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Mutagenic screening of some commonly used medicinal plants in Nigeria

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ABSTRACT

The uses of medicinal plants have always been part of human culture. The World Health Organization estimates that up to 80% of the world's population relies on traditional medicinal system for some aspect of primary health care. However, there are few reports on the toxicological properties of most medicinal plants especially, their mutagenicity and carcinogenicity. Therefore, this research is to determine the mutagenic potentials of *Morinda lucida* [Oruwo (Root)], *Azadirachta indica* [Dongoyaro (Leaf)], *Terapluera tetraptera* [Aridan (Fruit)], *Plumbago zeylanica* [Inabiri (Root)], *Xylopia aethiopica* [Erunje (Fruit)], *Newbouldia laevis* [Akoko (Leaf)], *Alstonia boonei* [Ahun (Bark)], *Enantia chlorantha* [Awopa (Bark)], and *Rauvolfia vomitoria* [Asofeyeje (Root)] using the *Allium cepa* Linn. model and the modified Ames assay.

Allium cepa model was used to determine the mean root length, mitotic index and chromosomal aberrations effects of these plants on onion bulbs using 0.1, 1, 5 and 10 mg/ml concentration of the plant extracts. The modified Ames test which is a modification of the standard Ames test as described by Ames et al. [Ames, B.N., McCann, J., Yamasaki, E., 1975. Methods for detecting carcinogens and mutagens with the Salmonella/mammalian microsome mutagenicity test. Mutation Research 31, 347–364] was done using *Escherichia coli* (0157:H7) that has the phenotypic characteristics of glucose and lactose fermentation, motile, urease negative, indole positive and citrate negative. The results obtained from *Allium cepa* assay showed increasing root growth inhibition with increased concentration, decreasing mitotic index with increased concentration and chromosomal aberrations. The modified Ames test showed an alteration in the biochemical characteristics of *Escherichia coli* (0157:H7) for all plants except *Rauvolfia vomitoria* and *Plumbago zeylanica*. Three of the medicinal plants altered at least three of the normal biochemical characteristics thus demonstrating mutagenic potentials. The results of internationally accepted *Allium cepa* were comparable with the modified Ames test. However, a long term in vivo and dose dependent study should be carried out to validate these results and the findings should be communicated to drug and food regulatory body and also to the general public.

1. Introduction

The uses of medicinal plants have always been part of human culture and it began from the era of early civilization as evidenced by the earliest recorded uses found in Babylon (1770 BC) and in ancient Egypt (1550 BC) (Veilleux and King, 1996). The World Health Organization (WHO) estimates that up to 80% of the world's population relies on traditional medicinal system for some aspect of primary health care (Farnsworth et al., 1985). WHO directive encourages developing countries to supplement their health programme with traditional herbal preparation provided they are proven to be non-toxic (WHO, 1985).

The National Agency for Food, Drugs, Administration and Control (NAFDAC) has started listing ethnomedicinal preparation on the market in Nigeria. Thus, some of the commonly used Nigerian medicinal plants *Morinda lucida* [Oruwo (Root)], *Azadirachta indica* [Dongoyaro (Leaf)], *Alstonia boonei* [Ahun (Bark)] have been demonstrated to possess analgesic, anti-inflammatory and antipyretic properties (Olajide et al., 2000). More so, a recent interview of the traditional practitioner and market survey of the use of ethnomedicinal plants conducted by our research team revealed that *Morinda lucida* [Oruwo (Root)], *Azadirachta indica* [Dongoyaro (Leaf)], *Terapluera tetraptera* [Aridan (Fruit)], *Plumbago zeylanica* [Inabiri (Root)], *Xylopia aethiopica* [Erunje (Fruit)], *Newbouldia laevis* [Akoko (Leaf)], *Alstonia boonei* [Ahun (Bark)], *Rauvolfia vomitoria* [Asofeyeje (Root)] and *Enantia chlorantha* [Awopa (Bark)] are commonly and regularly used by Nigerians especially the Yorubas for so many types of disease conditions.

Despite the profound therapeutic advantages possessed by some of the medicinal plants, some constituents of medicinal plants have been shown to be potentially toxic, mutagenic, carcinogenic and teratogenic (Gadano et al., 2006).

In view of the above facts, this present study is aimed at investigating the mutagenic potentials of the above listed medicinal plants

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that are commonly used in Nigeria especially by the Yorubas using a modified Ames test and *Allium cepa* assay.

2. Methodology

2.1. Plants selection

Market survey and oral interview were carried out in Mushin market (a local market in Lagos, Nigeria) among the traditional medical practitioners to know the most commonly used medicinal plants in this locality and plants were also selected based on the published investigation on medicinal plants.

2.2. Plants material

Fresh parts of *Morinda lucida* [Root], *Azadirachta indica* [Leaf], *Terapluera tetraptera* [Fruit], *Plumbago zeylanica* [Root], *Xylopia aethiopica* [Fruit], *Newbouldia laevis* [Leaf], *Alstonia boonei* [Bark], *Rauvolfia vomitoria* [Root] and *Enantia chlorantha* [Bark], were collected from secondary forest in Ikire Osun state – Nigeria. They were authenticated by T.K. Odewo, a senior superintendent of the Forestry Research Institute of Nigeria (FRIN) where voucher specimens FHI 108271, FHI 108276, FHI 108269, FHI 108274, FHI 108270, FHI 108272, FHI 108275 and FHI 108273 respectively were deposited for reference. Confirmation was done by A. Adeleke of the Pharmacognosy Department, University of Lagos-Nigeria.

