

Evaluation of anti-proliferative activities of ethanolic extracts of *Sarcocephalus latifolius* fruit and *Paullina pinnata* leaf

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Abstract

Scientific investigations over the years have indicated herbal preparations to be effective against wide range of diseases with little or no side effects associated with their use. Herbal preparations have very special features that differentiate them from other chemicals. The present study was carried out to investigate the anti-proliferative activities of ethanolic extracts of *Sarcocephalus latifolius* fruit and *Paulina pinnata* leaf on radicle length of fast growing *Sorghum bicolor* seeds. The extracts were prepared at three concentrations of 1000, 2000 and 3000 µg/ml. The radicle lengths of seeds were measured at 24th and 48th hours of the experiment. The extracts produced significant anti-proliferative activity against the radicle length of the *Sorghum bicolor* seeds. The anti-proliferative activity of the extracts do not follow concentration dependent pattern. The extracts significantly slowed down the proliferation of the *Sorghum bicolor* at the applied concentrations. The anti-proliferative activities of the extracts were significant ($P < 0.001$) at the 48th hours of the experiment. The standard reference methotrexate produced significant ($P < 0.01$) anti-proliferative activity at the 24th and 48th hours compared to the extracts groups. The results of the experiment elucidate the anti-proliferative activity of ethanolic extracts of *Sarcocephalus latifolius* fruit and *Paullina pinnata* leaf.

Keywords: Anti-proliferative activity, *Sarcocephalus Latifolius*, *Paulina pinnata* *Sorghum bicolor*, methotrexate.

INTRODUCTION

Cancer has been ranked as one of the dreaded diseases sapping into the long term vitality of many nations of the world. The incidence and prevalence of cancer is on a steady increase. Presently, effort is geared toward the use herbal medicine in stemming the scourge of cancer disease. Scientific investigations over the years have indicated herbal preparations to be effective against wide range of diseases with little or no side effects associated with their use. Herbal preparations have very special features that differentiate them from chemical agents; a single plant may contain a wide variety of bioactive ingredients and a combination of plants even more [1].

Herbal medicine now form integral parts of drugs commonly used in different parts of the world, especially in developing countries. Herbal medicine comes in different forms that include extract, tablet, essential oil or ointment. About 28% of all modern drugs

in use today are derived directly from naturally occurring substances in plants [2]. Substances derived from plants remain the basis for large proportion of commercial medication. The widespread use of herbs may stem from their natural origin giving them reputation of being somehow safe and better rated than prescribed drugs. An estimated 80% of the world population depends on herbal medicine to cater for their primary health care [3]. Herbal medicine can help treat a varying disease conditions and may have fewer side effects than orthodox medications [4]. The present study was designed to investigate the anti-proliferative activity of ethanolic extracts *Sarcocephalus latifolius* fruit and *Paullina pannata* leaf on fast growing radicle length of *Sorghum bicolor* seeds. The choice of the use of *Sorghum bicolor* in this study was borne out of the fact that meristematic cells of seeds just like cancer cells have the tendency to proliferate when exposed to

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favourable environmental conditions. This explains the use of *Sorghum bicolor* seeds.

MATERIALS AND METHODS

Materials

Methotrexate injection (Korea United Pharm Inc.) was purchase from a local pharmacy store, *Sorghum bicolor* (guinea corn) seeds were obtain from a local market in Jos, Nigeria.

Viability Test

The *Sorghum bicolor* seeds were subjected to viability test by submerging in water and observed their ability to remain submerged. Those that remain submerged in water were selected and dried. The seeds were deaned with absolute alcohol and allowed to dry before use.

Plant Material

The plant materials were obtained from Makurdi, Benue State, Nigeria. The plants were identified and authenticated by Ikehukwu Chijioke of Federal College of Forestry Jos. The collected fruits were sliced, washed and air-dried at 25°C for two weeks, then crushed into coarse powder.

Extraction of Plant Materials

40 g of powdered fruit and leaf materials were measured separately and dissolved in sufficient quantity of ethanol for 48 hours with mechanical shaking (4h/day). At the end of 48 hours, the mixture was filtered with ashless filter paper. The extract was concentrated using rotary evaporator at a temperature of 4°C. The concentrate was heated over a water bath to obtain a solvent free extract which was later stored in the refrigerator at 4°C.

Phytochemical Screening

Phytochemical screening of the ethanolic extracts of *Sarcocephalus latifolius* fruit and *Paulina pinnata* leaf was carried out using standard procedure[5].

Anti-proliferative Model

Anti-proliferative activity of the plant extracts were investigated using *Sorghum bicolor* seeds [6]. The extracts were prepared at concentrations of 1000, 2000 and 3000 µg/ml, as well as methotrexate at concentration of 50 and 100 µg/ml respectively. Twenty (20) viable seeds were spread on each plate laid with cotton wool and filter paper (Whatman No. 1). 5 ml of each extract at different concentrations were poured into 9 cm wide Petri dishes. The control seeds were treated with distilled water containing no extract. The radicle lengths of the fast growing seeds were measured at 24 and 48 hours. The experiments were carried out in triplicates.

