

## Phytochemical Characterization and Biological Activities of *Docynia indica* (wall) Fruit Extracts

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### Abstract

With increase in awareness about adverse effects of chemical use in food, there is a continuous effort for search of natural alternatives. Several plant extracts and their purified compounds are being explored for their biological efficacy. In the present study, *Docynia indica*, which grows as a wild tree in North Eastern region of India has been explored for its possible use as a source of natural preservatives. Although some reports about biological activities of *D. indica* fruit exist in literature, not much is known about its phytochemical profile. Various fruit extracts prepared at different maturity stages of *D. indica* fruits were analysed for their *in vitro* antioxidant and antimicrobial potential, and phytochemical profile of their methanolic extracts were determined by high performance liquid chromatography. The immature fruit extract showed high concentration of total phenolics (upto 196.15 mg gallic acid equivalent/ g extract) and flavonoids (up to 100.49 mg rutin equivalent/ g extract) depending on the extraction solvent; and also showed higher antioxidant activity in *in vitro* assays. Although no definite trend was observed for antibacterial activity based on maturity stages, extraction with the mixture of methanol, acetone and water (1:1:1) was found to show least minimum inhibitory concentration for all the maturity stages. Catechin and ferulic acid were the major phenolics present in *D. indica* fruits. The antioxidant and antibacterial compounds present in various extracts of *D. indica* indicate its potential for utilization as food preservative.

**Keywords:** *Docynia indica*; Antioxidant; Antibacterial; Phenolics; Preservative

### Introduction

Fruits and vegetables are reported to contain varying amount of biologically active compounds, which give them value beyond their nutritional importance. Antioxidants present in various plants have the capacity to scavenge free radicals and thereby reduce the risk of certain diseases [1,2]. Currently, wild plants are being explored to discover potential antioxidant. Many wild fruits are still unknown or inadequately exploited despite their good nutritional and nutraceutical values. The dietary intake of these fruits has a strong inverse correlation with the risk of developing coronary heart disease and cancer. In fruits, vitamins C, A and E, and polyphenols are known to be responsible for their antioxidant activity, with polyphenols being the most active [3]. Furthermore, fruits and vegetables are rich source of antimicrobial compounds. Several plant parts showing high phenolic content has been reported to possess good antimicrobial properties. Plants contain certain bioactive compound which hampers the growth of microorganism [4,5], and in developing countries, people still use plants as a medicine to treat infectious bacterial diseases [6]. Extensive studies are going on to establish antimicrobial activity of several plant extract, and there is a possibility to find out encouraging alternative for synthetic medicine [7,8].

*Docynia indica* (wall.) is a wild edible plant, and the fruits of this plant are well known in North-eastern area of India for their nutritive value. The fruit of *D. indica* are sour in taste, and are reported to contain sugar, organic acid, phosphorus and iron. For a long time the fruit has been used as natural remedy for treatment of infectious diseases, digestive aid and it also showed hypoglycemic and hypolipidemic effect [9]. Although some reports about biological activities of *D. indica* fruit exist in literature [9,10], they provide a very limited information. Further, not much is reported about the phytochemicals present in *D. indica* fruits. Therefore, in the present investigation, a systematic effort was made to analyse various biological activities of *D. indica* fruits at different stages of maturity. The phytochemical profile of fruit methanolic extracts was also determined using high performance liquid

chromatography (HPLC) in order to identify compounds responsible for various biological activities.

### Materials and Methods

#### Reagents

Most of the solvent used for extraction (AR grade) were purchased from Qualigens Fine Chemical (Mumbai, India). Diethyl ether and ammonium acetate were purchased from Rankem (New Delhi, India), and Folin ciocalteu (FC) reagent was from Sisco Research Laboratory (Mumbai, India). 1,1-diphenyl-2-picrylhydrazyl (DPPH) and standards used in HPLC (gallic acid, catechin, chlorogenic acid, p-coumaric acid, quercetin, ferulic acid, caffeic acid, syringic acid, tannic acid, cinnamic acid and rutin) were purchased from Sigma-Aldrich (Bangalore, India).

