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Research Paper

Potent nutraceuticals having antioxidant, DNA damage protecting potential and anti-cancer properties from the leaves of four *Ficus* species

Short Title – Potent nutraceuticals from leaves of four *Ficus* species

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Abstract

In the present study, we have evaluated fractionation based phytochemical constituents, antioxidant activity, DNA damage protecting potential and anticancerous properties of leaves of common *Ficus* species (namely *Ficus virens, Ficus benghalensis, Ficus religiosa, Ficus elastica*) along with GCMS analysis for identification of major bioactive constituents. Methanol fraction of *F. virens* contained maximum amount of phenolics (1267.35 mg GAE/g dry extract) and flavonoids (1080.61 mg QE/g dry extract) whereas hexane fraction of *F. religiosa* possessed highest amount of tannins (123.76 mg TAE/g dry extract). Least amount of phytochemicals was recovered from *F. elastica*. Highest DPPH radical scavenging activity (IC_{50} = 108.28 μg/ml) was detected by methanol fraction of *F. benghalensis* whereas highest ABTS activity (IC_{50} = 105.56 μg/ml) by *F. benghalensis* and highest ferric reducing power by *F. virens* (359.44 mg QE/g dry extract). Leaf methanol fraction of *F. virens, F. religiosa* and *F. elastica* were able to prevent oxidative DNA damage at 0.1 mg/ml. 0.2 mg/ml and 0.3 mg/ml respectively. Viability of normal breast cells was unaffected by methanol fraction of tested *Ficus* species at doses less than 160 μg/ml, whereas survival of breast cancer cells was decreased by *F. benghalensis* at 5 μg/ml. GCMS analysis of the purified methanol fraction of tested species revealed the presence of potent bioactive compounds such as carvacrol, phytol, tocopherol, benzophenone, dibutyl phthalate, lycopersen etc. All our experimental results along with the identification of the bioactive compounds supported the fact that leaves of tested *Ficus* species as rich source of phytochemicals with nutraceutical potentialities.

Key words: *Ficus* species, Antioxidant activity; DNA Protection potential; cytotoxic activity; Phytochemicals.

ABBREVIATIONS

Ficus virens – FV; *Ficus benghalensis* - FB; *Ficus religiosa* - FR; *Ficus elastica* – FE; Hexane Fraction – HF; Ethyl acetate Fraction - EF; Acetone Fraction - AF; Methanol Fraction - MF.

1. INTRODUCTION

Phytochemicals are secondary metabolites which not only have physiological functions in plants but also exert significant pharmacological effects especially for preventing oxidative damage to cells. Extensive research is going on in plant derived natural antioxidants which are largely used in treating damages caused by oxidative stress that leads to permanent cellular injury (Cassidy et al, 2020). It may weaken immune function leading to ulcer, diabetes, proliferation of cancer cells, neurodegenerative diseases, inflammation in joints, aging and other genetic disorders (Darkwah et al, 2018). Synthetic antioxidants may cause some adverse effects on liver function, DNA impairment and induce premature senescence and carcinogenesis etc. (Kornienko et al, 2019). Recently great interest have been focused on using natural antioxidants from plants for drug discovery due to the possible adverse effects of synthetic antioxidants.

Ficus is largest genus belonging to Moraceae or fig family; possesses large varieties of chemical constituents that are responsible for oxidative defense mechanism (Chaudhary et al, 2012; Abdel-Aty et al, 2019). These plant species have wide range of medicinal uses in treating several disorders related to respiratory, cardiovascular system and nervous system (Lansky et al, 2008; Singh et al, 2011). These figs are culturally and economically important plants and considered as edible food for a vast wildlife. They constitute more than one thousand members and are enormously distributed throughout the tropical and subtropical zones (Hendrayana et al, 2019). The main distribution of *Ficus* is seen in Asian-Australian

region consisting of 500 species comprising 66% of world species. Among the Indian provinces, Meghalaya in the north east part have about 43 species which can be considered as a hotspot for *Ficus* species. Maximum diversity however observed in the north-east and peninsular regions (Chaudhury, et al, 2012). Plants belonging to *Ficus* genus are used in various ways throughout tropical and subtropical regions of the world. Traditional ethnobotanical studies revealed many *Ficus* species having great health benefits throughout the world. There are about 735-755 species from the genus of *Ficus* that have been identified by the researchers (Shi, et al, 2018).

The bark of *F. benghalensis* and *F. religiosa* exhibited antidiabetic activities (Gayathri and Kannabiran, 2008; Pandit et al, 2010) while methanolic extract of *F. elastica's* bark showed antiplasmodial and antitrypanosomal activities (Teinkela et al, 2018). The bark methanolic extract of *F. virens* revealed anti-breast cancer and anti-mucositis activities along with inhibitory activity against HMGR (β-hydroxy-β-methylglutaryl-CoA) enzyme (Chen, et al, 2017; Iqbal et al, 2014).

Young leaves of many *Ficus* species are used as traditional medicines and leafy vegetables by tribal and local people (Kumari, H Solanki, 2019). Plant leaves are the rich source of valuable phytochemicals and tribal communities of Asian countries consume over 60 species of green leafy vegetables to fight against hunger, malnutrition and under nourishment (Kubmarawa et al., 2008). Soup made from the young leaves of *F. asperifolia* improves the breast feeding potential of pregnant women (Nkafamiya et al, 2010). The conventional young leaves plays essential role in everyday cooking in rural areas. People of Michika, Hong and Song Local Government areas of Adamawa State, Nigeria consumed young leaves of *F. asperifolia* and *F.sycomorus* which provide substantial nutrients to their normal diet (I. I. Nkafamiya et al, 2010). Since the young leaves of fig trees are reported to have great

nutritional as well as numerous medicinal values. So in our present work, we have selected four *Ficus* species namely *F. virens, F. benghalensis, F. religiosa* and *F. elastica* that are very common throughout the tropics from our nearby locality and evaluated that which fraction is most biologically active from each selected *Ficus* species that can be employed for specific extraction and designing nutraceuticals, having notable antioxidant, DNA damage protecting potential anti-cancer properties and also determination of the main phytocompounds responsible for the activities by undergoing GCMS analysis.

Henceforward detailed characterization of the phytochemical constituents of leaves of four *Ficus* species was performed by liquid–liquid extraction process depending on the specific solubility properties which makes the extraction more accurate and less troublesome. This provides great opportunity to pharmaceutical industries for desired extraction of bioactive compounds in a rapid and simplified manner. Beside elaborative polarity based extraction process, phytochemical profiling and in-vitro antioxidant assays, DNA damage protecting potential and anticancerous activity of the most potent bioactive fraction were done which are distinctive approach to confirm their therapeutic potency. The purified methanol fraction of tested *Ficus* species were subjected to GCMS analysis to identify the major bioactive compounds that can be utilized as a parent moieties for new drug development. Data obtained from all our experiments were validated statistically.

2. Materials and Methods

2.1. Collection of Leaves

Leaves of four *Ficus* species (namely *F. virens*, *F. benghalensis*, *F. religiosa* and *F. elastica*) were collected from the nearby areas of Kolkata (22.6482°N, 88.3768° E), West Bengal, India, from the month of January to March, 2019. These species were identified by Professor Nanda Dulal

Paria, (Former President of Botanical Society of Bengal, Professor and Renowned Taxonomist, Botany Department, Calcutta University) and voucher specimens (No. FV-001, FB-001, FR-001and FE-001) were submitted to the Head, AERU, Indian Statistical Institute, Kolkata, India for keeping record.

2.2. Extraction and isolation of different fractions from leaves of four *Ficus* species

The collected and dried leaves of four *Ficus* species were ground separately into fine powder by keeping the samples into Sample Miller Machine (Cyclotec 1093, TECATOR). Fine powder of leaves of each *Ficus* species were taken separately in each extraction flask (capacity 1000ml) and soaked in 600 ml of methanol. The mixture was stirred by Mechanical Stirrer (NZ-1000s, EYELA) at 3000 rpm for 2 h and clear filtrate was recovered by filtering through sintered disc funnel. Deep brown coloured extract having both polar and nonpolar compounds was collected and concentrated in a rotary vacuum evaporator (Rotavapor: R-3, BUCHI) and considered as a crude extract. This crude extract was further extracted sequentially by hexane, ethyl acetate, acetone and methanol depending on elutropic series. Four fractions were recovered from the crude extract of four *Ficus* species [namely *Ficus virens* (FV) - FVHF, FVEF, FVAF, FVMF; *Ficus religiosa* (FR) – FRHF, FREF, FRAF, FRMF; *Ficus benghalensis* (FB) – FBHF, FBEF, FBAF, FBMF; *Ficus elastica* – FEHF, FEEF, FEAF, FEMF]. It was then purified by consecutive runs through column chromatography with solvent systems. The four fractions (about 5 gm of each fraction) were soaked separately in activated silica gel G (mesh size 60-120) and loaded on to the glass column of 46×2 cm and eluted with firstly in hexane followed by ethyl acetate: hexane with increasing polarity. All the collected fractions were subjected to TLC silica gel 60 F254 plate using suitable solvent system and spots were detected under UV light (365 nm) and in iodine vapour chamber. The purified compounds were measured and kept in air-tight containers at 4°C for further study (Bhattacharya et al, 2019).

