Comparative Antiproliferative Activity of Leaf and Stem Bark Extracts of *Detarium senegalense* and Leaf of *Cymbopogon citratus*

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Abstract: Malignant cancer cells exhibit uncontrollable high proliferation similarly to meristematic cells of seeds, this led to the establishment that agents capable of producing antiproliferative effects are potential anticancer agents. The experimental plant used for this research was *Sorghum bicolor* seeds. The sole aim of this research work is to unveil the antiproliferative potential of *Detarium senegalense* and to evaluate the antiproliferative activity of leaf extract of *Cymbopogon citratus* on radicle length of *Sorghum bicolor* seeds. The three extracts were prepared at concentrations of 10, 20, 40, 60, 80 and 100 mg/cm³ as well as methotrexate (reference standard) at concentration of 0.05 mg/cm³. The growth lengths were measured at 24, 48 and 72 hours of the experiment and expressed as percentage inhibition and percentage growth. The extracts produced considerable amount of antiproliferative effect on the radicle length of the seeds. The antiproliferative activity of the three extracts were concentration (dose) dependent, as the concentration of the extracts of leaves of *D. senegalense* increases, the percentage inhibition also increases, with a percentage of 89.47 % at an optimum concentration of 100 mg/cm³. Similarly, *D. senegalense* stem bark and *C. citratus* leaves showed percentage inhibition which corresponds to 73.68 % at optimum concentration of 100 mg/cm³ and 86.84 % at an optimum concentration of 80 mg/cm³ after the 72 hours of the incubation period. This research work however, has unveiled the use of *D. senegalense* as potential therapeutics for cancer treatment especially in developing countries, and has added a new-found knowledge to science.

Keywords: Anticancer Agent, Antiproliferative Activity, *Sorghum bicolor*, Phytochemicals

1. 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 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 the normal property of cells. Malignant cancer cells exhibit uncontrollable high proliferation, this led to the establishment that agents capable of producing antiproliferative effects are potential anticancer agents. Though various anticancer agents have been discovered and are in use currently, cancer treatment and management is a

challenge due to complexity of the disease, toxicity of chemotherapy, unaffordability of treatment and adverse side effects [2].

This has left the scientific community with the task of discovering and developing better agents with high efficacy and minimal adverse effect. History have shown that herbal medicine has 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].

Detarium senegalense, J. F. Gmelin, is a native of tropical Africa, found close to river bank. It is planted as fruit tree and as ornamental shade tree. It is commonly known as 'Taura' in Hausa, it is called 'Ofo' in Igbo and it is known as 'Ogbogbo' in Yoruba. The leaves are eaten as vegetable and are used traditionally as wash for itch, enema for dysentery and eye wash for conjunctivitis. A bark decoction is given to women at childbirth to expel the placenta. It is macerated in palm in Senegal for bronchitis, pneumonia and all internal complaints. The fruit is globular and slightly flattened occur in two forms, one commonly known as 'Ofo' is popularly eaten and used as a soup thickener in Eastern Nigeria [4]. The stem bark, seeds, leaves, and root of *Detarium senegalense* are widely used in herbal medicine in Nigeria [5-6].

The roots are part of a medicomegical treatment for mental conditions and for protection against evil spirits [7]. An anthocyanidin alkaloid (2-methoxyamine-3, 4, 5, 7-tetrahydroxy anthocynadine) with antibacterial activity has been isolated from the stem bark of the plant [8]. In veterinary ethnomedicine, the leaves and roots are used to treat diarrhea in cattle.

Cymbopogon citratus commonly called lemon grass is an aromatic perennial grass that belongs to the family poaceae. The name of lemon grass is derived from its lemon-like odour of essential oil present in the shoot [9]. *Cymbopogon citratus* (*C. citratus*) flourishes in sunny, warm, humid conditions of tropics. Lemon grass flourishes in a wide variety of soil type ranging from the rich loamy soil to poor laterite soil. Calcareous and water-logged soils are unsuitable for its cultivation [9]. *C. citratus* is used traditionally in different parts of the world to treat varying degree of illness. This plant is used in different parts of the world to treat broad spectrum of illnesses like Rheumatism and Other joint pains [2].

