

## Studies on Local Knowledge and *In vitro* Cytotoxicity of *Moringa oleifera* L., *Andrographis paniculata* N. and *Asystasia vogeliana* B. Extracts

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**Abstract:** Multi-Purpose Medicinal Plants (MMPs) are gaining unprecedented attention apparently because of their potency to contribute to the prevention and treatment of myriads of diseases. As such the toxicity of many herbs including the MMPs has always been a concern particularly relating to internal organs. The present study investigates the local knowledge use and *in vitro* cytotoxicity against two cancer cell lines; BGC-823 and HeLa cells on three (MMPs); *Moringa oleifera* (Lam) (Moringaceae) *Andrographis paniculata* (Burm. f) and *Asystasia vogeliana* (Benth) (Acanthaceae). The local knowledge was collated through Participatory Rural Appraisal (PRA) approach from selected locations in the Oke-Ogun area of Oyo state, Nigeria. Ethanolic extracts were used for the *in vitro* cytotoxicity study. The extracts were screened in two cancer cell lines (BGC-823 and HeLa cells) using the Sulpho Rhodamine B (SRB) assay. For the treatment of diseases, the local knowledge shows that *A. paniculata* recorded higher fidelity level on the treatment of malaria (95%), diabetes (80%), high blood pressure (87.5%), cancer and tumor (65.7%). The infusion of *A. vogeliana* in combination with the leaves of *Cassia alata*, *Cymbopogon citratus* and fruit juice of *Citrus aurantifolia* recorded higher fidelity level in the treatment of malaria and chronic fever (74.8%), gonorrhoea (65%) and leprosy (40%) suggesting better alternative to *M. oleifera* and *A. paniculata*. The medicinal relevance of the species in the treatment of diseases such as malaria, fever, high blood pressure, cancer, diabetes among others in local herbal medicine were revealed. Cytotoxicity assay on the two cell lines, BGC-823 and HeLa cells revealed that only the ethanolic extract of *A. paniculata* exhibited some level (moderate) of cytotoxic activity with IC<sub>50</sub> values of 24.7 and 23.1 µg/mL, respectively. *M. oleifera* and *A. vogeliana* did not show any significant activity on the cell lines. The study highlights the importance of local knowledge in finding cost effective, potent and safe herbs for people and screening of the plant species for their activities against cancer cell lines.

**Key words:** *Moringa oleifera* (Mo), *Andrographis paniculata* (Ap), *Asystasia vogeliana* (Av), cytotoxicity assays, BGC-823, HeLa cells

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### INTRODUCTION

The use of plants has long been part of local cultures and traditions as source of food, medicine and other derivable products (World Health Organization, 2013; Chekole *et al.*, 2015). The role of indigenous knowledge in the identification, conservation and utilization of plant species cannot be over-emphasized. Globally, there is a growing interest in traditional medicine in finding cost effective and potent herbal preparations with bioactive compounds that will be efficient in the treatment of many diseases (Lambert *et al.*, 2005; Ferreira *et al.*, 2009, 2011). Natural products, particularly of plant origin remain the most important, reliable and cheaper source of new drugs

and supplements capable of combating infections and diseases (Odugbemi *et al.*, 2007). Several reports have shown that >85% of the world populations especially in Sub-Sahara African (SSA) countries depend on traditional use of plants in the treatment of diseases such as malaria, yellow fever, diabetes, hypertension, sickle cell anemia and HIV/AIDS infections (WHO, 2003; Maroyi, 2011; Dike *et al.*, 2012; Wodah and Asase, 2012).

Plants with multi-uses are receiving attention and considered very effective in the treatment and prevention of many diseases (Anwar *et al.*, 2007; Hossain *et al.*, 2014). Multi-Purpose Medicinal Plants (MMPs) are species with a range of medicinal values that have been and are still being successfully exploited and used by

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people and their livestock for treating various ailments (Lambert *et al.*, 2005). MMPs possess many medicinal values for human and livestock as well as nutritional advantage that contribute to food security especially during the periods of extreme drought (Lambert *et al.*, 2005). According to World Health Organization (2001), herbal medicines have an estimated global market value of US \$65 billion to which SSAs population can exploit. Interestingly, recent reports indicate that MMPs compare favorably with cash crops such as coffee, oil palms, cocoa and cotton as income generators (Lambert *et al.*, 2005). Thus, if MMPs are properly harnessed in SSAs, they can effectively enhance the healthcare sector and generate more income to rural dwellers. Similarly, with increased cultivation of MMPs, conservation, management and most importantly utilization will improve with added economic values. Since, over 70% of populations in the SSA depend on traditional medicines, especially from MMPs, research efforts need to target mode of administration, dosage and toxicity/pharmacological effects which constitutes major challenges facing traditional medicine in Africa (Wangchuk *et al.*, 2013).

