

ANTIOXIDANT AND ANTICANCER ACTIVITIES OF SELECTED INDIAN MEDICINAL PLANT VIZ., *ARTOCARPUS LAKOOCHA*, *KIGELIA PINNATA*, AND *AMARANTHUS VIRIDIS*

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ABSTRACT

In this study, we evaluated the total phenolic content, total flavonoids content, antioxidant and anticancer effects of methanol extracts of selected medicinal plants namely *Artocarpus lakoocha*, *Kigelia pinnata*, and *Amaranthus viridis* from Uttar Pradesh region of India. Fruits of *Kigelia pinnata* (KPF), seeds of *Artocarpus lakoocha* (ALS), and flowers of *Amaranthus viridis* (AVF) were used in this study. Methanol extract was prepared by Soxhlet extraction method. The total phenol content and total flavonoids activity were performed by (AlCl_3) colorimetric assay and Folin-ciocalteu (FC) reagent respectively whereas antioxidant potential asses by FRAP and DPPH. Cytotoxicity against the cancer cell line was done by MTT assay. Three cancerous cell lines, Hela, A549, and MCF-7 were tested in this study. The highest total phenol content and total flavonoids content were present in KPF ($127.97 \pm 1.17 \mu\text{g GAE/mg extract}$) and KPF ($57.97 \pm 1.25 \mu\text{g Quer/mg extract}$). The significant ferric reducing antioxidant power (FRAP) activity was shown by methanol extract of AVF ($56.09 \pm 0.51 \mu\text{M AAE/mg extract}$) and 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) activity was shown by methanol extract of and KPF (93.63%) respectively at $100 \mu\text{g/ml}$. Moreover, methanol extract of AVF exhibited 48.13% inhibition of MCF-7 cancerous cell line at $100 \mu\text{g/ml}$. The selected plants could be used with antioxidant and anticancer activities as new natural therapeutic sources.

KEYWORDS: *Artocarpus lakoocha*, *Kigelia pinnata*, *Amaranthus viridis*, Total phenolic content, Total flavonoids, Antioxidant activity, Anticancer activity.

INTRODUCTION

Reactive oxygen species (ROS) are derived from normal cell metabolism to generate energy or external sources such as smoking, different types of pollutants, industrial waste and chemicals(1). Free radicals are the highly reactive species and have capabilities to interact with nucleus and membranes of cells of biologically relevant macromolecules including DNA, proteins, carbohydrates, and lipids. In consequence, they attack macromolecules as well as other kinds of molecules of body leading to cell damage and homeostatic disruption(2). At high concentration, they generate a condition known as oxidative stress which plays a major role in the generation of chronic and degenerative diseases such as cancer, autoimmune diseases, aging, cardiovascular and neurodegenerative diseases(3). Natural antioxidants such as vitamin A, ascorbic acid and carotenoids reduce the oxidative stress by scavenging the free radical and minimize the risk of diseases caused by this condition. Moreover, medicinal plants also have numerous phenolic and

flavonoids type of molecules as natural antioxidants namely catechins, quercetin, kaempferol, Caffeic acid, gallic acid etc.(4). Phenols and flavonoids have also been shown an important role in the prevention of chronic diseases such as type 2 diabetes, tumor, Alzheimer and neurobiological diseases (5).

It is well established that free radicals take part in DNA damage by mutation and transformation of bases, sugar lesions, breaking of strands or oncogene activation and finally leads to carcinogenesis or cancer formation (3). Free radicals abruptly increase the DNA damage by reacting with double bond of pyrimidine bases and abstraction of hydrogen from the sugar moiety. A group of natural molecules like polyphenols and flavonoids that can reverse or suppress the carcinogenic progression has become a potential target to prevent the cancer and related diseases(6). Polyphenols and flavonoids have C6-C3-C6 structural unit that is favorable to molecular interactions with proteins along with their antioxidant potential. They also prevent the DNA damage by scavenging the free radical (7).