It is used as traditional folk medicine in the treatment of nervous condition, gastrointestinal disturbances, fever and hypertension. Lemon grass is also a folk remedy for coughs, elephantiasis, flu, gingivitis, headache, leprosy, malaria, ophthalmia, pneumonia and vascular disorders. It is principally taken in the form of tea as a remedy for digestive problems, diarrhoea and stomach ache. As a medicinal plant, lemon grass has been considered a carminative and insect repellent [10-12]. Studies on extracts from *C. citratus* leaves have demonstrated anti-inflammatory, vasorelaxing, diuretic and valuable remedy in treating ringworm as local application [11, 13]. Lemongrass oil was claimed to have antihelmintic activity [11]. In traditional medicine, lemon grass is usually prepared from the fresh herbs in the form of

infusions and decoctions also, the dried leaves of lemon grass could be given in form of tea [14]. Lemon grass contains mainly Citral [15]. and 1 to 2 % essential oil on a dry basis [16-17]. Essential oil and citral of lemongrass were detected to gather at parenchyma tissue cells, specifically in the adaxial surface of leaf mesophyll [18]. Citral of lemon grass is a natural combination of two isomeric aldehydes, namely isomers geranial (α -citral) and neral (β -citral) [19]. Other unusual active components are limonene, citronellal, β -myrcene and geraniol [15, 17]. Plants with antimalarial, anthelmintic and anti-inflammatory properties have been of immense ethnomedicinal use to mankind. In view of the widespread use of herbal products, important technical aspects such as standardization and quality control will be of immense benefit in order to enhance their efficacy and improve patients' compliance [20-22].

2. Methods

2.1. Collection and Authentication of Plant Material

The plant samples for study were collected from Isana village, Magama Local Government Area of Niger state, Nigeria in April, 2017 by Mr. Bitrus Imirsa. The plants were identified by the Taxonomist of Biological Sciences Department, Usmanu Danfodiyo University Sokoto in person of Abdulazeez Saihu (Herbarium Officer) with a voucher specimen number deposited at the Herbarium for each of the Samples.

The leaves of *D. senegalense* and *C. citratus* were cut off from the stalk, were washed to remove sand or dust, and the samples were shade dried at room temperature for two weeks. And then the three (3) samples were pulverized into powder using mortar and pestle.

2.2. Extraction of Plant Material

200 g of powdered plant materials were macerated in separating funnel with 600 cm³ of methanol for 24 hours at room temperature with occasional shaking, at the end, the extracts were then filtered with filter paper. The extracts were concentrated using water bath at a temperature of 60°C to obtain a solvent free extract which was later stored in the refrigerator at 4°C for future use.

2.3. Preliminary Phytochemical Studies

The Phytochemical screening procedure was carried out on the methanol extracts using standard procedures [23-26] as outlined below.

2.3.1. Test for Carbohydrate

Molisch Test;

To 2 cm³ of the extract in a test tube, few drops of molisch reagent and sulphuric acid was added and the production of violet colour indicates a positive test and was recorded.

2.3.2. Test for Tannins

Ferric Chloride Test;

0.5 cm³ of the extracts were dissolved in 10 cm³ of water

each and filtered. Few drops of 10 % ferric chloride were added to the filtrate and the color reaction was observed for brownish green, blue green or blue black coloration indicating the presence of tannins and the result was recorded.

Lead Acetate Test;

3 drops of 1 % Lead Acetate solution was added to the extract solution and the reaction was observed and recorded. A yellowish precipitate indicates the presence of tannins.

2.3.3. Test for Saponins

Frothing Test;

About 2 cm³ of the extract was dissolved in 10 cm³ of water and shaking vigorously for 30 seconds and allow to stand for 30 minutes. The test is based on the production of persistent foam, indicating positive test for Saponins. The reaction was observed and recorded.

