PHYTOCHEMICAL AND ANTIOXIDANT QUALITIES OF PHALANTUS AMARUS FOR USE IN LIVESTOCK FARM

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Abstract

Phyllanthus amarus has been used long time ago to cure diseases for human, this study try to extend the use of P.amarus to livestock by studying, the phytochemical and antioxidant characteristics of *P. amarus* methanol extract. Standard procedures were used to analyze: 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) percentage (%) inhibition. The highest the concentration the the higher the inhibition and at 300 µg/ml, 44.58 inhibition was obtained with least in 25 µg/ml 28.66 was obtained. The proximate composition also shows that the energy and crude fat was higher when compared to other parameters. The most prevalent compound was flavonoid, terpenoid and phenol Owing their antioxidant and phytochemical qualities, *P. amarus* methanol extract may potentially find application in medicine.

Keywords: Inoculated, performance, Phyllanthus amarus, sporulation and treatments

Introduction

Poultry birds especially broilers are known to come down with disease either contacted in the farm where they are raised or coming with the disease from the hatchery, the farmer should therefore be ready to monitor their health status throughout their stay on the farm. This is why the use of antibiotics among poultry farmers is common, but the use of this antibiotics has side effect as it causes residual effect and drug resistance to drugs by the birds. This is why the use of herbal plants come to play because African's are known to have a lot of herbal plants that are medicinal, they are readily available all year round because they are locally sourced, they are cost effective, rich in vitamins and minerals, all this makes them qualify to replace most of the drugs we use for our livestock. P. amari belong to the genus is one such significant medicinal plant that grows throughout the world including India. This plant has been known for its usage in the 'Ayurvedic' system of medicine for over 2000 years. *P. amarus* have been used for treating multi-faceted diseases like hepatitis B, jaundice, diarrhoea, dysentery, dropsy, intermittent fevers, Herpes Simplex virus, inflammation, oxidative stress, hypotensive, urinary disorders, etc. Adeney et.al,. (2006).

Coccidiosis is an infective disease of manyspecies of mammals and birds caused by protozoa of the Phylum Apicomplexa which causes diarrhoea, retarded growth, a slower feed conversion and increased mortality especially inchickens. The disease is caused by parasites of the genus Eimeria,Isospora and Cryptospora with a complex life cycle, affecting mainly the intestinal tract, especially in chicken it is one of the most serious diseases in chicken production as economic losses are possible even before the manifestation of clinical signs of the disease. These parasites cause damage in chicken production, chickens reared in large numbers and high densities (Peek, 2010). Management has always been important to control coccidiosis in poultry, however, it is very difficult to keep chickens coccidia free as oocytes are omnipresent and spread widely in the poultry house, and requires the administration of various drugs through feed and water. This parasitic disease is an expensive disease and is estimated to cost the poultry industry by 3.2 billion USD annually (Dalloul and Lillehoj, 2006). In Nigeria where poultry farming is less developed, the disease is more serious and causes heavy economic losses which could be in the region of millions of Naira (Maikai et al., 2007; Fornace et al., 2013). The major part of these costs, around 80%, is due to the losses in growth performance, and the rest is due to the costs of prophylaxis and treatment (Vermeulen et al., 2001). Coccidiosis infection led to decreased body gain and deteriorate feed conversion ratio, as well as

increased incidence of diarrhea and intestinal hemorrhage (Williams, 2005). All of these symptoms are economically significant to the poultry industry. Additionally, Williams, (2005) found other pathophysiological effects associated with coccidiosis which include, poor feed efficiency, reduced water intake, increased intestinal passage time, decreased digesta viscosity, intestinal mal-absorption, villus atrophy, intestinal leakage of plasma proteins, and increased intestinal activity owing to the widespread occurrence of drug resistant parasites.

