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ANTIOXIDANT ACTIVITY AND CYTOTOXIC POTENTIALS OF ETHANOLIC EXTRACT OF *K. AFRICANA (LAM) BENTH*

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ABSTRACT

Plants are an excellent source of compounds with biological activity. Since plants have been used in traditional medicine for a long time and also some plant substances have shown cytotoxic, anticoagulant and antiplatelet activity, they have been extensively studied. The present study focusses on antioxidant activity and cytotoxic potentials of ethanolic extract from *K. africana*. The results showed at 1µg/mL concentration the aqueous fruit extracts of *K. africana* showed best result. In cytotoxic studies at 24 hours, 50µg/mL concentration the number of dividing cells was found to be 63 and mitotic index was found to be 118 and it showed the best result compared to other concentration.

KEYWORDS: *K. africana*, compounds, biological activity.

INTRODUCTION

Secondary metabolites of plant are commercially important and find use in a number of pharmaceutical compounds. The presence of these secondary metabolites in plants probably explains the various medicinal and antioxidant activities of these plants (Singh, 2012). Antioxidants help to prevent the free radical damage that is associated with cancer and heart disease. Natural antioxidants of the host such as catalase, superoxide dismutase, and glutathione peroxidase. Therefore, antioxidants prevent the activity of the free radicals and thereby prevent the damage to cells and tissues, providing protection to human and animal subjects, and also enhance healing of infected and non infected wounds (P. J. Houghton *et al*., 2005).

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals, leading to chain reactions that may damage cells. The term "antioxidant" is mainly used for two different groups of substances. Oxygen radicals and lipid peroxidation are thought to be involved in the ethanol induced gastric damage and antioxidant compounds can have gastro protective properties (Ineu *et al.*, 2008).Of particular pharmacological importance, *K. africana* has both in vitro and in vivo antioxidant properties.(Olaleye and Rocha, 2007).

Antioxidant vitamins or antioxidant dietary supplements do not improve health nor are they effective in preventing diseases. Antioxidant contents of medicinal plants may contribute to the protection they offer from diseases. Natural antioxidants either in the form of raw extracts or their chemical constituents are very effective to prevent the destructive processes caused be oxidative stress.

Cytotoxic activity can be easily monitored by calculating the mitotic intex and aberrations. This is done by *Allium cepa* root tip squash test using various concentration of *Kigelia* sp. plant extract. This would directly ensure that the medicinal plant does not have side effects.

MATERIALS AND METHODS

Collection of plant material

K. africana.fruits were collected from Queen mary's college campus Chennai. The healthy plant material was selected. Fruits were washed well with tap water and dried under shade till they dry at room temperature. The dried fruit was made into fine powder. The fine powder was kept in a air tight container and stored.

Preparation of plant extract

Ten gram of dried fruit powder of *K. africana* (LAM.) Benth. was extracted separately with 50 mL of ethanol and aqueous for 24h in 3 days . It is soaked overnight at room temperature. The sample was then filtered through whatmann filter paper in a Buchner funnel .The filtered solution was evaporated under vacumm in a rota - vator

at 40^0c to a constant weight and then dissolved in ethanol and aqueous. The solution was stored at 18^0c until use.

Antioxidant Activity

Catalase Assay

Catalase assay has been checked for the provided extracts. Various concentration of extracts namely 100, 75,50,25 µg/mL has been taken to which 2mL of phosphate buffer solution and 1mL of H_2O_2 was added. Absorbance was read at 240nm.

Mitotic Cell Division in Onion Root Tips

The antimitotic activity of the test plant extract was screened using *Allium cepa*root tip meristematic cells which have been used extensively in the screening of drugs with antimitotic activity. The bulbs were germinated over water before being transferred to each of the test plant extracts. When the roots were about 5 mm long, the bulbs were placed on beakers containing the fruit extracts of four different concentrations (25,50,75,100µg/mL and control), such that the roots were immersed in the extracts. The duration of treatments for each extract was 24hr and 48hr. The sprouted roots were also treated with distilled water, which served as control. The experimental set up had five replicates.