#### Preparation of fruit extract

*Docynia indica* (wall.) fruits at different maturity stages (mature and immature) were collected from Manipur, India. Fruits were cleaned and cut into slices and dried in hot air oven at 50 to 55°C. One set of mature fruits were treated with potassium metabisulfite (KMS, 0.5%) and dried under similar conditions. Dried fruit slices were powdered in a grinder, and passed through a screen (250 µ). Fruit powder (2 g) was then used for the preparation of extract in 50 ml of solvent (Methanol, Acetone, Ethanol, mixture of Methanol: Acetone: Water (1:1:1), and Water) by placing over a magnetic shaker (2 h, 50°C). Fresh fruit extract

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was prepared by macerating fresh mature fruit followed by extraction in the above mentioned solvent (1:5; weight by volume). Extract thus obtained were filtered through Whatman filter paper No. 1, and the filtrate was concentrated in vacuum at 55°C on rotary evaporator (Buchi, Model R-205, Germany). These crude extract were dissolved in known volume of solvent used for their extraction (50 mg/mL), and stored under refrigerated conditions until further analysis.

### Determination of total polyphenol content

The total polyphenol content (TPC) was estimated by spectrophotometry, using Gallic acid as standard [11]. Briefly, 100 µL of the diluted sample extract was transferred to separate tubes containing 5.0 mL of a 1:10 dilution of Folin-Ciocalteu's reagent in water. Then, 4.0 mL of a sodium carbonate solution (7.5% w/v) was added. The solution was made up to 10 mL with water, mixed and all the tubes were then allowed to stand at room temperature for 45 min. Then absorbance was measured against reagent blank at 765 nm using UV-visible spectrophotometer (Shimadzu UV-1601 PC, Kyoto, Japan). TPC was expressed as mg Gallic acid equivalents (GAE)/g extract by comparing with a standard gallic acid (5-100 µg) curve.

### Estimation of flavonoid content

Flavonoid concentration in *D. indica* fruit extract was measured by the Aluminium chloride colorimetric assay [12]. Standard used was Rutin prepared in a concentration of 0.1 mg/mL. Aliquot of all extract or standard (0.1 mL-0.9 mL) was added to 10 mL volumetric flask, mixed with 0.3 mL of 5% NaNO<sub>2</sub>. After 5 min, 0.3 mL of 10% AlCl<sub>3</sub> was added. At 6<sup>th</sup> min, 2.0 mL of NaOH (1N) was added and total volume was made up to 10 mL with distilled water. The solution was mixed well in a vortex and the absorbance was read against reagent blank at 510 nm. All the extracts/standard (rutin, 10-90 µg) were taken in triplicate, and total flavonoid content was expressed as mg Rutin equivalents (RE)/g of extract.

### Determination of the free radical scavenging activity by 1,1-diphenyl-2-picrylhydrazyl (dpph) free-radical scavenging assay

The antioxidant activity of different extract of the *D. indica* fruit was evaluated using the DPPH free radical scavenging assay [13]. Test sample (20-500 µg) was added to 4 mL of 100 µM methanolic DPPH. It was incubated for 20 min at room temperature in the dark and the absorbance was measured at 517 nm using methanol as blank. Ascorbic acid (10-100 µg) was used as standard. Control was prepared without adding standard or test compound. The capability to scavenge the DPPH radical was calculated using the following equation.

$$\% \text{Inhibition} = \frac{OD \text{ of control} - OD \text{ of sample}}{OD \text{ of control}} \times 100 \quad (1)$$

The IC<sub>50</sub> was calculated from the graph of concentration versus % inhibition, and it was determined in triplicate.

