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Antioxidant, antibacterial activity, and phytochemical characterization of *Carica papaya* flowers

Manish Kumar Dwivedi[1], Shruti Sonter[1], Shringika Mishra[1], Digvesh Kumar Patel[2] and Prashant Kumar Singh[1*]

Abstract

Background: *Carica papaya* is an eminent medicinal plant used all over the world to treat several diseases like malaria, dengue, inflammation, and skin infections. In this study, preliminary phytochemical screening for *C. papaya* flowers was carried out using different methods as TLC screening and UV-spectroscopy along with evaluation of antioxidant and antibacterial activities. Methods were also developed for TLC and UV-visible spectroscopic analysis of the extracts.

Results: Results of phytochemical screening revealed that the methanol extract contains alkaloids, flavonoids, saponins, and tannins as major components. Saponins and tannins were present in chloroform and *n*-hexane extracts; however, steroids and flavonoids were additionally found in *n*-hexane extract. Flavonoids, saponins, and tannins were present in aqueous extract of papaya flower. TLC and UV-visible spectroscopy also confirmed the presence of phenolics and flavonoids in different plant extracts. The total phenolic content (0.76 ± 0.04 mg GAE/g dry weight) and total flavonoid content (1.53 ± 0.10 mg QE/g dry weight) were the highest in the *n*-hexane extract of the flower. Antioxidant activity using DPPH free radical scavenging assay was the highest in *n*-hexane extract (64.07%). Antibacterial screening was carried out using well diffusion method against two pathogens Gram-negative bacteria *Escherichia coli* and Gram-positive bacteria *Bacillus subtilis*. The antibacterial assays of the extracts displayed the highest activity in methanolic extract against both *E. coli* (4.00 ± 0.08) and *B. subtilis* (01.00 ± 0.05).

Conclusion: This is the first report for the presence of alkaloids and saponins in *C. papaya* flowers. Also, it is the first report for determination of total phenolics and total flavonoids in *C. papaya* flowers. Methanolic extract displayed considerable antibacterial activity against *E. coli* and *B. subtilis*. The antioxidant and antibacterial properties of phytochemical extracts make them attractive alternative complementary medicines. More chemical investigation for chemical constituents is important for further drug development programs.

Keywords: Antibacterial activity, Antioxidant activity, *Carica papaya* flower, Phytochemical analysis, Well diffusion, Total phenolic content

1 Background

Medicinal plants have been reported to have antioxidant, antibacterial, or antimicrobial activities that have been credited to the presence of secondary metabolites such as alkaloids, flavanols, flavones, tannins, saponins, steroids, and other secondary metabolites [1]. Many studies have also reported that medicinal plants contain a wide variety of free radical scavenging molecules, which act against bacterial diseases. Current estimates indicate that about 80 million people worldwide still depend on plants for their health needs [2], and approximately 95% of modern drugs have been isolated from traditional medicinal plants [3]. The World Health Organization (WHO) estimated that around 80% of rural

* Correspondence: prashant.singh@igntu.ac.in
[1]Department of Biotechnology, Indira Gandhi National Tribal University, Amarkantak, Madhya Pradesh 484887, India
Full list of author information is available at the end of the article

patients seek alternative treatment options in many countries and are dependent on different medicinal plants for curing different diseases.

Carica papaya are herbaceous plants belonging to the member of the *Caricaceae* family [4]. It is a dicotyledonous, polygamous, and diploid species comprising 31 species in four genera, first three belong from the USA such as *Carica, Jacaritia*, and *Jarilla* and one from equatorial Africa such as *Cylicomorpha*. It is a fast growing herb with short life span with all parts of the plant such as fruit, root, stem, seed [5], leaves, and flower being significantly used for the treatment of different types of diseases. The flower of papaya is tiny, yellow, funnel-shaped, solitary, or clustered in the leaf axils. It is useful for the treatment of cough, bronchitis, chest asthma, and cold from the ancient time [6, 7]. *C. papaya* plants have medicinal value due to the presence of natural metabolites found in leaf, bark, and twigs that possesses both anti-tumor and pesticidal properties [8]. The whole plant is being used for the production of plant biomass that can be utilized for the development of the anticancer drugs [9, 10]. The plant is also used as a natural pesticide and the approval is pending for the Food and Drug Agency [11]. The high level of natural self-defense compounds in the plant makes it highly resistant to insect and disease infestation [12]. Traditionally, the flowers of *C. papaya* are used as a fresh vegetable to supplement the diet of our society and support higher levels for the growth of the individuals. The *C. papaya* flowers have useful medicinal properties and can prevent cancer, increase digestion and appetite, and delineate heart problems [13]. The tannins, flavonoids, and antioxidants in papaya flowers that have been described previously to knock out free radicals from the body. Consumption of papaya flowers helps the body to neutralize the free radicals and modulate the immune system increasing disease susceptibility.

