Journal of Medicinal Plants Research Vol. 4(19), pp. 2016-2020, 4 October, 2010
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR10.416
ISSN 1996-0875 ©2010 Academic Journals

Full Length Research Paper

Studies of phytochemical constituents and anti-trypanosomal properties of fermented wheat germ and garlic bulbs extract on *Trypanosoma brucei* – infected rats

Oluwatosin K. Yusuf[1]* and Justine T. Ekanem[2]

[1]Department of Biochemistry, Federal University of Technology, P. M. B. 65, Minna, Nigeria.
[2]Department of Biochemistry, University of Uyo, Uyo, Nigeria.

Accepted 14 September, 2010

Ethyl acetate extract of wheat (*Triticum aestivum*) and methanolic extract of garlic (*Allium sativum*) were obtained by fermenting powdered wheat germ and garlic bulbs. The extracts were assessed for their active constituents. The result of the quantitative phytochemical analysis shows that the plant contain secondary metabolite with high percentage of glycoside (19.513%), alkaloids (4.017%) and saponins (7.992%) for wheat extract and glycoside (21.088%), alkaloids (3.570%) and saponins (0.696%) for garlic extract. The extract exhibit antit-rypanosomal activity by showing decrease in the proliferation of parasite and extension of surviving days of *Trypanosoma brucei* - infected rats from 8 days of the control (infected-untreated) to 14 days of infected treated with wheat and 17 days for infected treated with garlic extract. This study scientifically demonstrates the potential of fermented wheat germ ethylacetate extract and garlic bulbs methanolic extract in the management of Africa trypanosomiasis.

Key words: Anti-trypanosomal, wheat germ, garlic bulbs, Africa trypanosomiasis, phytochemicals

INTRODUCTION

Chemotherapy against African trypanosomiasis, a disease caused by *Trypansoma brucei* species which affected humans and animal, is best with problems. Therefore, the expensive nature of current trypanocides, coupled with the unbearable side effects necessitates the search for better drugs, in which natural products may offer unlimited source of chemical diversity for identification of new drug leads (Camacho et al., 2000; Fournet and Munoz, 2002). Medicinal plants are widely used worldwide to address a variety of health problems. About 25 to 50% of current pharmaceuticals are derived from plants (Cowan, 1999; Goh et al., 1995). Plants are rich in a wide variety of phytochemical metabolites which are divided into two groups: Primary and Secondary metabolite. Primary metabolite comprises of common sugars, amino acids, proteins and chlorophyll while Secondary metabolite consist of glycosides, alkaloids, saponins, phenolic compounds, terpenes steroids, anthraquinone etc (Mitcher et al., 1988; Habtermariam, 1993). The increasing demand for medicinal plant products has stimulated research in this field.

Fermented wheat germ extract called avemer was chosen for this work because it has been reported to control cell growth and proliferation mainly by inhibiting ribonucleotide reductase needed to make new DNA to

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

support replication (Sukkar and Edoardo, 2004). It had also been reported that avemer limit the access to glucose, needed to make the ribose sugar for DNA and RNA for new cancer cells (Boros et al., 2002; Boros et al., 1997) but the actual secondary metabolite constitute has not been fully studied. Garlic bulb *(Allium sativium)* was also chosen for study because of its easily availability and of is important part of diet of many population with long – held belief in their health enhancing properties. Garlic bulb *(A. sativum)* has been reported to contain two classes of antioxidant components namely flavonoids and polyphenol derivatives which are naturally occurring compound of gallic acid. Gallic acid had been reported to inhibit ribonucleotide reductase by causing imbalance of deoxynucleotide triphosphate (dNTP) (Sibylle et al., 2006). Garlic has also been found to reduce platelet aggregation and hyperlipidemia (Silagy and Neil, 1994; Gardner et al., 2007). It had also been reported to possess cancer fighting properties.The studied of the presence of phytochemical in the extracts and investigation of trypanocidal properties would explain their possible use in the control or management of African sleeping sickness.

MATERIALS AND METHODS

Collection of plant material

Wheat germ (Triticum aestivum) and fresh bulbs of A. sativum L., commonly known as garlic were purchased from Minna Central Market, Niger State, Nigeria and authentication was carried out at Federal College of Forestry, Ibadan, Oyo state.

Chemicals

Suramin and berenil were obtained from Sigma Aldrich, United state.