Recently, ethnobotanical survey and geographical distribution of *Moringa oleifera* was carried out in the Oke-Ogun area of Oyo State, Nigeria by Popoola and Obembe (2013) during which two other plants with similar indigenous uses were encountered. The two plants include *Andrographis paniculata* (Burm. f) and *Asystasia vogeliana* (Benth). Majority of dwellers in the area are mainly farmers who possess unique knowledge on plant usage which require proper documentation to avoid loss of relevant indigenous knowledge. Around the world, indigenous people are known to retain rich knowledge of plant resources to which they depend on for food, medicine, income, general uses and botanical expertise (Omonhinmin, 2014). This was not strange among the people of the area who primarily depend on agriculture for survival and had influenced domestication of many plant species, development of specific knowledge on uses and conservation of plant species. During our initial survey, we observed that the three species were commonly grown in home gardens and backyards of the people of the area with special interest and preference to other plant species, thereby eliciting curiosity why the interest on the use of these three MMPs. This indicates that the plant species are versatile in the prevention and treatment of certain diseases in the area. It is necessary to collate and document such traditional medicinal knowledge uses of the species in the area. Though, several ethnobotanical and chemical composition/toxicity studies have been reported on *Andrographis paniculata* and *Moringa oleifera*

(Thurber and Fahey, 2009; Akbar, 2011; Popoola and Obembe, 2013; Inta *et al.*, 2013; Hussain *et al.*, 2014; Leone *et al.*, 2015), such studies are scanty and limited on *Asystasia vogeliana*. And where available, such knowledge has not been reported from the Oke-Ogun area of Oyo State, Nigeria.

The ethnobotany, phytochemical, pharmacological activities, toxicity profile and therapeutic usage of *Andrographis paniculata* were separately reviewed by Hossain *et al.* (2014) and Okhuarobo *et al.* (2014) with preponderance of wide usage around the world. Traditionally, *A. paniculata* has been known as an immune system booster, blood cleanser and as herbal medicine to treat and cure several diseases such as infectious diseases, fever-causing diseases, colic pain, loss of appetite, irregular stools, diarrhea, cancer and viral infections among others (Saxena *et al.*, 1998; Okhuarobo *et al.*, 2014; Shalini and Narayanan, 2015). Also, different phytochemical constituents including diterpenoids, diterpene glycosides, lactones, flavonoids, xanthenes, noriridoides among others have been reported from the parts of *A. paniculata* (Hossain *et al.*, 2014; Okhuarobo *et al.*, 2014). In addition, pharmacological activities including anticancer, antidiarrheal, antihepatitis, anti-HIV, antihyperglycemic, anti-inflammatory, antimicrobial, antimalarial, antioxidant, cardiovascular, cytotoxic, hepatoprotective, immunostimulatory and sexual dysfunctions have been reported and reviewed for the species (Jarukamjorn and Nemoto, 2008; Okhuarobo *et al.*, 2014; Hossain *et al.*, 2014). Generally, the safety and toxicity effects of *A. paniculata* had proven to be safe in various studies on mice, rats and rabbits as well as in *in vitro* assays and some clinical trials (Akbarsha and Manivannan, 1993; Akbarsha and Murugaian, 2000; Kamal *et al.*, 2003; Hossain *et al.*, 2014). With respect to *Moringa oleifera*, several authors have also reported the local uses, commercial applications, phytochemical, pharmacological, safety and toxicity profile comparable to *A. paniculata* (Anwar *et al.*, 2007; Stevens *et al.*, 2013; Popoola and Obembe, 2013; Leone *et al.*, 2015; Koul and Chase, 2015; Daba, 2016). Globally, *Moringa oleifera* has received unprecedented scientific and commercial attention as many fortified *Moringa* products have been or are being tried and used for the treatment and cure of many diseases such as diabetes, cancer, malaria, jaundice, sickle cell anemia and several others. Studies have shown that *Moringa oleifera* is naturally safe and can be used as an anti-neoproliferative agent to inhibit cancer cell growth (Nair and Varalakshmi, 2011; Gopalakrishnan *et al.*, 2016). Contrarily, there is paucity of scientific information on *Asystasia vogeliana* particularly relating to pharmacological, toxicity activities and phytochemical profiles.

Cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells (Safarzadeh *et al.*, 2014; Zhang *et al.*, 2015). Treatments involving surgery, chemotherapy and radiation are however expensive and with side effects (Gopalakrishnan *et al.*, 2016). Thus, there is growing interest and consistent attempt in the efforts to find naturally safe and cost effective therapeutic solutions to cancer disease using plant species and such research efforts should be intensified until the objective is achieved. Therefore, our attempt in this study is to provide additional information on the local uses of the three species (*Andrographis paniculata*, *Asystasia vogeliana* and *Moringa oleifera*) as herbal medicine being used locally in the treatment of diseases particularly cancer with a view to propelling further studies. Our effort is directed toward future research needs especially in the quest to find lasting solution to the problem of cancer affecting human race. The present study therefore, aims to investigate the *in vitro* cytotoxicity of the extracts of the three plant species against human gastric cancer (BGC-823) and human cervical cancer (HeLa) cells as a preliminary step to further elucidate future studies on cancer.

## MATERIALS AND METHODS

**Study site description:** The study area covers six local government areas; Saki West, Saki East, Atisbo, Itesiwaju, Iseyin and Orelope in Oke-Ogun area of Oyo State, Nigeria. The area lies between 7.19083N, 3.41383W and 8.83249N, 3.74960W located within the Guinea Savannah Zone (Northern part of the state). Figure 1 shows the study site and areas of collection of voucher specimens and ethnobotanical information. The Oke-Ogun area shares borders with states like: Kwara, Niger, Ogun, Osun and Benin Republic (a neighboring country). The annual rainfall varies between 700-1100 mm. The mean annual temperature is 37°C and the vegetation is characterized by grassland, open shrubs and savannah trees. The area is recognized as the “food basket” of the Southwestern, Nigeria. The population is composed of Yorubas who are mostly agriculturists, transporters, civil servants, artisans, business men and women (Fig. 1).