The cells of living organisms generate free radicals against parasites or during diseased states creating an imbalance in the formation and neutralization of pro-oxidants. The biological macromolecules such as proteins, lipids, and DNA are the target of these free radicals that lead to oxidative stress in the physiological system [14]. If there is continuous exposure to these free radicals, then cellular damage may occur with the development of chronic diseases such as cancer, diabetes, atherosclerosis, cardiovascular, inflammation, and other degenerative diseases in humans [15]. The antioxidant is a substance that is present at low concentration, significantly delays or prevents oxidation of that substrate. Antioxidants are able to associate with decreased cellular damage and inhibited malignant transformation of cells [16]. Many plants are known to possess several antioxidants that may contribute to the total antioxidant activity of plant materials including carotenoids, polyphenols, and traditional antioxidant vitamins C and E [17]. Phenolic compounds are the major bioactive phytochemicals found in plants with human health benefits [18].

In the present study, preliminary phytochemical investigation of *C. papaya* flowers is carried out. Determination of total phenolic and total flavonoid contents of *C. papaya* flowers as well as evaluation of antioxidant and antibacterial (Gram-positive and Gram-negative) activity of different flower extracts was evaluated in methanolic, chloroform, *n*-hexane, and aqueous extracts of *C. papaya* flowers. Biological activity of papaya flower is first described in the study area and in Madhya Pradesh, India.

2 Methods

2.1 Chemicals and equipment

1,1-Diphenyl-2-picrylhydrazyl (DPPH) (HiMedia), ascorbic acid (HiMedia), gallic acid (Merk), Folin-Ciocalteu reagent (CDH), quercetin dihydrate (CDH), aluminum chloride (Merk), and all the chemicals were of high-purity analytical-grade reagents. Methanol (analytical grade), chloroform (analytical grade), and *n*-hexane (analytical grade) were throughout used for the phytochemical analysis, TLC, UV, antioxidant, and antibacterial activity. Milli-Q Ultra-pure water was obtained from a Millipore water purification system (Millipore, Milford, MA, USA). All other reagents were of analytical grade. The major equipment used were Soxhlet apparatus (Borosilicate Genuine, JSW) and UV-visible spectrophotometer (Shimadzu UV-Vis spectrophotometer-1800).

2.2 Plant materials and preparation of extract

The *C. papaya* flowers were collected as a fresh sample in the campus of IGNTU, Lalpur, and Amarkantak in the month of February–September (2017–2018). The plant was identified and authenticated by the botanist of the department of botany, IGNTU (Herbarium no. BT_C-20). The *C. papaya* flowers were washed, filled into Soxhlet apparatus and extracted with 150 ml methanol, chloroform, *n*-hexane, and aqueous solvent at a specific boiling point of the solvent for 3–4 h. The extracts were filtered through Whatman filter paper no. 1, and the filtrate was concentrated under reduced pressure at a specific temperature. The extracts were dried, weighed, and yield percentage were calulated for each extraction. Focused extracts were stored at 4 °C for further experimental use.

2.3 Phytochemical investigation

The analysis of *C. papaya* fresh flower extract in different solvents were carried out according to standard procedures [19] with minor modifications as follows.

2.3.1 Test for alkaloids

HCl was added in the plant extracts (3 ml) and then allowed to steam bath for a few minutes. Then few drops of Mayer regent were added to the mixtures. Turbidity indicates the presence of alkaloids.

2.3.2 Test for flavonoids

Few drops of diluted sodium hydroid solution were added to the stock solution of *C. papaya* extracts (0.5 ml). An intense yellow color appeared in the plant crude extract, which becomes colorless upon the addition of a few drops of diluted H_2SO_4 that show the presence of a flavonoid.

2.3.3 Test for saponins

A stock solution from each crude extract *C. papaya* flowers (0.5 ml) was diluted with distilled water (20 ml), and the test tube was shaken by hand for 15 min. The formation of foam layer on the top of the test tube showed the presence of saponin.

2.3.4 Test for steroids

The plant extracts (2 ml) were dissolved in chloroform (10 ml) and added concentrated sulfuric acid (1 ml) into the test tube by wall sides. The color of the upper layer turned red and the sulfuric acid layer turned yellowish with green fluorescence. This indicated the presence of steroids.

2.3.5 Test for tannins

Two milliliter of extracts were added in 2 ml of distilled water and stirred. Few drops of ferric chloride solution were added. The formation of green precipitate showed the presence of tannins.

2.3.6 Test for phlobatannins

Two milliliters of extracts were hydrolyzed with 1 ml HCl and the mixture was boiled for a few minutes. The deposition of red precipitation indicates the presence of phlobatannins.

2.3.7 Test for glycosides

Two milliliters of extracts were dissolved in chloroform and to the mixture 2 ml of acetic acid was added followed by few drops of sulfuric acid in the mixtures and observed color change from blue to green indicate the presence of glycosides.

2.4 Thin layer chromatography

Thin layer chromatography was carried out using solvent system chloroform: methanol (80:20) used as mobile phase. TLC runs were made in the laboratory conditions at room temperature (RT) and 60% relative humidity. The TLC plates (Merck-silica gel 60 F_{254}) were placed in UV chamber (254 nm) for few minutes for visualizing different spot position of the compounds. The R_f value of plant extract was calculated using the standard formula [20].

