International Journal of Biotechnology and Biochemistry
ISSN 0973-2691 Volume 13, Number 1 (2017) pp. 11-18
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In-vitro antioxidative, cytotoxic activity and phytochemical screening from stem bark of *Kigelia pinnata*

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Abstract

The study was designed to evaluate the phytochemical profile, antioxidant and cytotoxic activity of a methanolic extract from the stem bark of *Kigelia pinnata* (MEKP). The *In-vitro* anti-oxidative activity was investigated using DPPH assay, superoxide scavenging assay and cytotoxic activity was studied using cell viability (MTT) assay on non small lung cancer cell-line (A-549). MEKP displayed prominent anti-oxidative and cytotoxic activity against A-549 cells. On the basis of the *In-vitro* studies it could be concluded that there is huge scope for future investigation on *K. pinnata* as a source of potential anti cancer drug.

Keywords: Antioxidative activity, Cytotoxicity, *K. Pinnata*, MTT-assay, Phenolic and flavanoid content.

INTRODUCTION

Kigelia pinnata (family, Bignoniaceae) also known as Sausage Tree in sight to its huge typically shaped fruits, has a range of traditional medicinal uses all through Africa where it grows as a prevalent variety in different habitats [1, 2] as well as in India and the Middle East [3]. Secondary metabolites isolated from *K. pinnata* include

iridoids [4], naphtoquinoids [1, 5], flavonoids, lignans, terpenoids, coumarins, phenylethanoids, phenylpropanoids and sterols [2, 5, 6] considered to play major role in biological and pharmacological activity. *K. pinnata* have been studied for its antimicrobial effects [6, 7] and its cytotoxicity against cancer cell lines [8, 9]. Diferent cytotoxic agents such as lapachol (regarded as a potential anti-cancer drug), norviburtinal, kigelinone, and γ-sitosterolamong others have been isolated from *K. pinnata* through bioactivity-guided fractionation [9, 10]. Present study conducted to study methanolic extract from *Kigelia pinnata* stem bark for its antioxidative property and cytotoxicity against lung cancer (A-549) cells.

MATERIALS AND METHODS

Plant materials

The fresh sample of stem bark of *K. pinnata* was collected from Pune, in August 2013 under the supervision of a botanist and all material were authenticated and submitted in organ form to Dr. Subhash Sadhu Deokule, Head of Botany department, Pune.

Preparation of plant extracts

The powdered sample stem bark of *K. pinnata* was extracted using 99% methanol by cold percolation method. The powdered material was soaked in methanol (1:4) for 48 hours at 37ºC. Then the filtrate was filtered and distilled for recovering the solvent and then it was evaporated under reduced pressure at 50ºC.

Phytochemical screening

Phytochemical evaluation were performed for all the extracts as per the standard methods [11, 12].

Total phenolic and flavonoid content

Folin–Ciocalteu method was used to study total phenolic content in methanolic extract from stem bark of *K. pinnata* [13]. Total phenolic content was expressed in terms of µg of gallic acid equivalents per mg of dry extract. Total flavonoid content was measured as described previously [14]. Total flavonoid content of the different extracts was expressed in µg of quercetin equivalents per mg of extract.

In vitro Anti-Oxidant Activity

Antioxidant activity in methanolic extract from stem bark of *K. pinnata* was measured by using DPPH radical scavenging assay method [15] and superoxide scavenging method [16]. Tests were carried out in triplicate for every experiment. The amount of

extract needed to inhibit free radicals concentration by 50%, IC_{50}, was graphically estimated using a nonlinear regression algorithm.

Cell viability assay

The cell viability after treatment with MEKP was assessed using the MTT-dye reduction assay, based on the bioconversion of the yellow dye 3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyl tetrazolium bromide to a violet formazan product via the mitochondrial succinate dehydrogenase in viable cells [17]. The capability of cells to endure toxic concentration is the principle behind all cytotoxicity assays. This assay is based on the theory that dead cells or their products do not reduce tetrozolium. The MTT assay depends on the mitochondrial activity per cell and amount of cells present.1×10^4 cells were plated in 96-well plates to check toxicity of extracts towards A-549 (lung cancer cells). After adherence of cells, the medium was removed and replaced by media having the plant extracts. In 5% CO_2 incubator the plates were incubated for 24h at 37°C. Colorimetric assay with the tetrazolium salt MTT was used to determine cell viability. Absorbance of the formed purple formazan was measured at wavelength of 570 nm. Results were expressed as percentage cellular viability of the extracts.

% Cytotoxicity = O.D of control sample -O.D of treated sample/O.D of control sample $\times$100.