**Voucher specimen identification and description of the MMPs used for this study:** The three MMPs; *Moringa oleifera*, *Andrographis paniculata* and *Asystasia vogeliana* were photographed and herbarium samples authenticated at the Forest Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria. Figure 2 shows the voucher specimens of *Moringa oleifera* and *Andrographis paniculata* collected for this study.

***Moringa oleifera* Lam (Mo) (FHI No: 110373):** Mo belongs to the family, Moringaceae. Mo is a small to medium-sized tree, usually deciduous and grows up to 12 m in height. The leaves are bipinnate and tripinnate compound, triangular in outline, alternate and spirally arranged. The fruit pods are linear containing seeds which are dark brown in colour. Common names ascribed to Mo include: ‘ewe igbale’, ‘ewe ile’ and ‘gbogbonise’ (describing the multi-purpose medicinal activities). All parts are useful as food and medicine.

***Andrographis paniculata* (Ap) (Burm. f) with FHI No. 110374:** Ap is a bitter annual herb. It belongs to the family, Acanthaceae. The leaves are dark green in colour, simple, opposite, lanceolate, glabrous with acute apex and entire margin. It is an introduced species to the area of study.

***Asystasia vogeliana* (Av) (Benth):** Av belongs to the family, Acanthaceae. Av is a herb/shrub. The leaves are simple, opposite, ovate and decussate without stipules. The flowers are bisexual, zygomorphic and usually associated with coloured bracts.

**Local knowledge study and data collection:** Preliminary investigations and ethnobotanical survey of *Moringa oleifera* showed that the people of Oke-Ogun preferred to use MMPs to treat many diseases (Popoola and Obembe, 2013). Participatory Rural Appraisal (PRA) approach was employed to extract information from people of the area. Five households/people were randomly sampled in each of the local government areas. Questions were asked about the availability, how they knew the species, planting methods and medicinal uses of the three species. Information collected were pooled into data for analysis. The age range of 30-80 years of respondents were adopted. Traditional herbalists, herb sellers, teachers, farmers and artisans were engaged and each participated based on knowledge possessed. Selections of these groups were based on social status, occupation and informed knowledge on the ethnobotanical uses of plants in the sampled areas. Demographic survey of members of the communities sampled is presented in Table 1.

**Disease treatment:** Indigenous knowledge was investigated on the three MMPs based on the previous information gathered and observations on their usage to treat diseases such as malaria, gonorrhoea, hypertension, diabetes, epilepsy, gastric disorder, cancer and tumor.