2.3. Quantitative phytochemical screening

2.3.1. Estimation of total phenolic content

Folin-Ciocalteu method was carried out (Meda et al, 2005) for estimation of total phenolic content. Firstly, 100 µl of leaf extract (2 mg/ml) was mixed with 2 ml of 10% Folin-Ciocalteu reagent and 1.6 ml of 7.5% Na_2CO_3. The resultant reaction solution was kept for 30 min incubation at room temperature. The spectrophotometric readings were taken at 765 nm. A standard curve was prepared using Gallic acid at a concentration range of 0.03-0.3 mg/ml was used for standard curve preparation. The experiment was replicated thrice and mean was calculated from three readings. The total phenolic content was estimated as gallic acid equivalents (GAE) mg/g dry extract. Gallic acid standard curve follows the resulting equation: $y = 0.223x + (-0.005)$, $R^2 = 0.990$

2.3.2. Estimation of total flavonoid content

Measurement of total flavonoid content was done by using the method of Zhishen et al (1999) with slight modifications (Bhattacharya et. al, 2021). Firstly, 0.4 ml of 5% sodium nitrite ($NaNO_2$) was added to 1 ml of the sample extract (2 mg/ml), mixed uniformly and incubated for 5 min at room temperature. After incubation period, 0.6 ml of 10% $AlCl_3$ solution was mixed to it, followed by further incubation of 5 min at room temperature. 2 ml of 1 M sodium hydroxide (NaOH) solution was used to stop the reaction. The absorbance was read at 510 nm. A calibration curve was prepared by using quercetin in the concentration of 0.03-0.3 mg/ml ($y = 0.5425x + 0.0192$, $R^2 = 0.9599$). Total flavonoid content was calculated as quercetin equivalent (QE) mg/g dry extract.

2.3.3. Estimation of tannin content

Burns method with minor alterations (Burns, 1971) was performed for measuring the tannin

content using tannic acid as standard. At first, 200 µl of extract (2 mg/ml) was mixed with freshly prepared 200 µl of 0.35% ferric ammonium citrate and after that 200 µl of 0.8% ammonia solution was added to it.The volume of the resultant solution was made up to 4 ml by adding water. The absorbance of the resultant solution was assessed at 525 nm. The results were represented as tannic acid equivalent (TAE) mg/g dry extract. Tannic acid standard curve was prepared based on the following equation: y=0.086x + (-0.015), R^2 = 0.960.

2.4. Antioxidant activities

2.4.1. DPPH radical scavenging assay

The free radical scavenging activity for all the fractions was measured in vitro using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical as described by Pavithra and Vadivukkarasi (2015). At first, DPPH solution (0.025 mg/ml) in methanol was prepared and then 3.9 ml of DPPH solution was mixed with 0.1 ml of sample. Plant extract concentration of 2mg/ml were used for each fraction. The mixture was shaken vigorously and left to stand for 30 min and the absorbance was measured at 517 nm. Butylated hydroxy toluene (BHT) was used as standard. All analyses of the samples were done in triplicate and IC_{50} of each was calculated. DPPH radical scavenging capability of the samples was calculated using the following equation:

$$\textbf{DPPH radical scavenging activity (\%)} = \left(\frac{\mathbf{Ac-At}}{\mathbf{Ac}}\right)\mathbf{\times 100}$$

A_c: the absorbance of the blank, A_t: the absorbance in the sample extracts.

2.4.2. ABTS scavenging capacity assay

ABTS radical cation decolorization assay (Re et al, 1999) by all the fractions of leaves of each *Ficus* species was tested to detect ABTS scavenging activity. The ABTS cation radical is reduced by the addition of extract containing antioxidant properties that follows an electron transfer mechanism resulting in decolorization. A mixture of ABTS (7 mM) in water

and potassium persulphate (2.45 mM) was prepared in 1:1 ratio and incubated at room temperature for 12-16 h in dark before use. After incubation 3.9 ml of this solution was taken in a test tube and in that 0.1 ml of sample at 2mg/ml concentration was added. Absorbance was recorded spectrophotometrically at 734 nm after 30 mins of incubation. Quercetin was used as a standard and the degree of decolourization was evaluated to calculate the inhibition percentage of the ABTS cation radical which indicated the antioxidant nature of each extract of the sample.

$$\textbf{ABTS scavenging effect (\%)} = \left(\frac{\mathbf{AB-AA}}{\mathbf{AB}}\right)\mathbf{\times 100}$$

Where AB is absorbance of blank reaction; AA is absorbance in the presence of sample extract. All analyses of the samples were done in triplicate and IC_{50} for each was calculated.

2.4.3. *Reducing Power Assay (RPA)*

Ability of the different fractions of leaves of each *Ficus* species to reduce ferric ions was detected following the modified method described by Oyaizu (1986). Stock sample concentration was 2 mg/ml. Briefly, 1 ml of each extract at different concentration was mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of $K_3Fe(CN)_6$ (1% w/v) and incubated at 50°C for 20 min, to reduce ferricyanide to ferrocyanide. Trichloroacetic acid (10% w/v) of about 2.5ml was utilized to stop the reaction and then centrifuged the reaction solution at 3000 rpm for 10 min. Finally, 2.5 ml of the supernatant was added in the mixture of 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (0.1% w/v). The absorbance was detected at 700 nm. Quercetin (0.3 mg/ml to 2 mg/ml) was taken as standard. The results were expressed as QE/g dry extract (Benslama & Harrar, 2016).

2.5. DNA protection assay against peroxide radical induced damage

DNA protection assay was carried out with the biologically active fraction of leaf extracts of four *Ficus* species using Lambda phage genomic DNA (promega). The oxidative DNA damage (H_2O_2/UV) was induced in presence of leaf extract of each species along with positive (gallic acid) and negative control (deionized water) described by Russo et al (2003). Briefly, the reaction mixture contained 10 µL of Lambda DNA (0.263 µg/mL) which was added in microfuge tubes containing 10 µL each of tris buffer (50 mM, pH 7.4) and H_2O_2 (30% v/v) followed by addition of 10 µL of different concentration (0.1 mg/ml to 0.5 mg/ml) of leaf extract of each species. The tubes were UV irradiated using UV transilluminator (Fischer Scientific) for 45 mins at room temperature. After irradiation 5 µL of 6X Loading dye (bromophenol blue) was added to each tube. The reaction mixtures were loaded on 1% agarose gel in TAE buffer (pH - 8.0) and electrophoresis was performed at 75 V for 1 h followed by ethidium bromide staining.

2.6. Cytotoxicity study on human cell lines

Cytotoxic activities was performed by the most potent fraction of leaves of four *Ficus* species were tested on two breast cell lines MCF-10A (normal cell line) and MDA-MB-468 (breast cancer cell line). These cell lines were obtained from ATCC and preserved in Dulbecco's Modified Eagle Medium with 5% fetal calf serum and 5% antibiotic (penicillin) in incubator at 37°C. Cells were sown in 24 well culture plates in DMEM growth medium at a density of 2.5×10^4 cells/well and incubated overnight in 37°C at 5% CO_2. After 18 h, cells were treated with different concentrations of leaf extract of each *Ficus* species (0 – 160 µg/ml i.e. seven set of experiments starting from concentrations 5 µg/ml, 10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml, 160 µg/ml including control) dissolved in DMSO (Dimethyl sulfoxide), where the final concentration of DMSO was kept below 1%. Further, after 24 h cells were washed

with 1X PBS and then incubated with 0.5 mg/ml of MTT solutions in 1X PBS for 2.5 h (Mosmann, 1983; Stockert, et al, 2018). About 400 µl of DMSO was used to dissolve the Formazan crystals formed within the cells and then absorbance of the solution was measured on a multi well plate reader at 570 nm (Biotech Instruments, USA).

2.7. GCMS analysis of the most potent methanol fractions from each *Ficus* sp

Purified methanol fraction of each *Ficus* sp was subjected to GC-MS Analysis (Model No. AccuTOF GCV Agilent Technologies, GC-6860N Network GC System with 5973 inert Mass Selective Detector) for identification of bioactive compounds. The GC-MS analysis was done at the Sophisticated Analytical Instrument Facility (SAIF) in Indian Institute of Technology, Bombay, HP-1MS column (25 m $\times$ 0.33 mm, i.d. 0.25 µm) was used. Methanol fraction (0.1µl) of each *Ficus* sp dissolved in chloroform was injected into GC in the split mode for analysis at an injector temperature of 280°C. A constant flow of helium as the carrier gas was maintained at a rate of 1 mL/min. The oven temperature was programmed as follows: 50^{O}C(1 min hold), 50°C to 200°C at 7^{O}C/min, 200°C to 300°C at 6°C/min, 200°C (2 min). The mass spectrometer employed the electron ionization mode with an ionization energy of 70 eV. A full scan mode was used with an ion source temperature of 280°C and an acquisition rate of 0.2 s. The mass range was adjusted to 50-350 Da.