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Therefore, it is urgent need to search out the natural sources in the form of polyphenols and flavonoids for the treatment of oxidative stress and cancer. In consequence, we have selected three medicinal plants viz., *Artocarpus lakoocha*, *Kigelia pinnata*, and *Amaranthus viridis* having numerous types of polyphenols and flavonoids. These plants have extensively been used in Indian traditional system of medicine like Ayurveda to prevent or treat the various diseases including fever, constipation, tuberculosis, bronchitis, pain, pneumonia, liver and abdominal ailments in the form of decoction, infusion, paste or as formulation (8-10). Our literature survey revealed that there was a paucity of study on the anticancer and antioxidant properties of selected plants. Hence, in our study, we assessed the anticancer activity of methanol extracts of *A. lakoocha* seeds, *K. pinnata* fruits, and *A. viridis* flowers against Hela, A549 and MCF-7 cell lines and antioxidant activity against DPPH and FRAP assay along with the evaluation of total phenol content and total flavonoids content

MATERIAL AND METHOD

Reagents and Chemical

Dulbecco's Modified Eagle's Medium (DMEM) (1x), 0.4% trypan blue, phosphate buffered saline (PBS) (pH 7.2, 1x), 0.25% trypsin EDTA (1x), and antibiotic/antimycotic solution (100x) were purchased from Gibco India, Life Technologies whereas fetal bovine serum (FBS) and MTT were purchased from Himedia India. Quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent and 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ) were obtained from Sigma-Aldrich Co India. Further necessary chemicals such as ascorbic acid, Dimethyl sulphoxide (DMSO), Aluminium Chloride, Potassium acetate, Sodium carbonate, Ferric chloride, Ascorbic acid, Gallic Acid etc. used were of analytical grade and were acquired from Indian commercial sources.

Plants Collection and Identification

Kigelia pinnata (fruit), *Artocarpus lakoocha* (seed) and *Amaranthus viridis* (flower) were collected in June 2018 from local places of Lucknow

Preparation of Extracts

The plant material ALS (50.0 gm) was dried in shade, powdered and percolated with methanol (2 liters) solvent. Percolated system was remained at room temperature for twenty-four hours after which the percolate was collected. It was repeated five times, till the plant material was extracted exhaustively. The total percolate was pooled off, filtered and concentrated under vacuum using a rotavapor at 40-45 °C. The extract of this plant was found to weigh 6.0 g.

This procedure of extract preparation was repeated similarly for each of the plant's samples viz. KPF (90.0 gm), and AVF (49.0 gm). All the methanol extracts were found in good yield as 11.0 gm, and 5.1 gm for KPF and AVF respectively

Cell Lines

Hela (cervical cancer cells), A549 (human lung epithelial cancer cells), MCF-7 (breast cancer cell) and one normal cell line Vero (Monkey kidney epithelial cells) were purchased from NCS Pune, India. They were maintained in DMEM medium with 5% FBS at 5% CO₂ and 95% humidity in FormaSteri-cycle CO₂ Incubator (Thermo Fisher Scientific).

Estimation of Total Flavonoids Content

Total flavonoids content was determined by using Aluminium chloride (AlCl₃) colorimetric assay with slight modification as previously reported by Kamble et al. (2019) Methanol extract (1.0 mg/ml) were incubate with 200 µl 10% AlCl₃, 200 µl 1M CH₃COOK and 3.8 ml of distilled H₂O for 30 minutes. Absorbance was recorded on UV-spectrophotometer at 420 nm. Estimation of flavonoids content calculated with the help of Quercetin standard curve, and total flavonoids measured as µg Quer.E/mg extract (11).

Estimation of Total Phenol Content

The quantitative estimation of total phenol content was determined with Folin-ciocalteu (FC) reagent with the help of UV-spectrophotometer as previously reported by Ahamad et al. (2017) with some modification. 1.0 mg/ml concentration of methanol extract was thoroughly mixed with 1.5 ml (10%) FC reagent and 3.0 ml of 7.5% sodium carbonate (Na₂CO₃). After 30 minutes incubation period, absorbance of solution measured at 760 nm on spectrophotometer. To calculate the total phenol content, a standard curve of gallic acid was prepared. Total phenol content was measured as µg GAE/mg extract (12).

Ferric Reducing Antioxidant Potential (FRAP) Assay

FRAP activity of plant extract was determined as per method proposed by Benzie et al. (1999) with some modification. This reagent was prepared by mixing acetate buffer (300 mM; pH 3.6), TPTZ solution (10 mM) and FeCl₃ solution (20 mM) in the ratio of 10:1:1. FRAP reagent was kept for 30 minutes before use. Ascorbic acid (10-250 µM/ml) was used as a standard to prepare curve. 2.0 mL volume of FRAP reagent were incubated with extract (1.0 mg/ml) or ascorbic acid (1.0 ml) for 30 minutes in a test tube in the absence of light. The absorbance was measured at 593 nm. The results were expressed in FRAP value µM of ascorbic acid equivalents (AAE)/mg (13).