### Determination of ferric reducing antioxidant potential (FRAP)

FRAP method is based on the principle of reduction of a ferric-tripyridyltriazine complex to its coloured ferrous form in the presence of antioxidants [14]. The FRAP reagent [2.5 mL of a 10 mM/L TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM/L HCl plus 2.5 mL of 20 mM/L FeCl<sub>3</sub> and 25 mL of 300 mM/L acetate buffer, pH 3.6] was prepared freshly and warmed at 37 °C. Aliquots of 0.1 mL sample supernatant were mixed with 0.2 mL distilled water and 1.8 mL FRAP reagent, and the reaction mixture was kept for incubation (10 min at

37 °C), after which the absorbance of reaction mixture was measured at 593 nm spectrophotometrically. The FeSO<sub>4</sub> solution (200 mg/L) was used as the standard solution. Results were expressed as mM Fe<sup>2+</sup>/g extract using a FeSO<sub>4</sub>·7H<sub>2</sub>O calibration curve (5-20 µM).

### Antibacterial activity

The antibacterial study of extracts was carried out against *Bacillus cereus* (F 4810, Public Health Laboratory, London, UK), *Staphylococcus aureus* (FRI 722, Public Health Laboratory, Amsterdam, The Netherlands), *Listeria innocua* (CFR 1309, maintained in culture stock at CSIR-CFTRI, Mysore), *Escherichia coli* (ATCC 25922, American Type Culture Collection, Minnesota, USA), *Pseudomonas aeruginosa* (ATCC 27853, American Type Culture Collection, Minnesota, USA) and *Yersinia enterocolitica* (MTCC 859, Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India) by broth microdilution assay [15]. The bacterial culture was prepared by transferring a loop full of individual culture from stored slants at 4°C to sterilized Muller Hilton Broth (MHB) and it was incubated at 37 °C overnight. The grown culture was diluted to 7 log cells using serial dilution method in MHB for use in experiments. The known concentration of extract (0.5-15 mg/ mL) diluted in MHB was added into the 96 well plates. Wells without extracts were used as control and well with equal quantity of solvent served as negative control to check the effect of solvent on bacterial growth. All the wells were inoculated with 5 log cells of bacterial culture. The plate was incubated at 37°C for 24 hours and observed for growth. The minimum inhibitory concentration (MIC) was defined as the concentration at and above which no visible growth of the organism was observed. The MIC was confirmed in four separate experiments.

### Chromatographic conditions

A Shimadzu chromatograph (LC-10A Japan) with a Shimadzu SPD-M10A PDA detector at 280 and 320 nm was used for HPLC analysis. The phenolics present in *D. indica* fruits extracted in methanol were separated by liquid chromatography [16]. A supelco C18 (5 µm) column of 15 cm length and 4.6 µm id (Supelco, Bellefonte, PA, USA) and diode array detector operating at 280 and 320 nm were used for separation and identification of phenolics. The samples were eluted according to gradient program with flow rate of 1 ml/min. Acetonitrile (A) and 2% acetic acid in water (B) were used as mobile phase, and the following conditions for HPLC were applied: 0.01-30 min 100% of B; 30-50 min 85% of B; 50-55 min 50% of B; and 55-60 min 100% of B. Total duration for chromatographic analysis was 60 min. All solvent used for HPLC were degassed using vacuum filter. Mixture of standards of phenolics (2-10 µg) were used for identification and quantification of individual phenolics.

### Statistical analysis

Data were presented as mean value ± standard deviation. ANOVA was performed and means were compared using Turkeys test. Significance was determined at 5% level. The correlation coefficients (R) to determine the relationship between two variables were calculated using MS Excel software (CORREL statistical function).

## Results and Discussion

### Total phenolic and flavonoid content

The total phenolic content (TPC) and total flavonoid contents (TFC) were investigated in fresh mature fruits, powders obtained from immature (IDIFE), mature (MDIFE) and KMS treated fruit (KDIFE), and the results are presented in Tables 1 and 2, respectively. Except