2.3. Preparation of plant extract

The various fresh parts of the medicinal plants *Morinda lucida* [Root], *Azadirachta indica* [Leaf], *Terapluera tetraptera* [Fruit], *Plumbago zeylanica* [Root], *Xylopia aethiopica* [Fruit], *Newbouldia laevis* [Leaf], *Alstonia boonei* [Bark], *Enantia chlorantha* [Bark] and *Rauvolfia vomitoria* [Root] were air dried and grounded to a fine powder. The fine powder was then dissolved in distilled water (10 g/l) and the liquid was decanted 24 h later. The filtrate was evaporated to dryness in an oven set at 40 °C giving a yield that range between 12 and 13.5%. The dried extracts were stored in the refrigerator and dissolved in distilled water just before use. The pHs of the extracts were determined to be between 7.6 and 7.9 using a pH meter.

2.4. Modified Ames test

2.4.1. Media preparation

The various media that were used for this assay are MacConkey agar, Kliger iron agar, motility indole urea, Simmon citrate, brain heart infusion agar, nutrient agar and brain heart infusion broth. The preparation of these media was in accordance with the procedure of the LAB M^{TM} (Topley Home, 52 Washlane Bury, Lancashire, BL96AU, UK).

2.4.2. Preparation of the rat microsomal liver enzyme (S9)

Two Sprague-Dawley rats weighing about 180 g each were obtained from the animal house of the College of Medicine of the University of Lagos. The rats were injected intra-peritoneally with 10 mg/kg of phenobarbitone for 3 days to induce the liver microsomal enzymes as suggested by Maron and Ames (1984). After the third day, the animals were sacrificed and the liver was extracted aseptically and then macerated using a prior sterilized mortar and pestle. To every 1 g of the macerated liver, 5 ml of 1.65 M KCl solution was added. The resulting solution was centrifuged (1200 revolutions per minute) and the supernatant was filtered using a sterile membrane filter to obtain the rat microsomal enzyme.

2.4.3. The S9 mix

The S9 mix was freshly prepared using the method of Maron and Ames (1984). Thus, 20 ml of S9 mix contains 2 ml of rat liver enzyme, 10 ml of 0.2 M phosphate buffer at pH 7.4, 5.6 ml of distilled water, 1 ml of 80 mM NADP sodium salt hydrate, 1 ml of 120 mM glucose-6-phosphate, and 0.4 ml of potassium and magnesium salts solution. The mixture was stirred properly before 2 ml of the rat liver enzyme (S9) was added to the S9 mix.

2.4.4. Inoculation

The MacConkey agar plates were sub-cultured with strain of *Escherichia coli* (0157:H7) obtained from the Genetics Department of the Nigerian Institute of Medical Research, Yaba-Lagos (NIMR) and incubated at 37 °C for 24 h to obtain discrete colonies. Thus, the discrete colonies of the organism were re-sub-cultured into new MacConkey plates and incubated at 37 °C for 24 h to ensure the use of standard strain of the organism and not contaminants. The organism was then subjected to the method of identification of Enterobacteriaceae as described by Edwards and Ewing (1972) and Cowan (1973). The organism has biochemical characteristics of lactose and glucose fermenting, motile, urease negative, indole positive and citrate negative.

2.4.5. Bacteria mutation assay

This is a modification of the standard Ames assay as described by Ames et al. (1975) and as previously used in our study of Akintonwa et al. (2007). The assay was performed using *Escherichia coli* (0157:H7) obtained from Nigerian Institute of Medial Research, Yaba-Lagos, which have been grown on MacConkey plates to obtain discrete colonies and which the biochemical characteristics have been pre-determined. The experiment was performed in the presence and absence of metabolic activation of the rat liver enzyme. The fraction of the liver enzyme (S9) was used at a concentration of 10% (v/v) in the S9 mix. The S9 mix was freshly prepared for the experiment according to the method of Maron and Ames (1984). Test agents, negative control and positive control were tested for possible mutation with this strain of organism. Ethidium bromide which is a known intercalating agent was used as the positive control while distilled water was used as the negative control. Fresh culture of the organism obtained from MacConkey plate was inoculated into the brain heart infusion broth. The brain heart infusion broth containing the organism was incubated for 10–12 h at 37 °C in order to ensure adequate circulation of the organism into the media. 0.1 ml of the brain heart infusion broth that contains the organism was mixed with 0.5 ml S9 mix and 0.1 ml of the test sample. The mixture was incubated at 37 °C for 72 h and later seeded into the brain heart infusion agar plates, while the other portion without S9 was also seeded on other brain heart infusion agar plates. The plates were incubated at 37 °C for 24 h. The revertant/mutant strains and the wild strains produced by ethidium bromide (and other medicinal plants with mutagenic potentials) and distilled water (and other medicinal plants with no mutagenic potentials) obtained from the incubated brain heart infusion agar plates respectively were inoculated into the Kliger iron agar (KIA), motility indole urea (MIU) and citrate agar to re-examine the organism's biochemical characteristics and compare with the normal biochemical characteristics of the organism as previously obtained. An alteration in at least 3 biochemical characteristics out of the 7 normal biochemical characteristics of the organism (*Escherichia coli* 0157:H7) used in this study will be arbitrarily taken as the benchmark for mutagenicity and this decision of taking three as the benchmark is as a result of three (3) being a significant value ($P \leq 0.05$) out of seven (7). However, alteration in biochemical characteristics lesser than 3 may illustrate a weak mutagenicity or no mutagenicity, thus, further study may be done using different mutation model to ascertain their mutagenicity.