DPPH Antioxidant Assay

Antioxidant activity of each extract was determined by DPPH assay as previously described by Ayoola et al with some modification. DPPH (2.0 ml, 0.1mM) solution was incubated in 1.0 ml of different concentrations (25µg, 50µg, 100µg and 250µg) of each extract for 25 minutes in dark. Ascorbic acid was used as standard whereas absorbance was measured at 517 nm to compare the antioxidant results. The inhibition of DPPH radical was calculated as follow

$$\% \text{ Inhibition of DPPH} = \frac{\text{ABS}_{\text{control}} - \text{ABS}_{\text{sample}}}{\text{ABS}_{\text{control}}} \times 100$$

Where $\text{ABS}_{\text{control}}$ = absorbance of DPPH radical + methanol and $\text{ABS}_{\text{sample}}$ = absorbance of DPPH radical + extract/standard (14).

MTT Assay

The anticancer property of each extract was determined by using in-vitro MTT assay as described earlier with some modification against cancer cell lines MCF-7, HeLa, A549 as well as normal cell lines. The exponentially cells growth was trypsinized and seeded 200µl in each well of 96 well plates with cell density 0.5×10^5 cells/ml. The 96 well plates were incubated at 37°C and in 5% CO₂ atmosphere for 24 hours. After, cell lines treated with different concentration (25µg, 50µg, 100µg and 200µg) of each extract and incubated again for 48 hours. After the incubation period, 20µl MTT reagent was added in each well at concentration of 5.0 mg/ml and again incubated for 2 to 4 hours. During incubation period, formazan crystals were formed. Dimethyl sulphoxide (DMSO) was added to dissolve these crystal and absorbance measured at 570 to with the reference filter 650nm on iMarkmicroplate absorbance reader (Bio-Rad). The percentage cell viability was calculated as

$$\% \text{ cell viability} = \frac{\text{AT} - \text{AB}}{\text{AC} - \text{AB}} \times 100$$

Where, AT is the absorbance of the treatment well; AB is the absorbance of the blank and Ac is the absorbance of the control well(15, 16).

RESULTS

Estimation of Total Phenol Content

Total phenol content in all the extract was determined using gallic acid as standard. The results were demonstrated as gallic acid equivalents per gram of dry weight of extract (µg GAE/g DW) with the help of gallic acid standard curve and correlative coefficient

$R^2=0.924$ (Fig. 1). The significant amounts of phenol content were present in KPF (127.97 ± 1.17 µg GAE/mg extract) followed by AVL (67.39 ± 4.29 µg GAE/mg extract) (Table.1). The highest total phenol content was present in KPF

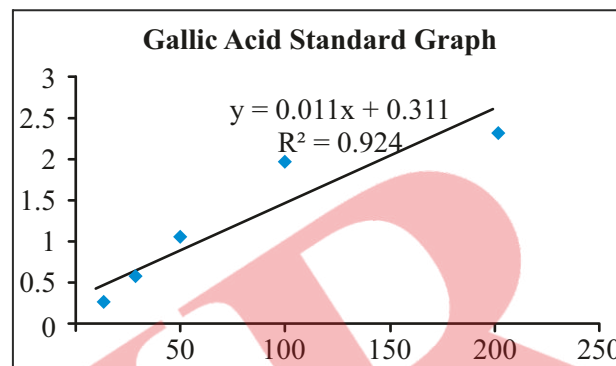


Fig 1: Gallic Acid Standard Graph

Estimation of Total Flavonoids

The estimation of total flavonoids in all extracts was determined using quercetin as standard. Results were demonstrated as quercetin equivalents per gram of dry weight of extract (µg Quer/g DW) with the help of quercetin standard curve and correlative coefficient $R^2=0.986$ (Fig. 2). The significant amount of flavonoids was found in the extract of KPF (57.97 ± 1.25 µg Quer/mg extract) and AVF (58.30 ± 2.14 µg Quer/mg extract). The highest flavonoid content was present in AVF (Table.1).

