

Preliminary Phytochemical Screening and Antioxidant Activity of Ethanolic Leave Extract of *Azadirachta indica*.

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Abstract: The phytochemical constituent and some antioxidant indices of ethanolic leaf extract of *Azadirachta indica* were evaluated. *A. indica* was found to contain some secondary metabolites like alkaloids, phenolic compounds, terpenoids, flavonoids, steroids and reducing sugar.

The total phenolic content, reducing power and H_2O_2 scavenging activity are 3.04 ± 0.03 mg/g garlic acid equivalent, 0.45 ± 0.01 and 10.15 ± 1.14 respectively. This shows that the plant has antioxidant activity, the phytochemical constituent explain why the plant has been used as antimicrobial agents for preventing and treating micro-organisms infections.

Keyword: *Azadirachta indica*, phytochemical analysis and antioxidant activity.

I. Introduction

Medicinal plants are used by 80% of the world population as the only available medicines especially in developing countries (EL-Kamali & EL-amir, 2010).

Herbal medicines already form the basis of therapeutic use in the developing countries, but of recent, there has been an increase in the use of herbal medicines in the developed world today (De and Ifeoma, 2002, El-Mahmood et al., 2010).

Practically, every part of the *A. Indica* (leaves, bark, fruit, flowers, oil and gum) has been reported to be associated with various remedial properties such as the treatment of general body pain after child delivery, pyorrhea, intestinal worms, antimicrobial effects, storage behaviour, in vitro antiviral activity and antibacterial agent (Biu et al., 2009; Taha et al., 2008).

The medicinal value of plants have assumed a more important dimension in the past few decades owing largely to the discovery that extracts from plants contain not only minerals and primary metabolites but also a diverse array of secondary metabolites with antioxidant potential. Antioxidant substances block the action of free radicals which have been implicated in the pathogenesis of many diseases including atherosclerosis, ischemic heart disease, cancer, Alzheimer's disease, Parkinson's disease and in the aging process (Aruoma, 2003; Dasgupta and D'abrosca, 2007; Coruh et al., 2007).

Hence, scientists are now searching for naturally occurring antioxidants in plant sources for food or medicinal materials as alternative for synthetic antioxidants (D'Abrosca et al., 2007; Stanojevic et al., 2009).

Natural antioxidants endogenous to food of plant origin can scavenge reactive oxygen and nitrogen species (RONS); evidence suggests that these may be of great importance in preventing the onset of oxidative disease in the human body (Amarowicz et al., 2010).

Phenolic compounds contribute largely to the colour and sensory characteristics of fruits and vegetables. In addition, phenols participate in growth and reproduction processes, and provide protection against pathogens and predators. At the cellular level, it participate in cell protection against the harmful action of reactive oxygen species (ROS), mainly oxygen free radicals, produced in response to environmental stresses such as salinity, drought, high light intensity or mineral nutrient deficiency, because of the imbalance between the production and scavenging of ROS in chloroplasts. These cytotoxic activated oxygen species can seriously disrupt normal metabolism through oxidative damage to lipids, proteins and nucleic acids. Accordingly, plants containing high concentrations of antioxidants show considerable resistance to the oxidative damage caused by the ROS, as shown in the case of salt stressed plants (Meot-Duros & Magne, 2009).

The present work was designed to investigate the phytochemical and the antioxidant potential of the leave extract of *Azadirachta indica*.

II. Materials And Methods

COLLECTION OF PLANT MATERIALS

The leaves *Azadirachta indica* of were obtained from Sabo Market in Ikorodu Local Government, Lagos State. The leave were identified and authenticated by a botanist in the Department of Botany, University of Lagos, Nigeria.

PREPARATION OF PLANT MATERIAL

The leave of *Azadirachta indica* were dried at room temperature for a period of one week and powdered with aid of a mechanical grinder (blender).

ETHANOLIC EXTRACTION OF AZADIRACHTAINDICA

100g of the powdered leaves was weighed and soaked in a 1L Pyrex beaker containing distilled water/ethanol (80%) in ratio 2:8 respectively (i.e 200ml/800ml) for 72hours (three days) under intermittent stirring. The solution was filtered after three days using Whatman paper and concentrated in an oven at reduced pressure (40°C) and completely dried until a deep –brown aromatic residue was obtained. The residue was stored in a refrigerator at -4°C under air tight condition to prevent moisture until use.

PHYTOCHEMICAL SCREENING OF THE EXTRACT

The powdered leaves were subjected to qualitative chemical tests using the methods of Sofowara (2008). The components analyzed for are saponins, cyanogin glycosides, steroid,

Antraquinones, Tannins, Flavonoids, alkaloid and reducing sugars.

Tannins: 0.2g of the plant materials was dissolved in 10ml of distilled water and then filtered. A 2mL of filtrate was pipetted into a test tube after which 2mL of 15% FeCl₃ was added.

Colour change was observed. Blue-black presence indicated the presence of Tannins.

Alkaloids: 0.2g of the plant materials was extracted with 200ml of methanol for 20minutes on a water bath and then filtered. To 2mL of cold water extract in different tubes, was added 6 drops of alkaloid reagents (Meyer's reagent).