The mass spectra with the spectral data of the NBS75K library provided by the GC/MS control and data processing software were compared for the identification of compounds

2.7. Statistical Analysis

All the samples were evaluated in triplicate in all the experimental parameters and the results were enumerated as mean ± standard deviation (SD). Analysis of data was completed using

231 one-way analysis of variance (ANOVA) and two tailed Students T-test. The criterion of
232 significance was pre-determined at values of *p≤0.05 and **p≤0.01. SPSS Statistics 21
233 software was used for all the statistical analyses.

234 # 3. Results

235 ## *3.1. Quantitative Phytochemical Screening*

236 Significant variation in total phenolic, flavonoid and tannin content was observed between
237 different fractions of leaves of four *Ficus* species. The most common and remarkable
238 characteristic among them is that the amount of phytochemicals were found to be maximum
239 in the methanol fraction of leaves of each species as compared to other fractions except
240 hexane fraction of *F. virens* and *F. religiosa* which contained highest amount of tannins (Table
241 1).

242 ### *3.1.1. Total phenolic content*

243 Highest quantity of phenolic was detected in MF of leaves of *F. virens* (1267.35 mg
244 GAE/g of dry extract) followed by *F. benghalensis* (966.05 mg GAE/g of dry extract), *F. religiosa*
245 (925.76 mg GAE/g of dry extract) and finally *F. elastica* (631.71 mg GAE/g of dry extract). Least
246 quantity of phenolics was noticed in EF (86.59 mg GAE/g of dry extract) and HF (66.09 mg
247 GAE/g of dry extract) of *F. elastica.*

248 ### *3.1.2. Total flavonoid content*

249 All the MF of leaves of four *Ficus* species contained maximum amount of flavonoid
250 ranging from 438.22 mg to 1080.61 mg QE/g dry extract in comparison to other fractions.
251 Highest quantity of flavonoids (1080.61 mg QE/g dry extract) was recorded from MF of *F.*
252 *virens* while significant amount (688.91 mg QE/g dry extract) from AF of *F. religiosa*. Least
253 amount of flavonoid (40.98 mg QE/g dry extract) was estimated from HF of *F. elastica.*

3.1.3. *Total tannin content*

Maximum amount of tannin was noticed from HF of leaves of *F. religiosa* (123.76 mg TAE/g dry extract) followed by *F. virens* (105.10 mg TAE/g dry extract). Considerable amount of tannins (84.80 mg TAE/g dry extract) was detected from MF of *F. virens*. Very small amount of tannin (11.57 mg TAE/g dry extract) was found in EF of *F. religiosa.*

3.2. Antioxidant activities

Antioxidant activities of all the fractions of leaves of four *Ficus* species measured by different in-vitro assays are shown in Table 2.

3.2.1. DPPH Radical Scavenging Activity

Highest DPPH radical scavenging activity with low IC_{50} value was exhibited by MF of leaves of all the four *Ficus* species in comparison to other fractions. Maximum activity (IC_{50} value of 108.28 µg/ml) was noticed by MF of *F. benghalensis* followed by *F. virens* (IC_{50} value of 127.11 µg/ml), *F. religiosa* (IC_{50} value of 187.62 µg/ml) and *F. elastica* (IC_{50} value of 217.57 µg/ml).

3.2.2. ABTS radical scavenging activity

Methanol fraction of leaves of all the four *Ficus* species showed greater ABTS radical scavenging activity with low IC_{50} value as compared to other fractions. Highest activity exhibited by the MF of *F. benghalensis* (IC_{50} value of 105.56 µg/ml) followed by *F. virens* (IC_{50} value of 119.31 µg/ml). Moderate ABTS radical scavenging activity was revealed by MF of *F. elastica* (IC_{50} value of 125.17 µg/ml) and *F. religiosa* (IC_{50} value of 282.56 µg/ml).

3.2.3. *Reducing power assay (RPA)*

Maximum ferric reducing power with a value of 359.44 mg QE/g dry extract was exhibited by MF of leaves of *F. virens* followed by *F. benghalensis* (268.34 mg QE/g dry

277 extract) and *F. religiosa* (237.26 mg QE/g dry extract). Other fractions (AF, EF and HF) of
278 both *F. virens* and *F. benghalensis* also showed activity ranging from 144.51 to 193.32 mg
279 QE/g dry extract. Fractions of *F. elastica* did not reveal any significant ferric reducing power.

280 ***3.3. Relationship between the total antioxidant capacity and the total phytochemical***
281 ***content:***

282 Linear correlation between antioxidant capacity with that of total phenol and flavonoids
283 content were reported by many studies. In our study, a strong negative correlation occurred
284 between total phenol (r= -0.996, p<0.01) and flavonoid content (r= -0.987, p<0.01) with that
285 of DPPH activity for all the fraction of leaves of *F. virens,* however tannin content (r= -0.967,
286 P<0.01) showed a strong negative correlation with ABTS activity. Strong positive correlation
287 detected between total phenol (r=-0.925, P<0.01) and flavonoids content (r=-0.932, P<0.01)
288 with that of reducing power assay of *F. virens* (Table 3).

289 In case of *F. benghalensis*, strong negative correlation was observed between total
290 phenolic content with that of DPPH (r=-0.934, P<0.01) and ABTS (r=-0.918, P<0.01) activity
291 however a strong positive correlation was found with reducing power assay (r=0.879, P=0.00)

292 *F. religiosa* showed a strong negative correlation between phenol with that of DPPH (r=-
293 0.968, p=0.01) and ABTS (r=-0.961, p=0.01) and flavonoid content with that of DPPH (r= -0.878,
294 p= 0.00) and ABTS (r=-0.869, p=0.00) activities but a strong positive correlation occurred with
295 reducing power assay (r=0.989, p<0.01 for phenol); (r=-0.869,p<0.01 for flavonoid).

296 In case of *F. elastica*, all the phytochemical content showed a strong negative correlation
297 with ABTS and DPPH activity whereas strong positive correlation revealed between total
298 phenol (r=0.768, p<0.01) and flavonoids (r=0.717, p<0.01) content with that of reducing
299 power assay.

A negative correlation indicates an inverse relationship between IC_{50} and antioxidant potential which means lower the IC_{50} value higher the antioxidant potential of the samples. In order to compare the content of phytochemicals and antioxidative potential of all the leaf fractions in all four *Ficus* species, a one-way ANOVA test was done using Post hoc Duncan test to compare means of all the fractions in each species based on their phytochemical content and antioxidant potential.

3.4. *DNA damage protective activity by the most biologically active leaf methanol fraction of four Ficus species against hydrogen peroxide:*

Depending on the phytochemicals constituents and antioxidant activities, DNA damage protective activity was performed with the leaf methanol fraction of four *Ficus* species. Figure 1 shows the electrophoretic pattern of DNA on subsequent UV-photolysis with H_2O_2 (100 mM) in the presence and absence of leaf methanol extract of all four *Ficus* species at different concentrations ranging from 0.1 mg/ml to 0.5 mg/ml concentrations. DNA untreated with leaf extract of *Ficus* species did not reveal any band as it is completely degraded when exposed to UV photolysis. Methanol fractions of all the four *Ficus* species exhibited strong DNA damage protecting potential at varying concentration range. DNA damage protecting activities was observed at 0.1 mg/ml concentration by *F. virens,* at 0.2 mg/ml concentration by *F. religiosa* and at 0.3 mg/ml concentration by *F. elastica. Ficus benghalensis* showed complete DNA damage protecting activities at 0.4 mg/ml but below this concentration it failed to protect DNA. The differential banding patterns of each leaf extract tested showed considerable magnitude of DNA protection against oxidative stress.

3.5. *Cytotoxicity study on human cell lines*

Cytotoxic activity on human cell lines was done by the leaf methanol extract of four *Ficus*

species based on their phytochemical constituents and antioxidant activities. Figure 2 shows the result of cytotoxic effects of leaf methanol fraction of four *Ficus* species at varying concentrations on both normal (MCF-10A) and breast cancer (MDA-MB-468) cell lines. Viability of the cancer cells was found to be declining profoundly from 5 μg/ml concentration in case of *F. benghalensis* whereas in *F. religiosa* and *F. virens*, viability started decreasing from 20 μg/ml and 40 μg/ml respectively. In *F. benghalensis*, at 5 μg/ml cell survivability decreases up to 85% at 10 μg/ml 78%, at 20 μg/ml 66% and at 40 μg/ml it is 56%. But the extract of *F. elastica* did not show any reduction on the survivability of the cancer cells.

On the other hand, in MCF-10A cell line the survivability percentage were unaffected at doses less than 160 μg/ml of the tested compound for *F. benghalensis*, *F. religiosa*, *F. virens* and *F. elastica*.

3.6 *GC-MS analysis of the purified leaf methanol fraction of each Ficus* sp.

GC-MS spectra of purified leaf methanol fraction of *Ficus virens* (Figure 3a) displayed three main compounds namely 2,4-Bis(1-phenylethyl)phenol (29.33 min), Lycopersen (33.29 min) and Vitamin E (35.32 min).

Ficus bengalensis revealed four major peaks of the compounds (Figure 3b) namely carvacrol (13.37min), caryophyllene oxide (19.09min), phytol (28.51min) and di-n-octyl phthalate (33.81min).

Ficus religiosa showed three major peaks of the compounds (Figure 3c) namely benzophenone (22.06min), 4-[(1E)-3 hydroxy-1 propenyl]-2-methoxyphenol (22.54min) and dibutyl phthalate (25.94min).

