Pharmacognostic evaluation and Antioxidant properties of the leaf and fruit extract of *Azanza garkeana*

Msc proposal

By

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Introduction

*Azanza garkeana* is a semi delicious tree/shrub with round medium crooked stem (palgrave et al., 1985) . It is commonly called the African chewing gum, wild hibiscus, snot apple and locally called Goron Tula in Hausa. The tree can grow to a height of 3-15meters, depending on the environment in which it grows (palgrave et al,). The twigs are hairy when young but smooth with age and branches have woolly hairs (ICRAF, 1992). The leaves have 3-5 lobes, which are covered in a brown star shaped hair, and have longitudinal fissures in the midrib (FAO, 1983). The flowers have many stamen and five (5) petals which are yellow or purplish (palgrave, 1985). The flowers are bisexual with all floral parts. The fruits are hairy, spherical, hard and about 2.5 to 4cm long in diameter, internally divided into 4 to 5 longitudinal sections which are eatable. They are brownish green when matured (ICRAF, 1992).

*Azanza* is widely distributed in east and south African countries like Botsuwana, Kenya Malawi, South Africa, Zambia, Zimbabwe, Tanzalia. The species was reported to be growing from Sudan to south Africa (Mbuya et al,. ). It is also found in Gombe state, Nigeria, west Africa which grows naturally in all types of woodlands from sea level to 1700m above sea level (Mulofwa et al,.). It grows in semi-arid areas receiving lowest annual rainfall of 250mm and highest of 1270mm (FAO , 1983). Over the range of its entirety, the species grows in a variety of soils and its found on or near termite moulds and desert village fields (White F, 1962). In Nigeria it grows in open woodland in savannah area, in Tula district of Kaltungo local government area of southern Gombe State of Nigeria.

**Statement of Problem**

*Azanza garkeana* is used in traditional medicine as remedy for various ailments without scientific validation.

There is paucity of information on the phytochemistry and phytomedicine of *Azanza garkeana.*

**Justification**

*Azanza garkeana* is an important plant which has attracted much interest from scientist in recent time. These includes the establishment of some pharmacological and pharmachemical activities.

**Hypothesis**

The bioactive constituents of the *Azanza garkeana* are responsible for its uses as a remedy for ailment.

**Aim**

To establish scientific bases for using the fruits and leaves of *Azanza garkeana* as an antioxidant. This can be achieved through the following specific objectives;

1. To extract the samples using Hexane, ethanol and ethyl acetate
2. To determine the antioxidant properties of the extract.

**Materials and methods**

**Methods**

**Collection and preparation of plant**

The fruits and leaves of *Azanza garkeana* will be collected from Tula yiri in kaltungo LGA of Gombe state, plant will be identified in the federal school of forestry, Jos, Plateau state Nigeria.

The leaves and fruits will be dried before using a mortar to pound into fine powder. A specimen will be deposited at the Herbarium, Department of Pharmacognosy , university of Jos.

**Macroscopical examination**

Various microscopical examinations will be carried out on the fruits and leaves which include observation of the colour, taste, odour, and size.

**Microscopical examination**

The quantitative and qualitative microscopy will be carried out to determine the stomatal index, vein, palisade ratio. The upper and lower epidermises will be observed also.

**Phytochemical screening**

This will be carried out on the extracts using the standard methods and procedures described by Sofowara, 2008 for the presence of secondary metabolites such as alkaloids, glycosides, saponins, tanins.

**TLC Profiles**

TLC profiles of the extracts will be studied on pre coated plates (merck F 254) and visualized with appropriate spray reagents, the qualitative will be done to determine the presence of compounds.

 **Determination of phenolic content**

Folin-ciocalteu method described by Adedapo et al., 2008 will be adopted

Samples containing phenols are reduced by folin-ciocalteu reagent to produce blue colour complex

The phenolic concenteration of the extract is evaluated from the gallic acid calibration curve.

TPC will then be expressed.

**Determination of flavonoid**

Flavonoid quantification will be done using aluminium chloride colorimetric method described by Choudahary et al., 2012

**Antioxidant assay**

The antioxidant activity of extracts on the stable radical 1,1-diphenyl-2-picrythydrazyl (DPPH) will be determined using the method described by Brand-williams et al., 1995

**Statistical analysis**

DPPH experiment will be carried out in triplicate studies (n=5). The result will be presented as mean +- standard error of mean (SEM). Student’s t-test will be used.

**Appendix I: Budget and Costing**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **s/No** | **Item/activity** | **quantity**  | **Unit cost** | **Total cost** |
| 1 | Plant material | 6kg | 2000 | 12,000 |
| 2 | Hexane  | 4 x 2.5L | 9000 | 36,000 |
| 3 | Ethyl acetate | 4 x 2.5L | 10,000 | 40,000 |
| 4 | Methanol | 4 x 2.5L | 9,000 | 36,000 |
| 5 | Acetone | 2 x 2.5L | 9,000 | 18,000 |
| 6 | Silica gel(column) | 3 x 50kg | 8,000 | 24,000 |
| 7 | Sample collectors | 300 | 250 | 75,000 |
| 8 | Elemental Analysis |  |  | 80,000 |
| 9 | GCMS Analysis |  |  | 25,000 |
| 10 | Structure elucidation |  |  | 60,000 |
| 11 | Thesis production |  |  | 50,000 |
|  | *Total*  |  |  | 456,000 |

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