Parasite inoculum

T. brucei was obtained from the Veterinary and Livestock Studies Department of the Nigerian Institute for Trypanosomiasis Research, Vom, Plataeu State of Nigeria. The parasite would be maintained through a passage of other rats.

Preparation of plant extract

Wheat germ powder of 70 g was fermented using 30 g of *Saccharomyces cerevisiae* (baker's yeast) for 48 h and the paste would be extracted using 250 ml ethyl acetate. The filtrate was concentrated using rotary evaporator and stored at room temperature. Garlic bulbs *(A. sativium)* were opened to reveal its fleshy sections called cloves. The cloves were peeled and blended.100 g of *A. sativium* was soaked using 250 ml methanol for 24 h and filtered. The solvent was removed using rotary evaporator. The crude extract was used in subsequent studies.

Experimental animals

Albino rats weighing approximately 200 g were obtained from the animal breeding unit of the department of Biochemistry, University of Ilorin, kwara state and fed with animal feed obtained from Bendel Feeds and Flour Mill Ltd, Ewo, Edo state.

Phytochemical analysis

Identification was conducted on the crude extracts using the method of Sofowora (1993).

Quantitative analysis on phytochemical constituents

Quantitative analysis of the phytochemical was carried out on the crude extracts. Flavonoid was quantified using the method of Allen's commercial organic analysis, 1979. Glycoside, steroids, phlobatannin and terpene were quantified using the method of Analytical Committee of Royal Society of Chemistry. Tannin was quantified using the method of Association of Analytical Chemistry (A.0.A.C). Alkaloid was quantified using the method of Henry (1993). Saponin was quantified using the method of Brunner (1984). Anthraquinones was quantified using the method of Lewis and Elvin-Lewis (1977). Phenol was quantified using the method of Harborne (1978).

Parasitaemia determination

Evaluation of parasitaemia was carried out 24 h interval to monitor infection progress. This was done by counting the number of parasite under the light microscope at X100 magnification from thin blood smear freshly obtained from the tip of the tail of infected rats.

Administration of extracts

Infected rats were administered intraperitoneally with 0.5 ml solution of extract containing 300 mg/kg body weight on the first day of sighting parasite in the blood (normally 3 days post infection) of infected rats. Administration of the extract continued on daily basis until the rats died. Infected untreated rats were considered as the control against the infected treated suramin, infected treated berenil, infected treated wheat and infected treated garlic.

Statistical analysis

Data were statistically analyzed and differences compared using the Student's't' test (Student, 1908) as described by Adamu and Johnson, 1997 while the level of statistical significance was taken at 5% confidence.

RESULTS

Phytochemical constituents of fermented wheat and garlic bulb extracts

The result of phytochemical analyses of 24 h fermented ethylacetate wheat extract showed appreciable amount of glycoside, alkaloids and saponins; moderate amount of phenol, tannins, flavonoid, steroids, terpenes and

Table 1. Phytochemical constituents of fermented wheat and garlic bulbs extracts.

Phytochemical	WheaT (%) ± S.D	Garlic (%)+ S.D
Tannins	0.071 ± 0.001	0.058 ± 0.000
Phenol	0.074 ± 0.005	0.075 ± 0.001
Flavonoids	0.0790 ± 0.000	0.052 ± 0.001
Steroid	0.073 ± 0.001	0.086 ± 0.004
Saponins	7.992 ± 0.031	0.696 ± 0.184
Phlobatannins	0.020 ± 0.002	0.025 ± 0.001
Terpenes	0.0844 ± 0.002	0.063 ± 0.001
Alkaloids	4.017 ± 0.259	3.570 ± 0.014
Glycosides	19.513 ± 0.111	21.088 ± 0.877
Anthraquinone	0.150 ± 0.001	0.092 ± 0.001

Each value is a mean of four determinations.