RESULTS AND DISCUSSION

Phytochemical screening

Preliminary phytochemical screening of the MEKP revealed the presence of various bioactive components like flavonoids, phenol, alkaloids, saponin, steroids, glycosides, terpenoids and tannin in MEKP. Anthroquinone is absent in MEKP. These phytochemicals are known to support bioactive activities in medicinal plants and may therefore be responsible for the antioxidant activities of the plant extracts. *K. pinnata* partly supports the common traditional use of plant in the treatment of cancer.

Total phenolic and flavonoid content

Presence of phenolic and flavonoids serve as health promoting compound as a results of its anion radicals [18]. Moreover, a few studies onthe *K. pinnata* revealed that they are good dietary sources of antioxidants. Thus, we studied the total phenolic and flavonoid contents of the MEKP. The total phenolic and flvanoid content found in stembark of MEKP was (272 ± 2.45µg GAE/mg and 98 ± 1.79µg of QE/mg) respectively.

Antioxidative activity

DPPH activity

In free radical scavenging (DPPH assay) activity, methanolic extract of stem bark of *K. pinnata* revealed significant antioxidant property with IC_{50} value 32.93µg/ml (r^2=0.986) as shown in figure 1. BHT was used as standard for comparison. IC_{50} value for BHT was 39.66µg/ml (r^2=0.998). Table 1 shows the antioxidant potential of MEKP at different concentration. The concentration dependent inhibition was observed in figure 1. The scavenging effect was increased with increasing concentration. DPPH assay is one of the most widely used methods for screening antioxidant activity of plant extracts [19].

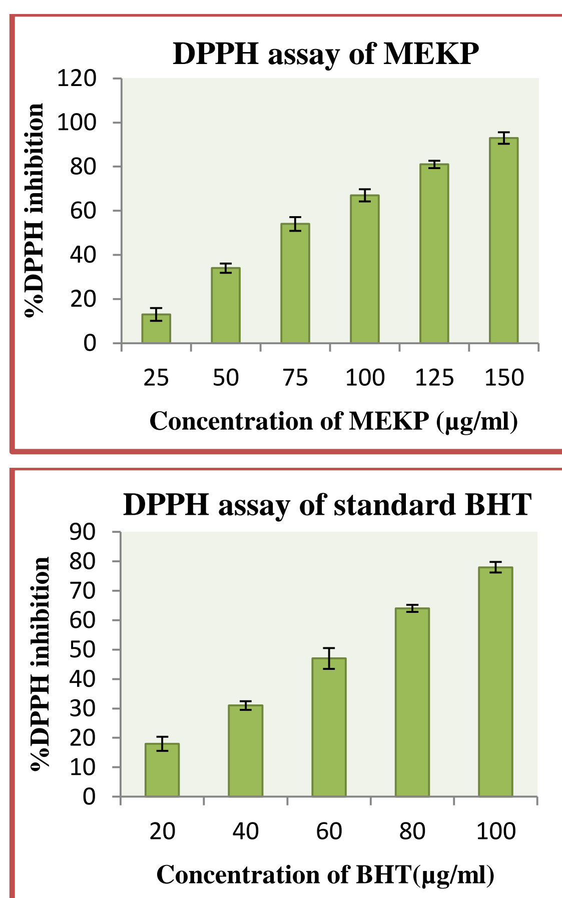


Fig 1: (a) DPPH scavenging assay of MEKP (b) Standard BHT.The results are expressed as mean ± SEM of three replicates

Superoxide scavenging activity

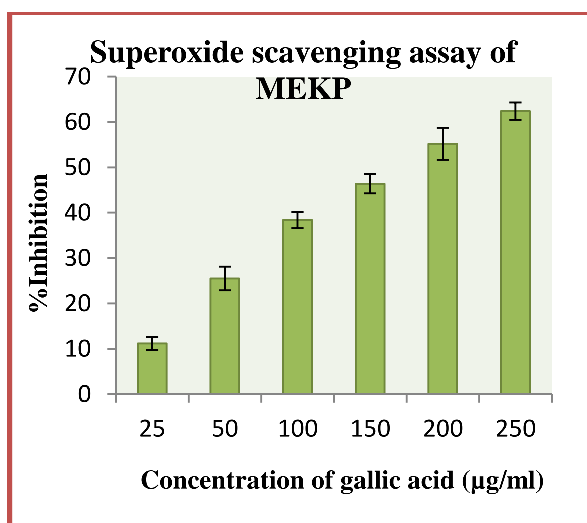
Superoxide ($O_2^{\bullet-}$) radical is recognized to be very harmful to cellular components as a precursor of the extra reactive oxygen species, cause harm to tissue and because of

that various diseases occur. The percentage of superoxide anion scavenging activity of MEKP was presented in Table 1 and figure 2. IC_{50} value for MEKP was 177.4/ml (r^2=0.95) in comparison with standard gallic acid (IC_{50}- 103.54μg/ml) (r^2=0.985). Superoxide scavenging ability of plant extract might primarily be due to the presence of flavanoids [20].

