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Antimutagenic Activity of Seeds of *Moringa Oleifera*

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ABSTRACT

Moringa oleifera has medicinal and nutritional values. This study was aimed at assessing the antimutagenic potential of *M. Oleifera* seeds. Ethanol was used for the extraction. Chemical analysis was done following standard methods. Antimutagenicity was determined against nitrate treated 1-amino- pyrene in acid solution using the *Salmonella assay.* Preliminary phytochemical screening of seed extract *M. Oleifera* revealed tannin, alkaloid, flavonoid, terpenoid, saponin, cardiac glycoside, anthraquinone, and steroid; phlobatanin was not detected. Tannin was found to be in the highest amount (28.2± 0.63) mg/100g, flavonoid (23.21± 0.44) mg/100g, steroid (20.50± 2.15) mg/100g, alkaloid (18.68 ± 2.15) mg/100g and saponin (15.22 ± 0.63) mg/100g. The extract showed a strong percentage inhibition in both strains at higher doses. The observed antimutagenic effect of the seed extract could be adduced to some phytochemical constituents therein.

1. INTRODUCTION

The second principal cause of death after cardiovascular diseases is cancer, which is a group of over hundred diverse diseases which results in uninhibited growth of cells, confined tissue incursion and spreading to other remote parts of the body, with genetic and epigenetic levels changes [26, 43]. Mutagens are substances capable of altering the hereditary material in living things, thereby leading to build-up of genetic changes that exceed normal fundamental rate [7]. Mutagens cause alterations in the DNA which upon accumulation may lead to cancer as detrimental mutation can result in anomalous, damaged or non-functional gene [41]. An example of a mutagen is 1-Amino pyrene (1-AP) [22].

Cancer can be linked to mutagens that induce oxidative stress in the cells as a result of overproduction of free radicals [21]. These free radicals are generated in living systems as electrons escape from the electron transport chain to oxygen in the mitochondria [31]. They can damage biomolecules as they are implicated in mutagenesis, genotoxicity and carcinogenesis [35]. A widely employed test for identifying mutagens at the early stage is Ames test. It involves the use of *Salmonella typhimurium* bacterial strains which lack the ability to produce histidine [29].

Antimutagenic agents are natural or synthetic compounds that can reduce or eliminate the genotoxic properties of mutagenic and carcinogenic factors [14]. This can be by inactivating the mutagen-DNA reaction or by preventing the conversion of a mutagenic compound into mutagen [9].

Plants are rich sources of nutrients and in addition also contain various phytochemicals employed in the treatment of a wide range of diseases. Medicinal plants are now being utilized more than before as a source of potential chemotherapeutic drugs. Compounds from plants could behave as defensive substances against cancer-formation processes in humans [16] or, perhaps, by destroying or inhibiting the mutagens that destroy DNA outside the cells, thereby forestalling cell mutations [38].

1.1 ORIGIN AND DISTRIBUTION

Moringa, popularly tagged "miracle tree", belongs to *Moringaceae* family, grown for a long time especially, in bush thickets, and flourishes more in the tropics. Every of its component is utilized in different ways in earth regions at the middle of the globe [5, 17]. *M. oleifera* has fruits that resemble drumstick, tiny white flowers, and leaves with round shape (Figure 1).

FIGURE 1: *Moringa* seeds.

1.2 PHARMACOLOGICAL PROPERTIES OF *M. OLEIFERA*

Diverse components of *M. Oleifera* plant possess pharmacological properties. It has been demonstrated that its leaves have potent hypotensive [18], useful against diabetes, [28], anti-microorganisms, spasms, and diuresis [46], and also reported to be helpful against inflammation in anaphylaxis, helminthesis, and scurvy [11]. The pits are reported to be useful against microbes [10], demonstrate anti-fungal [12], anti-fertility [34], anti-hepatotoxic [39], analgesic [36], anti-arthritic [27], anti-tumor [20], anti-pyretic, and anti-inflammatory effects [48]. Dried *M. oleifera* seeds have been utilized as purgative, tonic, eye ointments and in the treatment of veneral infections [5].

2. METHODOLOGY

2.1 ASSORTMENT AND IDENTIFICATION

The seeds of *Moringa* were obtained in a local market. It was then identified at the Biological science Department, University of Lagos, Akoka, Lagos State, and allotted the specimen numeral APR/2018/MO-2564.