**Data analyses:** Information collected were transformed into data and entered into excel sheet. Data were analyzed

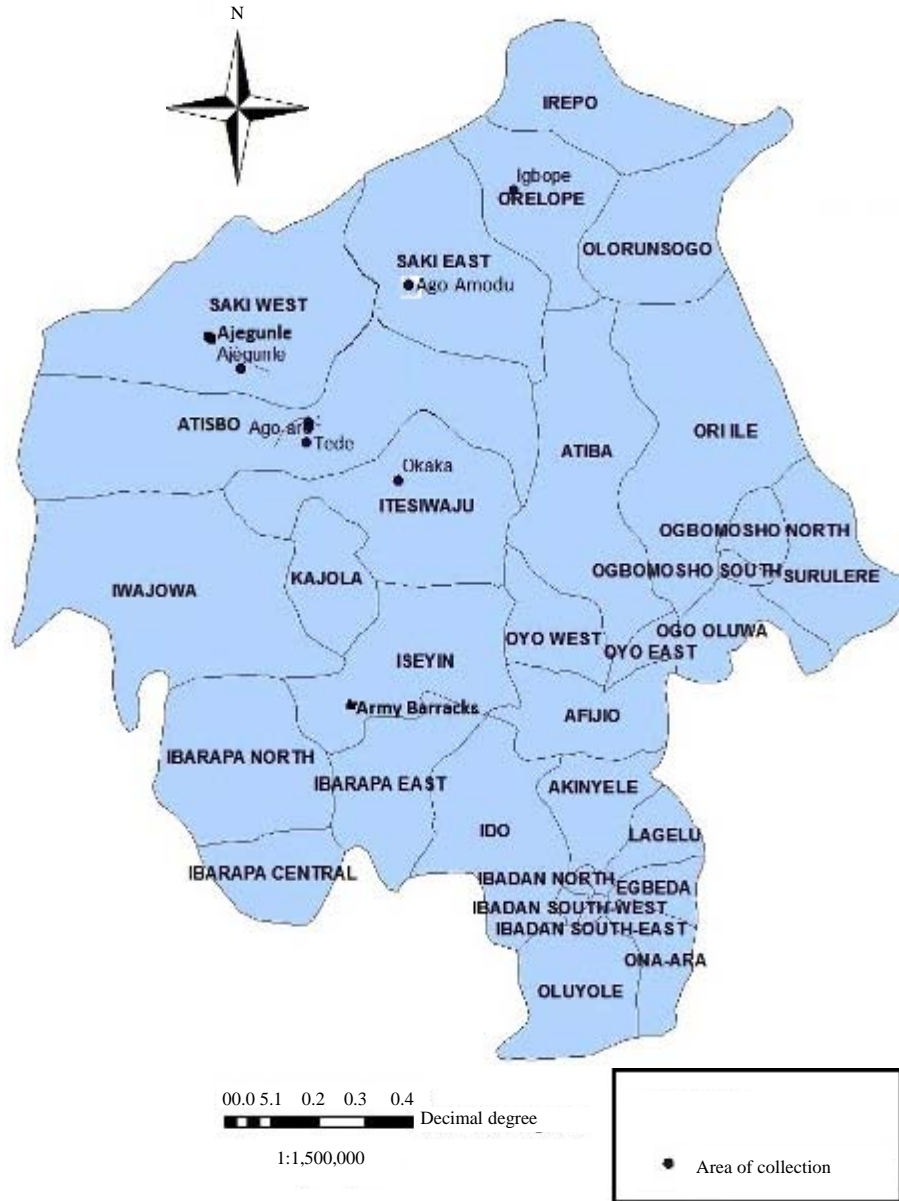


Fig. 1: Study site and areas of collection

| Parameters/class of respondents | Percentage |
|---------------------------------|------------|
| <b>Occupation</b>               |            |
| Traditional herbalists = 10     | 25.0       |
| Herbal seller/dealer = 15       | 37.5       |
| Artisans = 5                    | 12.5       |
| Teachers = 5                    | 12.5       |
| Others = 5                      | 12.5       |
| Total = 40                      |            |
| <b>Age (years)</b>              |            |
| 31-40                           | 15.0       |
| 41-60                           | 45.0       |
| >60                             | 40.0       |
| <b>Sex</b>                      |            |
| Male = 26                       | 65.0       |
| Female = 14                     | 35.0       |

using tables and expressed as percentages. The percentage of respondents who had knowledge on the three MMPs and in the treatment of various diseases was evaluated using fidelity level of use. According to Friedman *et al.* (1986) Fidelity Level (FL) can be computed as:

$$FL = \frac{S}{T} \times 100$$

Where:

S = Respondent interviewed citing species for treatment of specific disease  
 T = Total number of respondents

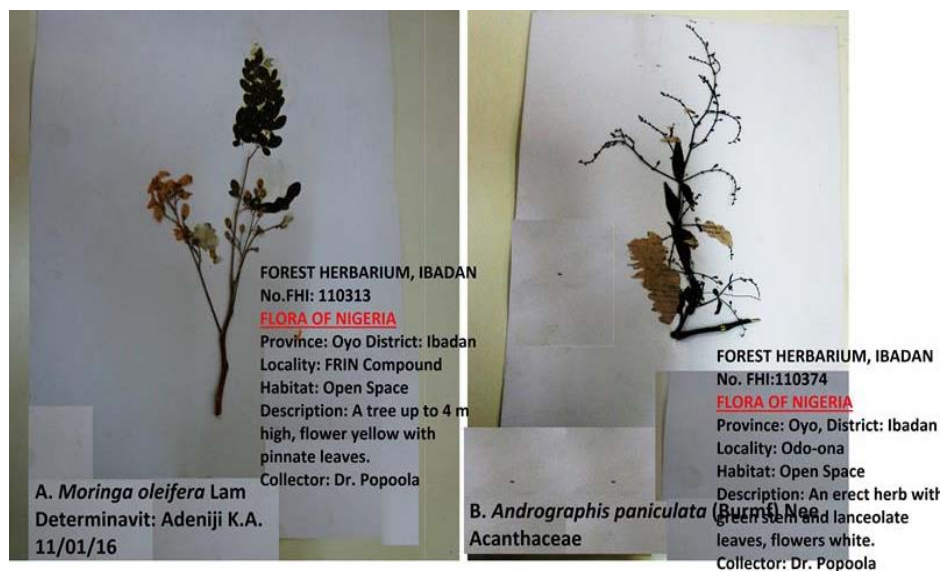


Fig. 2: Voucher specimen of the parts of the plants used for the study: a) *Moringa oleifera* and b) *Andrographis paniculata*

Preference Ranking (PR) method was employed to determine the level of effectiveness of each species in the treatment of diseases by the respondents. The PR was evaluated using a coding system; 3 = highly effective, 2 = fairly effective, 1 = effective but weak, 0 = not effective.

#### **In vitro cytotoxicity study of the MMPs extracts**

**Preparation of plant extracts:** The samples of the three MMPs; Mo, Ap and Av were air dried for four weeks under room temperature. Russell household electrical blender was used to blend the dried leaves of samples to reduce the surface area. The leaf powders were stored in sealed labeled reagent bottles. Alcoholic extracts were prepared from all the three plants leaves as follows: a 10 g portion of the leaf powder of each plant was weighed into 100 mL of absolute ethanol. Extraction was effected at room temperature by placing the mixture (in covered beakers) in a cool dry place for 72 h. After these hours of extraction, the filtrates were each concentrated to dryness using a rotary evaporator at 50°C and the yield obtained was 0.7, 0.8 and 0.75 g, respectively of extracts of Mo, Ap and Av.

**Reagents and cancer cell lines:** All the extraction reagents used were of analytical reagent grade. Trypsin, trizima base, sulforhodamine B (SRB), trichloroacetic acid, RPMI-1640 culture medium and trypan blue solutions were purchased from Sigma Chemicals Co., Ltd. (St. Louis, MO, USA). The two cell lines BGC-823 and HeLa cells were obtained from Shanghai Institute of MateriaMedica, Chinese Academy of Sciences, Shanghai, China.