Coccidiosis is generally controlled by using anticoccidial drugs which are administered in feed of chickens (Blake and Tomley, 2014; Shivaramaiah et al., 2014) and use of live vaccines, new approaches of controlling coccidiosis are urgently needed especially natural plant extract to treat and control Eimeria (Innes and Vermeulen, 2006; Williams, 2006; Morris et al., 2007). Management focuses on decreasing coccidial numbers to keep infection at a minimum until immunity is established in young birds proper hygiene use of anti-coccidial drugs and vaccines play major roles in controlling coccidiosis in commercial broiler chicken production. Coccidiosis is traditionally treated by chemotherapy but the appearance of drug resistance types of coccidia suggests the need of using alternative strategies such as the natural products from plant extract. P. amarus is a small erect tropical herb that grows to a height of 10-60cm. It is an annual and widespread throughout the tropics and subtropics. It is found in sandy regions as a weed in cultivated and wastelands. The plant can be found along roads, valleys, on riverbanks and near lakes. The broad objective of this study was to evaluate the efficacy of P. amarus leaf extract as alternative anticoccidial for broiler chicken production. While the specific objectives were to determine the effect of P. amarus leaf extract treatment on growth performance and carcass characteristics of broiler chickens infected with Eimeria oocysts. This project their will try to study the antioxidanr, phytochemicals, minerals and vitamins content of the Phyllantus amarus

Material and methods

Sample collection and preparation

Leaves of Phylantus amarus obtained from surrounding of Kwara State University, Malate. The leaves was manually washed and air-dried for 10 h separately and then milled into fine powder.

Extraction and phyto-constituents determination

Extraction

The bioactive compounds of the leaves of Phylantus amarus was extracted with ethanol to obtain the extracts of the two herbs. Abioye and Ama (2015)

Alkaloids

One milliliter of aqueous extract of the sample was suspended in 1 ml of distilled water and drops of diluted hydrochloric acid (2 M). To the resulting mixture 1 mL Dragendorff's reagent was added. An orange-red precipitate shows the presence of alkaloids . Jongrungruangchok *et al*., (2017)

Flavonoids

To 5 g of the sample, 5 ml of methanol was added and filtered. To a portion of the filtrate, 2 mL of sodium hydroxide solution was added. Three drops of diluted hydrochloric acid were added to the resultant mixture. The appearance of a yellow solution when the sodium hydroxide solution was added which became colourless when dilute hydrochloric acid was added, this confirmed that flavonoids are present He et al., (2018)

Saponins

Two grams of powdered plant material were suspended in 5 mL distilled water. The mixture was vigorously shaken for 1 min. Persistent copious lather for more than 5 min shows the presence of saponins Fahey, (2015)

Phenols

To 1 g of powdered plant material, 2 mL of distilled water was added after which the mixture was filtered. Ferric chloride (10 %) solution was then added to the filtrate dropwise. A dark green color inferred a positive result for the presence of phenols DanMalam et al., 2011

Terpenoids

Five grams of the samples was suspended in chloroform (2 mL) after which 3 ml of concentrated sulfuric acid was added to the resulting solution. The formation of a reddish-brown colored interface showed a positive inference of terpenoids Jimoh and Olatidoye, 2009

Tannins

To 5 g of the sample, 10 ml of distilled water, was added heated, and filtered. One milliliter of FeCl f iltrate. A blueish-green precipitate shows the presence of tannins Hekmat et al., 2015

DPPH free radical scavenging assay

A DPPH solution (0.2 mM) was prepared (0.0078864 g of DPPH in 100 ml methanol). In a microtiter plate, 150 µl of 0.2 mM DPPH was added and followed by a 50 µl of standard (ascorbic acid; , ranging 40 µg/ml to 200 µg/ml) or extract (BO/DBEE/BFWE; ranging 200 µg/ml to 1000 µg/ml). The mixture was left to stand in the dark at 27 ◦C for 30 min. From the resulting solution absorbances were taken against a blank at 517 nm with a SPECTROstar NANO, spectrophotometer. The scavenging activity of the extracts against DPPH radical was calculated as scavenging activity (%) =(DPPHabs – Extractabs/DPPHabs) ×100 Jongrungruangchok et al., (2017)