2.5 UV-visible spectroscopy

The plant extracts were scanned using UV-Vis spectrophotometer (Shimadzu, UV-1800). Methanol, chloroform, *n*-hexane, and aqueous solvents were used as blank and plant extracts were prepared in specific solvents at final concentration of 0.10 mg/ml. The λmax was scanned at 800–200 nm range.

2.6 Determination of total phenolic content

The total phenolic contents of the *C. papaya* flowers were determined by the Folin-Ciocalteu procedure [21]. The Folin-Ciocalteu (F-C) reagent is sensitive to reducing compound, polyphenols and thus produces a blue color [22, 23]. Plant samples (2 ml, triplicates) were introduced into test tubes; 1.0 ml of (1:10) Folin-Ciocalteu's reagent and 0.8 ml of sodium carbonate (7.5%) were added. The sample mixtures tubes were mixed and allowed to stand for 30 min. Absorption at 690 nm was measured (Shimadzu UV-Vis spectrophotometer). The standard curve of gallic acid solution (1, 1.5, 1.8, 3, and 3.4 mg/ml) was prepared using a similar procedure. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligram per gram dry material. All the experiments were performed in triplicate.

2.7 Determination of total flavonoid content

The total flavonoid contents in the examined flowers were determined using a spectrophotometric method, aluminum chloride ($AlCl_3$) complex forming assay. *C. papaya* extracts (1 mL, triplicates) were introduced into test tubes and then added 0.30 ml (5% $NaNO_2$). After that, wait for 5 min for the reaction, and 0.30 ml of 10% aluminum chloride solution was added and allowed to stand for later than 5 min, 2 ml solution of 1 M sodium hydroxide was added sequentially, and volume made up 10 ml with distilled water. The absorbance of this reaction mixture was recorded at 283 nm on UV spectrophotometer. The sample tubes were mixed and allowed to stand for 30 min. Absorption at 283 nm was measured (Shimadzu UV-Vis spectrophotometer-1800). The standard quercetin dihydrate solution in different concentrations (0.1, 0.2, 0.3, 0.4, and 0.5 mg/ml) was prepared in methanol. A calibration curve for quercetin dihydrate was drawn and determined as quercetin dihydrate equivalent.

2.8 DPPH radical scavenging assay

The antioxidant and free radical scavenging activities of the *C. papaya* flower were determined by using standard method 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay, as described earlier with some modification [24]. DPPH is able to oxidize by the decolorization of methanol solution gives deep violet color, and an antioxidant compound donates the electron causing its reduction and reduced from its color changes from deep violet to yellow [25]. The 0.1 mM DPPH solution was prepared in 95% methanol. The stock solution of the extracts was also prepared in 95% methanol. After that from the stock solution, 2 ml, 4 ml, 6 ml, 8 ml, and 10 ml were taken in five test tube and diluted with the same solvent to get a final concentration of 20 μl/mg, 40 μl/mg, 60 μl/mg, 80 μl/mg, and 100 μl/mg respectively. The sample extracts were taken 1 ml in each test tube and added 2 ml fresh DPPH solution each of these test tube. As a control, we used 95% methanol. After 30 min incubation in darkness at room temperature, the absorbance was recorded at 517 nm by using spectrophotometer. Ascorbic acids were used as a standard. The percentage inhibition of DPPH by extracts was calculated by using the following formula:

$$\text{DPPH scavenging effect} = \frac{[1-(\text{Abs sample}-\text{Abs blank Sample})]}{\text{Abs Control}} \times 100$$

2.9 Antibacterial activity

2.9.1 Bacterial strains

The antibacterial activity of the *C. Papaya* was tested individually on Gram-positive and Gram-negative bacterial strains. The well diffusion method of antibacterial test was performing on two bacterial strains *Bacillus subtilis* (Gram-positive) (MTCC code, 441) and *E. coli* (Gram-negative) (MTCC, 1687) both strains are purchased from Institute of Microbial Technology, Chandigarh, India. Bacterial strains were maintained on nutrient agar at 4 °C and sub-cultured every month in our laboratory.

2.9.2 Culture media and plates preparation

Culture media Muller Hinton (MH) was used in antibacterial activity as nutrition for bacterial growth. For the preparation of the media, we took powder (weight 3.4 g) and dissolved it in 100 ml distilled water. The media was autoclaved and after some time poured in Petri plates and left for solidification. Keep all plates in a cool, clean, and dry place when ready to use for experiments.

2.9.3 Culture preparation

2.9.3.1 Nutrient broth media for culture of *B. subtilis* strain 1.3 g of nutrient broth (NB) media was taken and mixed in 100 ml distilled water. The media was autoclaved and cooled. *B. subtilis* was added (powder form) and to 5 ml of NB media. The tubes were kept in an incubator for incubation at 35 °C (24 h).

2.9.3.2 LB media for culture of *E. coli* strain 3.4 g of LB media was taken and mixed in 100 ml distilled water. *E. coli* (lyophilized powder form) was taken in falcon tube in few amounts and added 5 ml LB media; the tubes were left for overnight incubation at 37 °C for optimum *E. coli* growth.

2.9.3.3 Standard preparation The antibiotic kanamycin was used as the standard for the antibacterial activity. Five milligram powder was added to 10 ml distilled water in a 15-ml falcon tube and was used as a stock solution.