**Cancer cell growth inhibition assay:** The procedures described by Newman and Craig (2007) and Adebayo *et al.* (2010) were followed. In brief: the sulphorhodamine B (SRB) assay was adopted for a measurement of cell growth and viability (Shukla and Kalra, 2007). Human gastric cancer (BGC-823) and human cervical cancer (HeLa) cells were seeded in 96-well micro titer plates at 3000-7000 cells per well. After 24 h, compounds were added to a final concentration of 10 µg/mL and incubated for 48 h. Cells were then fixed by the addition of 50% ice-cold CCl<sub>3</sub>COOH and then left at 4°C for 1 h. After washing, air-drying and staining for 15 min with 100 µL, 0.4% SRB in 1% glacial ACOH, excessive dye was removed by washing with 1% glacial ACOH. The absorbance values of re-suspended SRB in 10 mM Tris buffer were read at 560 nm on a microplate spectrophotometer (SPECTRA MAX 340, USA). If the cell growth inhibition was >50% at the highest tested concentration of 10 µg/mL, further assessment was carried out with at least 4 diluted concentrations (dilution ratio 1:2) to calculate the IC<sub>50</sub> values (50% inhibitory concentration). Taxol was used as the positive control compound.

## **RESULTS AND DISCUSSION**

**Species and demographic data:** The three MMPs selected for this study belongs to two families. *Moringa oleifera*. Mo belongs to *Moringaceae*, genus *Moringa* and

Table 2: Medicinal values of *A. paniculata*, *A. vogeliana* and *M. oleifera* investigated

| Plants name                    | Parts used   | Mode of preparation                                    | Form of use               | Purpose of use                   | Fidelity level (%) | PR |
|--------------------------------|--|--|---------------------------|----------------------------------|--------------------|----|
| <i>Andrographis paniculata</i> | Leaves and whole plant   | Boil in water  | Drink the extract         | Malaria and body weakness        | 95.00              | 3  |
|                                |  |  |                           | Diabetes                         | 80.00              | 3  |
|                                | Whole plant  | Squeeze in water and sieve the extract                 | Drink the extract         | High blood pressure/hypertension | 87.50              | 3  |
|                                |  |  |                           | Cancer and tumor                 | 65.70              | 3  |
|                                | Leaves   | Grind and mix with fruit of <i>Citrus aurantifolia</i> | Drink the concoction      | Hypertension                     | 90.00              | 3  |
|                                |  |  |                           | Gonorrhea                        | 56.00              | 2  |
| Diabetes                       |  |  |                           | 60.00                            | 2                  |    |
| Gastric disorder               |  |  |                           | 85.00                            | 3                  |    |
| <i>Asystasia vogeliana</i>     | Leaf and whole plant   | Squeeze solely in water                                | Drink the extract         | Malaria                          | 82.50              | 3  |
|                                |  |  |                           | Gastric disorder                 | 45.00              | 1  |
|                                | Boil <i>A. vogeliana</i> with leaves of <i>Cassia alata</i> , fruit of <i>Citrus aurantifolia</i> and leaves of <i>Cymbopogon citratus</i> | Drink extract and Infusion                             | Malaria and chronic fever | 74.80                            | 2                  |    |
|                                |  |  | Hypertension              | 50.00                            | 3                  |    |
|                                |  |  | Gonorrhea                 | 65.00                            | 2                  |    |
|                                |  |  | Gastric disorder          | 45.00                            | 3                  |    |
|                                |  |  | Cancer and tumor          | 25.70                            | 1                  |    |
|                                |  |  | Epilepsy                  | 40.00                            | 1                  |    |
|                                |  |  | Diabetes                  | 37.50                            | 1                  |    |
|                                |  |  | Hypertension              | 92.50                            | 3                  |    |
| <i>Moringa oleifera</i>        | Leaves   | Boil in water dry leaves and grind into powder         | Drink extract             | Cancer and tumor                 | 32.50              | 2  |
|                                |  |  |                           | Malaria                          | 90.00              | 2  |
|                                |  |  |                           | Diabetes                         | 75.00              | 2  |
|                                |  |  |                           | Epilepsy                         | 35.00              | 1  |
|                                |  |  |                           | Gastric disorder                 | 75.00              | 3  |
|                                |  |  |                           | Gonorrhea                        | 68.50              | 2  |

species *Oleifera*. *Ap* and *Av* belong to Acanthaceae. *Ap* belongs to the genus *Andrographis* and species *paniculata* while *Av* belongs to the genus *Asystasia* and species *vogeliana*.

In all the six local government areas sampled, different local names were ascribed to each of the species based on morphology and medicinal uses. *M. oleifera* was regularly referred to as 'Gbogbonise' or 'awogbaarun' which was more or less associated with multiple medicinal features/prowess to treat several ailments while 'agunmaniye' refers to its growth habit as a tall tree. *A. paniculata* was relatively foreign to the areas of study. The local name ascribed to *Ap* 'ewe Jogbojogbo' was more or less associated with the bitter taste attribute and ability to ameliorate the disease condition of diabetes mellitus and high blood pressure. On the other hand, *A. vogeliana* was identified and known based on resemblance to *Asystasia gagentica* and was referred to as 'ewe Lobiiri' (the leaf that turns).

A total of 40 respondents were encountered where 65% (26) were males and 35% (14) were females. Among the respondents, 25% were traditional herbalists, herbal seller (37.5%), artisans (12.5%), teachers (12.5%) and others including market and Motorbike riders (12.5%). In all, 55% dealt directly in herbs while 45% grew the three MMPs in their home gardens (Table 1).

