09 $\pm$ 0.39 as comparable to the untreated group. Of the fractions tested n- butanol fraction (F005) exhibited 100% activity at the dose of 500 $\mu\text{g/ml}$ and moderately active, 19.30 $\pm$ 1.07 lives trophozoites at the MIC value of 250 $\mu\text{g/ml}$. Aqueous fraction (F006) exhibited 100 % cytotoxicity at the dose of 250 $\mu\text{g/ml}$. and the number of live trophozoites 15.30 $\pm$ 0.37 was recorded at 125 $\mu\text{g/ml}$. Fractions F007-F011 prepared from the column chromatography of the aqueous fraction (F006), of these fraction F007 and F008 displayed 100% activity at the concentration of 1000 $\mu\text{g/ml}$, and the number

of *Giardia* trophozoites surviving at 500 $\mu\text{g/ml}$ were 32.76 $\pm$ 0.63 and 37.72 $\pm$ 0.30 respectively. F009 and F010 showed maximum activity with 100 % cytotoxicity at 500 $\mu\text{g/ml}$ and 250 $\mu\text{g/ml}$ and number of live trophozoites 39.16 $\pm$ 0.62 and 15.36 $\pm$ 0.82 at the MIC of 250 and 125 $\mu\text{g/ml}$ respectively. Fraction F011 exhibited promising activity and displayed 100% anti-giardial activity as comparable to the ethanolic extract (D002) and also showed good activity with the number of lives trophozoites 13.48 $\pm$ 0.97 at the concentration of 62.5 $\mu\text{g/ml}$. The n- hexane (F003) and chloroform (F004) were found to be inactive as the number of lives trophozoites recorded 55.76 $\pm$ 0.37 and 54.09 $\pm$ 0.71 respectively. The metronidazole was used as a reference drug showed 100% inhibition of the lives giardial trophozoites at 62.5 $\mu\text{g/ml}$., with the number of lives trophozoites of 25.11 $\pm$ 0.93 at 31.0 $\mu\text{g/ml}$.

3. Conclusion

Ethanollic extract of *P. Kunthianum* have been displayed significant anti giardial activity and its bioassay guided fractionation afforded the most active fraction F011. The isolation of the active constituent (s) from the fraction F011, activity optimizations of the active constituent (s) by the study of structural activity relationship and thereby the generations of lead molecule for giardial chemotherapy are under the way.

4. Acknowledgements

Authors are thankful to Director, Central Drug Research Institute, Lucknow, India for kind support and Dr. B. N. Mehrotra, Ex-Scientist-G and Head, Botany Division, CDRI, Lucknow for collection and identification of the plant materials. The financial assistance from ONR, USA is also gratefully acknowledged.

References

- 1- Hill DR. (1993) *Infect. Dis. Clin. North Am.* 7: 503-525.
2. Johnson PJ. (1993) *Parasitology Today*, 9 :183-186.
3. Farthing MJG. *In Giardia: From Molecules to Diseases* Thompson RCA, Reynoldson JA, Lymbery AJ. (1994) Eds: CAB International Wallingford, U.K. 15-37.
4. Kramer MH, Herwaldt BL, Craun GF, Calderon RL, Juranek DD. (1996) *Morbidity and Mortality Weekly Rep.* 45 :1-33.
5. Walia BNS, Ganguly NK, Mahajan RC. (1986) *Tropo. Geogr.* 38:367-370.
6. Davla C, Vesquer C, Trujillo B, Huerta M. (2002) *Am. J. Trop. Med. Hyg.* 66: 251- 254.
7. Morrone F, Carneiro J, Reis C, Cardozo C, Ubal C, Carli GD. (2004) *Rev. Inst. Med. Trop. Sao Paulo*, 46: 77-80.
8. Sullayman I, Nolder D, Warchurst D, Rossignol J. (2002) *J. Antimicrob. Chemother.* 49: 103-111.
9. Petri W. (2003) *Parasitol.* 19: 523-536.
10. Upcroft JA, Campbell RW, Benakli K, Upcroft P, Venelle P. (1999) *Antimicrob. Agents Chemother.* 43: 73-76.
11. Ellis JE, Wingfield JM, Cole D, Boreham PF, Lloyd D. (1993) *Int. J. Parasitol.* 23: 35-39.
12. Roe FJC. (1977) *J. Antimicrob. Chemother.* 3: 205-212.
13. Voogd CE. (1981) *Muta. Res.* 86 : 243-277.
14. Hill DR, *In Principles and Practice of Infectious Diseases*, 4th ed. Mandel GL, Bennett JE, Dolin R. (1995) Eds. Churchill Livingstone New York, 2: 2487-2493.
15. Singh DN, Verma N, Raghuwanshi S, Shukla PK, Kulshreshtha DK. (2006) *Bioorg. Med. Chem. Lett.* 16: 4512-4214.
16. Singh DN, Verma N, Raghuwanshi S, Shukla PK, Kulshreshtha DK. (2008) *Fitoterapia*, 79: 298-300.
17. Hooker JD. (1978) *The Flora of British India*, 4: 434.
18. Keister DB. (1983) *Trans. R. Soc. Trop. Med. Hyg.* 77: 487-488.
19. Tripathi DM, Gupta N, Laxami V, Saxena KC, Agrawal AK. (1999) *Phytotherapy Res.* 13 : 561-565.