2.9.3.4 Antibacterial activity The previously prepared Petri plates were used in the experiment. One hundred milliliter of overnight culture was taken and centrifuged, supernatant was discarded, and the pellet was resuspended in 1 ml of media and spread on LB agar plate. Wells were punched in the LB agar plates and the samples were loaded in the plates. The Petri plates were sealed with parafilm and kept in an incubator for 12 h at 37 °C for *E. coli* and at 35 °C for *B. subtilis*.

2.10 Statistical analysis

Primary phytochemical analysis, total phenolic content, total flavonoid contents, and antibacterial activity were carried out in triplicate and expressed as an average of three analyses ± standard deviation. All experimental measurements were calculated and presented in graphs by the using of MS-excel and SPSS 20 statistical software.

3 Results

3.1 Percentage yield of the plant extract

The yield percentages of papaya flower extract in different solvents were calculated by respective formula (W1/W2) × 100). The yield percentages of extracts in different solvents was obtained in methanol extracts (3.68% w/w), chloroform extracts (9.94% w/w), *n*-hexane (5.51% w/w), and aqueous extract (6.58% w/w). The highest yield percentage was obtained in the chloroform extract and the lowest yield was obtained in methanol extract (Table 1).

3.2 Phytochemical investigation

Phytochemical analysis was done to analyze the chemical constituents present in the methanol, chloroform, *n*-hexane, and aqueous extracts of fresh flowers (Table 2). Alkaloids, flavonoids, saponins, steroids, and tannins were

Table 1 Total phenolic content, total flavonoid content, and DPPH activity of fresh papaya flowers extracts

S. no.	Plant extract	Yield (% w/w) (W1/W2) × 100	Total phenolic content (mg GAE/g dry weight)	Total flavonoid content (mg QE/g dry weight)	DPPH (inhibition %)
1	Methanol	3.68	0.30 ± 0.01	0.61 ± 0.02	17.50
2	Chloroform	9.94	0.36 ± 0.07	0.73 ± 0.05	7.90
3	*n*-Hexane	5.51	0.76 ± 0.04	1.53 ± 0.10	64.07
4	Aqueous	6.58	0.29 ± 0.02	0.15 ± 0.08	26.20

present in the methanol extract, but glycosides and phlobatannine were absent (Table 2). Chloroform extract was found to contain saponins and tannins, but alkaloids, flavonoids, steroids, glycosides, and phlobatannine were absent. Saponins, flavonoids, steroids, and tannins were present in *n*-hexane extract while alkaloids, glycosides, and phlobatannine were absent in it. The aqueous extract contained flavonoids, saponins, and tannins while alkaloids, steroids, glycosides, and phlobatannine were also absent in it (Table 2).

3.3 TLC and UV-visible spectroscopy

TLC is most popular technique that can be utilized for identification and quantification of components in mixture. In the current study, different extracts of fresh papaya flowers were analyzed through TLC. In aqueous extract, three spots were observed in visible and UV region having R_f values of 0.35 (spot 1), 0.71 (spot 2), and 0.84 (spot 3) (Fig. 1 (W)). The methanol extract displayed two spots in visible region and UV region having R_f values of 0.35 (spot 1) and 0.91 (spot 2) (Fig. 1 (M)). The chloroform and *n*-hexane extracts displayed the presence of only one spot each with R_f value 0.91 for both extracts (Fig. 1 (C) and (H)).

The qualitative UV-visible spectrums of papaya flower extracts in different solvents were scanned at wavelength from 200 to 800 nm due to sharpness of the peaks and proper baseline. In the aqueous extract, two peaks at 385 nm and 671 nm with absorption values of 3.749 and 0.243 respectively (Fig. 1a). In methanol extract, spectrum peaks were observed at 370 nm and 664 nm having absorption values of 3.786 and 0.131 respectively (Fig. 2b). The chloroform extract displayed spectrum bands at 262 nm (3.528), 273 nm (2.814), 414 nm (1.360), 451 nm (0.925), and 667nm (0.328) (Fig. 2c), while the *n*-hexane extract displayed peaks at 412 nm (0.816), 441 nm (0.626), 468 nm (0.462), and 669 nm (0.178) (Fig. 2d).