**Fidelity level (FL%) and preference ranking of disease treatments of the species:** The three species; *M. oleifera*,

*A. paniculata* and *A. vogeliana* were used differently to treat several diseases. Seven major ailments that were being treated with the leaves of the plant species were selected and recorded in this study. The medicinal knowledge, fidelity level as well as preference ranking of the species often used for the treatment of diseases are presented in Table 2. *M. oleifera* was mostly mentioned with multiple uses. The ethno-medicinal knowledge on the treatment of diseases showed that *A. paniculata* and *M. oleifera* recorded higher fidelity levels in the treatment of malaria indicating 95 and 90%, respectively while *A. vogeliana* showed 82.5% fidelity level. These suggest that the species were very active and potent in the treatment of malaria in the sampled areas. Similarly, *A. paniculata* recorded higher fidelity value in the treatment of diabetes (80%), hypertension (87.5%) and very active when combined with the fruit juice of *Citrus aurantifolia* for the treatment of hypertension (90%), gonorrhea (56%), diabetes (60%) and gastric disorder (85%). *M. oleifera* also showed high fidelity levels of 92.5, 75 and 75% respectively with respect to treatment of high blood pressure, diabetes and gastric disorder while *A. vogeliana* recorded low values in the treatment of diabetes (37.5%) and high blood pressure (50%). Contrarily, both *A. paniculata* and *A. vogeliana* recorded a lower fidelity level for the treatment of gonorrhea (37.5%) and (25.7%) when compared with *M. oleifera* which recorded 68.5%. For the treatment of cancer and tumor, the extract of *A. paniculata* showed

Table 3: Cytotoxicity activities of extracts of *A. paniculata*, *A. vogeliana* and *M. oleifera* on BCG823 cells

| Plant extracts       | 50   | 10   | 2 ( $\mu\text{g/mL}$ ) | 0.4 | IC <sub>50</sub> |
|----------------------|------|------|------------------------|-----|------------------|
| <i>A. paniculata</i> | 75.1 | 17.7 | 7.3                    | 4.6 | 24.700           |
| <i>A. vogeliana</i>  | 19.6 | -    | -                      | -   | -                |
| <i>M. oleifera</i>   | 1.1  | -    | -                      | -   | -                |
| Taxol                | -    | -    | -                      | -   | 0.012            |

Table 4: Cytotoxicity activities of extracts of *A. paniculata*, *A. vogeliana* and *M. oleifera* on HeLa cells

| Plant extracts       | 50     | 10     | 2 ( $\mu\text{g/mL}$ ) | 0.4    | IC <sub>50</sub> |
|----------------------|--------|--------|------------------------|--------|------------------|
| <i>A. paniculata</i> | 82.560 | 14.397 | 2.741                  | 10.655 | 23.197           |
| <i>A. vogeliana</i>  | 6.125  | -      | -                      | -      | -                |
| <i>M. oleifera</i>   | 38.587 | -      | -                      | -      | -                |
| Taxol                | -      | -      | -                      | -      | 0.070            |

higher value of 65.7%, followed by *M. oleifera* (32.5%) extract while *A. vogeliana* recorded the least, 25.7%. This indicates that *A. paniculata* could be more effective as an anticancer agent than any of *M. oleifera* and *A. vogeliana*. This reflects in preference of ranking among the species in the treatment of the ailment (Table 2).

The infusion of *A. vogeliana* in combination with the leaves of *Cassia alata*, leaves of *Cymbopogon citratus* and fruit juice of *Citrus aurantifolia* recorded higher fidelity level in the treatment of malaria and chronic fever (74.8%), gonorrhoea (65%) and epilepsy (40%) which suggest better alternative to *M. oleifera* and *A. paniculata*. The concoction was also mentioned to be effective against HIV infection and anemia. The preference of ranking of the treatment of ailments showed that *A. paniculata* was most preferred and effective in the treatment of malaria, hypertension, cancer and tumor and gastric disorder (Table 2). The results also revealed that the decoction in either water or alcohol was the most common method of preparation of these medicinal plants. Other means of preparation such as the use of carbonated drink, grinding into powder and use of pap were mentioned by some of the respondents.

**In vitro cytotoxicity of the MMPs species:** The *in vitro* cytotoxicity activities of ethanol extracts of the three MMPs screened on two cancer cell lines (BGC-823 and HeLa cells) are presented in Table 3 and 4. The results showed that only the extract of *A. paniculata* exhibited some level (moderate) of cytotoxic activity with IC<sub>50</sub> values of 24.7 and 23.1  $\mu\text{g/mL}$ , respectively. *M. oleifera* and *A. vogeliana* did not show any significant activity on the cell lines. This suggests that among the three species investigated, *A. paniculata* can be considered as a potential source of anti-cancer agent.

## DISCUSSION

The use of plants as medicine and veritable source of new drugs and supplements is apparently on the increase.

The present study provides local knowledge on three selected Multi-Purpose Medicinal Plants (MMPs); *Moringa oleifera*, *Andrographis paniculata* and *Asystasia vogeliana* used in the Oke-Ogun area of Oyo state for the treatment of some diseases. The study also offers preliminary and basic information on *in vitro* cytotoxicity of the extracts of the three species on human gastric cancer (BGC-823) and cervical cancer (HeLa) cells. This will lay a foundation for future *in vitro* cytotoxicity studies particularly on *Asystasia vogeliana*.