2.3.4. Test for Sterols

Salkowski Test;

2 cm³ chloroform and few drops of concentrated sulphuric acid were added to about 2 cm³ of the extracts and the reaction was observed and recorded. Appearance of golden yellow color interface indicates the presence of steroid.

2.3.5. Test for Triterpenes

Liebermann-Burchard Test;

1 cm³ of acetic anhydride was added to 1 cm³ of the extracts. Few drops of concentrated sulphuric acid were carefully added to the solution above and the reaction was observed and recorded. Red color in the lower layer indicating the presence of triterpenoids.

2.3.6. Test for Anthraquinones

Bontragers Test;

2 cm³ of the extract to 10 cm³ of benzene and shaken. This was then filtered and 5 cm³ of 10 % ammonia solution was added to the filtrate and the reaction was observed and recorded. The presence of a pink or cherry red colour in the ammoniacal layer indicates the presence of free anthraquinones.

2.3.7. Test for Flavonoids

Shinoda Test;

About 0.5 g of the extract was dissolved in 2 cm³ of 50 % methanol. Few drops of Magnesium filings and 3 drops of concentrated hydrochloric acid were added and the reaction was observed and recorded. A pink-tomato red color indicated the presence of flavonoids.

Sodium Hydroxide Test;

Few drops of 10 % sodium hydroxide were added to 5 cm³ of the extracts and the reaction was observed and recorded. Formation of an intense yellow colour which becomes colourless on addition of few drops of dilute acid indicates the presence of flavonoids.

2.3.8. Test for Alkaloids

Mayer's reagent, Wagner's reagent, and Drangendoff's reagent were added to the different test tubes containing the

extracts solution and each of the reaction was observed and recorded. A creamish precipitate/brownish-red precipitate/orange precipitate indicates the presence of respective alkaloids. The formation of a precipitate in at least two of the test reagents is indicative of the presence of alkaloids.

2.4. Experimental Material

Sorghum bicolor also called Guinea Corn is the experimental plant. It was obtained from Sokoto Central Market.

Methotrexate injection was purchased from Passmark Pharmacy outlet in Sokoto.

2.5. Seed Viability Test

Seed Viability test was conducted on the seed by soaking it in water in a beaker for 5 minutes. The seeds that floats were considered to be non-viable and therefore, separated from those that have remained submerged in water. This test was to ensure that only viable seeds were selected and used for the assay. The viable seeds were further sterilized by soaking in methylated spirit for two minutes and washed immediately with distilled water five times to become prepared seeds and dried for use [27].

2.6. Determination of Growth Inhibitory Effect of Methanol Extracts on Sorghum Bicolor Seed Radicles Length

The modified bench top assay method was adopted for this research study [28].

Methotrexate was prepared to a concentration of 0.05 mg/cm³.

The extracts were prepared into different concentration of 10, 20, 40, 60, 80 and 100 mg/cm³ of the three different plants extracts. Eight (8) sterile petri-dishes were layered with cotton-wool and filter paper (Whatman No. 1). Twenty (20) viable seeds of *Sorghum bicolor* were placed into each layered petri-dish. The control seeds were treated with 10 cm³ of distilled water containing no extract, while the methotrexate group was treated with 10 cm³ of the prepared methotrexate solution. The test group seeds were treated with different concentrations of each of the plants extracts. The first group of test seeds was treated with 10 cm³ of 10 mg/cm³ concentration, the second group of test seeds was treated with 10 cm³ of 20 mg/cm³ concentration and the third group was treated with 10 cm³ of 40 mg/cm³. The fourth was treated with 10 cm³ of 60 mg/cm³ and another group of test seeds was treated with 10 cm³ of 80 mg/cm³ concentration. The last group was treated with 10 cm³ of 100 mg/cm³. 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 radicle emerging from the seeds in each group was taken after 24, 48 and 72 hours respectively.

The number of germinated seed were counted and expressed as % germination and % inhibition.