Extract	Fresh fruit	MDIFE	IDIFE	KDIFE
Methanol 80%	22.48 ± 0.27 <sup>a</sup>	36.82 ± 0.34 <sup>a</sup>	196.13 ± 3.07 <sup>a</sup>	96.39 ± 2.65 <sup>a</sup>
Ethanol 80%	13.34 ± 0.12 <sup>c</sup>	24.22 ± 0.69 <sup>d</sup>	127.93 ± 2.4 <sup>b</sup>	64.5 ± 1.46 <sup>c</sup>
Acetone 80%	20.97 ± 0.16 <sup>b</sup>	32.60 ± 0.29 <sup>c</sup>	104.87 ± 0.89 <sup>d</sup>	79.89 ± 0.92 <sup>b</sup>
MAW*	11.35 ± 0.10 <sup>d</sup>	34.81 ± 0.22 <sup>b</sup>	115.1 ± 3.74 <sup>c</sup>	74.71 ± 1.53 <sup>b</sup>
Water	4.90 ± 0.06 <sup>e</sup>	19.53 ± 0.28 <sup>e</sup>	55.21 ± 3.62 <sup>e</sup>	41.83 ± 0.94 <sup>d</sup>

Abbreviations: MDIFE: Mature *D. Indica* Fruit Extract; IDIFE: Immature *D. Indica* Fruit Extract; KDIFE: Potassium Metabisulfite Treated Mature *D. Indica* Fruit Extract. Values followed by different letter in a column are significantly ( $p < 0.05$ ) different from each other. \*mixture of methanol, acetone and water (1:1:1).

**Table 1:** Total phenolic content (Mg GAE/G of Extract) of *Docynia Indica* fruit extract.

Extract	Fresh fruit	MDIFE	IDIFE	KDIFE
Methanol 80%	11.76 ± 0.21 <sup>a</sup>	22.62 ± 0.21 <sup>a</sup>	87.97 ± 3.32 <sup>b</sup>	52.11 ± 1.33 <sup>a</sup>
Ethanol 80%	7.64 ± 0.05 <sup>c</sup>	21.39 ± 0.26 <sup>a</sup>	77.82 ± 1.46 <sup>c</sup>	38.16 ± 0.44 <sup>b</sup>
Acetone 80%	13.21 ± 0.65 <sup>b</sup>	22.80 ± 0.61 <sup>a</sup>	77.88 ± 3.55 <sup>c</sup>	58.10 ± 4.27 <sup>a</sup>
MAW*	5.47 ± 0.15 <sup>d</sup>	21.98 ± 0.46 <sup>a</sup>	100.49 ± 4.14 <sup>a</sup>	34.18 ± 0.64 <sup>b</sup>
Water	1.11 ± 0.11 <sup>e</sup>	15.49 ± 0.48 <sup>b</sup>	46.91 ± 0.40 <sup>d</sup>	39.37 ± 1.86 <sup>b</sup>

Abbreviations: MDIFE: Mature *D. Indica* Fruit Extract; IDIFE: Immature *D. Indica* Fruit Extract; KDIFE: Potassium Metabisulfite Treated Mature *D. Indica* Fruit Extract. Values followed by different letter in a column are significantly ( $p < 0.05$ ) different from each other. \*mixture of methanol, acetone and water (1:1:1).

**Table 2:** Total flavonoid content (Mg RE/G of Extract) of *Docynia Indica* fruit extract.

Extract	Fresh fruit	MDIFE	IDIFE	KDIFE
Methanol 80%	121.52 ± 0.47 <sup>d</sup>	49.05 ± 0.22 <sup>b</sup>	46.11 ± 0.84 <sup>b</sup>	36.78 ± 0.3 <sup>d</sup>
Ethanol 80%	185.58 ± 0.62 <sup>b</sup>	43.46 ± 0.98 <sup>c</sup>	42.3 ± 0.52 <sup>c</sup>	42.27 ± 0.74 <sup>c</sup>
Acetone 80%	116.17 ± 0.32 <sup>e</sup>	33.58 ± 0.46 <sup>d</sup>	32.89 ± 0.69 <sup>d</sup>	32.62 ± 0.45 <sup>e</sup>
MAW*	127.26 ± 0.75 <sup>c</sup>	45.18 ± 0.81 <sup>c</sup>	40.85 ± 0.90 <sup>c</sup>	47.85 ± 0.56 <sup>b</sup>
Water	209.99 ± 0.96 <sup>a</sup>	68.51 ± 0.78 <sup>a</sup>	70.05 ± 0.92 <sup>a</sup>	114.53 ± 1.42 <sup>a</sup>

Abbreviations: MDIFE: Mature *D. indica* Fruit Extract; IDIFE: Immature *D. indica* Fruit Extract; KDIFE: Potassium Metabisulfite Treated Mature *D. indica* Fruit Extract. Values followed by different letter in a column are significantly ( $p < 0.05$ ) different from each other. \*mixture of methanol, acetone and water (1:1:1).

