

Preliminary Assessment of the Antiproliferative Potential of the Herbal Formula "Ekpo Aganwo"

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ABSTRACT

Background: Cancer is a major global public health problem and has metamorphosed to a leading cause of death globally. Latest predictions by the World Health Organization suggest that cancer death rate may escalate to about 15 million deaths by 2030. Though various anticancer agents have been discovered and are in use currently, they have not been able to tame the disease effectively. This has left the scientific community with the task of discovering/developing better agents with high efficacy and minimal adverse effect. There have been increased interests by both developed countries and the scientific community towards the importance of herbs as a fountainhead in the development of newer drugs, as many classes of drugs now in use contain models from natural product. Ekpo aganwo (EA) is an herbal formulation prepared from the stem bark of *Khaya senegalensis* and is its main constituent.

Aim: This study was design as a preliminary assessment of the antiproliferative potential of EA.

Materials and Method: The modified Ayinde *et al.*, bench top assay method was employed for the study.

Result: Methotrexate compared with control, caused a significant ($P < 0.05$) decrease in radicle length throughout the study. The percentage inhibition was however highest after 72 hours, with a rate of 86.67%. EA 10 mg/ml did not produce any significant decrease in radicle growth after 48 hours, but however showed a significant ($P < 0.05$) decrease in radicle growth after 72 hours of experiment. The percentage inhibition after 72 hours was 24.56%. EA doses of 20 - 100 mg/ml produced significant ($P < 0.05$) decrease in radicle growth throughout the experiment. The percentage inhibition of EA doses of 20 - 100 mg/ml was 28.07 - 59.65 % after 72 hours.

Conclusion: The results obtained have favoured the postulation of EA having an anticancer potential, and this have been supported by the significant antiproliferative activity it produced during the experiment.

Keywords: Ekpo aganwo, antiproliferative potential, assessment

INTRODUCTION

Cancer is a major global public health problem and has metamorphosed to a leading cause of death globally. The World Health Organization has reported that the recorded cancer death rate for the year 2004 was about 7.4 million, while that of the year 2008 was about 7.6 million. However, latest predictions by World

Health Organization also suggest that cancer death rate may escalate to about 15 million deaths by 2030. ^[1] Cancer evolves from series of molecular events that fundamentally changes cells normal property. The hallmarks of cancer however are uncontrollable and sustained proliferation, others being evasion of apoptosis, angiogenesis and metastasis. ^[2] Though various

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anticancer agents have been discovered and are in use currently, they have not been able to tame the disease effectively. Most of these agents are known to elicit dangerous adverse effects, expensive and are not even readily available in most rural areas. This has left the scientific community with the task of discovering/developing better agents with high efficacy and minimal adverse effect. However, history have shown that herbal medicine have played a major role in the development of modern medicine. Herbal medicine has evolved to a common and widely used therapy for various ailments by a large proportion of the world's population.^[3] It has been stated by the World Health Organisation (WHO) that about 80% of developing countries population and 75% of the world population solely employ traditional medicines in the treatment/management of health issues.^[4]

There have been increased interests by both developed countries and the scientific community towards the importance of herbs as a fountainhead in the development of newer drugs, as many classes of drugs now in use contain models from natural product.^[5] There have been claims by herbal practitioners of successful use of herbal preparations in the treatment of certain ailments, but these are with minute scientific evidences.^[6] Therefore, the scientific assessment of such herbal preparations with the aim of affirming their folkloric usage should be an issue of top priority to the scientific community.

Ekpo aganwo (EA) is an herbal formulation prepared from the stem bark of *Khaya senegalensis* which is its main constituent. EA is used traditionally in the treatment of several ailments such as hypertension, diarrhea, stomach upset, back pain and fever. While there is also a belief that this formula can also be a useful anticancer agent. In the light of this, it is therefore important to screen this formula scientifically so as to ascertain if it is having an anticancer property. This study therefore, was

design as a preliminary assessment of the antiproliferative potential of EA, using bench top assay method, involving the antiproliferative effect of EA on guinea corn (*Sorghum bicolor*) seed radicle.