Lastly, from leaf methanol fraction of *Ficus elastica* four main compounds (Figure 3d) were

found namely 1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester (Phthalic acid) (22.65min), phytol (25.05), delta-tocopherol (26.44) and 7,8-Epoxylanostan-11-ol, 3-acetoxy (29.72).

4. Discussion

In recent times new drug development based on phytocompounds have gain importance in the field of natural products research (Dutt et al., 2019; AlSheikh et al., 2020). Isolated bioactive compounds from diverse plants species with significant therapeutic activities can be utilized in the management and treatment of various dreadful diseases with minimal side effects (Ashraf, 2020).

Leaf Methanol fraction of all the four *Ficus* species showed maximum amount of phenolic of which highest amount was recovered from *F. virens* (1267.35 mg GAE/g of dry extract), followed by *F. benghalensis* (966.05 mg GAE/g of dry extract), *F. religiosa* (925.76 mg GAE/g of dry extract) and *F. elastica* (631.71 mg GAE/g of dry extract). In our tested species, the amount of phenolic was much higher than earlier reported species namely *Ficus hispida* (285.42 mg/100 g dry wt), *Ficus carica* (412.37±57.9 mg GAE/100 g), and *Ficus deltoidea* (134.29 to 239.57 µg GAE/ml) (Ghazi et al, 2012; Wahid et al, 2010; Hlail et al, 2014). Significant pharmacological activities such as antioxidant, anticancer, antimicrobial, antiseptic and anti-inflammatory activity are exhibited by phenolic compounds (Mohammed et al., 2019). These are also active against the environmental stresses like wound healing, attack by pathogen, nutrient deficiencies, and temperature sensitivity as well as in the management of other diseases (Velu et al, 2018).

Maximum amount of flavonoids were detected from the methanol fraction of all the four *Ficus* species ranging from 438.22 mg to 1080.61 mg QE/g dry extract. Flavonoids are

structurally low molecular weight phenolic metabolites in plants, with multiple biological functions (Mondal & Rahaman, 2020). Besides having roles in regulating plant development, pigmentation and UV protection it also helps in defense mechanisms against different environmental stresses and exerts their molecular actions by scavenging free radicals and metal chelation (Karak, 2019).

In our study, a strong negative correlation was observed between total phenolic content with that of antioxidant activities. Several studies established a linear negative correlation between total content of phenols and flavonoids with antioxidant capacity (Kumaran, & Karunakaran, 2007) whereas some studies reported that there is no correlation among them.

Hexane fraction of *F. religiosa* and hexane and methanol fraction of *F. virens* contained greater amount of tannins. Tannins are polyphenolic compounds which are commonly present in forest trees and woody plants. They are oxidatively active due to their ability to precipitate proteins or to bind proteins via inhibition of cyclooxygenase (Zhang et al, 2004) by chelating properties of metal ions such as Fe (II). The most fascinating ecological functions of tannins are their roles as feeding deterrents for vertebrate herbivores, as modulators of decomposition and nutrient cycling in soil (Constabel et al, 2014).

Tannin content in case of *F. religiosa* did not show a significant correlation ($p > 0.05$) with antioxidant activity which may be due to the existence of non-hydrolysable condensed tannins or may be due to the ubiquity of complex tannins that are partially hydrolysable (Xiao et al, 2022).

The powerful antioxidant activities were shown by the leaf methanol fraction of all the four *Ficus* species which can be due to the presence of total phenols and flavonoids as they possesses a number of hydroxyl groups which are responsible for scavenging free radicals

(Cao et al, 1997). Though polyphenols are vital group of pharmacologically active compounds, the total antioxidant activities are conferred by communal activity of vast range of compounds that include phenolics, organic acids, peptides and other components (Abbas et al, 2014). The uptake of food with antioxidant benefits having high concentration of polyphenol which not only enhance the redox-active properties of the cells but also modify the activity and expression of antioxidant enzymes (Baranowska et al., 2021). As the methanol fraction of leaves of the tested *Ficus species* has the maximum amount of phytochemicals and greatest antioxidant or free radical scavenging activities in comparison to others, so we progressed with this fraction to undergo the DNA damage protective activity, cytotoxic activity and GCMS analysis to detect the bioactive compounds.

With regards to antioxidant activities and phytochemical constituent the methanol fraction of leaves of four tested species have protective activity against hydrogen peroxide and radiation induced DNA damage. This is the first report of the protective activity of leaf extract of our tested *Ficus* species against hydrogen peroxide and radiation induced DNA damage. Nitrogenous bases of DNA produces base radicals and sugar radicals when hydroxyl radicals react with DNA. The sugar moiety reacts with base radicals causing breakdown of sugar-phosphate backbone and the DNA reacts with hydrogen peroxide resulting in strand breakage (Soumya et al, 2019), sugar fragmentation, base modification, formation of malondialdehydes and various unsaturated aldehydes through oxidation of lipids. The resultant end products cause formation of mutagenic adducts by interacting with cellular DNA (Chaudhary et al, 1994).

Cytotoxic activities of methanol fraction of leaves of tested *Ficus* species on both normal cell line and breast cancer cell line showed that *F. benghalensis* (5 μg/ml), *F.religiosa* (20 μg/ml) and *F. virens* (40 μg/ml) were more effective on cancerous cells whereas no effects

were observed in case of normal cells. . So, this methanol fractions exhibited target specific activity towards breast cancer cell lines. In fact the leaves of *F. benghalensis* showed very good anticancer activity compared to other reports of its aerial roots which showed anti breast cancer activity at a dose of 97.89 μg/ml (Murugesu et al, 2021). Dried leaves of *F. religiosa* showed better cytotoxic activity compared to the fresh leaves which showed in vitro cytotoxic activity against MCF-7 human breast tumor cell line at concentration 100 μg/ml (Al-Snafi et al, 2017). Breast cancer cell line (MDA-MB-231) when treated with proanthocyanidin from stem bark of *F. virens* at 40 μg/ml concentration led to 50% cell viability which is comparable to our *F. virens* result (Chen et al,2017). One of most vital goal of cancer therapy is the specificity towards targeted cancer cells without displaying any toxicity towards normal cells. Hence selective toxicity is a major criteria that must be put into consideration during cancer treatment (Sylla et al, 2012). The high antioxidant activities might contribute to its cytotoxicity against the breast cancer cells. Various therapeutic activities such as anti-inflammatory, antitumor, analgesic and many more are possessed by bioactive phytochemicals (Singh et al, 2018). So, the phytochemicals & derivatives present in the leaves of selected *Ficus* species are promising alternatives for the improvised non-toxic cancer therapy.

The bioactive constituent present in the leaf methanol fractions of each *Ficus* sp has been reported to have anticancerous and antioxidant activities. Carvacrol (CV) is a monoterpenoid phenol found in the methanol fraction of *Ficus benghalensis.* This compound exhibits high antimicrobial, antioxidant activities and mainly associated with dietery phytoadditive to improve the antioxidant status in animals. In preclinical models of breast, liver and lung carcinomas, Carvacrol showed anticancer properties by inducing proapoptotic processes (Sharifi-Rad et al, 2018; Safaei-Ghomi et al, 2009). Caryophyllene oxide, a

440 constituent of *Ficus benghalensis* exhibited significant anticancer activities by altering the
441 growth and proliferation of cancer cells (Fidyt et al, 2016). Phytol, a bioactive compound in
442 *Ficus benghalensis* and *Ficus elastica* is responsible for ROS mediated apoptosis as reported
443 in *Schizosaccharomyces pombe* (Thakor et al, 2016). GCMS spectra of *Ficus religiosa* revealed
444 three major compounds, among them natural benzophenones are a class of compounds
445 containing more than 300 members that share a common phenol-carbonyl-phenol skeleton,
446 which have great structural variation. It exhibits an array of biological activities including
447 antifungal, anti-HIV, antioxidant, antiviral and cytotoxicity (Wu et al, 2014) another
448 compound from *Ficus religiosa* 4-((1E)-Hydroxy-1-propenyl]-2-methoxyphenol belongs to the
449 class of organic compounds known as methoxyphenols. It has antimicrobial, antioxidant and
450 anti-inflamatory activity (Muriithi, et al, 2016). Bioactive compound, Dibutyl phthalate
451 reportedly produced by a new soil isolate *Streptomyces albidoflavus* found from methanol
452 fraction of *Ficus religiosa* (Roy et al, 2006). Vitamin E (also known as tocopherol) from *Ficus*
453 *elastica* and *Ficus virens* belong to a class of phenolic antioxidants which can inhibit lipid
454 peroxidation by undergoing free radical scavenging and reacting with singlet oxygen (Frankel
455 et al, 1989). 2,4-Bis(1-phenylethyl)phenol from *Ficus virens* inhibit cell proliferation and
456 promote programmed cell death in cancerous cell as reported from butanol fraction
457 of *Cordyceps bassiana* (Kim et al, 2016). Lycopersen found in the methanol fraction of *Ficus*
458 *virens* is a secondary metabolite that is also reported from kari (*Murayya koeginii*) leaves
459 (Wirjosentono et al, 2019). Phthalic acid (1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl
460 ester) recovered from methanol fraction of *Ficus elastica.* Osuntokun et al isolated phthalic
461 acid with potent therapeutic and antimicrobial activity from *Spondias mombin,* a unique
462 medicinal plant with various medicinal properties (Osuntokun et al,2019). All this
463 phytoconstituents from each leaves of *Ficus* species revealed from GCMS analysis are

responsible for the potent antioxidant and cytotoxic activity. Therefore these reports are in accordance with the result of this study.

