



RESEARCH ARTICLE

# Antibacterial activity, free radical scavenging property and phytochemical screening of *Diospyros kaki* fruit of Kumaun Himalayan Region, Uttarakhand

Neelaxi Pandey<sup>1</sup>, Satpal Singh Bisht<sup>1</sup>, Mahendra Rana<sup>2</sup>, Rojita Mishra<sup>3</sup>

<sup>1</sup> Department of Zoology, D.S.B. Campus, Kumaun University, Nainital, Uttarakhand, India

<sup>2</sup> Department of Pharmaceuticals Sciences, Bhimtal Campus, Kumaun University, Nainital, Uttarakhand, India

<sup>3</sup> Department of Botany, Polasara Science College, Polasara, Ganjam, Odisha, India

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## ABSTRACT

*Diospyros kaki* is a deciduous tree found in the hills of Kumaun region up to an elevation of 1500 m, most of studies on the fruit of this plant have been carried out in the countries like China, Japan, Myanmar, Brazil, Turkey, Italy and South Korea very less attention has been paid to this less known wild fruit in India. This study primarily deals with total phenolic, Flavonoid, tannin content, antioxidant and antibacterial activity. Ascorbic acid was used as standard for the evaluation of antioxidant activity. The IC<sub>50</sub> value for ethanolic extract and aqueous extract was recorded 50.43; 81.9 ppm respectively. *Diospyros kaki* fruit was found to contain significant amount of antioxidants. Antibacterial activity was performed by disc diffusion method against the strain *Salmonella typhimurium* (MTCC 3224), *Klebsiella pneumonia* (MTCC 3384), *Bacillus subtilis* (MTCC441), *Aeromonas hydrophila* (MTCC 646), and *Pseudomonas aeruginosa* (MTCC 103). This study showed that the ethanolic extract of the fruit has significant amount of phytochemicals and antibacterial activity as compared to its aqueous extract.

**Keywords** *Diospyros kaki*, flavonoid content, phenolics content, tannin content, ic 50, antioxidant activity, antibacterial activity

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## INTRODUCTION

Plant-based nutraceutical research that deals with isolation and screening of new phytochemicals of plant origin useful in human health and counteract the risk of sickness and infections (Che and Zhang,2019;Jonas et al.,2013). Fruits, vegetables and functional foods are an important part of a balanced diet and rich source of secondary metabolites including dietary fiber, natural antioxidants and various phytochemicals (Butt et al., 2015; Caruso et al., 2015; Hamid et al., 2013). Vegetation biodiversity of Kumaun Himalaya endowed with a unique and diverse range of such phytochemical producers including the persimmon (*Diospyros kaki*). This is relatively unnoticed and underutilized wild edible fruit which is very rich in various phytochemicals including polyphenols, carotenoids, vitamins, sterols, terpenoids, tannins and dietary fiber contributing its taste, flavor, nutritive and medicinal value(Dalvi et al., 2018;Maulidiani.et al., 2018). Although the chemical composition differs with the variety and ripening stage of the fruit. Some phytochemicals have been used for different therapeutic purposes such as diuretic effect,

\* For correspondence: N. Pandey (Email: [neelaxhrdp@gmail.com](mailto:neelaxhrdp@gmail.com))

chemo-preventive, blood pressure-lowering capability, cough treatment, anti-mutagenic effects, viral and bacterial infections (Kashif et al., 2017; Yaqub et al., 2016)

*Diospyros kaki* has great economic value and widely cultivated in China, Japan, Myanmar, Brazil, Turkey, Italy and the South Korean. In India it is still treated as wild edible fruit (Giordani et al., 2011). China ranks first in persimmon production worldwide with an annual production of 1.65 million metric ton (Karaman et al., 2014). Recently researchers across the world have started intensive research on wild edible fruits and fruit products. It is imperative to study the wild edibles from Kumaun Himalaya to explore their nutraceutical potential for their possible exploration as a fruit of high nutraceutical and economic value.

