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ORIGINAL RESEARCH ARTICLE

Anti-Proliferative Potentials of Quail Eggs and Aqueous Root Extract of *Cymbopogon citratus* on Radicle Length of Fast Growing *Sorghum bicolor* Seeds

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ABSTRACT

Cancer is a multi-factorial disease comprises of over 100 different diseases that affect vast population of people in many countries worldwide. Presently, there is no ideal conventional treatment that can eliminate the whole cancerous cells. This is due to numerous pro-cancerous cells found in the human body. In this study, egg whites, egg yolks from quail egg and aqueous root extract of *Cymbopogon citratus* were examined for their possible anti-proliferative effect on radicle length of fast growing *Sorghum bicolor* seeds. Anti-proliferative effect was measured using *sorghum bicolor* seeds. The radicle lengths of the fast growing seeds were measured at 24th and 48th hour of the experiment and inhibition of growth was expressed as percentage inhibition. The extract caused significant ($P<0.001$) reduction in the radicle length at concentration of 250, 500 and 1000 g/ml at the 48th hour. 1000 µg/ml of the extract produced 64.44 and 72.31% inhibition at the 24th and 48th hour while 100 g/ml of reference drug methotrexate caused 67.33 and 85.12% inhibition. The egg white and yolks exhibited strong inhibitory effect on the radicle length of the germinating *Sorghum bicolor* seeds throughout the experiment. The egg yolks produced the highest percentage inhibition of 82.22 and 91.20 at the 24th and 48th hour of the experiment. The anti-proliferative effect of the egg yolks was far better than the egg whites and extract. This study implicates quail eggs and aqueous root extract of *Cymbopogon citratus* as potential anti-proliferative agents.

Key words: Anti-proliferative, *Cymbopogon citratus*, *Sorghum bicolor*, Methotrexate, Egg Whites, Egg Yolks, Quail egg.

INTRODUCTION

Cancer is a multi-factorial disease comprises of over 100 different diseases that affect vast population of people in many countries worldwide. Estimated 70% cases of cancer deaths in 2008 occurred in low and middle income countries of the world,[1] with 7.6 million deaths recorded worldwide in the same year.[2] Cancer is a term that describes a disease condition characterized by uncontrolled cellular proliferation and dedifferentiation,[3] of the body own cells. In biochemical terms, micro-organisms are both quantitatively and qualitatively different from human cells but cancer cells and normal cells are so similar in most respects that it is more difficult to find general, exploitable, biochemical, as well as explicit and clearly defined differences between them.[4] Cancer stem from series of molecular activities that hijack the normal signal that control cell division and growth.[5] Normal cells grow and divide in an orderly and well programmed pattern, but "mis-behaving" cancer cells disregard the normal rule of cell division,[5] within the internal body ecosystem. As these cells grow they develop new features such as uncontrollable proliferation, dedifferentiation and loss of function, invasiveness and metastases,[4] which distinguish them from the normal body cells. The prevailing causes of cancer are due to the combined effect of genetic changes and environmental factors,[5] and other carcinogenic agents like UV light, X-rays, viruses, tobacco products, pollutants, and other chemicals. The

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principal modalities of therapy used are cancer-surgical excision, irradiation and chemotherapy,[6] hormonal therapy and targeted therapies,[7] and the relative value of each of these approaches depends on the type of tumour and the stage of its development.[4] The use of chemotherapy in cancer treatment over the years has been greeted by unwanted side effects and toxicity not only to the tumour cells, but to the healthy tissue even at optimal dosages. This has prompted renewed interest in the search of potent active principles from medicinal plants.

Herbal medicine is generally accepted as a valuable alternative system of therapy in the form of pharmaceuticals, a trend recognized and advocated by the World Health Organization.[8] Plant preparations have very special features that distinguish them from chemical agents, a single plant may contain a wide variety of bioactive ingredients and a combination of plants even more.[9] *Cymbopogon citratus* commonly lemon called has been used in folkloric medicine to treat malaria, fever, stress and other diseases.