Natural product-based drug discovery for controlling various fatal diseases is one of the challenging scientific task to the modern medicinal practices (Wainwright et al, 2022). The drugs obtained from the plant secondary metabolites have a wide array of application in the prevention or treatment of numerous ailments. Pharmacogical activities may be augmented by slight structural alteration of the parent phytocompounds with no or very minimal side effects.

5. Conclusion

Methanol fraction of all the tested *Ficus* species possess maximum amount phytochemicals with potent antioxidant activities**.** In particular, the leaf methanol fraction of *Ficus virens*, *Ficus religiosa* and *Ficus benghalensis* are capable to prevent oxidative DNA damage at very low concentrations which facilitates cells for protecting themselves against oxidative stress. Anticancerous activity of *F. benghalensis, F. religiosa* and *F. virens,* also validated their role in cell proliferation. The identification of various bioactive compounds by GCMS analysis of the leaf methanol fraction of four *Ficus* sp justifies the fact that the leaves of these plants could become rich natural sources of bioactive compounds for the pharmaceutical industries to develop novel and effective drugs with almost no side effects. Moreover, young leaves of *F. virens, F. religiosa* and *F. benghalensis* may be suggested as health-promoting leafy vegetables whose therapeutic applications in various aspects are yet to be investigated.

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Conflict of interest statement

The authors who have engaged in this study declared that they have no conflict of interests among them regarding the publication of this paper.

Availability of data and material

Data and material will be available upon request to corresponding author.

Authorship Contribution Statement

Rajashree Dutta – Design of the experiment, investigation, collection of test data, formal analysis, drafting the article; **Ekta Bhattacharya** - Design of the experiment, investigation, formal analysis, revision of manuscript; **Suparna Mandal Biswas** – Critical revision, funding acquisition, project administration, validation, supervision. **Thomas Hughes** and **Arindam Pramanik** – Design of the experiment, investigation, formal analysis and made the critical revision of the article.

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Figure Captions:

Figure 1. Protective activity of the crude extracts of four *Ficus* species namely *Ficus virens, Ficus bengalensis, Ficus religiosa* and *Ficus elastica* at different concentrations ranging from 1mg/ml to 5 mg/ml against peroxide radical induced DNA damage. Lane marked '+' shows the effect of gallic acid (1 mg/mL) as the positive control. Negative control containing untreated DNA exposed to UV photolysis is loaded in the lane marked '-'.

Figure 2. MTT assay of crude extract of four *Ficus* species namely *Ficus virens* (FV), *Ficus benghalensis* (FB), *Ficus religiosa* (FR) and *Ficus elastica* (FE) at different concentrations on normal (MCF-10A) and cancer (MDA-MB-468) cell lines.

Figure 3a. GC-MS spectra of purified leaf methanol fraction of *Ficus virens.*

Figure 3b. GC-MS spectra of purified leaf methanol fraction of *Ficus benghalensis.*

Figure 3c. GC-MS spectra of purified leaf methanol fraction of *Ficus religiosa.*

Figure 3d. GC-MS spectra of purified leaf methanol fraction of *Ficus elastica*

Highlights

- All the four *Ficus* species possess maximum amount of phytoconstituents in the methanol fraction.
- Methanol fraction of all the four tested *Ficus* species showed strong antioxidant activity.
- *F. virens, F. religiosa* and *F. elastica* prevented oxidative DNA damage at very low concontrations.
- Significant antiproliferative activity was shown by *F. benghalensis*. *F. religiosa* and *F. virens.*
- GCMS analysis revealed chemical profiling of bioactive compounds from all the four *Ficus* species.

GRAPHICAL ABSTRACT

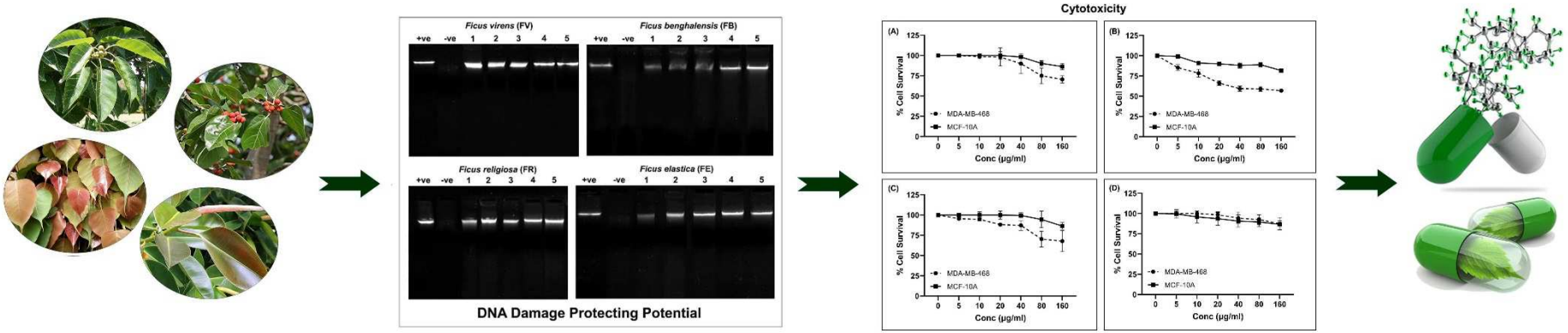

Figure 1.

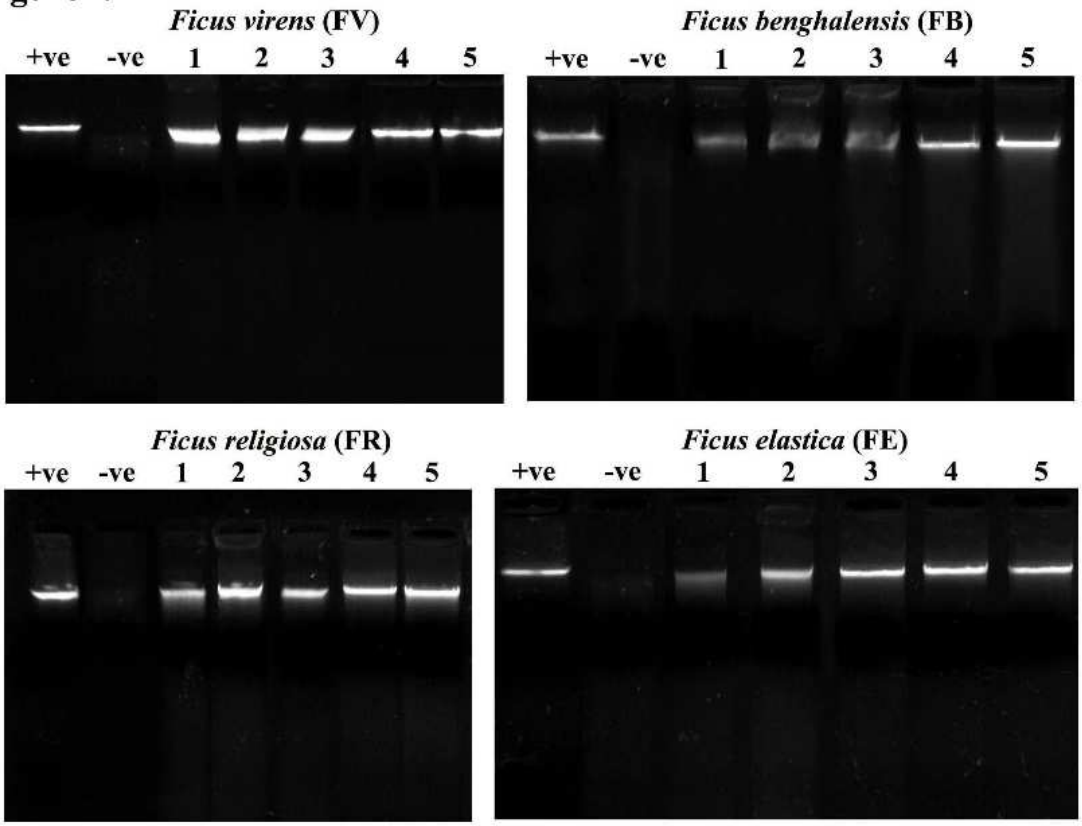

Figure 2.