## MATERIALS AND METHODS

### Plant Material

The *Diospyros kaki* fruits were collected from the forests of Nainital, Uttarakhand during the month of October-November 2018 and authenticated by Botanical Survey of India, Dehradun, Uttarakhand India, voucher specimen number 134. (Appendix-1)



Fig 1: (a) *Diospyros kaki* in nature

(b) *Diospyros kaki* fruit

(c) Fruit secondary metabolites

### Preparation of the raw material

The fruits were oven-dried at 40°C and finely chopped and subjected to extraction on an increasing solvent polarity (Ethanol and water) with the help of Soxhlet apparatus. Each extract was concentrated under vacuum using a rotary evaporator (IKA, Germany) followed by evaporation on a water bath. Fruit material was air-dried before extracting in every change in solvents. All extracts were stored at 4°C for further investigations.

### Estimation of total phenolic content

Total phenolic content was estimated by the Folin-Ciocalteu assay (Chandran and Indira, 2016). In this assay, gallic acid was used as a standard. 1 ml of plant extract of different concentrations was taken in a volumetric flask and 10 times diluted with 5 ml Folin-Ciocalteu reagent followed by 2-minute incubation and 5 ml of 7.5% sodium carbonate solution was added and the absorbance measured at 760 nm against the typical blank solution containing all reagents. The amount of phenolic content was expressed as mg of Gallic acid per gram of plant dried sample (mg GA/g).

### Estimation of total tannin content

Total tannins estimated by the Folin-Ciocalteu method (Chandran and Indira, 2016). Tannic acid was used as a standard. 100µl of plant extract, standard of different concentrations were taken in a volumetric flask containing 7.5 ml distilled water with 500µl Folin-Ciocaltaeu reagent, 1ml diluted solution of 35% sodium carbonate. The contents were mixed and kept at room temperature for 30 minutes; then the absorbance of the solution was measured at 700nm against the blank. Tannin content was expressed as mg of tannin per gram of dried sample (mg TAE/g).

### Estimation of total flavonoid content

Total flavonoid content was estimated using Quercetin as a standard (Chandran and Indira, 2016). 100µl of different concentration of quercetin, 4ml water and 300µl of 5% sodium nitrite was mixed in a volumetric flask. After 5 minutes, 300µl of 10% Aluminium chloride solution was added and after 6 minutes, 2 ml of 1M sodium hydroxide was added. The volume made up to 10ml with distilled water. The absorbance of the solution was measured at 510 nm against the blank i.e solution containing all reagents except aluminum chloride.

### DPPH scavenging activity

Free radical scavenging activity was estimated by DPPH assay (Williams et al.,1995). Ascorbic acid was used as standard. 200µl of fruit extract of different concentrations was prepared in volumetric flasks and added with 800 µl of DPPH solution (use methanol, DPPH molarity is 90 µM). The solution was kept at room temperature for 30 min; then absorbance of the solution was measured at 515 nm against the blank. A typical blank solution contained ethanol and DPPH solution.

$$\text{Scavenging Activity (\%)} = [(A \text{ control} - A \text{ sample}) / A \text{ control}] * 100.$$

The IC50 values were calculated using linear regression analysis and used to indicate antioxidant capacity.

### Antibacterial Assay using various bacterial Strains

Five bacterial strains were used i.e. *Salmonella typhimurium* (MTCC 3224), *Bacillus subtilis* (MTCC441), *Klebsiella pneumonia* (MTCC 3384), *Pseudomonas aeruginosa* (MTCC), *Aeromonas hydrophila* (MTCC 646).The disk diffusion assay was carried out to perform antibacterial activity (Ceylan et.al.,2018).The ethanolic extracts were dissolved in DMSO and water extract were dissolved in distilled water at two different concentrations 20mg/ml and 40 mg/ml. 20µl of bacterial inoculum was spread over the surface of a sterile muller hinton agar plates. Extracts were applied to filter paper disc (6 mm in diameter, Whatmann No.1,) and allowed to dry before placed on agar plate and plates were incubated at 37°C for 24hrs. After incubation, the diameter of inhibition zones was measured and antibacterial activity was calculated. Gentamycin (concentration 100µg/disc) was used as standard drug and ethanol, water and hexane were used as a negative control. Each test was performed in triplicate and results analyzed for statistical significance. Data was statistically analyzed and documented.