The root tips were harvested after the treatment duration and fixed in Carnoy's fluid (1 part of glacial acetic acid: 2 parts of absolute alcohol). The root tips were hydrolysed in 1N HCL for 5 minutes. The squashing was done over 2% aceto - orcein stain. The slides were then scanned under Leica DM 1000 trinocular research microscope and photomicrographs were taken. The numbers of cells, dividing and non- dividing, were recorded. Incidence of chromosome aberrations was calculated by expressing the number of aberrant cells as a percentage of total dividing cells for each treatment. Mitotic index was calculated by expressing the number of dividing cells as a percentage of total cells counted for each of the treatments and the control.

$$\text{Mitotic Index} = \frac{\text{Number of dividing cell}}{\text{Total number of cells}} \times 100$$

RESULTS

Antioxidant activity

The antioxidant activity – catalase assay of *Kigelia africana* fruit of aqueous extracts with various concentration of extracts were taken (100,75,50,25 µg/ mL) respectively. (table 1). The total activity was found to be higher at the concentration of 100 µg/ mL with 18.24 and 16.32 units/mL respectively

Cytotoxic analysis

In the present study the mitotic activity from the aqueous extracts of *K. africana* were checked for various concentration (25, 50, 75 and 100 µg/mL) respectively. At 24 hours and 48 hours studies were carried out. At 24 hours, 50 µg/ mL concentration the number of dividing cells was found to be 48.25 and mitotic index was found to be 91.46 and it is comparable to other concentration. During 48 hours at 100µg/ mL concentration the number of cells found to be dividing is 107.14 and mitotic index was found to be 93%. Compared to both extracts 48 hours study showed the best result at 100µg/ mL concentration respectively.(table 2,3).

The root length on 48 hours showed that 50 µg/ mL showed more number of roots and with maximum length. Further more chromosomal aberrations were found to be minimum. As the results clearly showed that the fruit had medicinal properties mainly at a concentration of 50µg/ mL. (table 4).

Table 1: Antioxidant activity- catalase assay of aqueous extract of *K.africana* fruit.

Plant Name	Concentration of Extract(µg/mL)	OD Value (240 nm)	Unit activity (units/mL)	Specific activity (units/mL)	Total activity (units/mL)
K. africana	100	0.76	4.56	9.12	18.24
	75	0.69	4.14	8.26	16.56
	50	0.66	3.96	7.92	15.84
	25	0.65	3.9	7.8	15.6

Table 2: Cytotoxic activity of aqueous extract of *K. africana* fruit.(24hours).

S. No	Concentration (µg/mL)	Total Number of Cells (%)	No. of Dividing Cells (%)	Mitotic Index(MI)
1	CONTROL	38.25	33.75	88.23
2	25	35	25.25	72.14
3	50	52.75	48.25	91.46
4	75	46.25	42	90.81
5	100	40.25	36.15	89.81

Table 3: Cytotoxic activity of aqueous extract of *K. africana* fruit.(48 hours).

S. No.	Concentration (µg/mL)	Total Number of Cells (%)	No. of Dividing Cells(%)	Mitotic Index(MI)
1	CONTROL	39.25	32.25	82.16
2	25	38.5	31.5	81.81
3	50	61.75	57.5	107.39
4	75	49	42.75	87.24
5	100	45	42	93.33

Table 4: Growth of root and shoot length of onion root tip from aqueous extract of *K. africana* fruit.