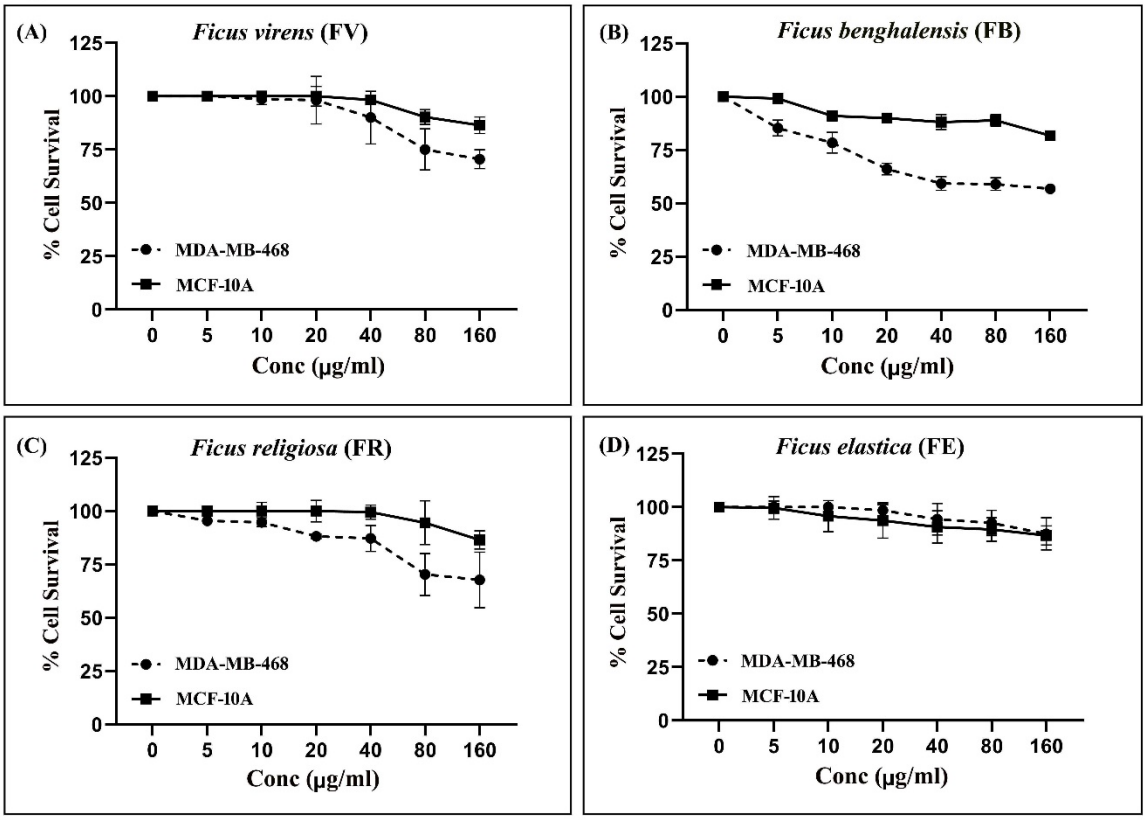

Figure 3a.

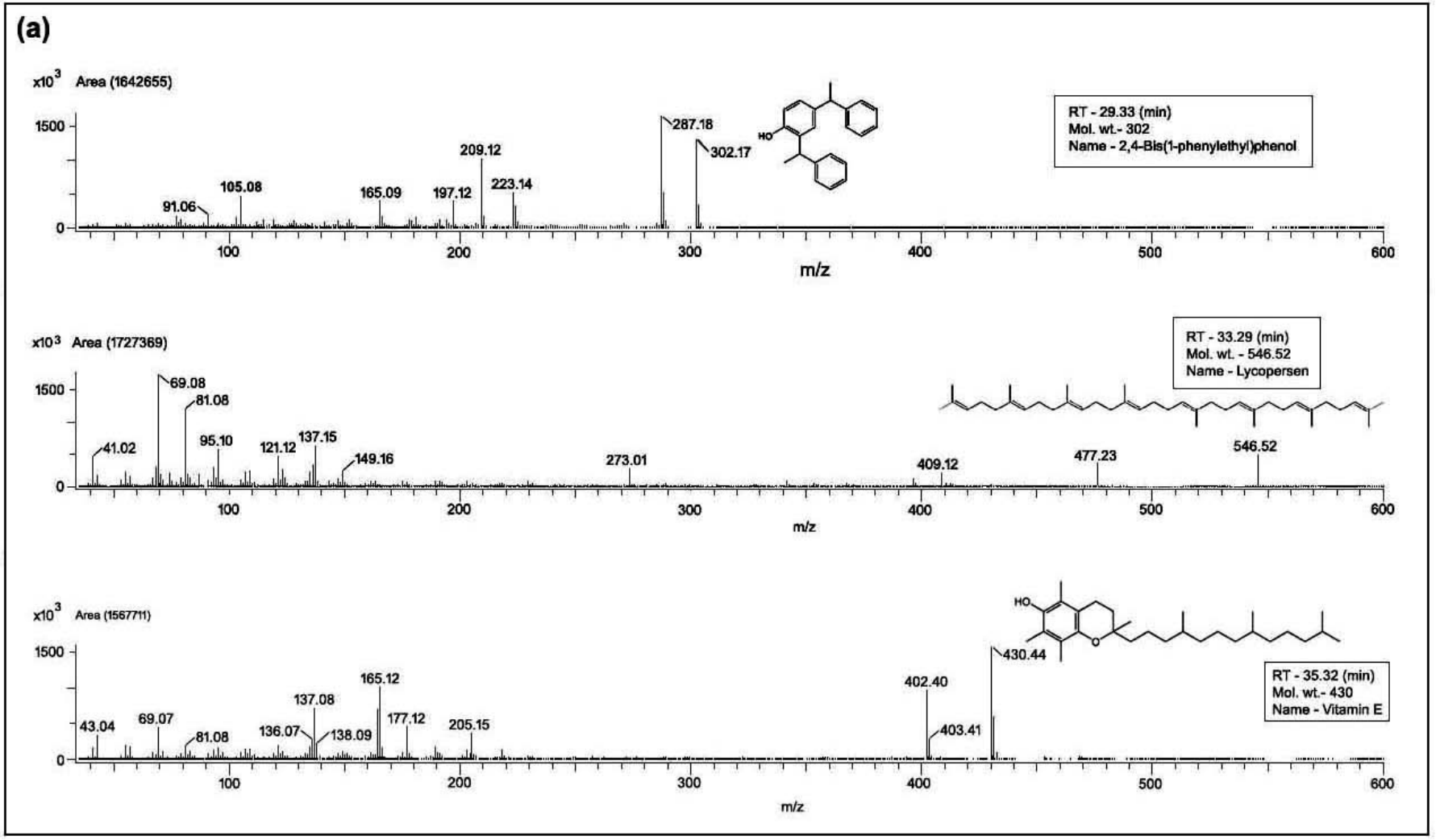
(a)
x10 3 Area (1642655)
1500
0
91.06
105.08
165.09
197.12
209.12
223.14
287.18
302.17
HO
RT - 29.33 (min)
Mol. wt.- 302
Name - 2,4-Bis(1-phenylethyl)phenol
100
200
300
400
500
600
m/z
x10 3 Area (1727369)
1500
0
41.02
69.08
81.08
95.10
121.12
137.15
149.16
273.01
409.12
477.23
546.52
RT - 33.29 (min)
Mol. wt. - 546.52
Name - Lycopersen
100
200
300
400
500
600
m/z
x10 3 Area (1567711)
1500
0
43.04
69.07
81.08
136.07
137.08
138.09
165.12
177.12
205.15
402.40
403.41
430.44
HO
O
RT - 35.32 (min)
Mol. wt.- 430
Name - Vitamin E
100
200
300
400
500
600
m/z

Figure 3b.