Proximate composition analysis

The nutritional composition such as moisture, ash, protein, fat, fiber, and carbohydrate content of the seeds and fruit pulp were determined using techniques certified by the Association of Official Agricultural Chemists (AOAC) with slight modifications Nkukwana et al., (2014). Each analysis was done in triplicates. A weighed sample (5 g) was placed in a preheated oven at 105 ◦ C and dried to a contant weight to measure the percentage moisture. The micro-Kjeldahl technique was used to determine the crude protein concentration . Percentage fatin the seeds and fruit pulp were determined by extracting from a 5 g sample in petroleum ether through the Soxhlet extraction method Nkukwana et al., 2014. After digesting a measured amount of the fat-free residue produced from the crude fat determination, the crude fiber was evaluated gravimetrically. With 1.25 % sodium hydroxide and 1.25 % sulfuric acid, the digestion was carried out under reflux. The sample was burned for 10 h at 600 ◦ C in a muffle furnace to ascertain its ash content. Carbohydrate percentages of the various samples were estimated as follows: Carbohydrate (%) = 100 - % yield [moisture + fat + ash + protein + fiber] The Total Energy values of the plant materials were obtained by calculations according to the Atwater factor as shown below: Energy value (kcal) = 4(% crude protein) + 9(% crude fat) + 4(% carbohydrate). The following analysis was carried out using standard method as indicated by the authors

Results and Discussions

Tabl 1 shows the phytochemical screening of phyllantus amari, Parameters like Tannin, saponin, Flavonoids, Terpenoids, Alkanoid and phenols were investigated in Phyllantus amarus leaves and it was discovered that, all were present except the saponin and alkaloids that are absent based on analysis that was conducted.

Table 2 shows the DPPH of the invitro assay of phyllanttus amarus leaves, it was discovered that significant difference occurred ($P<0.05$) in the Inhibition (%) of the sample, the higher the Conc. Sample (µg/ml) used then the higher the correspondent inhibition value, the highest value was obtained at 300 Conc. (µg/ml) .
Proximate composition shows that , phyllantus amarus has higher value of energy and fat in the leaves.

Table 1: Phytochemical screening of *Phyllathus amarus* leaves

Parameters	
Tannin	++
Saponin	-
Flavonoids	++
Terpenoids	++
Alkaloids	-
Phenols	++

N.B: + present, ++ much present and - absent

Table 2: DPPH Radical Scavenging Activity of P. amarus Leaves

Conc. Sample (µg/ml)	DPPH Inhibition (%)
25	28.66 ± 0.03^{c}
50	28.36 ± 0.08^{c}
75	31.05 ± 0.02^{c}
150	40.54 ± 0.13^{b}
300	44.58 ± 0.01^{a}

a,b & c are Means with different superscript on the same row differ significantly ($p< 0.05$).

Table 3; showing proximate composition of Phyllsantus amarus

Sample	**Moisture (%)**	**Ash(%)**	**Crude fibre(%)**	**Crude protein(%)**	**Crude fat(%)**	**C'ate(%)**	**Energy(kg/kcal)**
	5.15	2.00	0.22	12.05	4.05	76.54	390.79

Conclusions

The investigation reveals that Phyllantus amarus could be used to treat a number of livestock diseases because of both the phytochemicals and antioxidant capacity.

References

Adeneye, A.A., Benebo, A.S. and Agbaje, E.O. (2006): Protective effect of the aqueous leaf and seed extract of phyllanthus amarus on alcohol induced hepatotoxicity in rats. West African Journal of Pharmacological Drug Research. 23:42- 50.

Amadi, B.A., Agomuo, E.N. and Ibegbulen, C.O. (2004): Research methods in biochemistry, Supreme Publishers Owerri, Nigeria.

Anbudhasan, P., Surendraraj, S., Karkuzhali, S. and Sathishkumaran, S. (2014): Natural antioxidants and its benefits. International Journal of Food and Nutritional Sciences. 3(6). 225-232.

Antolovich, M., Prenzler, P. and Patsalides, E. (2002): Methods for testing antioxidant activity. Analyst, 127:183-198

Akubor PI (2016). Chemical composition and functional properties of african locust bean pulp flour and wheat flours 1 (1). FUW Trends in Science and Technology Journal 1(1):248-253.

Association of Official Analytical Chemists (AOAC) (2005). Official Methods of Analysis of the Association of Official Analytical Chemists, 18th edition. AOAC, Arlington, Va, USA.

Chadare FJ, Linnemann AR, Hounhouigan JD, Nout MJR, Van Boekel MAJS (2009). Baobab food products: a review on their composition and nutritional value. Reviews in Food Science and Nutrition 49(3):254-274. Dahouenon-Ahoussi E, Adjou ES, Lozes E, Yehouenou,LL, Hounye R, Famy N, Soumanou MM, Sohounhloue DCK (2012). Nutritional and microbiological characterization of pulp powder of locust bean (Parkia biglobosa Benth.) used as a supplement in infant feeding in Northern Benin'. African Journal of Food Science 6(9):232-238.