MATERIALS AND METHODS

Material

Methotrexate injection (Korea United Pharm. Inc) was purchased from a pharmacy outlet in Jos. The herbal formula "ekpo aganwo" was supplied by an herbal practitioner "Iya Abdul" who is based and practices in Jos, Nigeria. The herbal formula was supplied in powdered form and was reconstituted with distilled water before use.

Experimental Plant (*Sorghum bicolor*)

The experimental plant guinea corn (*Sorghum bicolor*) was purchased from Angwan-rukuba market, Jos. A viability test was carried-out on the seeds. This was done by pouring them into a bowl of water and observed for floating. The floating ones were discarded, while the submerged ones were dried and cleansed with absolute alcohol for usage.

Antiproliferative Evaluation

The modified Ayinde *et al.*,^[7] bench top assay method was adopted for this study. Methotrexate was prepared to a concentration of 0.05 mg/ml. The powdered herbal formulation was reconstituted with distilled water and different concentrations (10, 20, 40, 60, 80 and 100 mg/ml) of the herbal formulation were prepared. Eight (8) sterile petri-dishes were layered with cotton-wool and filter paper (Whatman No. 1). Twenty (20) seeds of guinea corn (*Sorghum bicolor*) were placed into each layered petri-dish. The control seeds were treated with 10 ml of distilled water, while the methotrexate group was treated with 10 ml of the prepared methotrexate solution. The test group seeds were treated with different concentrations of

EA. The first group of test seeds was treated with 10 ml of 10 mg/ml concentration, the second group of test seeds was treated with 10 ml of 20 mg/ml concentration and the third group was treated with 10 ml of 40 mg/ml. The fourth was treated with 10 ml of 60 mg/ml and another group of test seeds was treated with 10

ml of 80 mg/ml concentration. The last group was treated with 10 ml of 100 mg/ml. All seeds in the various groups were incubated in a dark room and observed for growth after 24 hours. The mean length (mm) of the radicles emerging from the seeds in each group were however taken after 48 and 72 hours respectively.

The percentage growth was calculated using the formula:

$$\% \text{ Growth} = \frac{\text{mean radicle length treated}}{\text{mean radicle length control}} \times 100$$

While the percentage inhibition was calculated using the formula:

$$\% \text{ Inhibition} = 100 - \% \text{ Growth}$$

Statistical analysis

Two way ANOVA (Analysis of Variance) and Bonferroni post-test were used to test for significance. $P < 0.05$ were considered significant.

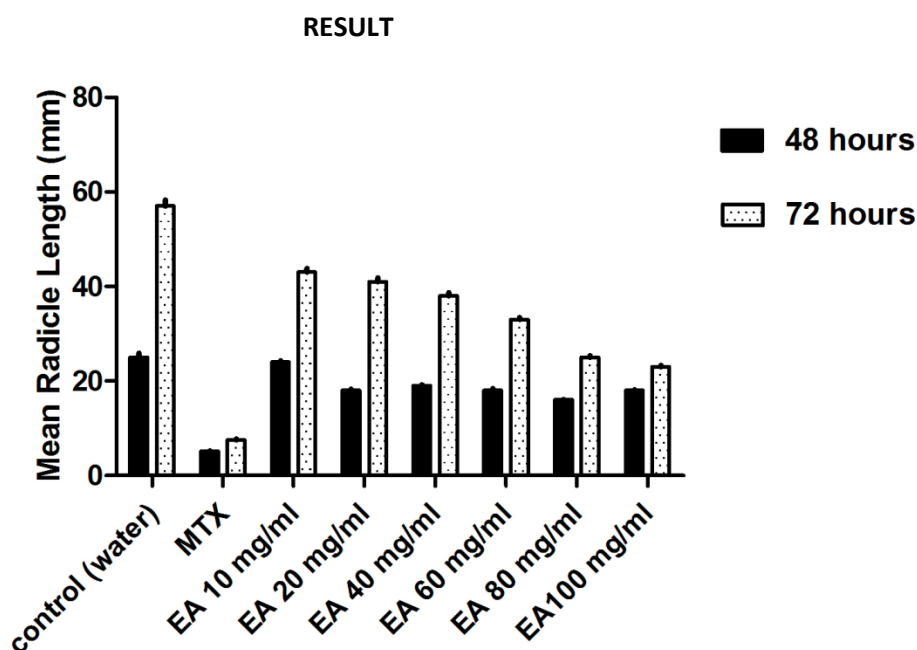


fig 1: Effect of the herbal formula "ekpo aganwo" on the growth of guinea corn (*Sorghum bicolor*) radicle. Values are mean \pm S.E.M. MTX=Methotrexate, EA=ekpo aganwo