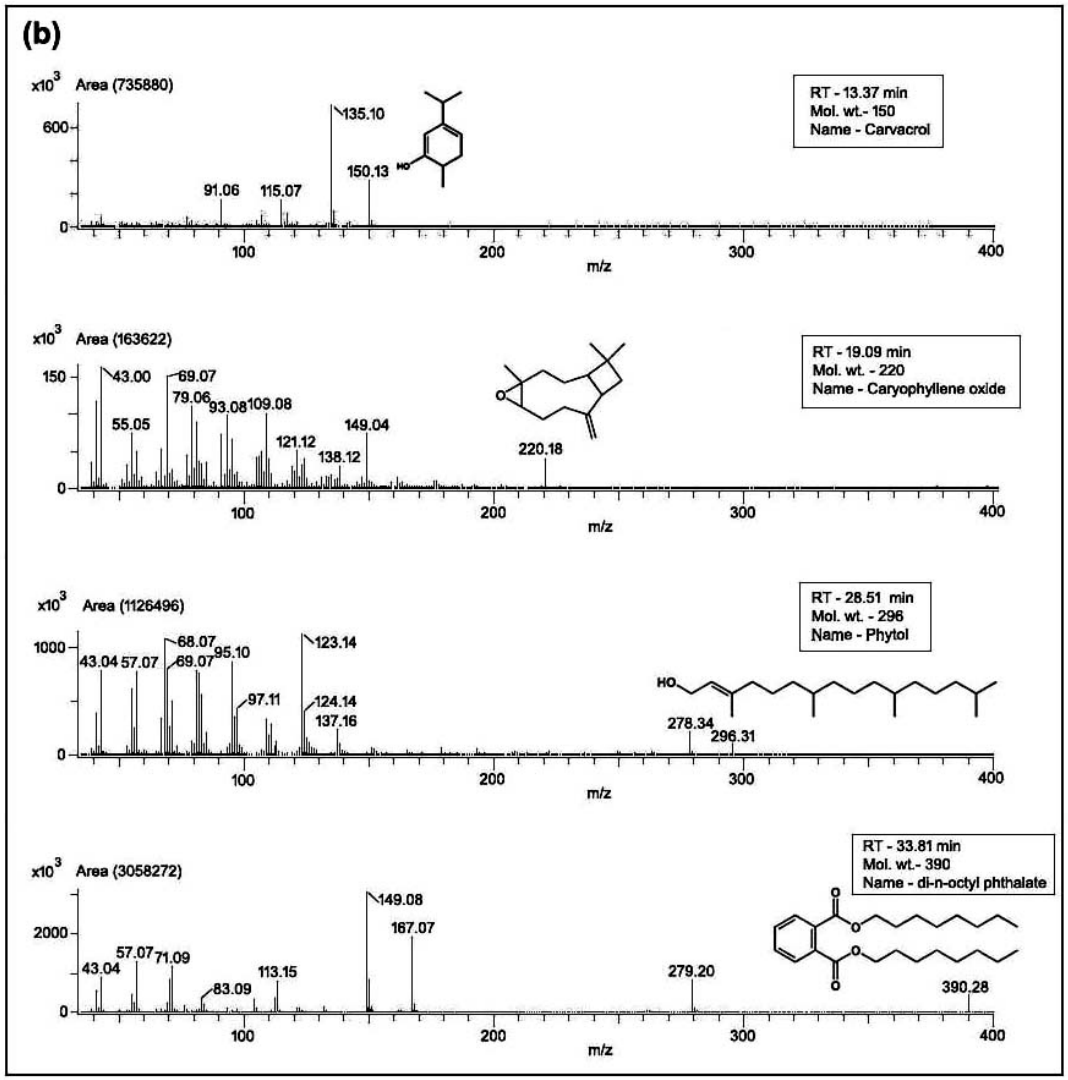
(b)
x10^3 Area (735880)
600
0
135.10
150.13
91.06
115.07
100
200
300
400
m/z
RT - 13.37 min
Mol. wt.- 150
Name - Carvacrol
x10^3 Area (163622)
150
0
43.00
69.07
79.06
93.08
109.08
55.05
149.04
121.12
138.12
220.18
100
200
300
400
m/z
RT - 19.09 min
Mol. wt. - 220
Name - Caryophyllene oxide
x10^3 Area (1126496)
1000
0
43.04
57.07
68.07
69.07
95.10
123.14
97.11
124.14
137.16
278.34
296.31
100
200
300
400
m/z
RT - 28.51 min
Mol. wt. - 296
Name - Phytol
x10^3 Area (3058272)
2000
0
43.04
57.07
71.09
83.09
113.15
149.08
167.07
279.20
390.28
100
200
300
400
m/z
RT - 33.81 min
Mol. wt.- 390
Name - di-n-octyl phthalate

Figure 3c.

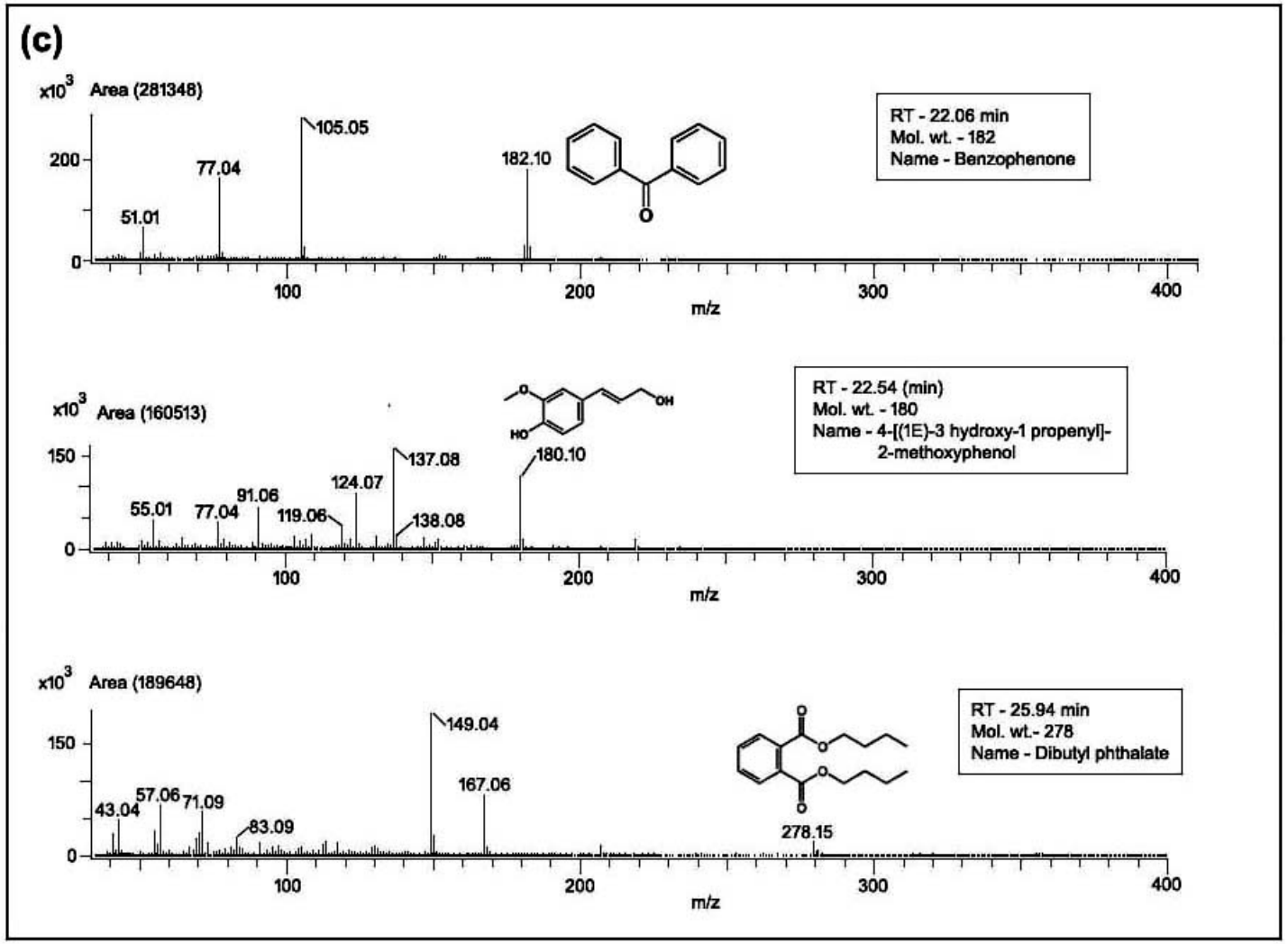
(c)
x10^3 Area (281348)
105.05
77.04
182.10
51.01
RT - 22.06 min
Mol. wt. - 182
Name - Benzophenone
m/z
x10^3 Area (160513)
137.08
180.10
124.07
91.06
55.01
77.04
119.06
138.08
RT - 22.54 (min)
Mol. wt. - 180
Name - 4-[(1E)-3 hydroxy-1 propenyl]-
2-methoxyphenol
m/z
x10^3 Area (189648)
149.04
167.06
57.06
71.09
43.04
83.09
278.15
RT - 25.94 min
Mol. wt.- 278
Name - Dibutyl phthalate
m/z

Figure 3d.

