Original Research Article

PHYTOCHEMICAL SCREENING AND ATOMIC ABSORPTION SPECTROSCOPIC ANALYSIS OF THE LEAF AND STEM OF AMARANTHUS SPINOSUS

Abstract

This research work was carried out to determine the phytochemicals and elemental constituents present in the leaf and stem of Amaranthus spinosus. The atomic absorption spectroscopic analysis was carried out using the crushed dry plant materials while the extraction of the phytochemicals was carried out using methanol in a Soxhlet extractor. From the analysis done, the following elements were detected in the plant parts: cobalt, lead, manganese, nickel, copper, cadmium, magnesium, iron, chromium and calcium but nickel was found absent in the leaf. Some of these elements are beneficial to health. The phytochemical screening done indicated the presence of the following: alkaloids, flavonoids, saponins, tannins, steroids, volatile oils, proteins, and carbohydrates.

Keywords: Phytochemicals, Amaranthus spinosus, spectroscopy, analysis, Elements.

Introduction

Plants or herbs have been found to have medicinal and therapeutic importance in the prevention, palliation, treatment or cure of diseases and ailment. This knowledge has been passed down from one generation to another either verbally or in writing (Sofowora, 2008)

Medicinal plants are well known for their role in complementing the fight against diseases in humans (Rabiu *et al*., 2020)

They play a significant role in providing primary health-care services to rural people and used by about 80% of the marginal communities in the world (Prajapati et al., 2002; Latif *et al*., 2003; Shinwari *et al*., 2006).

Medicinal plants components remain untapped reservoir for active compounds (phytochemicals) with properties that can potentially perform multiple biological activities such as anti-inflammatory, antimicrobial, antiviral, antioxidant and so on (Anyanele *et al*., 2022).

As at present a substantial number of drugs are developed from plants which are active against a number of ailments and diseases conditions such as hypertension, pains, fever, cancer, diabetes, arthritis, gastrointestinal diseases and so on (Olanipekun et al., 2021; Patiel *et al*., 2010; Anyanele *et al*., 2023).

Phytochemicals may also be described as non-nutritive plant chemicals that have protective or disease preventive properties. They are regarded as non-essential nutrients (Okwu and Okwu, 2004).

Normally, they are naturally occurring bioactive molecules produced by plants for protection from the elements of the earth and the sun's harmful rays. These amazing phytocompounds provide food resources for the human cells. Consumption of plant foods containing these compounds have been scientifically validated to help shadow the aging process and reduce the risk factors of many diseases including cancer, heart disease, stroke, high blood pressure, cataracts, osteoporosis, diabetes and urinary tract infections (Barjesteh *et al*., 2007).

The consumption of phytochemicals enhances reduction in the emergence of degenerating diseases (Allan, 1996).

The array of secondary metabolites produced by plants is daunting, with wide ranging chemical, physical and biological activities. These constitute a source of bioactive substances and presently scientific interest has increased due to the search for new drugs of plant origin (Ncube et al., 2008).

Amaranthus spinosus is a herb which belongs to Amaranthaceae family and used by ethnic people in the treatment of various infections and ailments. *Amaranthus spinosus* has a long history usage in traditional medicine against various ailments in the world (Barku *et al.,* 2013).

The work was aimed at investigating the pharmaceutical constituents present in the leaf and stem of Amaranthus spinosus

Materials and Methods

Amaranthus spinosus whole plants were uprooted from uncultivated farm land in Awo-Omamma in Oru East local government Area in Nigeria.

Sample preparation – The leaves were detached from the whole plant and the stems where cut off from the remaining parts. These plant materials were washed and the stems were sliced into smaller pieces. They were dried under room temperature for seven days for the leaves and fourteen days for the stems. The dried materials were differently crushed into powder with grinder. The samples were kept in different air tight containers for subsequent analyses.

Extraction of Plant Materials

500g of the powdered samples were placed in a round bottom flask containing 200ml of methanol, the extracting solvent. The flask was attached to the extractor. The Soxhlet extraction lasted for three hours. The crude methanol extracts were concentrated using water bath at 40℃, cooled and corked for phytochemical analysis.

Phytochemical screening

Phytochemical screening was done using standard methods (Ikan, 1996, Vishnoi, 1979, Sofowora, 1993, El-Olemy *et al.*, 1994; Harborne, 1998, Akpuaka, 2009, Cheesbrough, 2002)

The methanol crude extracts were reconstituted and the solutions were tested for the following compounds: saponins, tannins, steroids, flavonoids, volatile oils, alkaloids, glycosides, anthraquinones, resins, reducing sugars, protein.

Determination of Mineral Elemental Composition Using Atomic Absorption Spectroscopy.