FAO/WHO (2001). Human Vitamin and Mineral Requirements: Report of a Joint FAO/WHO Expert Consultation. In . Bangkok, Thailand. Food and Nutrition Division: FAO Rome.

Kayalto B, Zongo C, Compaore RW, Savadogo A, Otchom BB, Traore AS (2013). Study of the nutritional value and hygienic quality of local infant flours from chad, with the aim of their use for improved infant flours preparation. Food and Nutrition Sciences 4:59-68. Makalao MM, Savadogo A, Zongo C, Traore AS (2015). Composition nutritionnelle de 10 fruits sauvages consommés dans trois départements du Tchad. International Journal of Biological and Chemical Sciences 9(5):2385-2400.

Maranz S, Wiesman Z, Bisgaard J, Bianchi G (2004). Germplasm resources of Vitellaria paradoxa based on variations in fat composition across the species distribution range. Agroforestry Systems 60:71-76.

MECV (2007). Programme d'action national d'adaptation a la variabilité et aux changements climatiques (PANA du Burkina Faso). Minitsère de l'Environnement et du Cadre de Vie: Burkina Faso Tech. rep.

Montreuil J, Spik G (1969). Microdosage des glucides. I/Méthode colorimétrique de dosage des glucides totaux. Monographe du labochimie-biol. Fac. Sc. Lille (France).

Olujobi OJ (2012). Comparative evaluation of nutritional composition of african locust bean_regional variation. Nigerian Journal of Basic and Applied Science 20(3):195-198.

Ouédraogo AS (1995). Parkia biglobosa (Leguminosae) en Afrique de l'Ouest: Biosystématique et Amélioration. Thèse doctorat Wageningen University, Institute for Forestry and Nature Research, IBN-DLO. 205 p.

Ouoba LI, Diawara B, Amoa-Awua WK, Traore AS, Møller LP (2004). Genotyping of starter cultures of Bacillus subtilis and Bacillus pumilus for fermentation of African locust bean (Parkia biglobosa) to produce Soumbala. International Journal of Food Microbiology 90:197-205.

Pallo FJP, Sawadogo N, Zombré NP, Sedogo MP (2009). Statut de la matière organique des sols de la zone nord soudanienne au Burkina Faso. Biotechnology, Agronomy, Society and Environment 13(1):139-142.

Somé L, Sivakumar MVK (1994). Analyse de la longueur de la saison culturale en fonction de la date de début des pluies au Burkina Faso. Compte rendu des travaux n°1. Division du sol et agroclimatologie. INERA/Burkina Faso - ICRISAT/Niger 16 p.

Somé IT, Zagré MN, Kafando PE, Bendech MA, Baker SK, Deslile H, Guissou PI (2004). Validation d'une méthode de dosage des caroténoïdes par CLHP: application à la détermination de teneur en caroténoïdes dans dix variétés de patates douces (Ipomea batata). C. R. Chimie 7:1063-1071

Stadlmayer B, Charrondière UR, Eisenwagen S, Jamnadas R, Kehlenbeck K (2013). Nutrient composition of selected indigenous fruits from sub-Saharan Africa. Journal of Science, Food and Agriculture 93:2627-2636.

Teklehaimanot Z (2004). Exploiting the potential of indigenous agroforestry trees: Parkia biglobosa (Jacq.) Benth and Vitellaria paradoxa in sub-Saharan Africa. Agroforestry Systems 61:207-220.

Thiombiano DN E, Parkouda C, Lamien N, Séré A. Castro -Euler AM, Boussin IJ (2014). Nutritional composition of five trees species products used in human diet during food shortage period in Burkina Faso. African Journal of Biotechnology 13(17):1807-1812.

Thiombiano DNE, Lamien N, Castro-Euler AM, Vinceti B, Agundez D, Boussim IJ (2013). Local communities demand for food tree species and the potentialities of their landscapes in two ecological zones of Burkina Faso. Open Journal of Forestry 3(3):79-87.