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Qualitative phytochemical screening, micronutrients and heavy metal evaluation of Oat milk (*Avena sativa*)

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Abstract

Oat (*Avena sativa*), originally used as animal feeds is a cereal with both nutritional and therapeutic effects. This study as designed to investigate the phytochemical constituents, the presence of some micronutrients and heavy metals in oat milk. The Oat milk (Quaker oat®) was purchased in a supermarket in Abraka, Delta State, Nigeria and extracted using soxhlet apparatus with 80% methanol as soxhlet. The extract was then lyophilized slowly dried in an oven at 40°C and analyzed for the presence of phytochemicals using standard methods. Sample digestion for micronutrient and heavy metal determination was done with about 2 g of the sample measured into a digestion flask previously soaked in 20% HNO₃ for 24 hrs using a mixture of 2 ml of conc. HClO₄ and 12 ml of conc. NHO₃. The digested sample was analyzed for the presence of some micronutrients (Zn, Se, Cu, Co, Ni) and heavy metals (Pb, Cr, Cd, Fe) using atomic absorption spectrometry. The study shows the presence of the following phytochemicals; alkaloids, flavonoids, tannins, saponins, terpenoids, steroids, cardiac glycosides, reducing sugar, protein and carbohydrates. Micronutrients detected in the sample were Zn (12.02mg/kg), Se (0.08mg/kg), Cu (60±0.60mg/kg), Co (0.008±0.11mg/kg), Ni (0.10±0.02mg/kg) and heavy metals include; Pb (0.36±0.02g/kg), Cr (0.01±0.02 g/kg), Cd (0.04±0.01 g/kg), Fe (119.4±0.02g/kg). The micronutrient meets the requirements for the RDA and the detected heavy metals concentrations were above the permissible limit of WHO.

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Introduction

Avena sativa, popularly called oat is a cereal with both nutritional and therapeutic benefits. Oat is known to be a good source of carbohydrate and quality protein with good amino acid, balance, lipids and dietary fibre (Head *et al.*, 2010). Therapeutically, oat is reported to have antioxidant properties, hypolipidemic effect, and cardiovascular effect especially in hypertension when consumed with vitamin C, anti-obesity, antidiabetic, dermatological as antipruritic and anti-inflammatory activities (Sepideh Mirad *et al.*, 2010).

The therapeutic values of *Avena sativa* is as a result of its phytochemical content. Phytochemical screening is a fundamental tool used for the chemical constituents of medicinal plant. The bioactive constituents of medicinal plants include; Tannins, Alkaloids, Terpenoids, Steroids, Saponins, Flavonoids and Cardiac glycoside. These compounds are enzyme catalyzed secondary metabolism of living organisms. Secondary metabolites are very diverse compounds with definite functions. They are widely used in the human therapy, veterinary, agriculture and many scientific investigations.

Trace elements serve as cofactors in the body metabolism processes and their deficiency may result in metabolic disorders with pathologic consequences like diabetes. In developing countries dietary intake of micronutrients are grossly inadequate to meet their daily requirements in the body. These necessitate the need to investigate natural products for the presence of micronutrient to meet the requirement of the astronomical growth in population (Ramakrishimas *et al.*, 2011). Medicinal plants are known to contain heavy metals which may undermine their therapeutic values (Onyeloni and Nweke, 2022). Contamination of plants by heavy metal may result from their uptake in polluted soils agricultural and industrial processes. These heavy metals may cause oxidative stress when ingested as a result of the preponderance of free radicals over antioxidants; this may result in cellular damage including DNA which may result in cancer, autoimmune disorder and many other pathologic conditions. Thus continuous intake of heavy metals

may lead to their accumulation with attendant deleterious effect in the body.

Materials and methods

Sampling

Quacker Oat® is a brand of oat milk was purchased from a super market in Abraka, Delta State, Nigeria. About 200 g of the Oat milk sample was extracted with 400 ml 80% methanol using Soxhlet apparatus. The extract was filtered using Whatman No. 1 filter paper and lyophilized. The extract was further concentrated in an oven at 40°C until a constant weight was obtained and stored in a refrigerator to avoid microbial degradation.

Qualitative phytochemical screening

The extract was tested for the presence of bioactive compounds using standard methods as described by Harborne and Kokate.