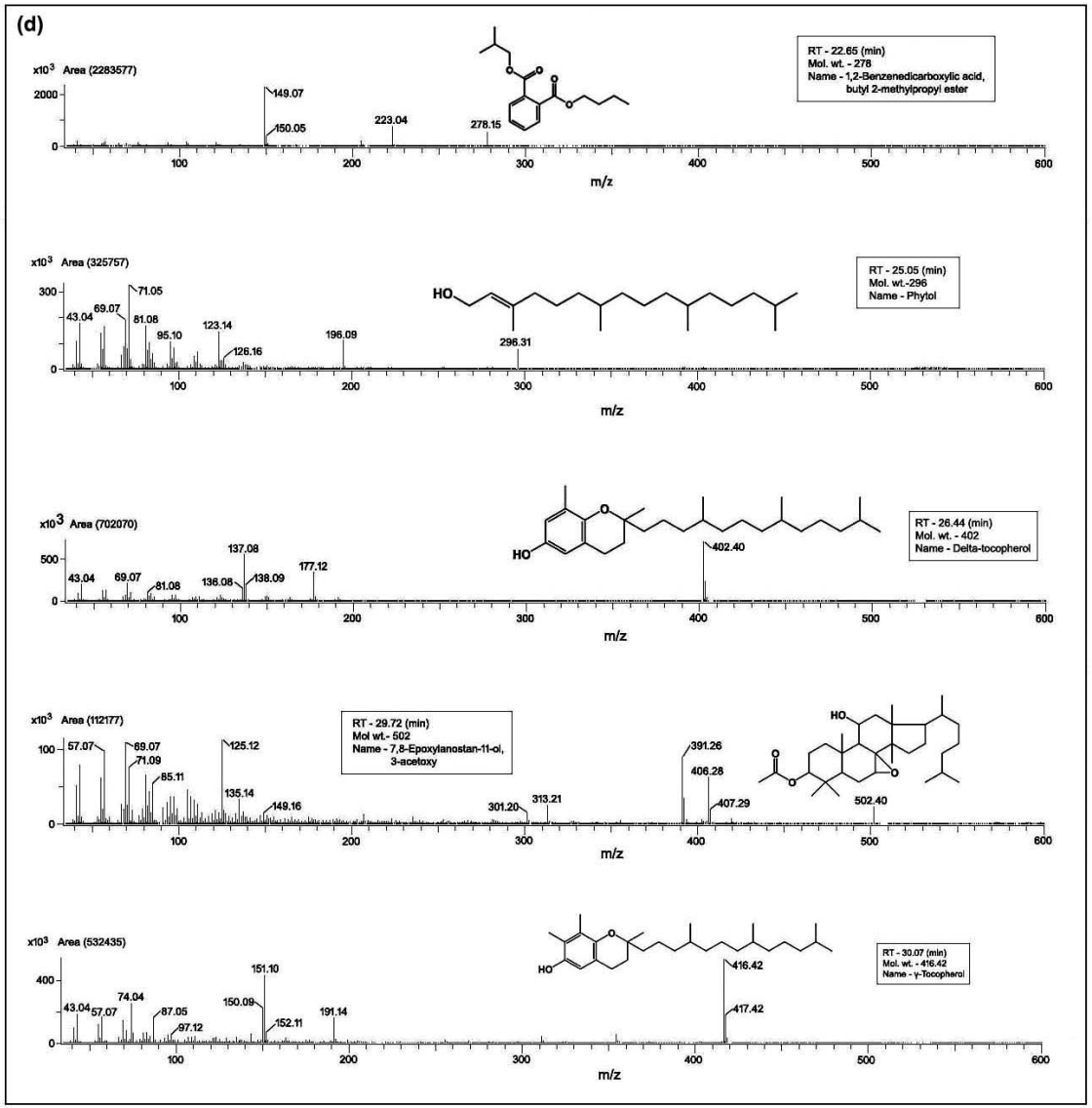
(d)
x10³ Area (2283577)
149.07
150.05
223.04
278.15
RT - 22.65 (min)
Mol. wt. - 278
Name - 1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester
m/z
x10³ Area (325757)
71.05
43.04
69.07
81.08
95.10
123.14
126.16
196.09
296.31
HO
RT - 25.05 (min)
Mol. wt.-296
Name - Phytol
m/z
x10³ Area (702070)
137.08
43.04
69.07
81.08
136.08
138.09
177.12
402.40
HO
RT - 26.44 (min)
Mol. wt. - 402
Name - Delta-tocopherol
m/z
x10³ Area (112177)
57.07
69.07
71.09
85.11
125.12
135.14
149.16
301.20
313.21
391.26
406.28
407.29
502.40
HO
RT - 29.72 (min)
Mol wt.- 502
Name - 7,8-Epoxylanostan-11-ol, 3-acetoxy
m/z
x10³ Area (532435)
43.04
57.07
74.04
87.05
97.12
150.09
151.10
152.11
191.14
416.42
417.42
HO
RT - 30.07 (min)
Mol. wt. - 416.42
Name - γ-Tocopherol
m/z

Table 1. Phytochemical composition in different fractions of four *Ficus* sp. namely *Ficus virens* (FV), *Ficus benghalensis* (FB), *Ficus religiosa* (FR), *Ficus elastica* (FE). HF: hexane fraction; EF: ethyl acetate fraction; AF: acetone fraction; MF: methanol fraction.

Phytochemicals	Name of the species	DIFFERENT FRACTIONS OF EACH *Ficus* sp.			
		MF	AF	EF	HF
PHENOLICS (mg GAE/g dry extract)	FV	1267.35±9.40^{a}	183.94±4.43^{c}	162.88±21.96^{c}	339.37±35.01^{b}
	FB	966.05±21.36^{a}	179.88±5.68^{b,c}	151.43±13.73^{b,c}	204.92±21.43^{b}
	FR	925.76±53.01^{a}	294.96±43.98^{b}	251.41±0.85^{b,c}	188.21±5.97^{b,c}
	FE	631.71±60.89^{a}	185.65±3.07^{b}	86.59±3.72^{c}	66.09±3.08^{c}
FLAVONOIDS (mg QE/g dry extract)	FV	1080.61±31.06^{a}	169.40±11.21^{b}	99.05±16.39^{c}	204.42±42.87^{b}
	FB	928.23±28.56^{a}	412.10±37.33^{b}	219.78±37.43^{c}	195.51±7.84^{c}
	FR	853.27±40.16^{a}	688.91±19.86^{b}	286.45±19.35^{c}	160.18±3.32^{d}
	FE	438.22±8.56^{a}	95.67±9.07^{b}	53.58±9.23^{c}	40.98±2.32^{c}
TANNINS (mg TAE/g dry extract)	FV	84.80±5.81^{b}	21.56±4.48^{c}	15.16±7.52^{c}	105.10±4.59^{a}
	FB	64.58±4.32^{a}	25.15±0.70^{b}	22.34±1.53^{b}	13.05±2.44^{c}
	FR	25.07±1.10^{b}	19.85±0.27^{c}	11.57±0.84^{d}	123.76±3.49^{a}
	FE	44.67±0.59^{a}	18.67±0.58^{d}	20.31±0.97^{c}	22.42±0.35^{b}

Values in each row with different superscripts (a, b, c, d) are significantly different (P<0.05). HF: hexane fraction; EF: ethyl acetate fraction; AF: acetone fraction; MF: methanol fraction.

Table 2. Phytochemical composition in different fractions of four *Ficus* sp. namely *Ficus virens* (FV), *Ficus benghalensis* (FB), *Ficus religiosa* (FR), *Ficus elastica* (FE). HF: hexane fraction; EF: ethyl acetate fraction; AF: acetone fraction; MF: methanol fraction.

Antioxidant Activities	Name of the species	DIFFERENT FRACTIONS OF EACH *Ficus* sp.			
		MF	AF	EF	HF
DPPH IC_{50} (µg/ml)	FV	127.11±42.27^{d}	439.04±57.05^{a}	421.57±99.01^{b}	377.82±66.08^{c}
	FB	108.28±54.11^{c}	314.87±54.85^{b}	324.86±97.52^{b}	477.89±89.68^{a}
	FR	187.62±10.09^{c}	316.04±50.55^{b}	386.06±51.64^{a}	407.25±33.61^{a}
	FE	217.57±30.46^{c}	318.15±97.38^{b}	411.23±63.79^{a}	435.32±78.91^{a}
ABTS IC_{50} (µg/ml)	FV	185.31±27.78^{c}	322.40±29.14^{b}	395.67±21.24^{a}	490.83±31.98^{d}
	FB	162.56±31.21^{d}	251.54±17.68^{c}	425.75±26.97^{b}	494.08±29.83^{a}
	FR	282.56±85.79^{c}	440.92±65.64^{b}	484.93±49.07^{a}	510.21±52.98^{a}
	FE	111.17±66.89^{b}	408.61±99.17^{a}	432.68±63.31^{a}	434.86±82.17^{a}
RPA (mg QE/g)	FV	359.44±46.77^{a}	172.77±42.53^{b}	157.34±28.25^{b}	144.51±8.13^{b}
	FB	268.34±17.99^{a}	193.31±18.41^{b}	175.91±15.45$^{b.c}$	147.01±11.06^{c}
	FR	237.26±9.64^{a}	84.01±3.71^{b}	59.79±7.35^{c}	66.13±6.06^{c}
	FE	99.92±10.51^{a}	88.64±6.58^{a}	30.05±11.57^{b}	4.45 ±1.66^{c}

Values in each row with different superscripts (a, b, c, d) are significantly different ($p<0.05$). HF: hexane fraction; EF: ethyl acetate fraction; AF: acetone fraction; MF: methanol fraction.

Table 3. **Correlation coefficients between antioxidant activities and phytochemical compounds of all four *Ficus* species namely *Ficus virens*, *Ficus benghalensis*, *Ficus religiosa*, *Ficus elastica*.**

Name of the Tested species	Antioxidant activity	Phytochemical compounds					
		Total Phenol		Total Flavonoid		Total Tannin	
		r	p	r	p	r	p
Ficus virens	DPPH	-0.996**	0.000	-0.987**	0.000	-0.560	0.058
	ABTS	-0.522	0.082	-0.464	0.129	-0.967**	0.000
	Reducing power	0.925**	0.000	0.932**	0.000	0.307	0.331
Ficus benghalensis	DPPH	-0.934**	0.001	-0.905**	0.000	-0.929**	0.000
	ABTS	-0.918**	0.009	-0.876**	0.000	-0.824**	0.001
	Reducing power	0.879**	0.000	0.932**	0.000	0.924**	0.000
Ficus religiosa	DPPH	-0.968**	0.000	-0.878**	0.000	0.407	0.190
	ABTS	-0.961**	0.000	-0.869**	0.000	0.430	0.163
	Reducing power	0.989**	0.001	0.792**	0.001	-0.273	0.390
Ficus elastica	DPPH	-0.922**	0.000	-0.897**	0.000	-0.779**	0.003
	ABTS	-0.927**	0.000	-0.918**	0.000	-0.877**	0.000
	Reducing power	0.768**	0.004	0.717**	0.009	0.530	0.076

** indicates $P<0.01$