**Table 3:** Antioxidant activities of *Docynia indica* fruit extract (DPPH assay, IC<sub>50</sub> value, µg/mL).

Extract	Fresh fruit	MDIFE	IDIFE	KDIFE
Methanol 80%	14.25 ± 0.46 <sup>b</sup>	80.68 ± 2.16 <sup>b</sup>	352.07 ± 2.49 <sup>b</sup>	151.87 ± 6.16 <sup>a</sup>
Ethanol 80%	8.28 ± 0.02 <sup>d</sup>	100.1 ± 2.02 <sup>a</sup>	405.87 ± 6.56 <sup>a</sup>	121.69 ± 4.93 <sup>b</sup>
Acetone 80%	19.52 ± 0.45 <sup>a</sup>	94.66 ± 4.03 <sup>a</sup>	346.37 ± 6.86 <sup>b</sup>	129.32 ± 1.55 <sup>b</sup>
MAW*	10.63 ± 0.07 <sup>c</sup>	94.83 ± 1.18 <sup>a</sup>	258.41 ± 6.14 <sup>c</sup>	130.49 ± 2.19 <sup>b</sup>
Water	5.92 ± 0.23 <sup>e</sup>	6.42 ± 0.24 <sup>c</sup>	9.14 ± 0.24 <sup>d</sup>	7.57 ± 0.51 <sup>c</sup>

Abbreviations: MDIFE: Mature *D. Indica* Fruit Extract; IDIFE: Immature *D. Indica* Fruit Extract; KDIFE: Potassium Metabisulfite Treated Mature *D. Indica* Fruit Extract. Values followed by different letter in a column is significantly ( $p < 0.05$ ) different from each other. \*mixture of methanol, acetone and water (1:1:1).

**Table 4:** Antioxidant activities of *Docynia Indica* fruit extract (FRAP Assay, Mm Fe<sup>2+</sup>/G of Extract).

a few cases, the TPC and TFC extracted by various solvent differed significantly ( $p < 0.05$ ). The maximum concentration of phenolic compounds was observed in IDIFE, and highest extraction of phenolics (196.13 mg GAE/g extract) was obtained with methanol which was significantly ( $p < 0.05$ ) higher than other solvents. Similarly, IDIFE showed highest concentration of flavonoids by extraction with mixture of methanol, acetone and water (100.49 mg RE/g extract), indicating no definite trend for extraction of phenolics and flavonoids with different

solvents. Phenolics and flavonoids in immature fruits were higher than mature fruits for all the solvent extracts. Significantly low amount of phenolics and flavonoids were extracted by water extraction, with exception of flavonoids for extraction with mixture of methanol, acetone and water. Flavonoids and polyphenol concentration in fruit extracts vary according to polarity of solvents used in the extract preparation [17,18]. However, there was a general trend of decrease in their extraction with increase in polarity of solvents.

The results showed that IDIFE showed highest phenolic and flavonoid content for all the solvents, which was followed by KDIFE. In many food industries, KMS is used for preservation and to prevent browning. Potassium in KMS inhibits enzyme polyphenol oxidase [19], which might be a possible reason of high phenolic content in KMS treated fruit powder extract. The fresh fruit extract had lowest phenolics and flavonoids, which is an expected result as other extracts were prepared from dry powder, where phenolics and flavonoids were concentrated.