The following elements were analyzed for: Co, Pb, Mn, Ni, Cu, Cd, Mg, Fe, Cr and Ca. The analysis was conducted using Varian AA240 Atomic Absorption spectrophotometer according to the method of APHA 1995 (American Public Health Association).

Each of the dried powdered plant materials (2.0g) was collected and heated in a furnace for 2hours at 550□C. The residue was diluted with 20ml, 20% H_2SO_4 and filter with filter paper. It was measured using FS240AA Agilent atomic absorption spectroscopy.

A series of standard metal solutions in the optimum concentration range was prepared, the reference solutions range were prepared daily by diluting the single stock element solutions with water containing 1.5ml concentrated nitric acid/ liter. A calibration blank was prepared using all the reagents except for the metal stock solutions.

Calibration curve for each metal was prepared by plotting the absorbance of standards versus their concentrations.

Results and Discussions

Table1: Results of the Phytochemical analysis of crude samples of Leaves and stems extracts of *Amaranthus spinosus.*

Phytochemical Compounds	Amaranthus Leaf	Amaranthus Stem
Alkaloids	-	+
Flavonoids	+	+
Saponin	-	+
Tannins	+	+
Steroids	+	+
Volatile oils	+	+
Cardiac Glycosides	-	-
Anthroquinones	-	-
Resins	-	-
Protein	+	+
Carbohydrates	+	+

Note : - = not detected

+ = present

The phytochemical screening of the leaves and stems extracts of Amaranthus spinosus indicated the presence of the following phytochemicals: alkaloids, flavonoids, saponins, Tannins, Steroids, volatile oil, protein and carbohydrates. Alkaloids were detected in the stem but not detected in the leaves. Choudhury, 2012 reported the absence of alkaloids in the leaves of Amaranthus spinosus.

Alkaloids are very important in medicine and constitutes most of the valuable drugs. They have marked physiological effect on animals (Akpuaka, 2009). They show considerable pharmaceutical activity (Dey *et al*., 2010).

Flavonoid was detected in both leaves and stems of Amaranthus spinosus. Flavonoids are anticoagulants (Morrison and Boyd, 2008). They also help in healing of wounds and in the treatment of skin diseases due to their ability to neutralize the acidity of wounds and inflammatory.

Saponins were detected in the stem and not in the leaves. Ishir et al., reported the presence of saponins in the methanolic stem extract of Amaranthus spinosus. The presence of saponins and alkaloids have been reported to be responsible for various pharmacological properties with alkaloids exerting toxic effects against cells of foreign organism (Fred-Jaiyessimi, 2010).

Tannins were detected in the two parts of plant tested for. Tannins have been found to form irreversible complexes with proline rich proteins (Akinpelu and Onakoya, 2006) resulting in the inhibition of the cell protein synthesis. Medicinally, this is important for the treatment of inflamed or ulcerated tissues (Akinpelu and Onakoya, 2006). Indeed, herbs that have tannins as their main component are astringent in nature and are used for treating of intestinal disorders such as diarrhoea and dysentery (Dharmanada, 2003).

Steroids were detected in both parts of the plant. It has been found that some of these Nigeria medicinal plants contain steroidal compounds. It should be noted that steroidal compounds are of importance and interest in pharmacy due to their relationship with such compounds as sex hormones (Okwu 2001).

Carbohydrates were detected in both the leaves and stems of Amaranthus spinosus. The primary role of carbohydrate is to provide energy to all cells in the body and dietary fibre (Slavin and Carson, 2014). The plant also contains protein. Functional food is usually derived from phytochemical and bioactive proteins from plants and animal sources.

The presence of these secondary metabolites in plants produce some biological activity in man and animals and it is responsible for their use as herbs. These compounds also serve to protect the plant against infection by microorganisms, predation by insects and herbivores (Abalaka *et al*., 2012). Some of these metabolites particularly the flavonoid might have been identified as vital in antimicrobial activity of medicinal plants (Mitra, 2013). Phytochemicals act in numerous ways to assist the body in combatting diseases and health problems. The consumption of

phytochemicals enhances reduction in the emergence of degenerating diseases (Odeleye *et al*., 2014).

Table 2: Result of The Atomic Absorption Spectroscopic Analysis of Amaranthus Spinosus' Leaf and Stem Extracts.

Metals(ppm)	Leaf	Stem
Cobalt	0.179	0.192
Lead	0.599	1.081
Manganese	1.873	1.475
Nickel	0.00	0.050
Copper	0.126	0.139
Cadmium	0.023	0.064
Magnesium	24.442	22.869
Iron	4.789	10.608
Chromium	1.052	1.089
Calcium	2.490	2.715

The Atomic Absorption Spectroscopic analysis result of Amaranthus spinosus leaves and stems in Table 2, showed the presence of cobalt, lead, manganese, nickel, copper, cadmium, magnesium, iron, chromium and calcium. Some of these elements have some health benefits to man and animals.

