Journal of Medicinal Plants Research Vol. 3(5), pp. 420-423, May, 2009
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875© 2009 Academic Journals

Full Length Research Paper

Ethanolic leaf extract of *Psidium guajava*: Phyto-chemical and trypanocidal activity in rats infected with *Trypanosoma brucei brucei*

Adeyemi, O. Stephen[1]*, Akanji, M. A.[2] and Oguntoye, S. A.[3]

[1]Bells University of Technology, College of Natural and Applied Sciences, Department of Chemical Sciences, P. M. B. 1015, Ota-112001, Nigeria.
[2]University of Ilorin, Department of Biochemistry, P. M. B. 1515, Ilorin, Nigeria.
[3]University of Ilorin, Department of Chemistry, P. M. B. 1515, Ilorin, Nigeria.

Accepted 13 May, 2009

The Phyto-chemical composition and trypanocidal activity of the ethanolic leaf extract of *Psidium guajava* (guava) was investigated in rats infected with *Trypanosoma brucei brucei*. Results showed that the extract contained a higher amount of flavonoids compared to tannins which is moderately present. Other phyto-chemicals present but in minute amounts included saponins, steroids, and terpenoids amongst others. The result of the test for trypanocidal effects showed that there was low to absolute zero parasitaemia in the treated rats compared to the infected and untreated control. Besides the attainment of low parasitaemia by the leaf extract, there was also an appreciable extension of life span of all infected and treated rats. The life span extension ranged from 30 days to 32 days post infection. The infected and untreated control animals died on the 8th day post infection. We have been able to show that *P. guajava* leaf extract has trypanocidal properties which could be attributed in parts to the broad antimicrobial and iron chelating activity of flavonoids and tannins respectively. Iron chelation has been suggested by several reports as an effective way of killing trypanosomes. The prime target is the enzyme, ribonucleotide reductase whose activity is central to DNA synthesis prior to cell division as obtained in trypanosomiasis infection. This result thus makes *P. guajava* leaf extract a probable agent for managing African sleeping sickness.

Key words: *Psidium guajava*, phytochemical, trypanosomes, antimicrobial, iron-chelation, ribonucleotide reductase.

INTRODUCTION

Chemotherapy and chemoprophylaxis, which form the most important and major aspect of the control and eradication of Trypanosomiasis in African countries is beset with problems. These include limited repertoire of compounds, resistance to drugs, drug toxicity and protracted treatment protocol (TDR, 1984). According to the world health organization, more than 80% of the world populations still rely on herbal medicines as their primary source of health care. Millions of Africans of all ages rely on herbal medicine for primary health care (McCaleb, 2000). Plants have provided the basis for traditional treatment for different types of diseases and still offer an enormous potential source of new chemotherapeutic agents. In northern Nigeria, where this disease is prevalent, medicinal plants are being used by traditional healers either singly or in combination in the treatment of different types of disease particularly Trypanosomiasis (Igweh and Onabanjo, 1989).

Extensive literature survey revealed that *Psidium guajava*, acclaimed as 'poor man's apple of the tropics', has a long history of traditional use for a wide range of diseases. The fruit as well as its juice is freely consumed for its great taste and nutritional benefits. Much of the traditional uses have been validated by scientific research (Kamath et al., 2008). Toxicity studies in mice and other animal models as well as controlled human studies

*Corresponding author. E-mail: yomibowa@yahoo.com.