### DPPH radical scavenging activity

Free radicals have crucial role in auto-oxidation of food. By contributing hydrogen from the phenolic hydroxyl groups, polyphenol interrupt the free radical chain of oxidation as stable radicals are formed, which cannot propagate further [20]. The ability of the polyphenol extracts to act as free radical scavengers against the DPPH radical was tested spectrophotometrically by measuring drop in absorbance at 517 nm after adding the different extract solutions. The results indicate that among the five solvents used, acetone and water exhibited the highest and the least scavenging activity ( $p < 0.05$ ), respectively. The IC<sub>50</sub> value in KDIFE and IMDIFE were found to be lower than fresh fruit extracts (Table 3). Similar IC<sub>50</sub> value for methanol extract of *D. indica* fruit has been reported in literature [10], however, variation in DPPH radical scavenging activity similar to *D. indica* among various extracts was also reported for *Garcinia cowa* [21].

### Ferric reducing antioxidant potential (FRAP)

The antioxidant potential of different extract of *D. indica* fruit was also determined on the basis of their ability to reduce ferric (III) iron to ferrous (II) iron using FRAP reagent [22]. At low pH, reactants reduce the ferric tripyridyl triazine [Fe (III) TPTZ] complex to ferrous tripyridyltriazine [Fe (II)-TPTZ], which has an intense blue colour. The change in absorbance was correlated to the reducing power of the electron donating antioxidants present in the *D. indica* fruit extract. The FRAP activities of all extracts of *D. indica* fruit were expressed as the concentrations of antioxidant having a ferric reducing ability equivalent to mM of FeSO<sub>4</sub> (Table 4). Among all the stages, the FRAP value was the highest for IDIFE, and among extracts, ethanol extract showed significantly ( $p < 0.05$ ) highest FRAP value. KDIFE also showed higher FRAP values, however, methanol was the best ( $p < 0.05$ ) solvent for KMS treated fruits. FRAP value is reported to change with change in bioactive compounds [23], and probably variable phenolics (Table 1) and flavonoids (Table 2) were responsible for differences in the variable FRAP values of *D. indica* fruit extract.