## RESULTS AND DISCUSSION

*Dysporus kaki* fruit was selected for the present study and investigation was done on its phytochemical evaluation, antioxidant, antibacterial potential and findings are summarized in the Table 1, 2,3,4,5 and 6. The findings revealed that this fruits is highly

nutritious as compared to many other cultivated and wild fruits (Chen et.al., 2008). Apart from the nutritional supplements this fruit can be explored as an economical alternative to local folk as it's a candidate fruit for nutraceuticals more especially as anti-mutagenic and anti-carcinogenic compounds with a large market in and outside the country (Celik and Ercisli, 2008).

### Preliminary phytochemical screening

The carbohydrates, protein, phenolics, flavonoids and alkaloids were presented in both ethanolic and aqueous extracts. Saponins were present only in ethanolic extracts. Oils, fats and Terpenoids were absent in both the extracts.

**Table 1: Organoleptic characters of fruits**

Fruit Name	Color	Size	Taste	Shape
<i>Diospyros kaki</i>	Orange-yellow	4-7 cm	Astringent	Spherical- oval

**Table 2: solvent used for the extraction and properties**

Extract type	Color	Odor	Consistency
Ethanolic Extract	Pale Brown	Reddish	Sticky
Water Extract	Brownish	Characteristic	Sticky

**Table 3: Preliminary phytochemicals of *Diospyros kaki*.**

Chemical Constituents	Ethanolic extract	Water Extract
Protein	+	+
Carbohydrates		
Molish test	+	+
Fehling test	+	+
Benedict test	+	+
Alkaloids		
Mayer's test	+	+
Dragendroff test	+	+
Phenolics and tannins	+	+
Oils and Fats	-	-
Flavonoids	+	+
Terpenoids	-	-

+ = Present, - = Absent

**Table 4: Total Phenolic and Tannin Content and total Flavonoid content of different extract of *Diospyros Kaki* fruit**

Sample	Total phenolics content GAE(mg/g)	Total tannic acid content TAE(mg/g)	Total flavonoids content quercetin equivalent(mg/g)
<i>Diospyros Kaki</i> (ethanolic extract)	43.08 ± 0.26	79.94±0.40	40.83±1.79
<i>Diospyros Kaki</i> (Aqueous extract)	5.26 ±0.404	18.15±0.10	4.52±0.47

### Estimation of total phenolic, flavonoid and tannin content

Fruits are rich source of phenolics, flavonoids and tannins. These constituents have ability to scavenge free radicals thus they work as immune boosters to the body (Velmurugan and Bhargava, 2014). These dietary supplements provide protection against the oxidative damage to cell membrane lipids, nucleic acids and proteins (Verma et al., 2011). To accept and prove *Diospyros kaki* fruit as a therapeutic fruit, its efficacy needs to be evaluated. During the present investigation the highest content of phyto-constituents were found in ethanolic extract as compared to aqueous solvent. Phenolic hydroxyl groups act as a reducing agent due to their electron donor property (Bendary et al., 2013). These compounds prevent oxidative disease burden (Babbar et al., 2015). The total phenolic content found in ethanolic and aqueous extract was  $43.08 \pm 0.26$  mg and  $5.26 \pm 0.40$  mg of gallic acid equivalent per gram of extract respectively. Similar kind of studies have been performed using peel and fruit flesh separately in four solvent systems and results varied significantly i.e.  $91.30 \pm 1.06$  to  $4.27 \pm 0.13$  mg GAE/g (Jang et al., 2010). Whereas Tannins are astringent polyphenolics plant secondary metabolites that typically act as astringents and are commonly found in fruits, legumes and grasses and play an important role in protection from predators and helps in plant growth (Saxena et al., 2013).