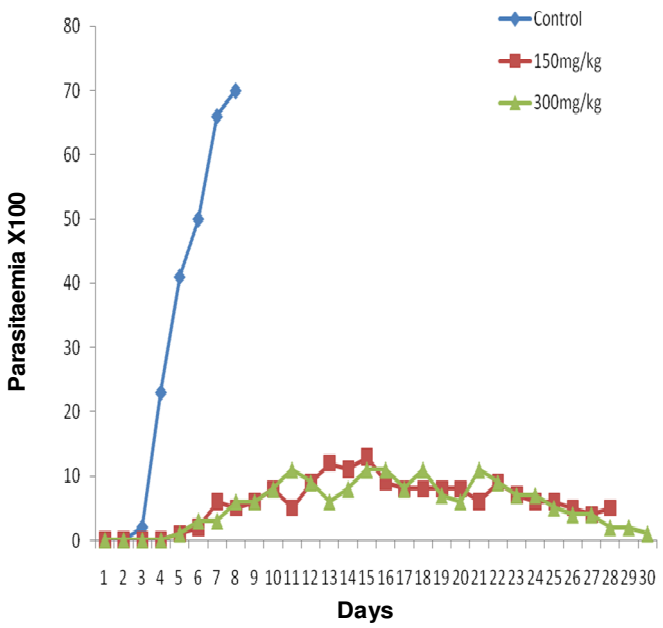

Figure 1. Parasitaemia of *T. brucei* infected rats treated with ethanolic extract of *P. guajava* leaf as the infection progressed until death of animals. Treatment started 72 h before infection. Each point is an average count from five rats.

show both leaf and fruit are safe without any side effects (Kamath et al., 2008). A number of chemicals isolated from plants like quercetin, guaijaverin, flavonoids and galactose-specific lecithins have shown promising activity in many human trials (Abdelrahim et al., 2002). The plant has been extensively studied in terms of pharmacological activity of its major components, and the results indicate potent anti-diarrheal, antihypertensive, hepatoprotective, antioxidant, antimicrobial, hypoglycemic and antimutagenic activities (Nwinyi et al., 2008).

P. guajava belongs to the family Myrtaceae. *P. guajava* may have been domesticated in Peru several thousand years ago (Rosa et al., 2008); Peruvian archaeological sites have revealed *P. guajava* seeds found stored with beans, corn, squash, and other cultivated plants (Hawrelak, 2003). *P. guajava* fruit is still enjoyed as a sweet treat by indigenous peoples throughout the rainforest region. The leaves and bark of *P. guajava* tree have a long history of medicinal uses that are still employed today (Nwinyi et al., 2008).

In view of the immense medicinal importance of *P. guajava* plant evidenced in the various studies mentioned above and also corroborated in a recent review article by Kamath et al. (2008), there is a strong incentive for further research into the pharmacological activities of *P. guajava* plant extract against common infectious diseases considering the fact that the plant is readily available in the tropics and within the reach of the local populace. In line with this an experiment was designed to assess the trypanocidal activity of leaf extract of *P. guajava.*

MATERIALS AND METHODS

Plant material

Fresh samples of *P. guajava* (guava) leaves which were used for the study were collected from a local farm in Ilorin, Kwara State, Nigeria. The plant was identified and authenticated at the Herbarium of the Department of Plant Biology University of Ilorin, Nigeria and voucher specimen has been deposited in the department for reference purpose.

Sample preparation

The ethanolic extract of the leaf was prepared according to the method of Viera et al. (2001). 500 g of fresh samples of *P. guajava* leaves were air dried and ground. The ground sample was soaked in ethanol and water ratio 8:2 (v/v) and left for 24 h. The mixture was filtered and the filtrate concentrated by evaporation at 40°C.

Parasite

Typanosoma brucei brucei was obtained from the Veterinary and Livestock Studies Department, Nigerian Institute for Trypanosome Research (NITR) Vom Jos, Nigeria. The parasite was injected intraperitoneally into rats and maintained by repeated passaging into other rats.

Phytochemical Screening

The phytochemical analysis of the plant extracts was carried out as described by Odebiyi and Sofowora 1978 to test for the presence of tannins, resins, glycosides, flavonoids, alkaloids and saponins among others.

Experimental animals

Adult white albino rats were sourced from the Department of Biochemistry Research Laboratory, University of Ilorin, Ilorin, Nigeria.

The rats were housed in cages and allowed to acclimatize for two weeks before the commencement of the experiment. Standard rat pellets and clean water was provided for the rats *ad libitum*.

Inoculation of rats with parasite

Parasite infected blood was obtained from the tail of infected rats at high parasitaemia and used to maintain parasite suspension in 0.90% saline solution which was inoculated into the peritoneal cavity of uninfected rats weighing approximately between 200 – 250 g. The suspension as described by Ekanem and Yusuf (2005) and Ekanem et al. (2006) contained 3 or 4 trypanosome per view at x100 magnification.

The results are shown in Figures 1, 2, 3 and Table 1.