### Antibacterial activity

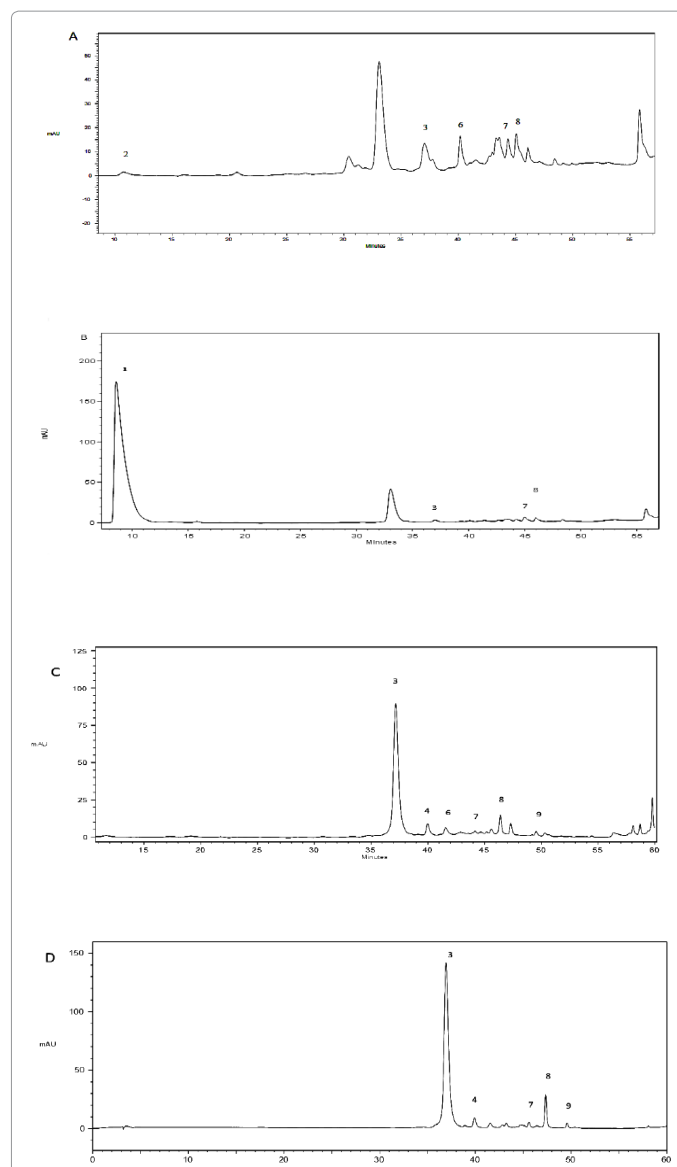
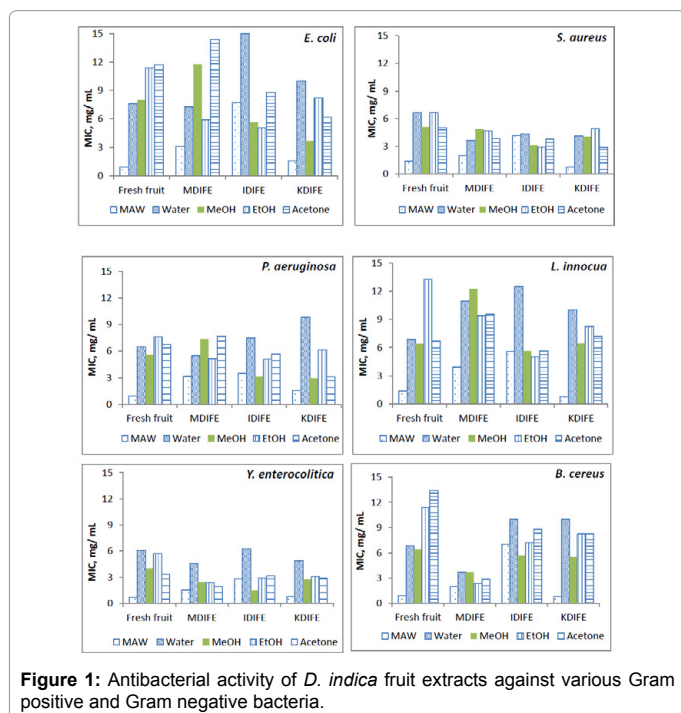
The antibacterial activity of various extracts (Figure 1) was determined against 3 Gram positive (*B. cereus*, *S. aureus*, and *L. innocua*) and 3 Gram negative (*E. coli*, *P. aeruginosa* and *Y. enterocolitica*) bacteria using broth micro-dilution method. Methanolic extract of IDIFE; and extraction of fresh fruit, MDIFE and KDIFE with mixture of methanol, acetone and water were most effective as they showed lowest minimum



inhibitory concentration (MIC) values against all the tested bacteria. Although, there was no definite trend for antibacterial effect among extracts, KDIFE showed the lowest MIC values; and among solvents used for extraction, extraction with water showed the highest MIC values. However, these MIC values were much higher than the standard Nisin. The lowest MIC values among various maturity stage and solvent used for extraction observed were 0.78, 0.78, 0.68 and 0.91 mg/ mL for *B. cereus*, *S. aureus*, *Y. enterocolitica* and *E. coli*, respectively, which were 8-10 times higher than Nisin for the same bacterial cultures (0.10, 0.09, 0.07 and 0.09 mg/mL, respectively) reported in our earlier study [24]. Use of methanol resulted in better extraction of antimicrobial compounds in this study, and higher activity of methanolic extract is also reported in literature for seabuckthorn seeds [18] and *Peltophorum ferrugineum* flowers [25]. Although various plant extracts are shown to be more effective against Gram positive bacteria than Gram negative bacteria [7], in the present study we did not observe a definite trend. Variable antibacterial activity has been reported against various bacteria for *D. indica* [26], and differences in antibacterial activity of among extracts of *Nigella sativa* [27] and *Plectranthus amboinicus* [28] also have been reported. Various mechanisms for antimicrobial effect of plant extracts are proposed [7], and probably fruit extract of *Docynia indica* are acting on genetic material of bacteria similar to the fruit and bark extracts of *Dillenia indica* [29].