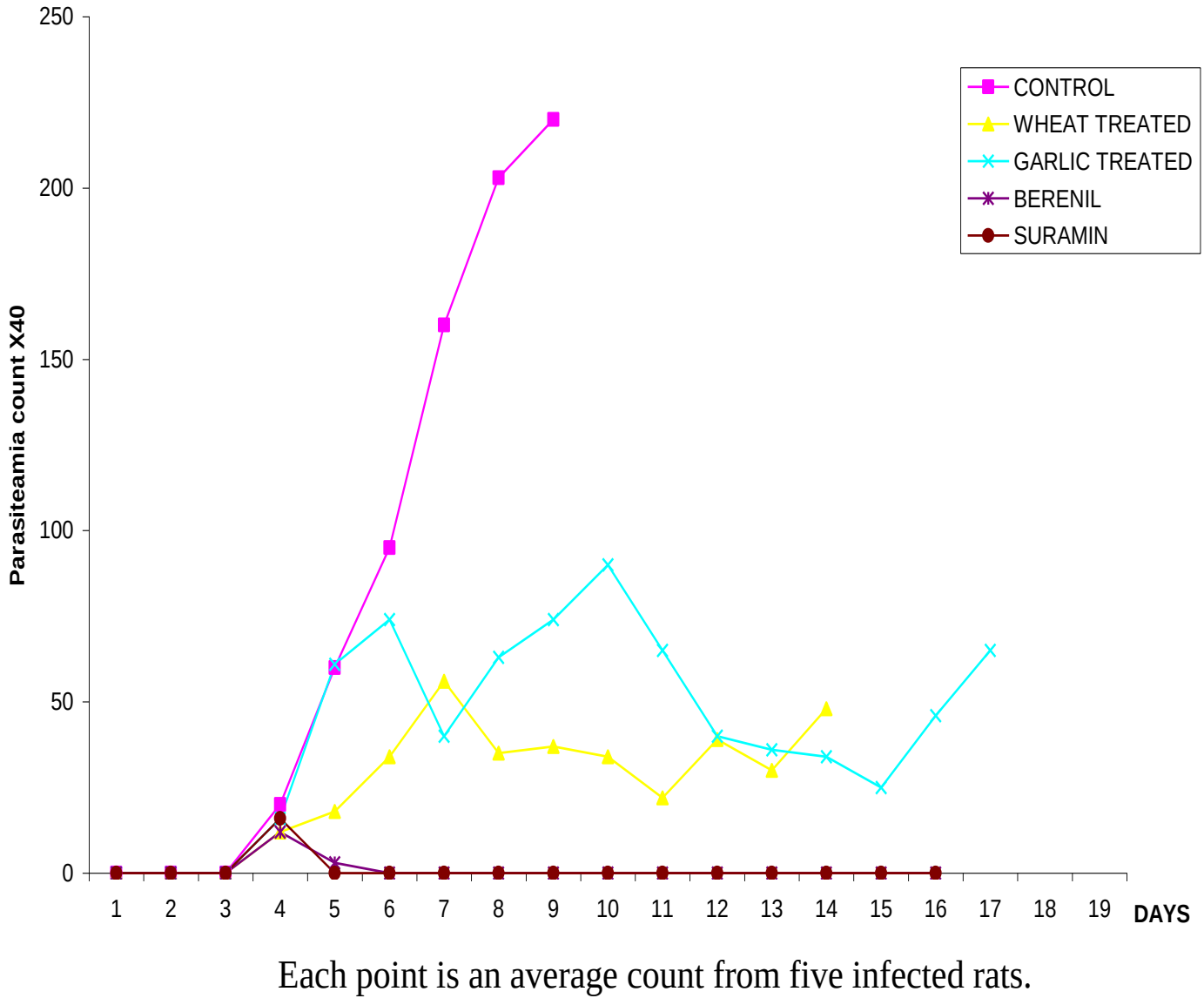

Figure 1. Parasiteamia count of rats infected with *T. brucei* and treated with standard drugs, fermented wheat and garlic extract.

anthraquinone and trace amount of phlobatannins (Table 1). Also, phytochemical analyses of 48 h fermented methanolic garlic extract show appreciable amount of glycoside, alkaloids and saponins; moderate amount of phenol, tannins, flavonoid, steroids, terpenes and anthraquinone and trace amount of phlobatannins (Table 1).