DISCUSSION

The results obtained from treatment with ethanolic extract of *P. guajava* leaf indicates that the leaf extract could be effective in the management of African Trypanosomiasis, since there was considerable extension of life span of infected animals compared with the control. There was

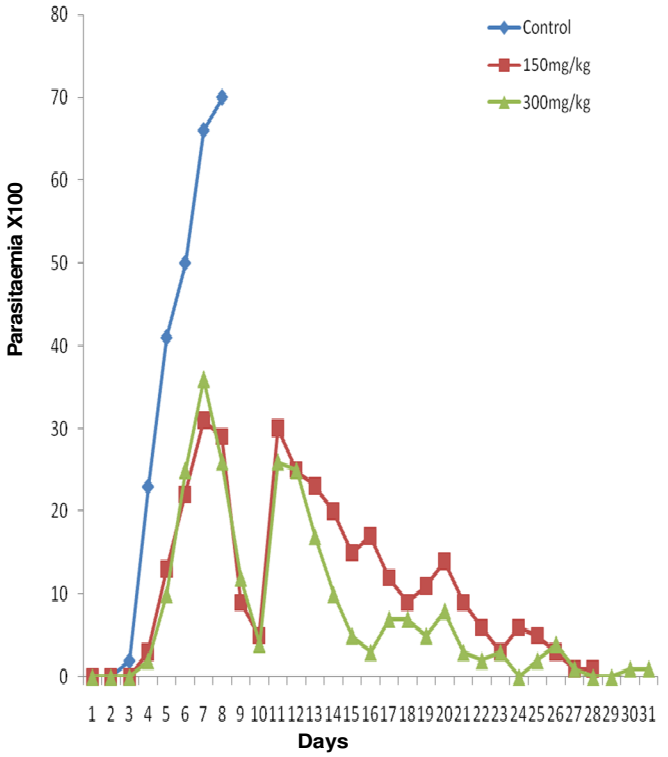

Figure 2. Parasitaemia of *T. brucei* infected rats treated with ethanolic extract of *P. guajava* leaf as the infection progressed until death of animals. Treatment started on the first day parasite was sighted in blood. Each point is an average count from five rats.

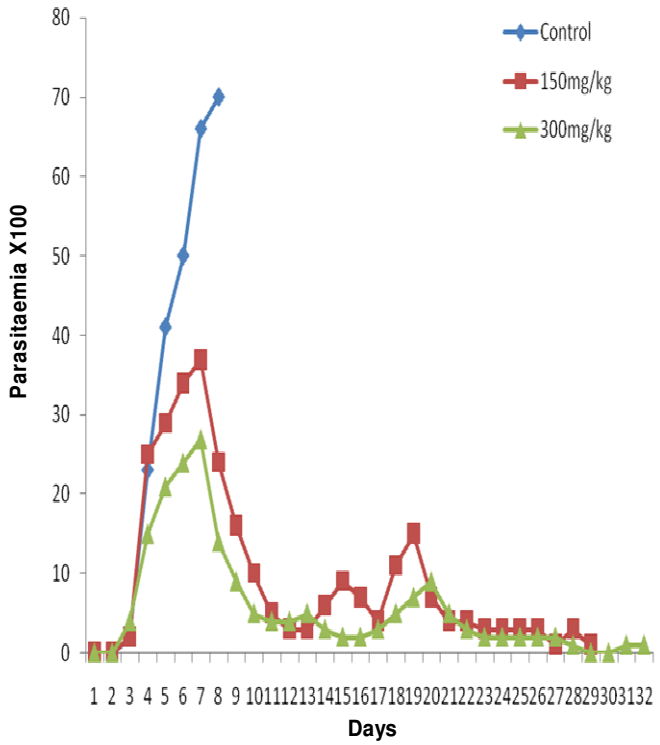

Figure 3. Parasitaemia of *T. brucei* infected rats treated with ethanolic extract of *P. guajava* leaf as the infection progressed until death of animals. Treatment started on the 7th day post infection. Each point is an average count from five rats.