The percentage germination was calculated using the formula:

$$\% \text{ Germination} = \frac{\text{number of germinated seed}}{\text{Total number of seeds used}} \times 100\% \quad (1)$$

The percentage inhibition was calculated using the formula:

$$\% \text{ Inhibition} = 100 - \% \text{ germination} \quad (2)$$

2.7. Statistical Analysis

All data were expressed as mean $\pm$ SEM and one way Analysis of Variance (ANOVA) statistical test using 'Data Analysis Add-ins' in Excel 2016 Version to test the significance. $P < 0.01$ was considered Significance.

3. Result

3.1. Phytochemical Screening

The results of the phytochemical screening of the leaves and stem bark extracts of *Detarium senegalense* and leaves of *Cymbopogon citratus* extracts revealed the presence of the following secondary metabolites and the respective antiproliferative activities of the three (3) samples as shown in the Table 1 below.

Table 1. Result of Phytochemical Screening of MeOH extracts of *D. senegalense* (leaves and stem-bark) and *C. citratus* leaves.

Test	Inference		
	<i>C. citratus</i> (leaves)	<i>D. senegalense</i> (leaves)	<i>D. senegalense</i> (stem-bark)
Test for Carbohydrate:			
Molisch test	+	+	–
Test for Tannins:			
Ferric chloride test	+	+	+
Lead Sub-acetate test	+	+	+
Test for Saponins:			
Frothing test	+	+	+
Test for sterols:			
Salkowski test	+	+	+
Test for triterpenes:			
Lieberman-burchard test	+	+	+
Test for Anthraquinones:			
Bontragers test	–	+	+
Test for Flavonoids:			
Shinoda test	+	+	+
Sodium Hydroxide test	+	+	+
Test for Alkaloids:			
Dragendoff's reagent test	+	+	+
Wagner's reagent test	+	+	+
Mayer's reagent test	+	+	+

Key: (+) indicates the presence of secondary metabolite

(-) indicates the absence of secondary metabolite

3.2. Results of Mean Radicle Length, Percentage Growth and Percentage Inhibition of *Sorghum Bicolor*

The mean radicle length of *Sorghum bicolor* seeds was calculated after 24 hours, 48 hours and 72 hours of the incubation period and their percentage growth and percentage inhibition were also calculated for the three extracts as shown appropriately in the Tables 2, 3, 4, 5, 6 and 7 below.

Table 2. Mean Radicle Growth Length of *Sorghum bicolor* in MeOH extract of *D. senegalense* Leaves.

S/No.	Concentration (mg/cm ³)	Mean Radicle Length (mm)		
		24hrs	48hrs	72hrs
1	Water (control)	5.00 $\pm$ 0.17	12.50 $\pm$ 0.17	38.00 $\pm$ 0.64
2	MTX (0.05 mg/cm ³)	0.60 $\pm$ 0.01	1.80 $\pm$ 0.02	2.10 $\pm$ 0.03
3	10	0.80 $\pm$ 0.01	7.30 $\pm$ 0.11	15.00 $\pm$ 0.15
4	20	0.80 $\pm$ 0.01	2.30 $\pm$ 0.10	10.00 $\pm$ 0.10
5	40	0.90 $\pm$ 0.01	2.30 $\pm$ 0.01	5.30 $\pm$ 0.07
6	60	0.70 $\pm$ 0.01	2.30 $\pm$ 0.04	4.10 $\pm$ 0.02
7	80	0.50 $\pm$ 0.00	2.30 $\pm$ 0.03	4.80 $\pm$ 0.08
8	100	0.50 $\pm$ 0.00	1.40 $\pm$ 0.02	4.00 $\pm$ 0.07

Values expressed as Mean $\pm$ SEM, MTX = Methotrexate, MeOH = Methanol

Table 3. Anti-proliferative effect of MeOH extract of *D. senegalense* leaves on the growth length of *Sorghum bicolor* seeds.