Anti-trypanosomal properties of fermented wheat germ and garlic bulbs extract in *T. brucei* – infected rats

Figure 1 showed the result of parasite count in infected rats treated with suramin, berenil (registered standard drugs), wheat and garlic bulbs extract at 300 mg/kg body weight compared with the control (infected untreated) rats. Suramin, berenil, wheat and garlic bulbs extracts were administered separately to infected rats to assess its activities against *T. brucei* infection. Suramin and berenil shows total clearance of parasite from the bloodstream after some days of treatment (24 h with suramin and 48 h with berenil) (Figure 1).The parasitaemia count of infected untreated group increased infinitely while infected treated with wheat extract shows a decrease in the proliferation (Figure 1). The graph shows low replication of parasite and extension of surviving days of rats treated with wheat extract from 8 days of the control (infected untreated) to 14 days for the infected treated group. Also, the parasitaemia of infected treated with garlic bulbs extract of 300 mg/kg showed a decrease in the proliferation of parasite and extension of surviving days of rats from 8 days of the control (infected untreated) to 17 days for infected garlic treated rats (Figure 1).

DISCUSSION

Many studies have been carried out in recent years on the pharmacological effects of wheat and garlic crude extracts (Suttle et al., 2000). Fermented wheat extract has been reported to have anti-proliferative action that target nucleic acid synthesis enzymes (Tian et al., 1999). The extract also has analgesic, antimicrobial, anti-inflammatory and immunological effects (Tsen, 1985). Garlic has been used as a remedy for infection (Koch and Lawson, 1996). It has been claimed to help in preventing heart disease, high cholesterol, high blood pressure and cancer (Mader, 1990; Block, 1992; Silagy and Neil, 1994; Gardner et al., 2007).

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 was 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).

Fermented wheat and garlic bulbs extract has anti-trypanosomal properties as well as the ability to extend the life span of *T. brucei*-infected rats (Figure 1). This may be as a result of phytochemical constituents of the extracts. Phytochemical analysis result showed that the extracts have appreciable amount of alkaloids, glycosides and saponins (Table 1). The presence of glycoside can explained the antioxidant properties of the extract.

Antioxidants neutralize highly unstable and extremely reactive molecules, called free radicals, which attack the cells of human body (Stauth, 2007). Free radical damage is believed to contribute to a variety of health problems, including cancer, heart disease and aging (Stauth, 2007). Also another reason for the medicinal properties of the extract may be due to cleanse and purify blood properties of saponins (Kenner and Requena, 1996).

Alkaloids, comprising of a large group of nitrogenous compounds are widely used as cancer chemotherapeutic agents (Chabner and Horwitz, 1990; Noble, 1990). Alkaloids also interfere with cell division. Therefore, the results suggest that the extracts probably have antitrypanocidal properties as well as the ability to reduce parasitaemia and the severity of the disease. Antioxidation is probably one of the ways by which the extracts achieves their results (Adsule et al.,1986; Paul et al.,1987; Holland et al., 1991; Andorfer et al., 2003; Lee et al., 2003). Methoxy – substituted benzoquinone, which are present as glycosides implicated as active component of fermented wheat (Tian et al., 1999; Suttle et al., 2000) and sulphur containing compounds of garlic, *allicin* (Lee et al., 2003) might be the cytotoxic constituent conferring trypanocidal properties. However, it can be suggested at this point that the extracts could be a useful cheap agent for the management of African sleeping sickness.

Conclusion

This research work has revealed further potential of fermented wheat and garlic bulbs extract. This study has provided the phytochemical constituents of the extracts and it usefulness in treatment of African sleeping sickness. The quantitative analysis of phytochemical constituents of fermented wheat and garlic bulbs extract shows that the extracts are rich in glycosides, alkaloids

and saponins which are popular phytochemical constituents and also scientifically demonstrates the antitrypanosomal properties of the extract on *T. brucei* - infected rats.

REFERENCES

Adsule RN, Kadam SS, Salunkhe Dk, Austin A (1986). Wheat products. New Delhi, Indian: Metropolitan Book Co, pp. 12-6.

Allen's commercial Organic Analysis (1979). Analysis of Analytical Methods Committee of Royal Society of Chemistry.AMC-RSC. 9: 156-189. pp. 222-239.

Andorfer JH, Tchaikovskaya T, Listowsky I (2003). Selective expression of glutathione S-transferase genes in the murine gastrointestinal tract in response to dietary organosulphur compounds. *Carcinogenesis,* November 21.

Block E (1992). The organosulfur chemistry of the genus Allium — implications for organic sulfur chemistry. *Angewandte* Chemie Int. Edition, 104: 1158-1203.

Boros LG, Puigjaner J, Cascante M, Lee WN, Brandes JL, Bassilian S (1997). Oxythiamine and dehydroepiandrosterone inhibit the nonoxidative synthesis of ribose and tumor cell proliferation. Cancer. Res., 57: 4242-8.