Statistical analysis

Data obtained from the results were expressed as Mean ± SEM. Statistical significance was determined by two way ANOVA followed by Bennefonni post-test and values of P<0.05 was considered significant. The analysis was performed using instant graph pad software (version 5.2).

RESULTS

Table 1. Phytochemical Screening.

Constituents	<i>Sarcocephalus latifolius</i>	<i>Paulina pinnata</i>
Saponins	+++	++
Flavonoids	++	++
Tannins	+	-
Alkaloids	:+	+++
Steroids	+	++
Carbohydrates	-	-
Cardiac glycosides	++	+

+Present, -Absent, ++Moderately present,

+++Abundantly present

Effect of *Sarcocephalus latifolius* fruit extract on radicle length of *Sorghum bicolor* seeds.

The fruit extract of *Sarcocephalus latifolius* does not exhibit a concentration dependent anti-proliferative activity against the radide lengths of *Sorghum bicolor* seeds. However significant (P<0.001) anti-proliferative

activity was observed at the 48th hours of the experiment.

Table 2. Anti-proliferative activity of ethanolic fruit extract of *Sarcocephalus latifolius* on radicle length of *Sorghum bicolor* seeds.

Concentration ($\mu\text{g/ml}$)	Mean Radicle Length (mm)	
	24 h	48 h
Control	9.13 \pm 0.62	27.53 \pm 2.38
Extract (1000 $\mu\text{g/ml}$)	6.67 \pm 1.06	11.33 \pm 1.64***
Extract (2000 $\mu\text{g/ml}$)	4.53 \pm 1.03	7.53 \pm 1.84***
Extract (3000 $\mu\text{g/ml}$)	4.80 \pm 1.28	10.20 \pm 2.47***
MTX (50 $\mu\text{g/ml}$)	2.60 \pm 0.42**	3.60 \pm 0.40***
MTX (100 $\mu\text{g/ml}$)	1.40 \pm 0.23***	3.00 \pm 0.35***

Values expressed as mean SEM, n=20, **($P<0.01$), ***($P<0.001$), MTX= Methotrexate

Effect of *Paullina pinnata* leaf extract on radicle length of *Sorghum bicolor* seeds.

The anti-proliferative activity of the extract does not follow a concentration dependent pattern. However, there were reductions in the radicle length in the extract treated groups compared to the control group. The extract produced significant ($P<0.001$) anti-proliferative effect on 48th hour of exposure.

Table 3. Anti-proliferative activity of *Paullina pinnata* leaf extract on radicle length of *Sorghum bicolor* seeds.

Concentration ($\mu\text{g/ml}$)	Mean Radicle Length (mm)	
	24 h	48 h
Control	9.13 \pm 0.62	27.53 \pm 2.38
Extract (1000 $\mu\text{g/ml}$)	4.69 \pm 1.13	13.92 \pm 2.38***
Extract (2000 $\mu\text{g/ml}$)	3.92 \pm 0.92	12.15 \pm 2.14***
Extract (3000 $\mu\text{g/ml}$)	4.54 \pm 1.23	13.69 \pm 1.62***
MTX (50 $\mu\text{g/ml}$)	2.60 \pm 0.42**	3.60 \pm 0.40***
MTX (100 $\mu\text{g/ml}$)	1.40 \pm 0.23***	3.00 \pm 0.35***

Values expressed as mean SEM, n=20, **($P<0.01$), *** $P<0.001$), MTX= Methotrexate.

DISCUSSION

The use of herbal formulations in the treatment of diseases have largely been supported by world health organization as the surest way of reaching local dwellers that cannot afford or access modern pharmaceutical products[3]. Herbal preparations over the years have

proven to be useful therapeutic agents in the treatment of different kind of diseases. Different bioactive ingredients found in them now form basis of conventional drugs [2]. The anti-proliferative screening of the plant extracts were carried out using *Sorghum bicolor* seeds. Previous studies have validated the use of *Sorghum bicolor* in the screening of plant extracts with anti-proliferative activity [7, 8]. The phytochemical screening of the extracts showed the presence of flavonoids, alkaloids, saponins, and anthraquinones. Previous studies have indicated these active principles as major cancer fighting agents present in medicinal plant extracts [9]. The extracts produced significant anti-proliferative activity against the radicle length of the *Sorghum bicolor* seeds. The ethanolic fruit extract of *Sarcocephalus latifolius* significantly slowed down the proliferation of the *Sorghum bicolor* seeds at the applied concentrations. The anti-proliferative activity of the extract was significant ($P<0.001$) at the 48th hours of the experiment. The standard reference produced significant ($P<0.01$) anti-proliferative activity at the 24th and 48th hour compared to the extracts groups. The anti-proliferative activity of *Paullina pinnata* leaf extract was similarly to that of the ethanolic fruit extract of *Sarcocephalus latifolius* extract. The anti-proliferative activity of the extract may be due to the interaction of the various active principles present in them. The extracts may as well interfere with various stages involved in the growth process of *Sorghum bicolor* seeds.

CONCLUSION

The results of the study showed the anti-proliferative activity of ethanolic extracts of *Sarcocephalus latifolius* fruit and *Paullina pinnata* leaf. Further investigations are needed to ascertain the mechanism and bioactive principles responsible for their anti-proliferative activity.

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