The total tannin content found in ethanolic and aqueous extract was  $79.94 \pm 0.40$  mg and  $18.15 \pm 0.10$  mg of tannic acid equivalent. Flavonoids are also known as Vitamin P, yellow and other pigments producing plant secondary metabolites. It consists of great pharmaceutical activity and readily ingested (Kumar and Pandey, 2013). The high flavonoid content found in ethanolic extract that is  $40.83 \pm 1.79$  mg quercetin equivalent per gram (Table 4.). Phenolic, Flavonoid and tannin content was observed much higher in ethanolic extract of *Diospyros kaki* fruit. Presence of high amount of phenolics reduce the risk of metabolic disorders and also reduces total cholesterol levels (Jyoti et al., 2020). Carotenoids have received attention because of their medicinal applications that is particularly associated with the maintenance of cell cycle and checks the risk of mutations. Chemo preventive effects of this fruit against various forms of cancer are due to carotenoid contents. Apart from that some other secondary metabolites of this fruit may also affect multidrug resistant (MDR) inhibiting activity (Takako et al., 2014). *Diospyros kaki* contains two ingredients- Lutein and Zeaxanthin that helps to improve eye health and prevent retina damage (Alba-Mir et al., 2015; Yaqub et al., 2016)

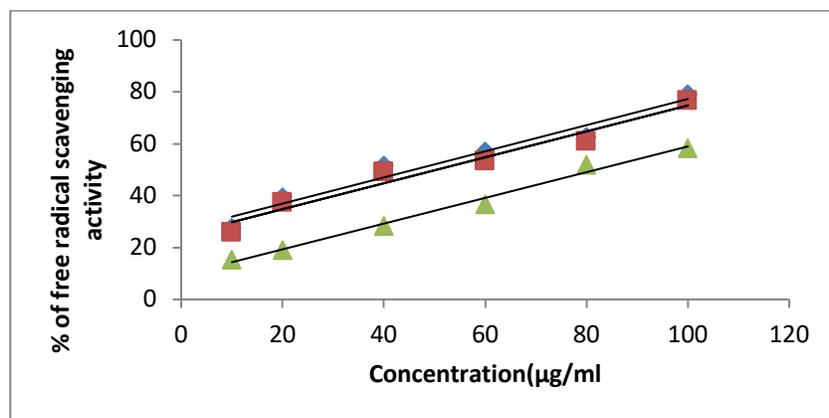
### Radical Scavenging Activity

Free radicle scavenging property of fruits received wide attention as they are safe and non-toxic (Ratnam et al., 2006). Antioxidant activity of *Diospyros kaki* was assayed using DPPH. The IC<sub>50</sub> value was calculated to determine the antioxidant capacity after the DPPH assay<sup>1</sup>. Higher is the IC<sub>50</sub> value lower is the antioxidant activity of the sample. The results showed that the IC<sub>50</sub> value of ethanol extract is lower compared to the aqueous. The values are tabulated in Table 5 and

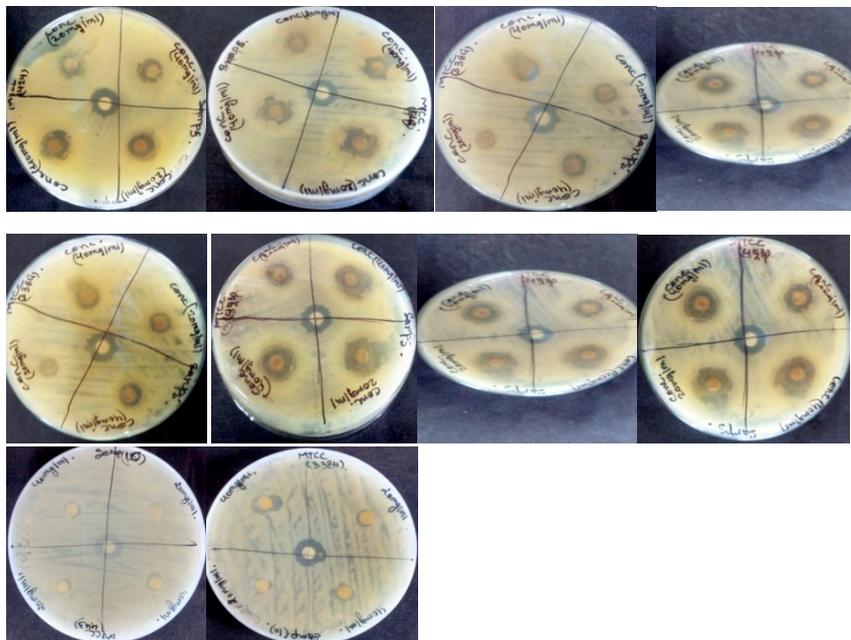
represented in Fig .2, IC50 of DPPH radical scavenging activity of ethanolic extract was found to be 50.43µg/ml and aqueous extract was 81.9µg/ml respectively.