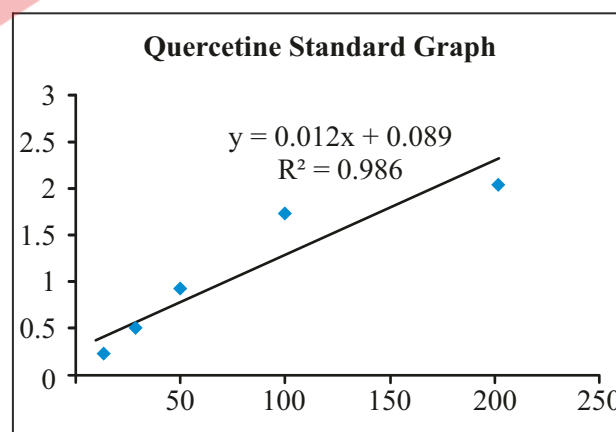


Fig 2: Quercetin Standard Graph

Antioxidant Activity

The antioxidant activity of all samples were evaluated by using FRAP and DPPH scavenging spectrophotometric methods as shown in Table 1.

Ferric Reducing Antioxidant Potential (FRAP)

The ferric reducing antioxidant potential in all extracts

were assayed using ascorbic acid standard curve with correlation coefficient $R^2=0.985$ (Fig 3). All the extracts were showed significant FRAP activity. Highest FRAP activity was found in AVF (56.09 ± 0.51 μ M AAE/mg extract), followed by KPF (52.89 ± 0.40 μ M AAE/mg extract) (Table 1)

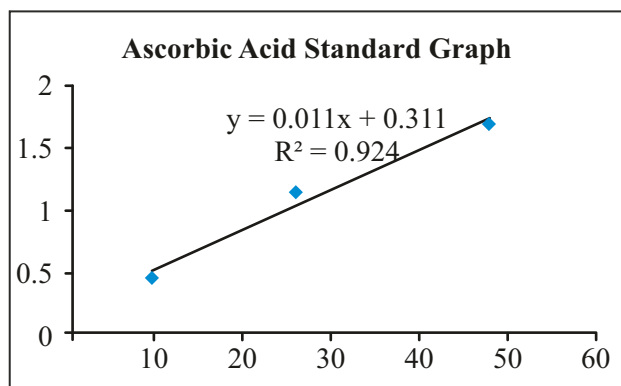


Fig 3: Ascorbic Acid Standard Graph

S.No.	Extract	Total Flavonoid Content(μ g Quer.E/mg extract)	Total phenol Content (μ g GAE/mg extract)	FRAP value in(μ MAAE/mg extract)
1	ALS	13.16 ± 0.59	17.60 ± 0.83	31.22 ± 0.89
2	AVF	58.30 ± 2.14	67.39 ± 4.29	56.09 ± 0.51
3	KPF	57.97 ± 1.25	127.97 ± 1.17	52.89 ± 0.40

Table 1: Estimation of Total Flavonoids Content, Total Phenol Content and FRAP Activity

S. No.	Sample	Cell lines	% Inhibition		
			25 μ g/ml	50 μ g/ml	100 μ g/ml
1	ALS	MCF-7	17.26	18.37	23.58
		A549	03.83	20.22	21.85
		HeLa	08.78	11.03	13.65
		Vero	1.67	4.89	8.11
2	AVF	MCF-7	24.68	33.99	48.13
		A549	04.15	12.70	24.50
		HeLa	02.48	13.19	14.21
		Vero	2.45	5.63	13.20
3	KPF	MCF-7	02.05	06.04	14.01
		A549	01.44	14.42	16.89
		HeLa	15.54	22.54	25.07
		Vero	0.89	3.90	7.12

Table 3: Percentage Inhibition of Extract on Different Cancer Cell Line

DPPH radical scavenging activity

All the extracts exhibited weak to significant DPPH radical scavenging activity. The methanol extract of KPF (93.63%) and AVF(62.49) showed significant

S. No.	Sample	% Inhibition			
		25 μ g/ml	50 μ g/ml	100 μ g/ml	250 μ g/ml
1	ALS	-18.28 ± 4.22	-12.5 ± 7.47	-1.05 ± 0.68	7.95 ± 0.72
2	AVF	-11.28 ± 2.68	5.25 ± 1.27	25.08 ± 1.12	62.49 ± 5.00
3	KPF	48.39 ± 0.42	75.77 ± 0.68	93.63 ± 0.16	94.01 ± 0.76
4	Ascorbic acid	91.3 ± 0.21			