S/No.	Concentration (mg/cm ³)	Percentage Inhibition (%)			Percentage Growth (%)		
		24hrs	48hrs	72hrs	24hrs	48hrs	72hrs
1	Water (control)	0.00	0.00	0.00	100.00	100.00	100.00
2	MTX (0.05 mg/cm ³)	88.00	88.00	95.66	12.00	12.00	4.34
3	10	84.00	41.60	60.53	16.00	58.40	39.47
4	20	84.00	81.60	73.68	16.00	18.40	26.32

S/No.	Concentration (mg/cm ³)	Percentage Inhibition (%)			Percentage Growth (%)		
		24hrs	48hrs	72hrs	24hrs	48hrs	72hrs
5	40	82.00	81.60	86.05	18.00	18.40	13.95
6	60	86.00	81.60	89.21	14.00	18.40	10.79
7	80	90.00	81.60	87.37	10.00	18.40	12.63
8	100	90.00	88.80	89.47	10.00	11.20	10.53

Figure 1. The growth inhibitory effects of the MeOH extract of *D. senegalense* leaves on the growth length of *Sorghum bicolor* radicle length. Values expressed as Mean $\pm$ SEM, MTX = Methotrexate.

Table 4. Mean Radicle Growth Length of *Sorghum bicolor* in MeOH extract of *D. senegalense* stem bark.

S/No.	Concentration (mg/cm ³)	Mean Radicle Length (mm)		
		24hrs	48hrs	72hrs
1	Water (control)	5.00 $\pm$ 0.17	12.50 $\pm$ 0.17	38.00 $\pm$ 0.64
2	MTX (0.05 mg/cm ³)	0.60 $\pm$ 0.01	1.50 $\pm$ 0.02	1.65 $\pm$ 0.03
3	10	0.90 $\pm$ 0.01	4.50 $\pm$ 0.14	20.00 $\pm$ 0.41
4	20	0.80 $\pm$ 0.01	5.00 $\pm$ 0.07	11.70 $\pm$ 0.16
5	40	1.60 $\pm$ 0.08	3.90 $\pm$ 0.07	11.80 $\pm$ 0.09
6	60	1.10 $\pm$ 0.01	3.50 $\pm$ 0.07	12.00 $\pm$ 0.08
7	80	0.90 $\pm$ 0.01	1.90 $\pm$ 0.02	10.70 $\pm$ 0.06
8	100	0.50 $\pm$ 0.00	2.90 $\pm$ 0.09	10.00 $\pm$ 0.07

Table 5. Anti-proliferative effect of MeOH extract of *D. senegalense* Stem bark on the growth length of *Sorghum bicolor* seeds.

S/No.	Concentration (mg/cm ³)	Percentage Inhibition (%)			Percentage Growth (%)		
		24hrs	48hrs	72hrs	24hrs	48hrs	72hrs
1	Water (control)	0.00	0.00	0.00	100.00	100.00	100.00
2	MTX (0.05 mg/cm ³)	88.00	88.00	95.66	12.00	12.00	4.34
3	10	82.00	64.00	47.37	18.00	36.00	52.63
4	20	84.00	60.00	69.21	16.00	40.00	30.79
5	40	68.00	68.80	68.95	32.00	31.20	31.05
6	60	78.00	72.00	68.42	22.00	28.00	31.58
7	80	82.00	84.80	71.84	18.00	15.20	28.16
8	100	90.00	76.80	73.68	10.00	23.20	26.32

Figure 2. The growth inhibitory effects of the MeOH extract of *D. senegalense* Stem bark on the growth length of *Sorghum bicolor* radicle length.

Table 6. Mean Radicle Growth Length of *Sorghum bicolor* in MeOH extract of *C. citratus*.

S/No	Concentration (mg/cm³)	Mean Radicle Length (mm)		
		24hrs	48hrs	72hrs
1	Water (control)	5.00 ± 0.00	12.50 ± 0.41	38.00 ± 0.27
2	MTX (0.05 mg/cm³)	0.60 ± 0.01	1.50 ± 0.03	1.65 ± 0.06
3	10	1.00 ± 0.00	2.40 ± 0.06	14.00 ± 0.11
4	20	0.90 ± 0.01	7.80 ± 0.17	15.50 ± 0.17
5	40	0.90 ± 0.01	5.50 ± 0.05	8.80 ± 0.05
6	60	0.60 ± 0.01	1.00 ± 0.00	13.00 ± 0.13
7	80	0.50 ± 0.00	1.00 ± 0.00	5.00 ± 0.10
8	100	0.50 ± 0.00	4.20 ± 0.22	8.80 ± 0.16

Table 7. Anti-proliferative effect of MeOH extract of *C. citratus* Leaves on the growth length of *Sorghum bicolor* seeds.