The concentration of Iron is higher in the stem than in the leaf. Iron is an essential element for almost all living organisms as it participates in a wide variety of metabolic processes, including oxygen transport, deoxyribonucleic acid (DNA) synthesis, and electron transport (Abbaspour *et al*., 2014). It is the key element for our growth and development. Iron is strictly required for the survival of most forms of life, including bacteria, plants and humans (Gozzelino, 2015). It helps the heart in the production of red blood cells and oxyhemoglobin distribution and it is also involved in cellular respiration.

The high concentration of magnesium in both the leaf and stem of Amaranthus spinosus is an indication that the plant is of great importance. Magnesium is an essential element for humans, animals and plants; it is required for growth, development and maintenance of health. Excessive exposure or intake may lead to a condition known as manganism (Avilat *et al*., 2013).

Magnesium is an essential nutrient involved in many important processes in living organisms, inducing protein synthesis, are cellular energy production (Castiglioni, 2021). Magnesium has several functions in the human body. It acts as a cofactor for more than 300 enzymes, regulating a number of fundamental functions such as muscle contraction, glycemic control, myocardial contraction and blood pressure (Abdullah et al., 2018). The results assured the usefulness of these elements in the physiological administration of the crude drug since some of the elements were of health benefit and equally at low concentration.

Calcium, present in significant concentration is a supportive element in bone and tooth structure. It is also important to good muscle tone. Research has confirmed that calcium is involved vascular contraction, vasodilation, muscle functions, nerve transmission, intracellular signaling and hormonal secretion (Beto, 2015).

The heavy metal ions that are known to be poisonous such as lead and nickel were present in low concentration.

Conclusion

The result of the investigation carried out on the leaves and stems of Amaranthus spinosus showed the presence of important phytochemicals which is an indication of the usefulness of the plant parts in the manufacture of drugs. The high content of iron and magnesium suggests that the leaves and stems of Amaranthus spinosus should be included in our daily diet.

References

1. Sofowora, A. (2008). Medicinal plants of Traditional Medicine in Africa. 3rd Edition, Spectum Books, Ibadan, Nigeria. Pp199-181
2. Okeniyi, S.O., Adedoyin, B.J. and Garba, S. (2012). Phytochemical Screening, Cytotoxicity, Antioxidant and Antimicrobial Activities of Stem and Leave Extracts of Euphorbia heterophylla. *Bulletin of Environment, Pharmacology and Life Sciences*. vol1(8):87-91
3. Rabiu, N., Haruna, S., Ahmed, A., Ridwan, N., Hussans, A., Abdulraheem, T.A and Y.I. (2020). Phytochemical analysis and antimicrobial activity of leaf extract of Amaranthus spinosus. *Communication in Physical science*, 5(1): 42-45.
4. Prajapati ND, Prajapati T. Sustainable cultivation of medicinal plants, multitier agriculture system-a new concept. In: Biochemistry. 3rd ed. United States: Benjamin Cummings; 2002.
5. Latif A, Almad H, Begun S, Adnan M, Hussain WM. Medicinal and Other Economic Plant as Substitute to Forest Logging in Miandam and Sulatat Valleys, Swat. Pakistan: *Proceedings of International Workshop on Conservation and Sustainable Use of Medicinal and Aromatic Plants in Pakistan*; 2003. p. 101-5.
6. . Shinwari ZK, Pehman M, Watanabe T, Yoshikawa T. A Pictorial Guide to Medicinal Plants of Pakistan. Kohat: Kohat University of Science and Technology; 2006
7. Anyanele, S.I., Ajiwe, V.I.E, Anyanele, W.C. and Umeokoli, B.O. (2022). Phytochemical and Atomic Absorption Spectroscopic Analysis of leaf, Stem and Root Extracts of Euphorbia heterophylla. *Asian Journal of Advances in Research*. 16(1); 1-4.
8. Olanpekun, M.K., Akirun, K. and Amoo, J.A. (2021). Phytochemical screening and proximate analysis of some Medicinal plants used in Irun Akoko, Akoko North West

Local Government Area Ondo State, Nigeria. *Europian Journal of Botany, Plant Sciences and Phytology*. 6(1): 1-13.