Boros LG, Cascante M, Paul Lee WN (2002). Metabolic profiling of cell growth and death in cancer: applications in drug discovery. Drug Discov. Today, 7: 364-372.

Brunner JH (1984). Direct Spectrophotometer determination of Saponin. Anal. Chem., 34: 1314-1326.

Cowan MM (1999) Plants products as anti-microbial agents. Clinical. Microbiol. Rev., 12: 564-582.

Chabner BA, Horwitz SB (1999). Plant alkaloids in: Meyer R, Pinedo HM, Chabner BA, Cancer Chemotheraphy and Biological Response Modifiers Annual 18. Elsevier Science Publ. Co. p. 632.

Gardner CD, Lawson LD, Block E, Chatterjee LM, Kiazand A, Balise RR, Kraemer HC (2007). The effect of raw garlic vs. garlic supplements on plasma lipids concentrations in adults with moderate hypercholesterolemia: A clinical trial. Archives. Int. Med., 167: 346-353.

Goh SH, Chuah CH, Mok JSL, Soepadmo E (1995). Malaysian Medicinal Plants for the Treatment of Cardiovascular Diseases. Selangor Darul Ehsan: Pelanduk Publication. Kaula Lumpur, Malaysia.

Habtermariam S, Gray AI, Waterman RG (1993). A new anti-Bacterial sesquiterpene from *Premma oligotricha*. J. Nat. Prod., 5(1): 140–143.

Harborne JB (1978). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis.Chapman A & Hall.London, 1973: 279.

Henry TA (1993). A textbook titled The plant Alkaloids.,pp. 6-466.

Holland B, Welch AA, Unwin ID, Buss DH, Paul AA, Southgate DAT (1991). In McCance and Widdowson's. The composition of foods. The Royal society of chemistry. Cambridge.

Lee YL, Cesario T, Wang Y, Shanbrom E, Thrup L (2003). Antibacterial activity of vegetables and juices. Nutrition. 19(11-12): 994-996.

Lewis WH, Elvin – Lewis MPF (1977). Plants Affecting Man's Health. Kidney/ Liver Medical Botany. p. 425.

McGee, Harold (2004). *On Food and Cooking (Revised Edition)*. Scribner. ISBN 0.684-80001-2. The Onion Family: Onions Garlic, Leeks., pp. 310-313.

Mitcher LA, Okwute SK, Gollapudie SR, Drake S, Anova E (1988). Antimicrobial pterocarpans of Nigeria *Erythrina midbreadii.* Phytochemical, 27(11): 3449-3452.

Noble RI (1990). The discovery of Vinca alkaloids chemothera-peutic agents against cancer. Biochem. Cell. Biol., 68(12): 1544–1551.

Paul AA, Southgate DAT, Russell J (1987). Supplement to McCance and Widdowson's. The composition of foods. Her Majestys Stationery Office, London.

Sibylle Madlenera, Christoph Illmera, Zsuzsanna Horvatha, Philipp Saikoa, Annemarie Losertb, Irene Herbacekb, Michael Gruschb, Howard L, Elfordc, Georg Krupitzad, Astrid Bernhausa, Monika Fritzer-Szekeresa, Thomas Szekeresa (2006). Gallic acid inhibits ribonucleotide reductase and cyclooxygenases in human HL-60 promyelocytic leukemia cells. 245(1): 156-162.

Silagy C, Neil A (1994).Garlic as a lipid – lowering agent- a meta – analysis, J. Royal College Physicians., 28(1): 2-8.

Sofowara A (1993). Medicinal Plants and Traditional Medicinal in Africa. 2nd Edition. Spectrum Books, Ibadan , Nigeria. pp. 26-100.

Sukkar SG, Edoardo R (2004). Oxidative stress and nutritutional prevention in autoimmune rheumatic diseases, Autoimmunity. Rev., 3: 199-206.

Tian WN, Brawnstein LD, Apse K, Pang J, Rose M, Tian X, Stamton RC (1999) Compositional value of wheat. Annual. J. Physiol., 276: 1121-1131.

Tsen CC (1985). Amino acid composition and biological value of cereal germs/protein. In Lasztity R, Hidvegi MD, Reidel Publishing Co. Boston. pp. 453-466.