S/No	Concentration (mg/cm³)	Percentage Inhibition (%)			Percentage Growth (%)		
		24hrs	48hrs	72hrs	24hrs	48hrs	72hrs
1	Water (control)	0.00	0.00	0.00	100.00	100.00	100.00
2	MTX (0.05 mg/cm³)	88.00	88.00	95.66	12.00	12.00	4.34
3	10	80.00	80.80	63.16	20.00	19.20	36.84
4	20	82.00	37.60	59.21	18.00	62.40	40.79
5	40	82.00	56.00	76.84	18.00	44.00	23.16
6	60	88.00	92.00	65.79	12.00	8.00	34.21
7	80	90.00	92.00	86.84	10.00	8.00	13.16
8	100	90.00	66.40	76.84	10.00	33.60	23.16

Figure 3. The growth inhibitory effects of the MeOH extract of *C. citratus* leaves on the growth length of *Sorghum bicolor* radicle length.

4. Discussion

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. This method has terrific advantages which includes reproducibility of result, simplicity of process, rapid time and cost effective. This method may also be used to screen and eliminate herbs that has been claimed to have anticancer activities, which however does not have such property.

The 200 g of each powdered sample which include leaves and stem bark of *D. senegalense* and leaves of *C. citratus* were observed to have yielded 15.05 %, 5.85 % and 6.73 % (w/w) respectively. The leaves of *D. senegalense* was observed in this work to contain Alkaloids, Flavonoids, Saponins, Sterols and Triterpenes, Carbohydrate, Tannins and Anthraquinones, whereas only Carbohydrate was absent in the stem bark extracts of *D. senegalense* and only Anthraquinone was absent in the extract of *C. citratus* as seen in Table 1, which are likely to be some of the constituents that contributes to the plants uses in ethnomedicine. The activities of plant extract in effecting any

therapeutic or biological changes in ailing of animals suffering from diseases or living tissues are a direct function of the chemical constituents that are present in them.³ The experimental result obtained from the study shows a high unrestrained proliferation of the control seeds radicle throughout the 72 hours of study.

The lengths of the seed radicals increased with the incubation period of 24 to 72 hours depicting germination.

Methotrexate compared with control as shown in figures 1, 2 and 3 caused a significant ($P < 0.01$) decrease in radicle length throughout the study. The percentage inhibition was however highest after 72 hours, with a rate of 95.66 %. This justifies its current use as an anticancer agent. The inhibitory effect of the extracts was not consistent, but decreased with time. The antiproliferative activity of the three extracts were concentration (dose) dependent, as the concentration of the extracts of leaves of *D. senegalense* increases, the percentage inhibition also increases, with a percentage of 89.47 % at an optimum concentration of 100 mg/cm³. Similarly, *D. senegalense* stem bark and *C. citratus* leaves showed percentage inhibition which corresponds to 73.68 % at optimum concentration of 100 mg/cm³ and 86.84 % at an optimum concentration of 80 mg/cm³ after the 72 hours of

the incubation period.

The leaves of *D. senegalense* showed strong antiproliferative activity compared to the leaves of *C. citratus* which is closest to it and also have a significant antiproliferative activity followed by the stem bark of *D. senegalense*.

5. Conclusion

The antiproliferative activity of *C. citratus* has been documented in some literature

This research work however, has unveiled the use of *D. senegalense* as potential therapeutics for cancer treatment especially in developing countries, and has added a new-found knowledge to science.

Recommendations

The three samples have a remarkable antiproliferative activity, it is therefore, recommended that further research should be carried out using human and suitable animal model to ascertain this claim. Further studies could be channelled towards isolation and characterization of the active ingredient responsible for their antiproliferative activity.

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