also appreciable decline in parasitaemia for all animals treated with the ethanolic extract. Especially for the prophylactic treatment in which the parasitaemia was extremely kept low. This result confirms the many pharmacological uses of *P. guajava* leaf extract (Kamath et al., 2008). The death of the treated animals even at such a low parasitaemia level may not have been unconnected with the release of extracellular factors by the trypanosomes. Studies have shown that these extracellular factors have pathological effects on the host rats (Nwagwu et al., 1987; Boutignon et al., 1990; Ekanem, 1989; Ekanem et al., 1994, 1996). Upon invasion of the mammalian system trypanosomes proliferate rapidly to establish its population in infected host (Poltera, 1985; Pentreath and Kennedy, 2004). Toxins are released into the mammalian system (Nwagwu et al., 1987; Boutignon et al., 1990; Ekanem, 1989; Ekanem et al., 1994, 1996).The antibodies produced by the host against the parasite are not effective because of the ability of the parasite to produce a large repertoire of antigens. The host defense mechanism is only partially specific and often lagging behind the progress of the disease in terms of antigen-antibody interaction (Sternberg, 2004). Eventually there is a breakdown of the host immune system coupled with parasite invasion of the central nervous system leading to coma and death. Removal of the parasite from the system and simultaneously boosting the host immune system could be very relevant in the control of African sleeping sickness (Hoet et al., 2004; Chibale, 2005). *P. guajava* has trypanocidal properties as well as the ability to extend the life span of *T. brucei*-infected rats (Figures 1 - 3). Despite the clearance of the parasites from the blood, the infected rats still died suggesting the involvement of agents that are not necessarily life parasites. Factors extracellularly derived from the parasites (Nwagwu et al., 1987; Ekanem, 1989; Boutignon et al., 1990; Ekanem et al., 1994, 1996) could be responsible for the death.

The Phytochemical screening (Table 1) showed that the extract contains an appreciable amount of flavonoids and tannins amongst others. The ability of the extract to maintain a low parasitaemia may in part be attributed to its capacity to chelate iron. A study by Thepanon et al. (2005) showed that the leaf extract has an excellent capacity to form coloured complex with iron. Iron chelation therapy has been shown to be anti-parasitic especially in African trypanosomiasis (Ekanem, 1989). The prime suspect in this regard is the enzyme ribonucleotide reductase (RNR). RNR which requires iron for its activity plays a key role in the production of ribonucleotides prior to cell proliferation. So removing available iron from the cell system could have deleterious effects on the

Table 1. Phyto-Screening of the Ethanolic Extract of *P. guajava* (guava) Leaf

Compounds tested	Results obtained
Carbohydrates	+
Reducing sugars	+
Lipids	+
Alkaloids	-
Steroids	+
Tannins/Polyphenols	++
Anthraquinones	-
Terpenoids	+
Flavonoids	+++
Saponins	+

- = absent; + = slightly present; ++ = moderately present; +++ = highly present

proliferation of the parasite since the parasites need iron to establish their infection. The flavonoids could also have contributed to the overall effect of the extract as it were in this study. Several studies have indicated flavornoids as the main constituent responsible for the antimicrobial activities of *P. guajava* leaf extract.

RESULT

The result of the qualitative phytochemical screening of *P. guajava* leaf extract is presented in Table 1. Phytochemical analysis of theof the *P. guajava* leaf extract revealed the presence of tannins, saponins, steroids, flavonoids and reducing sugars. The concentration of flavonoids and tannins were found to be much higher than the other phytochemical components. The trypanocidal result for the prophylactic test is as shown in Figure 1. Figures 2 and 3 shows the results for the trypanocidal activity for the early and late stage treatment of the infected animals respectively. The results showed that the extract extended the life span of the treated animals considerably.

Conclusion

This study has been able to show that guava leaf extract has trypanocidal activity thereby lending credence to the usage of the plants in African traditional medicine system. We are however suggesting that the active principles in this extract should be isolated for further study to establish their individual effects and synergisms if any.

REFERENCES

Abdelrahim SI, Almagboul AZ, Omer ME, Elegami A (2002). Antimicrobial activity of Psidium guajava. L. Fititerapia. 737-8: 713-715.

Boutignon F, Huet G, Demeyer D, Richet C, Degand P (1990) Study of proteolytic activities released by incubation of trypanosomes (*Trypanosoma brucei brucei*). Biochem. Biophys. Acta. 1035: 369-377

Chibale K (2005) Economic drug discovery and rational medicinal chemistry for tropical diseases. Pure Appl. Chem. 77: 1957-1964.