2.2 CHEMICALS

Standard chemicals used in this study were bought from Sigma Aldrich, Munich, Germany. The Lagos University Teaching Hospital Central research laboratory supplied the technical grade chemicals, and TA98 and TA100 variants of *Salmonella typhimurium*. The remaining chemicals utilized conformed to standard specifications.

2.3 PREPARATION OF PLANT EXTRACT

The method of [47] was used in preparing the extract. The seeds were washed and allowed to dry in the sun for seven days before being blended into finer particles using a milling machine. 1000 ml of ethanol was added to 100g of the powdered *Moringa oleifera* seeds in a flask which resulted in a mixture. This was stirred gently and covered for 72 hours followed by evaporation using a vacuum drier at 40^0C. A yield of 10.5% was obtained from the extract. It was then stored in the dark at +4ºC until when needed.

2.4 DETERMINATION OF THE CHEMICAL CONSTITUENTS

Initial qualitative investigation of the chemicals in the *Moringa oleifera* seed was done to identify the constituents using a method described by [45], followed by quantitative analysis of some of the chemicals present.

2.5 EVALUATION OF ANTIMUTAGENICITY

The ability of the seed extract at different concentrations to counteract the actions of a known mutagen was determined by employing TA 98 and TA 100 variants of *Salmonella typhimurium* using the method of [29] with slight modifications. The inhibitory effect (antimutagenic efficacy) of seed extract of *Moringa oleifera* was calculated and expressed in percentage as previously reported by [32] using the following equation:

$$\text{Inhibition (\%)} = [(A-B) / (A-C)] \times 100$$

A represents the number of colonies observed on the plate after being exposed to the mutagen;

B represents the number of colonies observed on the plate after being exposed to the mutagen and *Moringa oleifera* seed extract;

C represents the number of spontaneous colonies observed per plate

Antimutagenic effect was concluded to be efficacious if percentage inhibition is greater than 60, modest (40-60), small (20-40) and insignificant if less than 20.

2.6 STATISTICAL TEST

Results were expressed as average ± standard deviation. The results were subjected to statistical test by first employing one-way analysis of variance (ANOVA) and afterwards, Dunnett's test [Graphpad software, San Diego, CA, USA]. Probability (p) values at <0.05 were considered statistically significant when compared to the concentration of the ethanol extract (3.5, 7.5, 15, 30 mg) on the number of revertants/plate (1000 / Plate) and inhibition rate (%).

3. RESULTS

3.1 CHEMICAL ANALYSIS

Initial investigation revealed the presence of alkaloid, tannin, flavonoid, steroid, saponin, cardiac glycoside, anthraquinone, terpenoid in *Moringa oleifera* seed, while phlobatanin was not detected as shown in table 1. The amount of some of the chemicals contained in the seed is also shown in Table 1. As seen from the table, tannins was most abundant, followed by flavonoid, steroid, alkaloid and saponin.

TABLE 1. Qualitative Phytochemical Analysis of Seed Extract of *Moringa oleifera*

Phytochemical	***Moringa Oleifera* seed extract**	
	Present	**mg/100g**
Tannin	+	28.2±0.63
Alkaloid	+	18.7±2.15
Flavonoids	+	23.2±0.44
Phlobatanin	-	**n/a**
Terpenoid	+	**n/a**
Saponin	+	15.2±0.63
Cardiac glycoside	+	**n/a**
Anthraquinone	+	**n/a**
Steroid	+	20.5 ± 2.15
LEGEND: (+) is Present; (-) is not detected Values = Mean ± SD of triplicate experiments.		

3.2 ANTIMUTAGENIC ACTIVITY

As seen in table 2, a higher percentage inhibition and a lower number of revertants per plate were recorded at higher concentrations for both TA 98 and TA 100 variants. A decrease ($p < 0.05$) in the number of TA 98 and TA 100 revertants was recorded at *M. Oleifera* seed extract concentrations of 15 and 30 mg/ml as compared to those obtained at lower doses of the extract percentage, which resulted in a higher percentage of inhibition at those doses.