Table 1: Inhibitory effect of the herbal formula “ekpoaganwo” on the percentage growth of guinea corn (*Sorghum bicolor*) seeds radicle

Treatment	Percentage Growth (%)		Percentage Inhibition (%)	
	48	72	48	72
Control (Water)	100	100	0.00	0.00
MTX	20.80	13.33	79.20*	86.67*
EA 10 mg/ml	96.00	75.44	4.00	24.56*
EA 20 mg/ml	72.00	71.93	28.00*	28.07*
EA 40 mg/ml	76.00	66.67	24.00*	33.33*
EA 60 mg/ml	72.00	57.90	28.00*	42.10*
EA 80 mg/ml	64.00	43.86	36.00*	56.14*
EA 100 mg/ml	72.00	40.35	28.00*	59.65*

* = $P < 0.05$, MTX= Methotrexate, EA=ekpo aganwo

DISCUSSION

The search for new anticancer agents that are more effective and elicit less toxic effects has kindled great research interest in herbal medicine. This is because herbal medicines have grown to become the bed-rock of modern medicine development.^[8] Herbal medicine have longed been employed in the treatment of various ailments, especially in developing countries and even developed countries. This have been accompanied with some advantages which includes easy accessibility, less toxicity and even cheaper.^[9] There have been increased claims of success in the use of herbal medicine. However, this has been greeted with increased scientific investigation on these herbs, so as to validate these claims. Scientific screening of herbs for their likely anticancer effects require a lot of materials as well as efforts and time. Even in most times, the results obtained are not really promising and doesn't in any way reflect the said claims. These challenges have led to the establishment of various bench top procedures which have been reportedly employed by researchers as pointers of potentially promising anticancer herbs. In the same vain, McLaughlin *et al.*,^[10] David *et al.*,^[11] Shogbaiké *et al.*,^[12] and Ayinde *et al.*,^[13] have used germinating seeds radicles as parameters in preliminary screening of suspected potential

anticancer agents. This method has terrific advantages which includes reproducibility of result, simplicity of process, rapid, time and cost effective. It can be a very useful alternative in laboratories where suitable human cell lines are not readily available. It is also a priceless method that can be used to screen and eliminate several herbs that may be claimed to be anticancers, which however doesn't possess such property. Cancerous cells are known to show rapid proliferation and this is experienced also in meristematic cells of seeds (including *Sorghum bicolor*) under favourable conditions. This shows why this method was employed in this study.

The experimental result obtained from the study shows a high unhindered proliferation of the control seeds radicle throughout the 72 hours of study. This affirms the ability of *Sorghum bicolor* seeds to proliferate rapidly under favourable conditions. Methotrexate compared with control as shown in Fig. 1, caused a significant ($P < 0.05$) decrease in radicle length throughout the study. The percentage inhibition was however highest after 72 hours, with a rate of 86.67%. This justifies it's current use as an anticancer agent. EA 10 mg/ml did not produce any significant decrease in radicle growth after 48 hours, but however showed a significant ($P < 0.05$) decrease in radicle growth

after 72 hours of experiment. The percentage inhibition after 72 hours was 24.56%. EA doses of 20 - 100 mg/ml produced significant ($P < 0.05$) decrease in radicle growth throughout the experiment. The percentage inhibition of EA doses of 20 - 100 mg/ml as shown in Table 1, was 28.07 - 59.65 % after 72 hours. Its effect was however dose dependent, as higher doses gave better effects (i.e effect produced by EA increased with dose increase). This result shows a remarkable antiproliferative activity by the herbal formula "ekpo aganwo, thereby

favouring the postulation of it having an anticancer potential.

CONCLUSION

The results obtained have favoured the postulation of EA having an anticancer potential, and this have been supported by the significant antiproliferative activity it produced during the experiment. The authors are therefore considering furthering to another stage of this study on animal models and human cell lines.

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