The use of quail eggs date back to thousands year ago by local Chinese medicinal practitioners in treatment of myriads of illnesses and diseases like, fever, cough, different type of skin infections,[10] respiratory infections, diabetes mellitus, stomach ulcer. The eggs are used as immune booster, memory enhancement, nervous system stabilizer,[11] stimulate appetite, nervous disorders. Quail eggs are exceptionally different from other eggs with high nutrients content three to four times greater than chicken eggs.[11] Quail eggs contain bio-stimulator, biologically active ingredients indispensible to human. The eggs are packed with rich source of anti-oxidants,[12] lot of vitamins including Vitamin A, B1, B2, B6, D, E, mineral salts, enzymes and amino acids. In this study, egg whites, egg yolks from quail egg and aqueous root extract of *Cymbopogon citratus* were examined for their possible anti-proliferative effect on radicle length of fast growing *Sorghum bicolor* seeds.

MATERIALS AND METHODS

Materials

Methotrexate injection (Korea United Pharm.Inc) and quail eggs were used in the study. They were purchased from local firm in Jos, Nigeria.

Experimental Plant (*Sorghum bicolor*)

Guinea corn (*Sorghum bicolor* seeds) was obtained from a local market in Jos, Nigeria. The 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 cleaned with absolute alcohol and allowed to dry before use.

Plant Material

Fresh *Cymbopogon citratus* were collected in March, 2012 from Jos, Plateau State, Nigeria. The plant material was identified by Mr. Ikechukwu Chijioke of Federal College of Forestry Jos. Voucher specimen of sample was deposited in the Herbarium Unit of the College. The roots were air dried at room temperature 25^0C for two weeks, then crushed into coarse powder using mortar and pestle.

Extraction of Plant Materials

Sixty grams of the powdered root material was weighed and dissolved in sufficient quantity of water for 48 hours with mechanical shaking (4h/day), at the end of 48 hours; the mixture was filtered with ash less filter paper. The extract was concentrated using rotary evaporator at a temperature of 40^0C.The concentrate was heated over a water bath to obtain a solvent free extract which was later stored in the refrigerator at 4^0C.

Phytochemical Screening

Phytochemical screening of the powdered crude plant samples were carried out using the standard established procedure.[13]

Separation of Egg Whites and Egg Yolks from Quail Eggs

Egg sample was weighed for whole, and separated for egg whites, and egg yolks, respectively. 10 ml of each of the separated samples were applied directly on the *Sorghum bicolor* seeds spread on each plate laid with cotton and kept in a dark room. The radicle length of *Sorghum bicolor* seeds were measured at 24^{th} and 48^{th}.

Anti-proliferative Assessment of the Extract

Modified procedure of Ayinde was employed in the study.[14] The extract was prepared at various concentrations of 250, 500 and 1000 µg/mg, 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 the extract at different concentrations were poured into 9 cm wide Petri dishes. The control seeds were treated with distilled water containing no extracts. The radicle lengths of the fast growing seeds were measured at 24 and 48 hours. The experiments were carried out in triplicates. The radicle lengths of the germinated *Sorghum bicolor*

seeds were recorded and percentage inhibition was calculated as mean radicle length control-mean radicle length treated×100 /mean radicle length control

Statistical analysis

The results of the study were expressed as mean ± SEM (standard error mean). Statistical significance was determined by one-way ANOVA followed by Bonferroni post-test and values of $P<0.05$ were considered significant. The analysis was performed using instant graphpad prism (version 5.02)

RESULTS

Table 1: Phytochemical screening of aqueous root extracts of *Cymbopogon citratus*

Phytochemical constituents	Root
Alkaloids	++
Carbohydrates	++
Tannins	+++
Flavonoid s	+++
Anthraquinones	-
Cardiac glycosides	-
Steroids	++
Saponins	++

- = absent, + = present, ++ moderately present, +++ abundantly present.