Table 1: IC_{50} value of *K. pinnata* plant's stem bark for antioxidant and cytotoxic activity

Plant		DPPH radical scavenging assay activity [IC_{50}(μg/ml)]	Super oxide radical scavenging assay [IC_{50}(μg/ml)]	Cytotoxic activity [IC_{50}(μg/ml)]
1.	**MEKP**	32.93± 1.36	177.04± 2.71	102.74± 3.28
2.	**Standard**	BHT 39.66± 1.27	Gallic acid 103.54± 2.71	-

For antioxidant and cytotoxic activity IC_{50} value of MEKP. Each value represents mean value± SD of triplicate samples analysis, SD: standard deviation



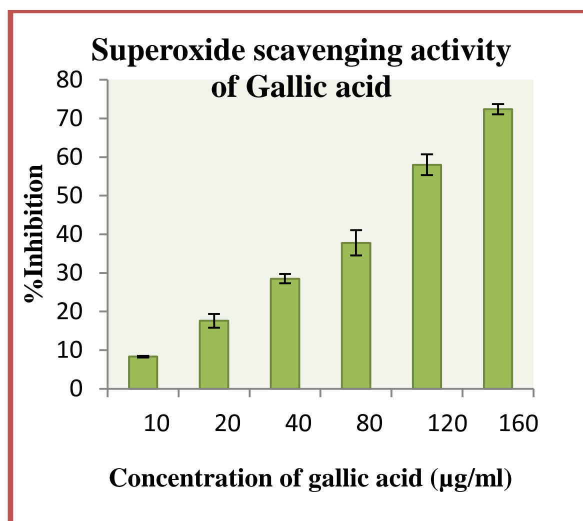


Fig 2: (a) Superoxide scavenging assay of MEKP (b) Standard BHT. The results are expressed as mean ± SEM of three replicates

Cytotoxic activity

The MEKP was tested for their cytotoxicity against A-549, lung cancer cells. Results showed that MEKP potentially inhibited viability of A-549 with their IC_{50} value (102.71µg/ml, R^2=0.97) where shown in Table 1. MEKP tested at different concentrations (200, 150, 100, 50 and 25µg/ml) as shown in figure 3. MEKP had significant concentration dependent inhibition on viability of the A-549 cells in cytotoxicity assessment (MTT-dye reduction assay). Cytotoxic activity of the methanolic extract further supported the earlier findings regarding the effects of *K. pinnata* dichloromethane, ethanol or water extracts against human cancer cells [8, 9].

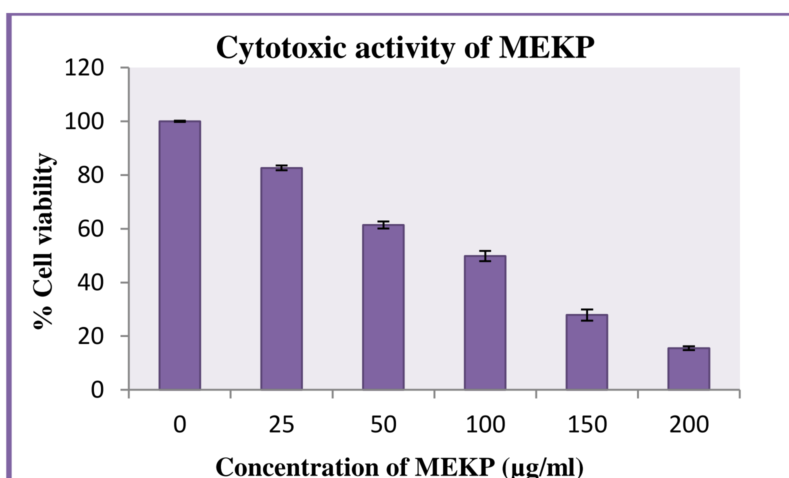


Fig 3: Cytotoxic activity of MEKP using MTT asaay. The results are expressed as mean ± SEM of three replicates.

The present study revealed that the phytochemicals present in MEKP possess potent antioxidant capacity and cytotoxic potential against lung cancer cells. The free radical scavenging ability probably is one of the mechanisms by which herbal medicines exhibit higher antioxidant activity. In addition crude methanolic extracts have capability to combat oxidative damage because of its DPPH activity, superoxide scavenging activity and iron binding ability. Knowing the exact compounds responsible for the plant's anticancer properties will help in formulating anticancer agents.

ACKNOWLEDGEMENT
Authors are grateful to Charutar Vidya Mandal (CVM) Vallabh Vidyanagar, Gujarat and Director of Ashok and Rita Patel Institute of Integrated Study & Research in Biotechnology and Allied Sciences (ARIBAS) New Vallabh Vidya Nagar, for providing platform for this research work.

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