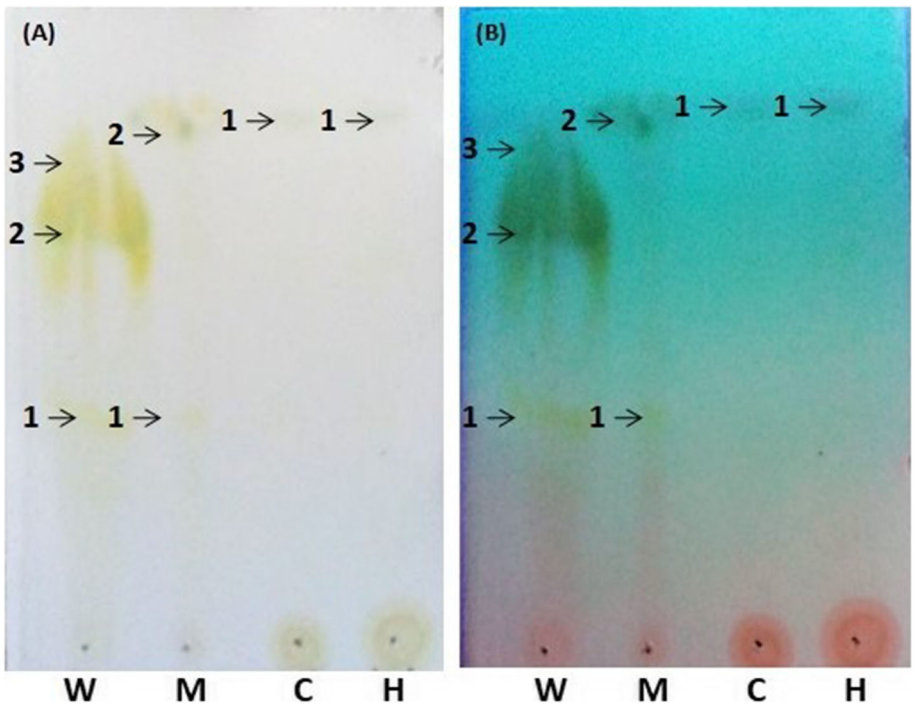

Fig. 1 Thin layer chromatograph of aqueous (W), methanol (M), chloroform (C), and *n*-hexane (H) extracts of fresh papaya flowers. **a** Visualization in visible region. **b** Visualization in UV-region (254 nm)

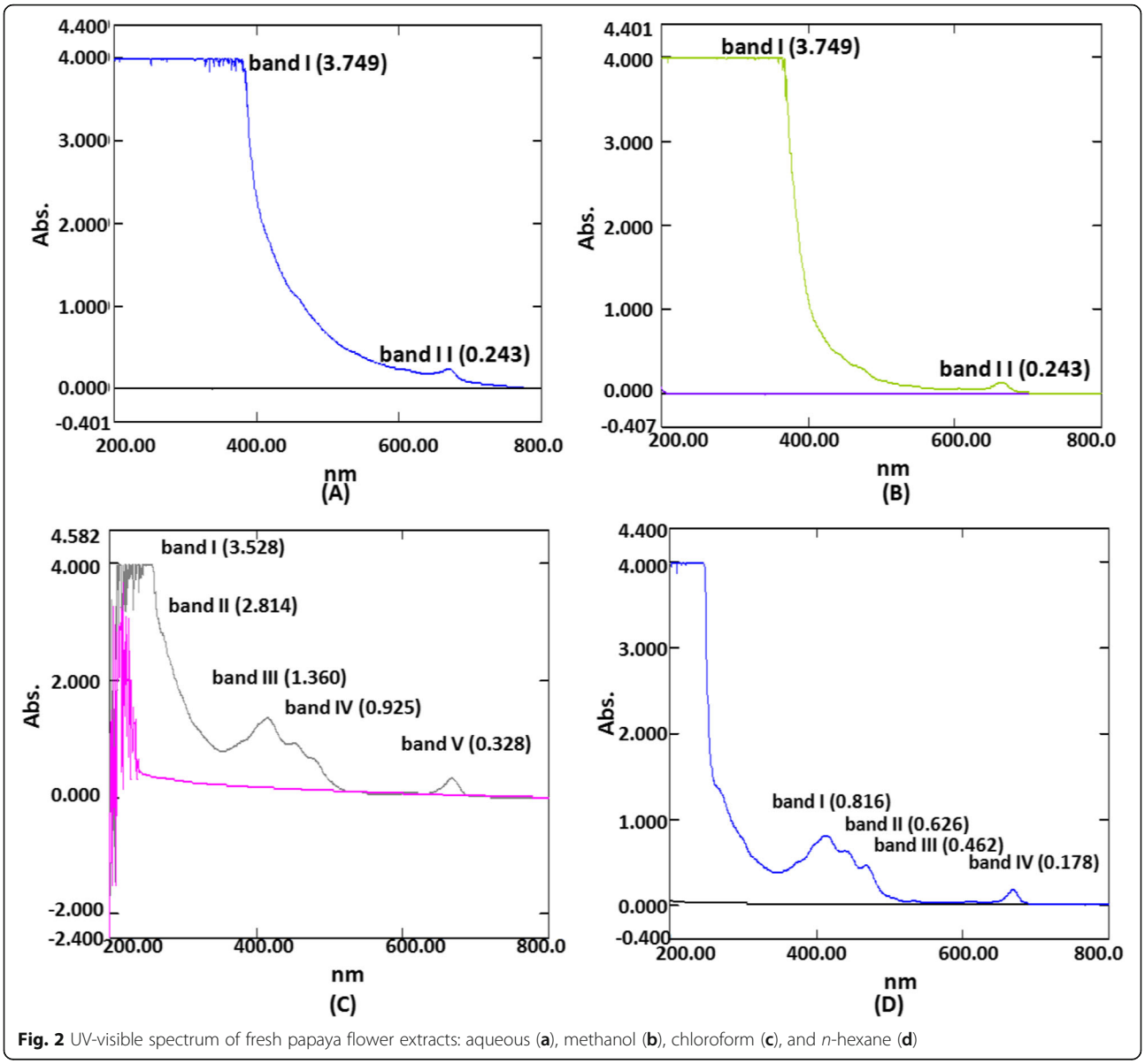

Fig. 2 UV-visible spectrum of fresh papaya flower extracts: aqueous (**a**), methanol (**b**), chloroform (**c**), and *n*-hexane (**d**)