Table 2: Estimation of Antioxidant Potential of Extract by DPPH Antioxidant Assay

inhibition of free radicals at 100 μ g/ml concentration as compared to ascorbic acid (91.3 ± 0.21) at 25 μ g/ml concentration (Table2)

In-vitro anticancer activity

In-vitro anticancer activity was done by using MTT assay against MCF-7, A549 and HeLa cell lines in percentage inhibition values as shown in table 3. Live cells reduced the yellow tetrazolium MTT in to the purple formazan crystal with the action of Dehydrogenase enzymes. The formazan was quantified by spectrophotometrically. The highest inhibition of cancer cell lines was found in extract of AVF extract (48.13%) on MCF-7 cell line at 100 μ g/ml concentration (Table 3). While the remaining plants extract ALS and KPF showed very weak or no inhibition against all cell lines. None of the extracts show the cytotoxicity on the normal cell line Vero.

DISCUSSION

In our study, three Indian medicinal plants namely *Artocarpus lakoocha*, *Kigelia pinnata*, and *Amaranthus viridis* have been investigated for their phytochemicals, total phenol content, total flavonoids, antioxidant as well as anticancer activities. The total phenol content and flavonoid content were examined with help of Folin-Ciocalteu (FC) reagent method and aluminum chloride method respectively. In quantitative estimation analysis, highest total phenol content was found in methanol extract of KPF ($127.97 \pm 1.17 \mu\text{g GAE/mg extract}$) whereas other plant samples showed good to poor total phenol content viz. AVF (67.39 ± 4.29) and ALS (17.60 ± 0.83) $\mu\text{g GAE/mg extract}$ respectively. We also estimated total flavonoid content and found that AVF and KPF plant samples contained significant amounts of total flavonoids as 58.30 ± 2.14 and $57.97 \pm 1.25 \mu\text{g Quercetin / mg extract}$ respectively. Total flavonoids content was poorly found in ALS as $13.16 \pm 0.59 \mu\text{g Quercetin / mg extract}$.

It is known that antioxidant potential of medicinal plants directly depends upon the presence of total phenol content as well as total flavonoid content. Therefore, keeping in mind we evaluated all selected plant extracts for their antioxidant effect by using FRAP and DPPH assays. As shown in the table 1, a significant FRAP values were found in AVF and KPF plant extract as 56.09 ± 0.51 and $55.00 \pm 0.47 \mu\text{M AAE/mg extract}$ respectively. ALS showed FRAP effect with $31.22 \pm 0.89 \mu\text{M AAE/mg extract}$. In addition, KPF showed potent DPPH percent inhibition ($93.63 \pm 0.16\%$) at $100 \mu\text{g/ml}$ concentration while AVF have been found to show $25.08 \pm 1.12\%$ free radical scavengers in DPPH assay at $100 \mu\text{g/ml}$ concentration. As a result, our experiments showed that the extract having higher phenol content with high flavonoid content exhibited the significant antioxidant effect in comparison to extracts having lower phenols as well as flavonoid content. Cytotoxic effect of all selected plant extract was assessed by using MTT assay against MCF-7, A549 and HeLa cell lines. Among the selected plant, effective cytotoxicity was shown by AVF methanol extracts on MCF-7 cancer cell line. AVF was found to show only 48.13% inhibition of MCF-7 cells at $100 \mu\text{g/ml}$ concentration. Moreover, remaining plant extract showed no significant cytotoxic effect on MCF-7, A549 and HeLa cell lines.

CONCLUSION

In concluding remarks, the present study revealed the potential use of selected plants with antioxidant and anticancer activities. Our study showed that highest flavonoid and phenol content were found in methanol extract of *Kigeliapinnata* as $127.97 \pm 1.17 \mu\text{g GAE/mg}$

extract and $57.97 \pm 1.25 \mu\text{g Quercetin/mg extract}$ respectively. While potent antioxidant effect was found in *Amaranthus viridis* against FRAP assay and *Kigelia pinnata* against DPPH assay. *Amaranthus viridis* exhibited significant percentage inhibition of MCF-7 cancer cell lines. The results obtained support that additional studies may be required to isolate bioactive compounds present in plant extracts that are responsible for antioxidant and anticancer activities.

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