Sample digestion for micronutrient and heavy metal evaluation

The digestion flask was thoroughly washed using a detergent, soaked in 20% HNO₃ for 24 hrs and rinsed with demineralized water, dried in an oven at 100°C. About 2g of the Oat milk sample was weighed into the flask. 2ml of conc. HClO₄ and 12ml of conc. HNO₃ was added. The mixture was then heated on a hot plate in a fume cupboard. Firstly at 60°C for few minutes and then 120°C until a dense white fume was observed. The flask was allowed to cool to room temperature. The concentrate was dissolved in 40ml 0.1N HNO₃ and demineralized water was added and further heated until clear solution was seen. The clear solution was filtered into a volumetric flask and the volume made up to 50 ml with demineralized water. The filtrate was immediately analyzed for the presence of micronutrients and heavy metals using Atomic Absorption Spectroscopy (AAS), blank was prepared similarly without the sample.

Statistical analysis

The result of micronutrients and heavy metals determination was analysed using one way analysis of variance (ANOVA) and level of significance was set at P<0.05.

Results

The results of the qualitative phytochemical analysis, micronutrients and heavy metals determination of *Avena sativa* are presented in Tables 1-3.

Table 1. Phytochemical analysis of methanolic extract of *Avena sativa*.

Phytochemical	Result
Alkaloids	+
Flavonoids	+
Tannins	+
Saponins	+
Terpenoids	+
Steroids	+
Cardiac glycosides	+
Reducing sugar	+
Carbohydrate	+
Protein	+

Key: + = present, - = absent

Table 2. Micronutrient determination of methanolic extract of *Avena sativa*.

Micronutrients	Concentration (mg/kg)
Zinc (Zn)	12.02
Selenium (Se)	0.08
Copper (Cu)	60±0.6
Cobalt (Co)	0.008±0.11
Nickel (Ni)	0.1±0.10

Table 3. Heavy metals analysis of methanolic extract of *Avena sativa*.

Heavy metals	Concentration (g/kg)
Lead (Pb)	0.36±0.02
Chromium (Cr)	0.01±0.02
Cadmium (Cd)	0.047±0.01
Iron (Fe)	119.4±0.02

Means±S.D

Discussion

Phytochemical analysis

The study shows the presence of alkaloids, flavonoids, saponins, terpenoids, steroids, reducing sugar, tannins, cardiac glycosides, carbohydrates and protein. These phytochemical present in oat milk may be responsible for the reported therapeutic values of *A. sativa* such as hypolipidemic, cardiovascular benefits, antidiabetic, antimicrobial dermatological and central nervous effects.

Alkaloids maybe responsible for the CNS effect of *A. sativa* such as improvement in active stress response and enhance shock avoidance, learning and an

increased functioning in social behavior. The dermatological benefits of *A. sativa* maybe the result of its content of tannins which have astringent properties hasten wound healing with anti-inflammatory effect on mucous membrane. The presence of flavonoids may enhance the anticoagulant and antidiabetic effect of the studied sample. Terpenoids found in the study sample have antimicrobial, anti-parasitic anti-inflammatory and immunomodulatory effect. A steroid in oat milk possesses anti-allergic, anti-inflammatory and regulates immune response. Saponins have hematologic and antidiabetic benefits. Cardiac glycosides have cardiogenic effect and may be useful in the management of congestive cardiac failure. *A. sativa* is regarded as a functional food as shown by its content of protein, carbohydrate and reducing sugar.

Micronutrients determination

Oat milk in this study contain copper, zinc, selenium, cobalt and nickel as shown in Table 2.

Zinc (Zn)

Zinc concentration in digested study sample is 12.02mg/kg. The RDA for Zinc is 11mg a day for man and 8mg for women. But higher quantity may be required during pregnancy and lactation. 200 g retail pack of Quaker oat will contain approximately 12mg of Zinc and 4 equal serves for breakfast will contain 3mg of Zinc each. This one source of daily intake of Zinc is appropriate considering its RDA the catalytic activities of hundreds of enzymes requires Zinc, enhance immune function and is involved in protein and DNA synthesis (Hashemiponr *et al.*, 2009).

Selenium (Se)

The investigated sample contains 0.08mg/kg. This concentration is appropriate in a diet of oat milk as the RDA for Se is 55 µg daily. Selenium play key roles in thyroid hormone metabolism, reproduction and DNA synthesis. In animal study Selenium is known to enhance carbohydrate metabolism and hypoglycemic action (Steinbrenner *et al.*, 2011).