3.4 Determination of total phenolic content

Total phenolic content was analyzed in methanol, chloroform, *n*-hexane, and aqueous extracts using of Folin-Ciocalteu method with the gallic acid as standard. The total phenolic content was calculated from the equation of the gallic acid solution with a regression co-efficient R_2 = 0.9990 and standard plot (y = 15.675x − 0.7129). The result showed that the total phenolic content in methanol extract was 0.30 ± 0.01 mg GAE/g of dry weight of the sample. In chloroform extract, *n*-hexane, and aqueous extract, the values obtained were 0.36 ± 0.07 mg GAE/g, 0.76 ± 0.04 mg GAE/g, and 0.29±0.02 mg GAE/g. The total phenolic content ranged from 0.29 mg GAE/g to 0.76 ± 0.04 mg GAE/g, and the highest total phenolic content was observed in *n*-hexane extract (0.76 ± 0.04 mg GAE/g) (Table 1).

3.5 Determination of total flavonoid content

The total flavonoid content was estimated by using aluminum chloride complex forming assay. Quercetin dihydrate was used as standard. The values were calculated using the equation of standard with regression co-efficient R_2 = 0.997. The plot has a slope of y = 0.106x − 0.019. The total flavonoid content of *C. papaya* flowers in methanol, chloroform, *n*-hexane, and aqueous extracts were 0.61 ± 0.02 mg QE/g, 0.73 ± 0.05 mg QE/g, 1.53 ± 0.10 mg QE/g, and 0.15 ± 0.08 mg QE/g respectively. The highest flavonoid content was observed in *n*-hexane extract (1.53 ± 0.10

Table 2 Phytochemical analysis of fresh papaya flowers

Phytochemicals	Methanol	Chloroform	*n*-Hexane	Aqueous
Alkaloids	+	–	–	–
Flavonoids	+	–	+	+
Saponins	+	+	+	+
Steroids	+	–	+	–
Tannins	+	+	+	+
Phlobatannine	–	–	–	–
Glycosides	–	–	–	–

+ present, – absent

mg QE/g); minimum flavonoid content was obtained in aqueous extract (0.15 ± 0.08 mg QE/g) (Table 1).

3.6 Free radical scavenging assay (DPPH)

The examination of antioxidant activities of papaya flower in methanol, chloroform, *n*-hexane, and aqueous extract was carried out. Ascorbic acid was used as standard in these assays. The antioxidant values varied from 7.9 to 64.07% (Table 1). The free radical scavenging activity inhibition for methanol, chloroform, *n*-hexane, and aqueous extract was 17.50%, 7.90%, 64.07%, and 26.20% respectively. The largest capacities to neutralize DPPH radicals were found in *n*-hexane extract, while chloroform extract displayed minimum DPPH reduction activity.

3.7 Antibacterial activity

The inhibitory effect of the *C. papaya* fresh flower extracts in different solvents was evaluated against different bacterial strains. The antimicrobial activity was determined using well diffusion method summarized in Table 3. The activity was quantitatively assessed on the basis of inhibition zone, and results were compared with activity of standard drug Kanamycin (11 mm). Against *E. coli* (MTCC, 1687), the inhibition zones in methanol, chloroform, and aqueous extracts were 4.00 ± 0.08 mm, 0.30 ± 0.04 mm, and 0.50 ± 0.10 mm respectively. Similarly, the methanol, chloroform, and aqueous extract also displayed inhibition zones against *B. subtilis* (MTCC code, 441). The values for inhibition zone were 01.00 ± 0.05 mm, 0.20 ± 0.02 mm, and 0.80 ± 0.05 mm respectively. The *n*-hexane extract was not active against *E. coli* or *B. subtilis*. Among the different extracts studied, methanol extract displayed maximum degree of inhibition zone against both strains, while the chloroform extract was least active (Fig. 3).

4 Discussion

The worldwide use of medicinal plants in human healthcare entails that their systematic phytochemical evaluation shall be undertaken for the reasons of safety and medicinal use. During the last few decades, considerable progress has been achieved regarding the therapeutic properties of papaya [13, 26]. The tribal communities in Anuppur district are routinely using the fresh flowers of papaya in food and beverages. Papaya flowers are also being used in many traditional therapies such as malaria, jaundice, joint pain, cough, cold, fever, and viral infections. It is thus imperative to analyze the phytochemical constituents of papaya flowers that are being used traditionally for the treatment of various diseases. According to the World Health Organization (WHO), phytochemicals naturally present in medicinal plants are the best sources for novel drug discovery [22]. These are non-nutritive chemicals having protective or disease preventive properties. Many such chemicals play vital role against asthma, arthritis, cancer, diabetes, malaria, jaundice, dengue, diarrhea, dysentery, fungal, and bacterial infections [23]. There are more than thousand known phytochemicals present in the fruits and vegetables that are being consumed daily. In the current study, secondary metabolites such as alkaloids, flavonoids, saponins, steroids, and tannins were present in different papaya flower extracts (Table 1). The alkaloids are a class of nitrogenous compounds and more than 10,000 alkaloids are known to be produced by the plants [27]. Approximately 8000 flavonoids have been reported from different plants species [28]. They play vital role in