TABLE 2. Antimutagenicity Assay of Seed Extract *Moringa Oleifera* against Nitrosated Products

***Moringa Oleifera* (mG/mL)**	**TA98 Number of revertants /plate)**	**TA98 Inhibition (%)**	**TA100 Number of revertants/plates**	**TA100 Inhibition (%)**
3.5	388.20±19.40**	11.92±4.50*	858.60±37.85*	12.36±4.40*
7.5	307.60±18.74**	30.61±4.35*	729.00±65.94*	27.43±7.67*
15	180.80±17.53***	60.02±4.16**	560.40±20.77**	47.06±2.42*
30	101.00±11.25***	78.53±2.61***	421.80±25.78*	63.18±3.00**
*Figures within the same column differ ($p < 0.05$); **, ***Figures differ greatly ($p < 0.05$)				

4. DISCUSSION

In this research on *Moringa oleifera* seed extract, results revealed the presence of medicinally active constituents. These results agree to that of [15, 35]. The explicit physiological action of diverse medicinal plants against an extensive array of diseases in the human body is due to the various phytochemicals in those plants [44].

The quantitative phytochemical analysis showed that seed extract of *M. Oleifera* is richer in tannins than other phytochemicals. Tannins are employed as caustic medicine for treating ailments that affect the intestine e.g dysentery, diarrhea [37]. They also prevent carcinogenesis and the treatment of swollen tissues [1].

The seed extract of *M. Oleifera* also contains considerable amounts of flavonoids which possess anti-oxidant properties, are also able to counteract allergies, inflammation, viruses, proliferation and carcinogenesis, thereby influencing some aspects of mammalian metabolism [3].

Saponins are used medically for treating high blood cholesterol and are valuable in managing arteriosclerosis, hypertension and the control of post-menopausal syndrome. Saponins impede the assimilation of surplus cholesterol in the intestine, thereby reducing the likelihood of cardio dysfunction of which hypertension is an example [4, 17]. Alkaloids are used to treat malaria, cold, cough, hypertension, diabetes, and cancer [4]. *Moringa* seeds contain saponins and terpenoids; hence, it is employed in managing hyperglycemia, which is a characteristic symptom of diabetes [24]. Steroids are significant signaling molecules and membrane components [25].

AMES test is a simple, cheap and convenient preliminary screening test for mutagens and it involves the use of bacteria that rely on amino acid and are able to revert genetically upon

exposure to mutagens a [30]. The cells cannot form colonies, when histidine is not available. However, on the reversion of mutation, the cells regain their ability to synthesize histidine and can produce colonies.

Table 2 shows the antimutagenic effect of ethanol extracts of *Moringa oleifera* seeds at various concentrations on *S. typhimurium* variants TA98 and TA100 exposed to 1-aminopyrene. As it pertains to TA98, an efficacious antimutagenic property of the extract was observed at a concentration of 30 mg/ml plate with 78.53% as the inhibition percentage. Regarding TA100, a relatively high inhibition percentage of 63.18% was also obtained at 30 mg/plate. This might imply that mutagenic substances that work directly are generated through contact with acidified nitrosated products such as 1-AP to suppress some components in the *Moringa oleifera* extract. The method involved in the antimutagenic property of the extract remains vague. An antimutagenic substance is able to hamper conversion of pro-mutagen to mutagenic substances, incapacitate the DNA changing agent or thwart the association of the agent with the genetic material. Enzymes involved in the pathways of the repair, recombination and replication of DNA may be stimulated by some antimutagens by direct or indirect mechanisms [9].

Moreover, the ability of the seed extract to counteract the effect of the mutagenic agent might be due to its ability to form a complex attributed to its constituents which nullifies the nutagen. One other theory for the antimutagenic property of the seed extract could be the activation of the transmembrane export system in bacteria which allows mutagens to be quickly eradicated from the bacteria thereby preventing contact between the DNA and the mutagen [2]. Specific chemicals in plants like flavonoids group [8, 16, 19] and tannins [13] have been linked to antimutagenic activities of certain plants.

Flavonoids do contribute hydrogens and behave as antioxidants [6] by clearing free radicals, thereby preventing oxidative cell damage. The relatively high amounts of tannins in seeds of *Moringa oleifera* could also be linked to the considerable amounts of tannins present in the plant, as tannins are known to demonstrate antimutagenic activity [23, 40, 42].

5. SUMMARY

Mutagenesis plays a significant role in carcinogenesis. *M. Oleifera* plant with antimutagenic properties can protect organisms against the initiation stage of carcinogenesis.

KEYWORDS

1-Amino pyrene (1-AP)

Antimutagenic

Cancer

Moringa oleifera

Mutagen

S. typhimurium

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Chapter 17 P09
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LIST OF ABBREVIATIONS IN THIS CHAPTER

1-AP	1-Amino pyrene
DNA	deoxyribonucleic acid