Brownish –red precipitate indicate the presence of alkaloid

Cyanogenic glycosides: 200 mg of powdered plant material was placed in each of 3 different test tubes labelled A, B and C, respectively. The powder in test tubes A and B were moistened with 5 mL of water, while that in test tube C was left dry. 3 pieces of freshly prepared sodium picrate paper were inserted into the mouth of each tube and stoppered. Test tube B was placed in a water bath while test tube A and C were kept at room temperature. After 30 minutes the colour of the picrate papers in each of the test tube were observed and recorded.

Cardiac glycosides:

- Salkowski's test: 200 mg of the extract was dissolved in 2 mL of chloroform. Concentrated tetraoxosulphate (VI) acid was carefully added to form a lower layer. A reddish-brown at the interface indicated the presence of a steroidal ring.

Reducing sugars:

- Fehling's test: ethanolic extract of the powdered material was obtained by boiling on water bath. To 2 mL of the extract, in the test tube was added, 1 mL each of Fehling's solutions A and B. The mixture was shaken and heated in a water bath for 10 minutes. The colour obtained was recorded.

Saponins:

- Frothing test: ethanolic extract was obtained by boiling on water both. The extract was transferred into a test tube and shaken vigorously then was left to stand 10 minutes and the result noted. Frothing persistence meant saponins were present.

Flavonoids: 200 mg of the powdered sample was boiled in 10 mL of absolute ethanol for 10 minutes. The solution was allowed to cool and then filtered. To 2 mL of the filtrate was added concentrated hydrochloric acid and magnesium ribbon. Pink-tomato red colour indicated the presence of flavonoids.

Antraquinone derivatives:

Bontrager's test: Chloroform extract of the powdered sample was obtained by boiling on the water bath. To 2mL of this extract, 1 mL of dilute (10%) ammonia was added and the mixture was shaken. Any colour change was recorded.

Evaluation of reducing power

The reducing power of *A. indica* was determined by the method of Yen et al 1995.

Evaluation of H₂O₂ scavenging activity

The H₂O₂ scavenging activity of *A. indica* and standard was assayed by the method of Zhao et al 2006.

Measurement of Total phenolic content

The total phenolic was determined by Folin-ciocalteu colorimetric method with little modification. Each extract 10mg was dissolved in distilled water and different concentration of gallic acid (0.0078- 1) were

prepared in water. Sample (40µl), 1M Folin-ciocalteu reagent (20µl) and 20% Na₂CO₃ 60 µl were mixed. The mixtures were kept in the dark at room temperature. Absorbance was measured at 760 nm using a UV-visible spectrophotometer. Total phenol content was determined from the standard calibration curve.

III. Results

Table 1. The Phytochemical analysis of Azadirachtaindica

Phytochemical analysis	Qualitative test	Methanolic extract A. indica
Saponin	Foam test	-
Tannins	with Ferric chloride	-
Phenolic compounds	with lead acetate	+
Alkaloid	with Mayer's test and Dragendoff's test	+
Flavonoids	with NaOH, with Mg/HCl	+++
Antraquinones	Bontrager's test	+++
Terpenoids	Thionyl chloride test	++
Cardiac glycosides	Legal test	-
Steroids	Liebermann Burchard test	+++
Reducing sugars	Benedict's test	+++
Non-reducing sugars	Molisch's test	++

- Absent, + Lowly present, ++ Moderately present and +++ Highly present

The Phytochemical analysis of ethanolic extract of Azadirachtaindica shows the presence of secondary metabolite like alkaloids, phenolic compounds, terpenoids, flavonoids, steroids and reducing sugar.

Table 2. Determination of reducing power, total phenolic content and H₂O₂ scavenging activity of Azadirachtaindica.

Test	Ethanolic extract of Azadirachtaindica
Reducing power	0.45±0.01
H ₂ O ₂ scavenging activity	10.15± 1.14
Total phenolic content	3.04±0.03mg/g garlic acid equivalent

IV. Discussion

Medicinal plants constitute an effective source of both traditional and modern medicines, herbal medicine has been shown to have genuine utility and about 80% of rural population depends on it as primary health care. Over years, the World Health Organization advocated that countries should interact with traditional medicine with a view to identifying and exploiting aspects that provide safe and effective remedies for ailments of both microbial and non-microbial origins. The phytochemical screening of Azadirachtaindica extract indicated the presence of tannins, saponins, alkaloids, flavonoids, etc (Table 1). Several studies have linked the presence of these bioactive compounds (secondary metabolites) in plants to produce some biological activity in man and animals and it is responsible for their use as herbs. These active constituents can be used to search for bioactive lead compounds that could be used in the partial synthesis of more useful drugs (Ogbonnia et al., 2008; El-Mahmood et al., 2010). 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. Nevertheless, these bioactive products from medicinal plants are presumed to be safe without any compromising health effect, and thus widely used as self medication (Vaghasiya). These compounds also serve to protect the plant against infection by microorganisms, predation by insects and herbivores, while some give plants their odours and or flavours and some are responsible for their pigments (El-Mahmood et al., 2008). Azadirachtaindica has a total phenolic content of 3.04±0.03mg/g garlic acid and a reducing power of 0.45±0.01. Both parameters indicate that the extract possess potent antioxidant activity.

H₂O₂ scavenging activity of A. Indica has a value of 10.15± 1.14 (Table 2) this value is high when compared to the control (garlic acid). This is an indication that the extract has the potential of scavenging free radicals.

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