Table 3 Antibacterial activity of fresh papaya flower extracts

S. No.	Extracts	Control	Diameter of inhibition zones (mm)		Diameter of inhibition zones (mm)	
			Target bacteria (extracts)		Target bacteria (control)	
			E. coli (MTCC, 1687)	*Bacillus subtilis* (MTCC code, 441)	*E. coli* (MTCC, 1687)	*Bacillus subtilis* (MTCC code, 441)
1	Kanamycin (standard)	Aqueous	11.10 ± 0.2	11.06 ± 0.05	0.00 ± 0.00	0.00 ± 0.00
2	PF-M	Methanol	4.00 ± 0.08	01.00 ± 0.05	0.00 ± 0.00	0.00 ± 0.00
3	PF-C	Chloroform	0.30 ± 0.04	0.20 ± 0.02	0.00 ± 0.00	0.00 ± 0.00
4	PF-H	*n*-Hexane	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
5	PF-W	Aqueous	0.50 ± 0.10	0.80 ± 0.05	0.00 ± 0.00	0.00 ± 0.00

PF-M papaya flower–methanol, *PF-C* papaya flower–chloroform, *PF-H* papaya flower–*n*-hexane, *PF-W* papaya flower–aqueous

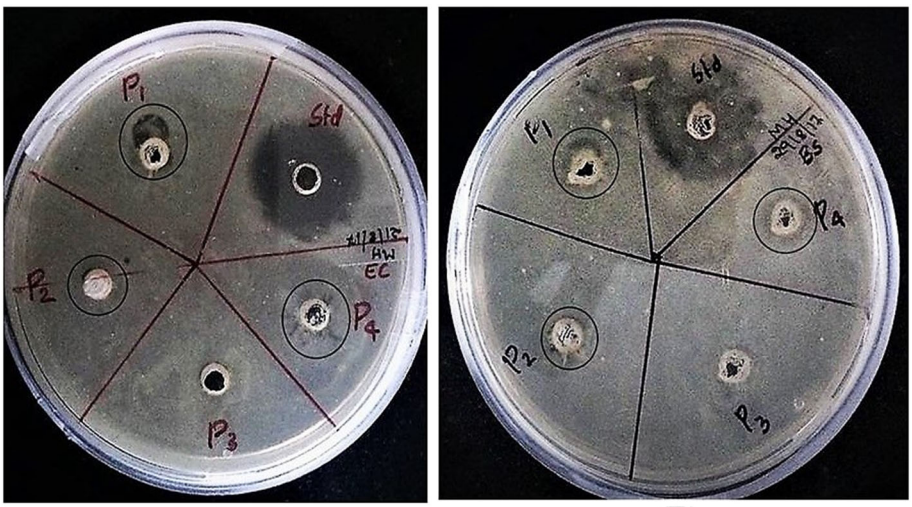

Fig. 3 Antibacterial activity of methanol (P1), chloroform (P2), *n*-hexane (P3), and aqueous (P4) extracts of fresh papaya flowers using well diffusion assay. **a** Activity against *Escherichia coli* (Gram-negative). **b** Activity against *Bacillus subtilis* (Gram-positive)

stimulation, protection, flavoring, communication, and pigmentation [29]. Flavonoids are also known to protect plants against stress and are valued as rich antioxidants [30]. The flavonoids are anti-allergic, anticancer, hepatprotective, anti-diabetic, antibacterial, anti-inflammatory, and anti-viral in nature [31–35]. The saponins are vast group of glycosides with foam forming and detergent properties [36–38]. Saponins are known to be antimicrobial, anti-malarial, anti-allergic, anti-diabetic, insecticidal, and anti-inflammatory in nature [10, 39–42]. The tannins are complex of polyphenolic compounds that are synthesized by plants [39]. These are known to protect plants against herbivores and insects [43, 44]. Tannins have also been reported to have antioxidant, antibacterial, anti-viral, anti-tumor, and anti-inflammatory activities [45–51]. The presence of all these compounds in the papaya flower highlights the importance of the papaya flower in the ailment of various diseases. The tribal communities are well aware of the health benefits of papaya flower as they are using it in various ethnomedicinal preparations.

Spectroscopic and chromatography techniques have become a powerful tool for secondary metabolite profiling as well as for qualitative and quantitative analysis of pharmaceutical and biological materials. The current study of flower extracts revealed that the presence of phenolic compounds, flavonoid compounds, and their derivatives which indicate the medicinal properties of *C. papaya*. The UV-visible spectrum data was compared with literature data, and the presence of phenolic, flavonoids, their derivatives, and other secondary metabolites was indicated in different plant extracts. It is known that the spectra of phenolic compounds and flavonoids typically lie in the range of 230–290 nm [52] or between 300–350 nm [53]. Thus, our result also confirms the presence of phenolics and flavonoids in different plant extracts.