### HPLC analysis

As the methanolic extract of fruits showed highest phenolic concentration at various maturity stages (Table 1), identification of phenolic compounds present in methanolic extract of fresh fruit, IMDIFE, MDIFE, and KDIFE was done by HPLC (Figure 2). Phenolic compound were tentatively identified by comparing with retention times of standards. Gallic acid was detected only in fresh fruit and tannic acid was present only in MDIFE. Catechin (82.85 mg/g of extract) was most predominant phenolic acid observed in fresh fruit extract, followed by rutin (5.89 mg/g of extract), ferulic acid (4.71 mg/g of extract) and gallic acid (3.36 mg/g of extract). KDIFE showed higher concentration of catechin (127.90 mg/g of extract) and ferulic



**Figure 2:** HPLC chromatogram of various *Docynia indica* fruit methanolic extracts. (A): Fresh fruit, (B): Mature *D. indica* fruit extract (C): Immature *D. indica* fruit extract, (D): Potassium metabisulfite treated mature *D. indica* fruit extract KDIFE 1-Tannic acid, 2-Gallic acid, 3-Catechine, 4-Caffeic acid, 5- Chlorogenic acid, 6- Syringic acid, 7- Rutin, 8- Ferulic acid, 9- Quercetin.

acid (1.83 mg/g of extract) than IMDIFE (113.98, 1.35 mg/g of extract) and MDIFP (1.69, 0.18 mg/g of extract), respectively. Caffeic acid was present only in IMDIFE (3.30 mg/g of extract) and KDIFE (2.11 mg/g of extract); similarly syringic acid was quantified in fresh fruit (11.21 mg/g of extract) and IMDIFE (0.64 mg/g of extract). Quercetin was recorded in IMDIFP and KDIFE, and it was found to be 0.43 and 0.40 mg/g of extract, respectively. Chlorogenic acid and cinnamic acid were not detected in any of the extract. There is no study on quantification of individual phenolics in *D. indica*, however variable levels of phenolics in stem [24] and leaf [30] extracts of *Plectranthus amboinicus* are reported in literature.

### Correlation among phytochemicals and biological activity

The correlation analysis between phytochemicals and biological activity gave mixed correlation coefficient. While there was a high

correlation between TPC and DPPH (0.86 for KDIFE), TPC and FRAP (0.82 for IDIFE, 0.87 for fresh fruit, and 0.91 for KDIFE), Flavonoid content and DPPH (0.90 for MDIFE), flavonoid content and FRAP (0.94 for MDIFE and 0.87 for fresh fruit), all other combinations showed coefficient below 0.80. Similarly, the correlation coefficient between TPC and antibacterial activity were 0.86 against *P. aeruginosa*, 0.85 against *E. coli* for KDIFE, 0.90 against *Y. enterocolitica* and 0.82 against *B. cereus* for IDIFE. Total flavonoid content showed high correlation only in case of IDIFE (*P. aeruginosa*, 0.93; *B. cereus*, 0.91; *Y. enterocolitica*, 0.87; *L. innocua*, 0.86; and *E. coli*, 0.79). Literature survey also reveals that relationship between polyphenol content and antioxidant activity is contradictory. While in some study a high correlation between the two has been observed [31], other study showed that there is no or very weak correlation [32].

## Conclusion

In the present study, it was observed that the immature *D. indica* fruit extracts had high concentration of total phenolics and flavonoids, and they exhibited higher antioxidant activity in DPPH and FRAP assay. Although no definite trend was seen in antibacterial activity assay, extraction with mixture of methanol, acetone and water showed higher antibacterial activity against tested bacteria. However, their potential as antifungal agent or antiviral agents need to be studied, as *D. indica* fruit extracts are rich in various phenolic compounds, which are known to be good antiviral agents. The antioxidant and antibacterial compounds present in various extracts of *D. indica* showed its potential for utilization as food preservative, however their behaviour in food system needs deeper study.

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