The study showed that the people of Oke-Ogun area of Oyo state, Nigeria have been using medicinal plants as a major component of their traditional medicine to treat and prevent diseases for many years. The area is rich in plant diversity consisting of herbs, shrubs, grasses and savannah trees. Over 70% of the population depends on traditional medication especially among the old people in the treatment of diseases. The knowledge of medicinal plants was observed to be rich and diverse among the old respondents in all the six local government areas sampled. Indigenous knowledge of medicinal plants is usually transferred along the family lines, among hunters, herbal healers and sellers. This study observed a shift in the transfer of indigenous knowledge of MMPs which was mainly on the search for plants that could treat and cure many disease symptoms. However, it was observed that the younger generation considered plant medication a sort of superstition, fetish and less efficient compared to modern medicine. Some older respondents (65-70 years) lamented the lack of interest among the youth in using and inheriting the knowledge and information available on medicinal plants and particularly on MMPs. Based on the age of older respondents, the information gathered are reliable since knowledge of use is usually perpetrated through generations, age and along professional lines such as farmers, hunters, herb sellers and traditional healers (Popoola and Obembe, 2013; Omonhinmin, 2014). The information was collected through consultation with older and knowledgeable individuals within and neighboring communities during the time of plant collection and preparations. The results of this study were therefore based on testimonies of those that have attempted the use of the plants for the treatment of one disease or the other. For instance, according to one of the respondents (Baba Bello) *Andrographis paniculata* was collected from herbal trader who had earlier sourced the plant from Ilorin, Kwara State, a neighboring state for the treatment of diabetes and cancer. According to this respondent *Andrographis paniculata* was found to be effective by many users in the community to treat diabetes and cancer, thus increasing the interest on the species. In addition, women in the communities sampled

often combine the leaves of *Andrographis paniculata* with vegetables to prepare vegetable soup. The knowledge of *Asystasia vogeliana* was perpetrated through regular use in treating malaria and other fever related conditions which spread to other members of the communities and thus became known. However, *Moringa oleifera* was well known as multipurpose medicinal plant based on sudden cultivation, popularity of use in most communities as leafy vegetable and medicine (leaf, seed and root). The claims on *M. oleifera* in this study are in consonance with the reports of several authors (Popoola and Obembe, 2013; Inta *et al.*, 2013; Hussain *et al.*, 2014; Leone *et al.*, 2015). *Asystasia vogeliana* and *Andrographis paniculata* on the other hand are not well known, very few respondents of 60 years and above who had knowledge on plant species acknowledged and provided useful information on local uses and preparations. The knowledge of use in the area is strong and in many cases, leaf extracts/infusions are taken orally early in the morning and to some extent in the night after meals.

The study revealed a multiple pattern of use for each of the species. The leaves/aerial parts and whole plant of *Andrographis paniculata* prepared in form of decoction/extracts are usually used by the people to treat gastric disorder, body weakness, cold, diabetes, cancer, malaria, high blood pressure, epilepsy and high fever. The leaves/whole plants when boiled was employed in the treatment of malaria and body weakness while when squeezed in water and sieved, the species was usually used to challenge high blood pressure, gonorrhea, cancer and tumor. The concoction of extracts of *Andrographis paniculata* and fruit juice of *Citrus aurantifolia* administered orally, was said to be very potent against malaria, gastric disorder, hypertension, gonorrhea and diabetes can be further verified and tested on animal models for confirmation and safety tests. These local uses of *Andrographis paniculata* are comparable to wider usage of the species in China, Indian, Pakistan and Africa (Yao *et al.*, 1992; Akbar, 2011; Yabesh *et al.*, 2014; Okhuarobo *et al.*, 2014). In traditional Chinese medicine *Andrographis paniculata* is considered active against cold and purge the body of heat and fever and to dispel toxins from the body (Okhuarobo *et al.*, 2014). The higher fidelity level of use value of 95% recorded on the use of *Andrographis paniculata* for the treatment of malaria indicates that the species was preferred and utilized more than the other two species in the treatment of malaria. This also suggests that future studies can target *Andrographis paniculata* as potential/possible source of malaria therapy. The preference of ranking on the treatment of ailments also revealed the medicinal potency and

effectiveness of *Andrographis paniculata* particularly in the treatment of cancer infections and therefore preferred by many respondents to *Asystasia vogeliana* and *Moringa oleifera* in the prevention and treatment of cancer infections.

The leaf extracts and powdered form of the whole leaf preparations of *Moringa oleifera* are commonly used to treat and cure malaria, stomachaches/pains, jaundice, hypertension/blood pressure, common cold, diabetes, gonorrhea, epilepsy, cancer and anemia. The recorded medicinal uses of *Moringa oleifera* to treat diseases are not quite different from several local medicinal uses already reported by several authors (Thurber and Fahey, 2009; Awodele *et al.*, 2012; Popoola and Obembe, 2013; Adedapo *et al.*, 2015; Zvinorova *et al.*, 2015). *Moringa oleifera* has a remarkable range of medicinal uses, high nutritional contents, phytochemical and probably postulated to have the highest antioxidant in food (Ali *et al.*, 2016).

With respect to the local uses of *Asystasia vogeliana*, the leaf and whole plant are usually squeezed in water either singly or boiled with the leaves of *Cassia alata*, *Cymbopogon citratus* and fruit juice of *Citrus aurantifolia*. These preparations administered orally are commonly engaged to challenge chronic fever, hypertension, gonorrhea, gastric disorder, anemia, cancer and tumor. Higher fidelity level of use values recorded for *Asystasia vogeliana* infusion in the treatment of diseases such as malaria and chronic fever (74.8%), gonorrhea (65%) and epilepsy (40%) indicate the potentials of the species to treat and prevent such ailments. Our result suggests the use of *Asystasia vogeliana* as a better alternative to *Moringa oleifera* and *Andrographis paniculata* on the treatment and prevention of chronic fever, gonorrhea and epilepsy diseases. This observation is crucial to future screening studies of the species which can be leveraged upon as first-hand information in the quest to finding cost effective treatment regime potent enough to cure malaria fever, gonorrhea and epilepsy in the area and Nigeria in general. To our knowledge, very little information is available on local knowledge of *Asystasia vogeliana* and as such it has not been reported from the area of this study. However, further studies are recommended in the area of chemical composition, phytochemical screening and conservation of the species.