Nutritional value of food is mainly based on total flavonoid and phenolic content. Both are considered as the index of medicinal values of natural products [51]. Maximum yield of phenolic compounds was observed in hexane extract. Aqueous extract displayed minimal presence of the phenolics that might be due to the presence of impurities [47]. The phenolic compounds have redox properties and have facilitated radical scavenging by their hydroxyl groups, which allow them to act as antioxidants [49]; however, the presence of phenolic contents did not correlate to the antioxidant properties of the different extracts [54]. Statistical analysis showed strong significant difference in total phenolics among different solvents extraction of *C. papaya* (Table 2). Flavonoids synthesized by the plants also possess antioxidant properties. Flavones and flavanols are the major compounds responsible for the antioxidant activity, which depends on the presence of free OH groups. In the current study, maximum flavonoid content was observed in *n*-hexane extract, while the minimum content was present in the aqueous of *C. papaya* flowers. Statistical analysis showed strong significant difference among flavonoid contents of different extracts of *C. papaya* flower. The general assessment of the results is that the *C. papaya* flowers are rich in flavonoid and phenolic compounds that are responsible for the medicinal properties of the flower.

Recently, much attention has been given to natural antioxidants with several health benefits. Antioxidant compounds are known to prevent or treat many complex

diseases. The medicinal plants produce many types of secondary metabolites with antioxidant properties. The antioxidant potential is based on redox properties that facilitate the activity as reducing agents, hydrogen donors, singlet oxygen quenchers, and metal chelators [55]. Polyphenols are considered the index of antioxidant potential of fruits and vegetables. Different assays are being carried to quantify the antioxidant strength. DPPH free radical scavenging assay is considered one of the best authentic assay for antioxidant study [25]. In the current study, the *n*-hexane extract of *C. papaya* flower displayed maximum antioxidant activity followed by the methanol, chloroform, and aqueous extracts. The difference in the activity might be due to the difference in polarity of extracted solvents and compounds. The DPPH free radical scavenging assay results displayed significant difference in scavenging activity among different solvents as shown in Table 2. The current study also analyzed the antibacterial activity of different papaya flower extracts. Until date, very little information is available regarding the antimicrobial activity of the papaya flowers. Results demonstrated that methanol extract to be the most potent active extract against *E. coli* and *B. subtilis* followed by aqueous, chloroform, and *n*-hexane extracts. The active secondary metabolites present in the methanol extract need to be identified for further development of antibacterial drugs. The quality and quantity of the therapeutic compounds vary according to the solvents and the chemotypical variation in different plants. Several studies report the antibacterial activity in papaya plants. Peter et al. reported inhibitory activity of 70% methanolic extract of *C. papaya* seeds against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *E. coli* [56]. The seeds of the papaya plant contain crude proteins, crude fiber, carpaine, benzylisothiocynate, benzylglucosinolate, glucotropacolin, benzylthiourea, hentriacontane, β-sistosterol, caricin, and an enzyme nyrosin [56]. Methanolic extracts of papaya leaves also displayed antibacterial activity against *S. aureus* and *E. coli* [9]. These studies demonstrate that importance of organic extracts against bacterial strains [57]. The papaya leaves are known to contain carpaine, which kills microorganisms that often interfere with the digestive function [26, 58]. Papaya leaf extracts have phenolic compounds, such as protocatechuic acid, *p*-coumaric acid, 5,7-dimethoxycoumarin, caffeic acid, kaempferol, quercetin, and chlorogenic acid [26, 51, 56, 59, 60]. Alkaloids carpaine, pseudocarpaine and dehydrocarpaine I and II, choline, carposide, and vitamin C and E are also present. The plant flowers also contain the vitamins such as thiamine (B1), riboflavin (B2), niacin (B3), and ascorbic acid (C) [5]. Flowers are reported to contain minerals such as for calcium, magnesium, manganese, zinc, copper, cadmium, cobalt, lead, iron, potassium, and sodium [5]. All these results demonstrate the importance of papaya as a medicinally important plant. Activities from leaves and seeds had displayed the presence of important molecules. The active flower extracts need to be characterized further for identification of the active molecules with antibacterial and antioxidant activities.

5 Conclusion

This study was undertaken to evaluate the antioxidant and antibacterial properties of the *C. papaya* fresh flowers. The metabolites present in the organic extracts displayed significant antibacterial and antioxidant activities. It was observed that the presence of phenolic compound or flavonoids leads to enhanced antibacterial activity. The *n*-hexane extracts displayed strong antioxidant activity. Our results strongly validate the traditional use of papaya flowers for treating different ailments by the tribal communities.

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Authors' contributions
MKD, SS, SM, and DKP: Collected the data, analyzed the data, and wrote the paper. PKS: Designed the study, analyzed the data, and wrote the manuscript. All authors have read and approved the final manuscript.

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The authors declare that they have no competing interest.

Author details
[1]Department of Biotechnology, Indira Gandhi National Tribal University, Amarkantak, Madhya Pradesh 484887, India. [2]Department of Environmental Science, Indira Gandhi National Tribal University, Amarkantak, Madhya Pradesh 484887, India.

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