Ekanem JT (1989) Extracellular fractions derived from *Trypanosoma brucei* activate erythrocyte Ca^{2+}- ATPase. Med. Sci. Res. 17:739-740.

Ekanem JT, Akanji MA, Odutuga AA (1994) Host and parasite derived factors during mammalian African trypanosomiasis. Biokemistri 4:103-116

Ekanem JT, Akanji MA, Odutuga AA (1996). Extracellular proteins of *Trypanosoma brucei* origin lyse erythrocytes of rats *in vitro*. Biokemstri. 6: 21-29.

Ekanem JT, Yusuf OK (2005) Activities of alkaline phosphatase, glutamate oxaloacetate transaminase and glutamate pyruvate transaminase in liver and serum of *Trypanosoma brucei*-infected rats treated with honey. Biochem. 17:185-191

Ekanem JT, Majolagbe OR, Sulaiman FA, Muhammad NO (2006) Effects of honey supplemented diet on the parasitemia and some enzymes of *Trypanosoma brucei*-infected rats. Afr. J. Biotech. 5:1557-1561

Hawrelak J (2003). Medicinal herb monogragh: Guava (Psidium guajava). J. Aust. Tradit Med. Soc. 9 (1): 25-29.

Hoet S, Opperdoes F, Brun R, Quetin-Leclercq J (2004) Natural products active against African trypanosomes: a step towards new drugs. Nat. Prod. Reports 21: 353-364

Igweh AC, Onabanjo AO (1989) Chemotherapeutic effects of *Annona senegalensis* in *T. b. brucei.* Ann. Trop. Med. Parasitol. 83: 527-534.

Kamath JV, Rahul N, Ashok Kumar CK, Lakshmi SM (2008). *Psidium guajava*: A review. Int. J. Green Pharm. 2:9-12.

McCaleb R (2000). McCaleb's Traditional Medicine Agenda added to National Plan of Action for Africa. A SNAPP UNDATA 8, A- SNAPP NEWS.

Nwagwu M, Okenu DMN, Olusi TA, Molokwu RI (1987) Trypanosoma brucei releases proteases extracellularly. Trans. Roy. Soc. Trop. Med. Hyg. 82:577.

Nwinyi OC, Chinedu NS, Ajani OO (2008). Evaluation of antibacterial activity of *Psidium guajava* and Gongronema Latifolium. J. Med. Plants Res. 2(8): 189-192

Odebiyi OO, Sofowora EA (1978). Phyto-chemical screening of Nigerian medicinal plants – Part II. Lloydia 41:234.

Pentreath VW, Kennedy GE (2004) Pathogenesis of human African trypanosomiasis. In *The Trypanosomiasis* (eds. I. Maudlin, P. H. Holmes and Miles) CABI Publishing pp. 283-301

Poltera AA (1985) Pathology of human African trypanosomiasis with reference to experimental African trypanosomiasis and infections of the central nervous system. Brit. Med. Bull. 41:169-174

Rosa MP, Sylvia M, and Rosario VS (2008). *Psidium guajava*: A review of its traditional uses, phytochemistry and pharmacology. J. Ethnophamacol. pp ????

Sternberg JM (2004) Human African trypanosomiasis: clinical presentation and immune response. Parasite Immunol. 26:469-476

TDR (1984) Tropical Disease Research, 7th programme report 1st January, 1984 – 31st December 1984. Chapter 5, 3-9. United Nations Development Programme/world bank/ world health organization.

Thapanon S, Supaporn KH, Somchai L, Jaroon J, Gary DC, Kate G (2005). Exploiting guava leaf extract as an alternative natural reagent for flow injection determination of iron. Talanta 68 (2): 262-267 13th International Conference on Flow Injection Analysis – ICFIA 2005.

Vieira RHS, Rodrigues D, Goncalves FA, Menezes FGR, Aragao JS, Sousa OV (2001). Microbicidal effect of medicinal plant extracts (*Psidium guajava* Linn. and *Carica papaya* Linn.) upon bacteria isolated from fish muscle and known to induce diarrhea in children. Instituto de Ciencias do Mar-UFC, Ceara, Braxzil. Revista do Instituto de Medicina Tropical de Sao Paulo. 43 (3): 145-148.