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Original Research Article

Phytochemical analysis of trunk bark with branch bark, wood and stored bark of *Ficus racemosa* and comparison with other *Ficus* barks

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ABSTRACT

The phytochemistry of *F. racemosa* bark was compared with other *Ficus* species available in South India (*F. religiosa, F. benghalensis* and *F. hispida* barks). Further, fresh bark was compared with stored bark of *F. racemosa*; wood with the trunk bark of *F. racemosa*; branch bark with the trunk bark of *F. racemosa*. Also, the protective potential of active fraction was evaluated against oxidative stress in *ex vivo* skin model. Qualitative and quantitative estimation of phytochemicals were done for *Ficus* species bark samples and also subjected to UPLC-QTOF-MS analysis. It was found that methanolic extract of *F. racemosa* has a high number of phytochemicals (Flavonoids: 4.10 mg/mL; phytosterols: 3.28 mg/mL; total phenols: 29.93 mg/mL and tannins: 8.98 mg/mL). Kaempferol-3-*O*-β-D-glucoside was noted as the major compound in *F. racemosa* bark. Trunk bark of *F. racemosa* was quantified to have high amount of phytochemicals than branch bark. Also, bark was found to contain higher concentration of different phytochemicals than *F. racemosa* wood. Stored bark of *F. racemosa* was found to exhibit lesser amount of phytochemicals than fresh bark. *F. racemosa* bark also exhibits moderate superoxide and hydrogen peroxide scavenging activities, high inhibition of lipid peroxidation and moderate inhibition of protein oxidation.

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1. Introduction

The plant *Ficus racemosa*, also known as *Ficus glomerata*, belongs to the family Moraceae. *F. racemosa* commonly called "Cluster fig" in English, "*Aththi*" in Tamil and "*Goolar*" in Hindi, and is distributed in Southeast Asia and Australia (Joy et al., 2001; Ahmed and Urooj, 2010a). It is a lactiferous and evergreen tree that grows to a height of 12 m in damp areas throughout India and is frequently planted for its edible fruit (Kobmoo et al., 2010). The leaves are simple, ovulated; the fruits are grouped together in woody branches; the flowers contain three ovate triangular basal bracts. The bark is reddish-grey in color and appears fractured and thickness of 0.8-1.4 cm (Paarakh, 2009).

In India's traditional medical system, all the parts of *F. racemosa* including the leaves, fruits, bark, latex and sap of the roots are used for various therapeutic purposes. Bark decoction is used to treat ulcers, inflammation, mouth infection, variety of skin conditions, diabetes, piles, dysentery, asthma, gonorrhoea, gleets, leucorrhea, urinary tract disorders and applied as a poultice to swellings and boils (Murti et al., 2010; Ahmed and Urooj, 2011). In Siddha and Ayurvedic systems of medicine, bark of *F. racemosa* is reported as *Attippattai* and *Udumbara*, respectively and is traditionally used for treatment of liver disorders, diarrhoea and respiratory diseases (SPI, 2008). Anti-microbial (Ahmed and Urooj, 2010a), antidiabetic (Keshari et al., 2016), antidiuretic (Ratnasooriya et al., 2003), anti-tussive (Bhaskara Rao et al., 2003), anthelmintic (Chandrashekhar et al., 2008), hepatoprotective activity (Mandal et al., 1999), anti-hyperglycemic (Ahmed and Urooj, 2010b), hypolipidemic (Keshari et al., 2016), antioxidant (Veerapur et al., 2009), anti-inflammatory (Dharmadeva et al., 2018), antipyretic (Bhaskara Rao et al., 2002), anti-diarrhoeal (Mukherjee et al., 1998) and anti-ulcer activities (Rao et al., 2008) of *F. racemosa* bark have also

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been reported. The main flavonoids in the stem bark are bergenin, coumarin, kaempferol, sitosterol, stigmasterol, amyrin acetate, lupeol, lupeol acetate, leucocyanidin-3-*O*-D-glucopyrancoside, leucopelargonidin-3-*O*-D-glucopyranoside and ellagic acid (Ahmed and Urooj, 2010b).

In spite of various medicinal applications, there are some problems associated with *F. racemosa* bark. With respect to distribution and is less frequently available in South India when compared to other *Ficus* species. When market demand for *F. racemosa* bark rises, bark material of other more readily available *Ficus* species such as *F. religiosa* (*Arasamaram* in Tamil), *F. benghalensis* (*Aalamaram* in Tamil) and *F. hispida* (*Peiaththi* in Tamil) could be marketed due to their similar morphology (Bhalerao and Sharma, 2014; Tripathi et al., 2015). As *F. racemosa* bark is cut, immediately dried, made into powder and marketed and if the demand is low, then the bark may be stored, but the data on change in phytochemical profile during storage is not available. Bark is the outer surface layer in the trunk region whereas wood is inner part and wood may also be supplied instead/along with *F. racemosa* bark. Usually the bark from trunk region of *F. racemosa* is being used, but there is a chance to collect the bark from branch also. Due to these alternative materials and variable conditions, the drug marketed in the name of *F. racemosa* bark may cause low efficacy and severe side effects and hence there is a need for comparison of phytochemical profile of related samples with the original drug.