Plant Length	Various Concentration of Extract (µg/mL)				
	Control	100	75	50	25
Shoot number	-	-	-	2	-
Root number	4	8	11	14	7
Shoot length(cm)	-	-	-	3	-
Root length(cm)	4	1.5	3.5	4.2	1

DISCUSSION

Catalase assay (antioxidant activity)

Catalase and superoxide dismutase are the main antioxidant enzymes in seminal plasma that prevent increases in reactive oxygen species concentration thus protecting the sperm cells against damage (lipid peroxidation) (Kawakami *et al.,* 2008). Sperm plasma membranes have high content of polyunsaturated fatty acids (PUFA) thereby making them sensitive to damage by free radicals resulting in loss of membrane integrity (Suzuki and Sofikitis, 1999). Low catalase activities closely relates to low motility of ejaculated spermatozoa (Kawakami *et al.,* 2008). Testicular catalase activity was higher in group B compared to the control but it was not significant. Catalase activity was significantly lower in group C after 4 weeks. By 8 weeks, catalase activity was significantly increased in group E compared to control. (Azu *et al.,* 2010). In the present study the antioxidant activity of catalase assay were checked for aqueous and ethanolic extracts in various concentrations namely 1, 0.75, 0.5, 0.25µg/mL respectively. At 1µg/mL concentration aqueous fruit extracts of *K. africana* showed best result and the total activity was found to be 18.24 units/ mL respectively. According to Amandeep *et al.,* 2013 the ethanolic and aqueous fruit extracts of *K. africana* were carried out for enzymatic catalase activity. Among the two extracts of *K. africana* the highest activity of catalase were observed in aqueous extract with 3.26 mg protein and lowest in ethanolic extract with 1.80 units /mg protein.

Antioxidants are defined as compounds that can delay, inhibit, or prevent the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress. Oxidative stress is an imbalanced state where excessive quantities of reactive oxygen and/or nitrogen species (ROS/RNS, e.g., superoxide anion, hydrogen peroxide, hydroxyl radical, peroxynitrite) overcome endogenous antioxidant capacity, leading to oxidation of a varieties of biomacromolecules, such as enzymes, proteins, DNA and lipids.

Mitotic analysis

Onion root tip cells are in the active stage of division and can be used to study the effects of various compounds on cell division or chromosomes by the number of dividing and non-dividing cells which gives the mitotic index. Reduction in mitotic index caused by AEPO indicates inhibition of actively dividing cells. The number of aberrant cells was also observed to be increasing with the concentration of extract. Cell division was normal in the root tips kept as control. The chromosomal aberrations induced in the treated onion root cells were definitely caused by the chemical ingredients in the aqueous leaf extracts of the tested plant species, since such aberrations were not observed in the control. The observation of cells with laggards, chromosome gaps, and giant cells in the treated onion cells is an indication that the extract, especially at high concentrations, is capable of causing changes in chromosome number and structure. The reduction of the mitotic index might be explained as being due to the obstruction of the onset of prophase, the arrest of one or more mitotic phases, or the slowing of the rate of cell progression through mitosis. Earlier reports suggest that the presence of nuclear lesions and nuclear dissolution offer cytological evidence for the inhibitory action on DNA biosynthesis.

According to Gowtham *et al.,* 2014 from aqueous and methanol extracts of *P. odoratissimus* the aqueous extract showed significant reduction in the mitotic index of squashed onion root tips with increase in concentration at 10mg/ mL.

In the present study the antimitotic activity from the aqueous extracts of *K. africana* were checked for various concentration (25,50,75,100µg/mL) respectively. At 24 hours and 48 hours studies were carried out. At 24 hours, 50µg/mL concentration the number of dividing cells was found to be 63 and mitotic index was found to be 118 and it showed the best result compared to other concentration. During 48 hours at 100µg/mL concentration the number of cells found to be dividing is 70 and mitotic index was found to be 111.2 compared to

both 48 hours study shows the best result at 5mL concentration respectively. According to Anjali *et al.*, 2013 shows the data on the mitotic indices and the percentage of chromosome aberrations observed in *A. cepa* root tip cells treated with the aqueous leaf extracts of the test plant material. The mitotic indices of all the extract treated roots were comparable lower than that of the control. Also, the mitotic index values were observed to be decreasing with increasing concentrations of the extracts (100µg/mL)

CONCLUSION

Furthermore this study can be carried out for targeting the active compound from the drug and production of new compound

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