Copper (Cu)

The Oat milk used in this study has a concentration of about 60±0.6mg/kg for adults the RDA for Cu is 900

μg daily. This value is significantly higher than the 13.00mg/kg reported by Onyeloni and Nweke (2022). Copper is a cofactor for several enzymes that play key roles in energy production, neuropeptide activation synthesis of connective tissues neurotransmitter synthesis and iron metabolism.

Cobalt (Co)

The concentration of Cobalt in the study sample is $0.008 \pm 0.11 \text{ mg/kg}$. Cobalt content of a meal of oat milk will be significantly lower than the 5 – 8 μg RDA of Cobalt. Cobalt is essential for cell function as a component of vitamin B12. It is also involved in red blood cell production. It plays a vital role in fat and carbon hydrate metabolism and in protein synthesis (Vasudevan and McNeill, 2007).

Nickel (Ni)

The concentration of Nickel in the Oat milk studied is $0.1 \pm 0.10 \text{ mg/kg}$. Although there is no established RDA for Nickel but it assist in breaking down of glucose for energy production and iron absorption. It is found in every cell as a component of DNA and RNA (Kazi *et al.*, 2008).

Heavy Metals

Lead (Pb)

The concentration of Pb in the digested sample is $0.36 \pm 0.02 \text{ g/kg}$. This value is significantly higher than the WHO permissible limit of 0.01 mg/L in water. This high value may be as a result of the uptake of Pb from contaminated soil from oat farm from agricultural machinery, contaminated water and processing methods of oat milk, chronic exposure to Pb may result in autism due to its effect on CNS and decrease in intelligence capacity in children. Pb may also be carcinogenic in humans, kidney damage that is now almost endemic in Nigeria. In humans, oxidative stress which is as a result of imbalance between the productions of free radical and generation of antioxidant (Matthew *et al.*, 2011).

Chromium (Cr)

In the digested sample Cr level is $0.01 \pm 0.02 \text{ g/kg}$. This is higher than the chromium permissible limit of 0.005 mg/L . Cr is considered to be one of the most

environmental toxic pollutants the world especially in its hexavalent form that is water soluble and mobile (Oliveira, 2012). The presence of Cr in the sample may occur through sewage and fertilizer and wide ranges of industrial and agricultural practices. Chronic exposure to chromium may result in cellular and protein damage through oxidative stress, liver and kidney damage, lung and liver cancer (WHO, 2003).

Cadmium (Cd)

In this study Cd concentration was found to be $0.047 \pm 0.01 \text{ g/kg}$. This value is higher than SON and WHO permissible limit and United State environmental protection agency recommended level of 0.03 mg/L (WHO, 2011). This presence of Cd in this sample may result from the high rate of transfer of Cd from contaminated soil to plant Cd is absorbed through the intestinal tract and may result in hepatotoxicity, nephrotoxicity and through tubular accumulation of iron deficiency (Rafati Rahimzadeh *et al.*, 2017).

Iron (Fe)

Iron concentration in the study sample is $119.4 \pm 0.02 \text{ g/kg}$. This level of Fe is significantly higher than the 1.0 mg/kg WHO recommended daily intake of Fe. The high content of Fe in the study may be the result of agricultural, industrial manufacturing processes and ground water. Iron is the second most abundant metal on the earth crust and is absorbed through the GIT when ingested. When this absorbed Fe fails to bind to the protein, a wide range of harmful free radicals are formed. This Fe produced hydrogen free radicals attack DNA, resulting in cellular damage, mutation and malignant transformations which may result in several pathological conditions (Grazuleviciene *et al.*, 2009) including necrotizing gastritis and hemorrhage (Baranwal and Singh, 2003).

Conclusion

The study revealed the presence of wide range of phytochemicals and some micronutrients giving credence to *A. sativa* as a functional food which have nutritional and therapeutic values. The phytochemical revealed in this study may be responsible for its reported therapeutic benefits.

However, more study need to be done on the isolation characterization and structural elucidation of compounds in the methanolic extract of oat milk that may result in the development of lead compounds for a possible discovery of drugs with the high therapeutic index. However, cognizance should be given to good manufacturing practice (GMP) in the processing and manufacturing of oat milk as some heavy metals found in this study are above the recommended tolerable limit.

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