It is remarkable to note that the regular users of these herbal concoctions and respondents claimed that they had never experience any form of contra-indications, poisoning or discomfort from the use of the three MMPs infusions/preparations. Herbal combinations in the form of concoctions have been reported to be more potent than single preparation in the treatment of diseases (Dike *et al.*,



2012; Popoola and Obembe, 2013; Zhai *et al.*, 2014). However, these claims need to be verified scientifically to ascertain the pharmacological and dosage effects as well as oral safety of the different herbal preparations in experimental animals.

Although, the results of the *in vitro* cytotoxicity of the three plant species on the two cell lines, human gastric cancer (BGC-823) and human cervical cancer (HeLa) did not reflect significant activities, the ethanolic extract of *Andrographis paniculata* exhibited moderate cytotoxicity activity with IC<sub>50</sub> values of 24.7 and 23.1 µg/mL, respectively. This indicates that *Andrographis paniculata* could be a potential source of therapeutic agent against cancer and tumor infections. This may not be unconnected to the recorded higher fidelity level of use in the treatment of cancer and tumor as this plant was observed to be commonly used among respondents of over 60 years of age. Recently andrographoline isolated from *Andrographis paniculata* possessing anticancer properties was reported to prevent breast cancer-induced osteoclastic bone loss through attenuated RANKL signaling (Zhai *et al.*, 2014; Wang *et al.*, 2015). The findings of Akbar (2011) also showed that the extracts from *Andrographis paniculata* exhibited modest *in vitro* activity against HIV 18, 23, 24 and 25 and therefore could be carefully screened for bioactive compounds responsible for its cytotoxicity activity. Previous studies have demonstrated the inhibitory and cytotoxicity activities of andrographolide against tumor specific angiogenesis and induction of apoptosis in animal and *in vitro* experiments using human cancer cells such as prostate, breast, cervical, colon, hepatoma, melanoma and lymphocytic leukemia (Yao *et al.*, 1992; Sheeja and Kuttan, 2007; Lim *et al.*, 2015). A new semisynthetic andrographolide derivative (SRS06) with improved anticancer potency and selectivity which inhibits nuclear factor-κB nuclear binding in the A549 non-small cell lung cancer cell line was reported by Lim *et al.* (2015).

Though, our findings on *in vitro* cytotoxicity of *Moringa oleifera* did not reveal any significant activity against the cell lines, studies have shown that crude and aqueous extract of *Moringa oleifera* possess antioxidants and anticancer agents which induce ROS (Nair and Varalakshmi, 2011; Tiloke *et al.*, 2013; Gopalakrishnan *et al.*, 2016; Wang *et al.*, 2016). Recent studies of Ali *et al.* (2016) on antiproliferative activities of *Moringa oleifera* extracts on two cancer cells (not BGC-823 and HeLa) but MCF7 (breast cancer cell line) and HepG2 (liver carcinoma cell line) showed that two-well characterized phytochemicals (Moringinine and quercetin) were actively responsible for the antitumor activity of the plant. Further research studies on *in vitro* cytotoxicity

of *M. oleifera* are needed in view of avalanche of reports on the potentials of *M. oleifera* as anti-cancer agent. In similarity to *Moringa oleifera* on *in vitro* cytotoxicity *Asystasia vogeliana* did not record significant cytotoxicity activity on the two cell lines which also reflected on the low fidelity level in the treatment of cancer and tumor infections in the communities sampled. However, based on higher fidelity level of use values of *Asystasia vogeliana* on some diseases, further research efforts are required in the area of phytochemical, pharmacological and *in vitro* cytotoxicity to ascertain the medicinal relevance of the species.

## CONCLUSION

Although, the three species are being used in the treatment of ailments in the Oke-Ogun area of Oyo state, the study shows that *Andrographis paniculata* and *Moringa oleifera* were preferred in the treatment of malaria, diabetes, high blood pressure and cancer infections. The combinations of *Asystasia vogeliana* leaves with the leaves of *Cassia alata*, lemon grass (*Cymbopogon citratus*) and fruit juice of *Citrus aurantifolia* are potent in the treatment of cancer and tumor, HIV infection, hypertension, gonorrhoea and anemia. Screening for their pharmacological properties, crude extracts, isolation and identification of bioactive principles will enhance development and production of new drugs to combat these dreaded diseases.

The practice of plant conservation for future use was observed to be poor and lacking in all the areas sampled for this study. Plants grown in home gardens were mainly for healing purposes by older generation who were interested in herbal medicine to treat diseases instead of using synthetic drugs. The study found out that many members of the communities particularly among the youth preferred to collect plant samples/parts from neighbors instead of cultivating the plants. Some of the respondents affirmed that many plants are fast disappearing from the area. The three MMPs used in this study are not well cultivated; *A. paniculata* and *A. vogeliana* are usually not available except in home gardens where regular water is being applied. Therefore, a conservation and management strategy needs to be encouraged via proper orientation on cultivation in view of the medicinal significance of the species.

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