Anti-proliferative effect of aqueous root extract of *Cymbopogon citratus* and methotrexate on radicle length of *Sorghum bicolor*

Proliferation of the radicle length of *Sorghum bicolor* seeds were less in the extract treated and methotrexate group compared to control group. At 24 hours of the experiment, the mean radicle length of the control group measured 4.50±0.34 mm while that of the extract treated groups were 4.10±0.28, 3.50±0.31, 1.60±0.37, and methotrexate groups measured 2.93±0.44, 2.93±0.44 mm respectively. The anti-proliferative effect of the extract followed a concentration dependent pattern. At 48 hours, 250, 500, 1000 µg/ml of the extract and 50, 100 µg/ml of methotrexate produced 24.21, 34.21, 72.31, 80.45 and 85.12 as percentage inhibition. The extract and methotrexate caused significant reductions in the mean radicle length at the 24th hours of the experiment.

Table 2: Effect of aqueous root extract of *Cymbopogon citratus* and methotrexate on radicle length of *Sorghum bicolor*

Concentration (µg/ml)	Mean Radicle Length (mm)		Percentage Inhibition (%)	
	24 hours	48 hours	24 hours	48 hours
Control	4.50±0.34	24.20±2.21	0.00	0.00
Extract (250 µg/ml)	4.10±0.28	18.10±0.57***	8.89	24.21
Extract (500 µg/ml)	3.50±0.31	15.80±1.16***	22.22	34.71
Extract (1000 µg/ml)	1.60±0.37	6.70±0.91***	64.44	72.31
MTX (50 µg/ml)	2.93±0.44	4.73±0.43***	34.89	80.45
MTX (100 µg/ml)	1.47±0.24	3.60±0.49***	67.33	85.12

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

Anti-proliferative effect of egg whites from quail egg and methotrexate on radicle length of *Sorghum bicolor* seeds

The mean radicle length of egg whites at the 24 hours measured 1.80±0.48 mm, less compared to the control group. Similarly, there were reductions in the mean radicle length of the methotrexate treated groups, significant (P<0.001) at the 48 hours of the experiment with 80.45, 85.12 as percentage inhibition. The egg whites compete favourable with the methotrexate treated group

Table 3: Effect of egg whites and Methotrexate on radicle length of *Sorghum bicolor* seeds

Concentration (µg/ml)	Mean Radicle Length (mm)		Percentage Inhibition (%)	
	24 hours	48 hours	24 hours	48hours
Control	4.50±0.34	24.20±2.21	0.00	0.00
Egg Whites (5 ml)	1.80±0.48	4.60±0.97***	60.00	80.99
MTX (50 µg/ml)	2.93±0.44	4.73±0.43***	34.89	80.45
MTX (100 µg/ml)	1.47±0.24	3.60±0.49***	67.33	85.12

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

Anti-proliferative effect of egg yolks from quail egg and methotrexate on radicle length of *Sorghum bicolor* seeds

The mean radicle lengths of the egg whites group at the 24th and 48th were 0.80±0.22, 2.13±0.50 mm. These values were much less compared to the control and methotrexate groups. Egg yolk produced 82.22 and 91.20 percentage inhibition at the 24th and 48th. 50 µg/ml and 100 µg/ml of methotrexate at the 48 hours produced 80.45 and 85.12 as percentage inhibition.

