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

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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
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 antimitogenic 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**

*Trypanosoma 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 passing 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
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; Penttreated 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
The extract extended the life span of the treated animals and infected animals respectively. The results showed that the extract considerably contributed to the overall effect of the extract as it have contributed to the overall effect of the extract as it were in this study. Several studies have indicated flavonoids 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 the leaf of *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


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

<table>
<thead>
<tr>
<th>Compounds tested</th>
<th>Results obtained</th>
</tr>
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<tbody>
<tr>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>Reducing sugars</td>
<td>+</td>
</tr>
<tr>
<td>Lipids</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>Tannins/Polyphenols</td>
<td>++</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+++</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
</tbody>
</table>

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

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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 flavonoids as the main constituent responsible for the antimicrobial activities of *P. guajava* leaf extract.