**Table 5: The IC50 values of different extract of *Diospyros kaki* fruit for DPPH radical scavenging assay (µg/ml)**

Sample	IC50 value
Ethanolic Extract	50.43
Aqueous extract	81.9



**Fig 2. The comparative curve of free radical scavenging activity of Ethanolic and Aqueous extract of *Diospyros Kaki* fruit with standard gallic acid**



**Fig3. Antibacterial activity of five bacterial strains against *Diospyros kaki* fruit extract.**

**Table 6: Antibacterial activities, indicated by diameter of inhibition zone (mm) at different concentration of fruit sample ethanolic and water extract against the selected bacterial species:**

Type of Fruit extract	Bacterial species Fruit extract Conc <sup>n</sup>	<i>Salmonella typhimurium</i>	<i>Klebsiella pneumonia</i>	<i>Bacillus subtilis</i>	<i>Aeromonas hydrophila</i>	<i>Pseudomonas aeruginosa</i>
Ethanolic Extract	20mg/ml	9.83±0.24	11.76±0.14	11.33±0.08	8.7±0.28	10.46±0.08
	40mg/ml	12.86±0.20	17.5±0.15	12.63±0.13	10.46±0.21	13.46±0.17
Water Extract	20mg/ml	7.78±0.17	11.36±0.18	M.A	8.86±0.18	N.A
	40mg/ml	10.81±0.34	15.03±0.26	8.16±0.12	12.3±0.17	6.8±0.23

### Antibacterial screening

Antibacterial activity of the fruit of *Diospyros kaki* was performed using ethanolic and water extracts against five bacterial strains i.e. *Salmonella typhimurium* (MTCC 3224), *Bacillus subtilis* (MTCC 441), *Klebsiella pneumonia* (MTCC 3384), *Pseudomonas aeruginosa* (MTCC), *Aeromonas hydrophilla* (MTCC 646) using disc diffusion method (Table 6; Fig. 3). The findings of ethanolic and aqueous extract are summarized in Table 6; water extract of fruit against *Pseudomonas aeruginosa* didn't show any antibacterial activity. Ethanolic fruit extracts with 40mg/ml concentration showed higher antibacterial activity against all bacterial strains used in the study. Maximum zone of inhibition was observed in case of ethanolic extract and water extract against *Klebsiella pneumonia* i.e. 17.5±0.15mm and 15.03±0.26mm and minimum zone of inhibition was observed in case of *Pseudomonas aeruginosa* 6.8±0.23 mm in aqueous extract. The average zone of inhibition at 100µg/ml concentration of standard antibiotic Gentamycin was observed 20mm, 18mm, 22mm, 16mm, 14mm against *B. subtilis*, *K. pneumoniae*, *P. aeruginosa*, *S. typhimurium*, *A. hydrophila* respectively.

### CONCLUSION

Phenolics, flavonoids and tannins, compounds show the significant linear correlation with antioxidant property determined by DPPH assay and these compounds were ubiquitously present in plants as secondary metabolite. The results obtained in this study showed a significant level of phenolics, flavonoids and tannins in ethanolic extract as well as in aqueous extracts of the *Diospyros kaki* fruit. The availability of phytochemicals was observed higher in ethanolic extract than aqueous extract. This fruit is a natural free radical scavenger and a very rich natural source of various phytonutrients those are considered very effective and safer as compared to their synthetic counterparts. The results clearly indicate that the free radical scavenging activity of *Diospyros kaki* is due to the significant presence of phenolic acids, flavonoids and its tannin content. Based on the results of investigations, *Diospyros kaki* is a candidate source of novel antioxidants. This unnoticed fruit in the study site is not only rich in nutraceuticals but also contains effective antibacterial activity and could be explored for therapeutic and commercial purposes.

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