Table 4: Effect of egg yolks and Methotrexate on radicle length of *Sorghum bicolor* seeds

Concentration (µg/ml)	Mean Radicle Length (mm)		Percentage Inhibition (%)	
	24 hours	48 hours	24 hours	48hours
Control	4.50±0.34	24.20±2.21	0.00	0.00
Egg Eggs (5 ml)	0.80±0.22*	2.13±0.50***	82.22	91.20
MTX (50 µg/ml)	2.93±0.44	4.73±0.43***	34.89	80.45
MTX (100 µg/ml)	1.47±0.24	3.60±0.49***	67.33	85.12

Values expressed as mean±SEM, n=20, * (P<0.05), ** (P<0.001), MTX= Methotrexate

DISCUSSION

The menace of cancer has become a serious global health burden, claiming millions of lives annually. Cancer present a great challenge due to the ways by which the diseases is symptomized in the affected individuals and also the resistance posed by the ailment to cancer therapy.[14] Presently, there is no ideal conventional treatment that can eliminate the whole cancerous cells.[15] This is due to numerous pro-cancerous cell found in the human body.[15] Nature strongly beacons on us to return to the ancient wisdom of traditional medicine. In a bit to address this problem, efforts are now directed toward the use of herbal medicine. Herbal preparations over the years have proven to be effective against wide range of diseases with little or no side effects. In this study, egg whites, egg yolks from quail egg and aqueous root extract of *Cymbopogon citratus* were examined for their possible anti-proliferative effect on fast growing radicle length of *Sorghum bicolor* seeds.

From the result of the study, percentage inhibitions of the extract groups were much higher compared to the control group. The extracts inhibited the growth of the radicle length of the *Sorghum bicolor* seeds at various concentration of the extract used in concentration dependent pattern. The inhibitory effect of the extract was not fairly constant but varies. The anti-proliferative effect of the extract was eminent at 24th hours of the experiment. The increase in percentage inhibition and reduction in radicle length of the extract treated groups clearly highlighting the anti-proliferative activity of the extract. The extract caused significant (P<0.001) reduction in the radicle length at various concentration of 250, 500 and 1000 µg/ml at the 48th hour (table 2). 1000 µg/ml of the extract produced 64.44 and 72.31% inhibition at the 24th and 48th hour while 100 µg/ml of reference drug methotrexate caused 67.33 and 85.12% inhibition, implicating the extract as a potential anti-proliferative agent. The anti-proliferative effect of the extract may be ascribed to the various bioactive principles present in the extract interacting with each other. Isolated bioactive ingredients of flavonoids and alkaloids from different plants have been reported to possess anti-proliferative activities.[16,17] Reduced cancer risks have been reported with the consumption of vegetables and fruits rich in flavonoids.[17,18] The proliferation of the radicle length was consistent in the control group throughout the study period. However, anti-proliferative effect of the extract decreased with time. Though the mechanism by which the extract exert it anti-proliferative effect cannot be ascertain but one may likely suggests that the extract mimic the action of methotrexate.

Quail eggs are highly packed with vitamins and minerals. The nutritional contents of quail eggs is much higher than those obtained from other eggs with rich sources of antioxidants, minerals, and vitamins, and give us a lot of nutrition than foods.[12] The egg white and yolks exhibited strong inhibitory effect on the radicle length of the fast growing *Sorghum bicolor* seeds throughout the experiment. The egg yolks produced the highest percentage inhibition of 82.22 and 91.20 at the 24th and 48th hour of the experiment. Study showed that good nutrients in quail eggs are concentrated in the egg yolks.[11] Egg yolk has been reported to be one of the few natural foods containing vitamin D.[19] This may explained the anti-proliferative effect of the egg yolks. The anti-proliferative effect of the egg yolks was far better than the egg whites and the extract. The increase in percentage inhibition in egg yolk treated group clearly showed it's potent anti-proliferative effect than the extract, methotrexate and egg white.

This study validates the anti-proliferative potentials of quail eggs, aqueous root extract of *Cymbopogon citratus*. Eggs yolks from quail eggs exhibited a better anti-proliferative effect than egg while, aqueous root extract of *Cymbopogon citratus* and the reference drug methotrexate. However, It would be worthwhile to further investigate the anticancer properties of the quail eggs and extracts using suitable animal models and human cells lines

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