9. Patiel, P.N., Suthar, M., Shah, T.G. and Patiel, L.J. (2010). Anticancer activity of *Nigella sativa* seeds against HL-60, U-937 and HEK-293T cell line. *Pharm Expt*.
10. Anyanele, S.I., Ajiwe, V.I.E, Anyanele, W.C. and Umeokoli, B.O. (2023). Structural Elucidation and Antimicrobial Analysis of Bioactive Compounds From Amaranthus Spinosus. *Asian Journal Applied Chemistry Research.* Vol 13(4), pp 31-39.
11. Okwu, D.E. and Okwu, M.E. (2004). Chemical composition of Spondius mombin plants. *Journal of sustain Agric Environ*. 6:140-147
12. Barjesteh Van Waalwijk Van Door-khosrovani S, Janssen J, Maas LM, Godschalk RW, Nijhuis JG, Van Schooten F.J. (2007). Dietary flavonoids induce MLL translocations in primary human CD34+ cells. *Carcinogenis*. 28(8): 1703-9.
13. Allan, L.M. (1996). Antioxidant flavonoids: Structure, Function and Clinical usage. *Alternative Medicine Review* 1(2):103-111
14. Ncube, N.S.,Afolayan, A.J. and Okoh, A.I(2008).Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. *African Journal of Biotechnology*, vol 7(12), pp1797-1806
15. Barku, V.Y.A., Opoku-boahen, Y., Owusu-Ansah, E. and Mensah, E.F. (2013). Antioxidant activity and the estimation of total phenolic and flavonoid contents of the root extract of Amaranthus spinosus. *Asian J. Plant Sci Res*.3(1):69-74
16. Ikan. R.C. (1996). Natural Products: A Laboratory Guide. Academic Press, London, United Kingdom. Pp 1-182.
17. Vishnoi, N.K. (1979). Advanced Practical Organic Chemistry, Vicas Publishing House, PVT Ltd, India, pp. 13-20,447-232.
18. Sofowora, A. (1993). Medicinal plants and traditional medicine in Africa. Spectrum books Ltd. Ibadan.pp95-238.
19. El-Olemy, M.M., Al-Muntadi, F.J. and Afifi, A.A. (1994). Experimental Phytochemistry: A Laboratory Manual, King Saud University Press 1415, Riyad, Saudi Arabia, pp. 6-8.
20. Harbonne , J.B. (1998). Phytochemical methods: A guide to modern techniques to plant analysis (3rd edition). Chapman and Hall, UK.pp1-290.
21. Akpuaka, M.U. (2009). Essentials of Natural Products Chemistry. Mason Publishers, Enugu, Nigeria, pp 1-110.
22. Choudhurry, A. (2012). Evaluation of Physicochemical and phytochemical parameters of Amaranthus spinosus leaves. *International Research Journal of Pharmacology.* 3(10).
23. Morrison, R.T and Boyd, R.N (2006). Organic chemistry, 16th edition. Allyn and bacon, INC, BOSTON. Pp1002-1005.

24. Fred-Jayesimi, A.A. and Abo, K.A. (2010). Phytochemical and Antimicrobial analysis of the crude extract, petroleum ether and chloroform fractions of Euphorbia heterophylla Linn Whole Plant. *Pharmacognosy Journal*. vol 2(16):1-4.
25. Akinpelu, D.A., Onakoya, T.M. (2006). Antimicrobial activities of medicinal plants used in forklore remedies in south western Africa. *Afr. J. Trad*. CAM 3: 112-115.
26. Dharmanada S. (2003). Gallnuts and the uses of tannins in Chinese medicine. J. Riol. Chem. 256: 4494-4497.
27. Okwu, D.E. (2001). Evaluation of the chemical composition of indigenous spices and flavouring Agents. Global J. Pure Appl Sci. 7(3): 455-459.
28. Slavin, J. and Carlson J. (2014). The primary role of carbohydrate is to provide energy to all cells in the body and dietary fiber. Advances in Nutrition . *An International Review Journal*. 5(6): 760-761
29. Abalaka, M., Oyewole O.A and Kolawole A.R. (2012). Antibacterial Activities of Azadirachdita indica against some Bacterial Pathogens. *Advances in life Sciences*. 2(2):5-8.
30. Mitra, P.k.(2013). Comparative evaluation of antigastric ulcer. Activity of root, stem and leaves of Amaranthus spinosus Linn in rats. *International Journal of Herbal Medicine*. 1,2, pp 1675-1680.
31. Odeleye, O.P., Oluyeye, J.O., Aregbosola, O.A. and Odeleye, P.O. (2014). Evaluation of preliminary phytochemical and antibacterial activity of Ageratum conyzoides(L) on some clinical bacterial isolates. *The International Journal of Engineering and Science (IJES)*, volume 3 (6): pp 01-05.
32. Abdullah, M.A., Sandawana W.M. and Henrik, F. (2018). Magnesium and Human health: Prospectives and Research Directions. *International journal of Endocrinology Article* ID 9041694, 17 pages.
33. Beto, J.A. (2015). The role of calcium in Human Aging. *Clinical Nutrition Research*, 4(1): 1-8.