To tackle the problems as stated above, the objectives of the project are devised to analyse and compare the phytochemical constituents of *F. racemosa* bark with other related *Ficus* species (*F. religiosa*, *F. benghalensis* and *F. hispida*); fresh and stored bark of *F. racemosa*; bark and wood of *F. racemosa* & Trunk and branch bark of *F. racemosa*. Further, it is also aimed to isolate and purify the phenolic compounds from *F. racemosa* bark to evaluate its protective potential against oxidative stress in *ex vivo* skin model.

2. Experimental

2.1. Collection and identification of samples

Trunk Bark, branch bark and wood of *F. racemosa* were collected from Medical College campus, Thanjavur, Tamilnadu (10°45′43.0″N & 79°06′19.9″E). Barks of *F. religiosa* and *F. benghalensis* were collected from SASTRA Deemed University campus, Thanjavur (10°43′43.6″N & 79°01′06.2″E). Bark of *F. hispida* was collected from Chettimandapam, Kumbakonam, Tamilnadu (10°58′37.0″N & 79°24′03.6″E) (Supplementary Fig. 1). The identity of the trees was authenticated by Rapinat Herbarium, Trichy and voucher specimen numbers of *F. racemosa* (V.M.L. 001), *F. religiosa* (V.M.L. 002), *F. benghalensis* (V.M.L. 003) and *F. hispida* (V.M.L. 004) were given (Supplementary Fig. 2). The trunk bark materials of all species, branch bark and wood materials of *F. racemosa* (Each sample with ten replicates) were shade dried. Then the materials were chopped and ground with the help of a pulveriser and made into a fine powder using an electric mixer.

2.2. Preparation of extracts

Based on the method of Akshaya et al. (2023), sequential extraction of plant material (Trunk bark, branch bark and wood samples of *F. racemosa* & bark sample of *F. religiosa*, *F. benghalensis* and *F. hispida*) was carried out. A 10 g powdered sample was taken and extracted in 100 mL of solvent with increasing polarity (hexane, chloroform, ethyl acetate, methanol and water). Mixture was shaken for 1 h with continuous stirring using a magnetic stirrer (300 rpm) (Supplementary Fig. 3). The contents were filtered through filter paper and the filtrates were then subjected to further phytochemical analysis.

2.3. Phytochemical screening

All the solvent extracts of barks, branch bark, and wood were tested for the presence of different classes of phytochemicals including alkaloids, flavonoids, terpenoids, phytosterols, saponins, phenols and tannins. To 500 µL of extract, 5 drops of Dragendroff's reagent was added which gives orange-red precipitate to confirm the presence of alkaloids (Sekar et al., 2016). To 500 µL of extract, 500 µL of NaOH added, which turn yellow color indicating the presence of flavonoids (Pant et al., 2017). Formation of red precipitate confirmed the presence of terpenoids when 500 µL of conc. H_2SO_4 was added to 500 µL of extract (Shaikh and Patil, 2020). To 500 µL of extract, addition of 500 µL of CCl_4, 500 µL of CH_3COOH and 500 µL of conc. H_2SO_4 forms reddish brown color in lower layer, which confirms the presence of phytosterols (Shaikh and Patil, 2020). Prolonged foam formation when 500 µL of distilled H_2O was mixed with 500 µL of extract, which indicated the presence of saponins (Santhi and Sengottuvel, 2016). To 500 µL extract, 500 µL of $FeCl_3$ (5%) was added to form dark blue color solution, which confirms the presence of phenols (Deyab et al., 2016). To 500 µL of extract, addition of 500 µL of $Pb(C_2H_3O_2)_2$ (1.0%) formed creamy precipitate, which confirms the presence of tannins (Ukoha et al., 2011). The qualitative results are expressed as + for the presence in low amounts, ++ for the presence in medium amounts, +++ for the presence of high amounts and − for the absence of phytochemicals.

2.4. Quantitative analysis

Total alkaloid content was estimated using the method of Sreevidya and Mehrotra (2003). To 500 µL of different extracts, 500 µL of Dragendroff's reagent was added. The precipitate formed was centrifuged for 5 minutes. Then, the supernatant was decanted completely and the precipitate was washed with 1000 µL of methanol and centrifuged for 5 minutes. The supernatant was discarded and the residue was then treated with 1000 µL of disodium sulphide solution (1.0%). The brownish-black precipitate formed was then centrifuged for 5 minutes and the precipitate was dissolved in 1000 µL of concentrated nitric acid. This solution was diluted with 1000 µL of distilled water, from which 500 µL

was then pipetted out and mixed with 1000 µL of thiourea solution (4.0%). The absorbance was measured using spectrophotometer at 600 nm against the blank containing nitric acid and thiourea. A standard calibration curve for berberine in the range of 100-1000 µg/mL was prepared in the same manner and results were expressed as mg/mL of extract.

Total flavonoid content was determined by following the method of Ahmed and Iqbal (2018). To 100 µL of each extract, 100 µL of sodium nitrate solution (5.0%) was added and incubated at room temperature for 5 minutes. Then, 100 µL of aluminium chloride (1.0%) was added, incubated for 5 minutes and further 700 µL of NaOH was added and incubated at room temperature for 5 minutes. Finally, 500 µL of distilled water was added and incubated for 10 minutes. After that, absorbance was read at 520 nm using spectrophotometer against the blank containing water instead of extract. Concentration of flavonoids of the extracts was calculated using the standard curve of catechin which ranged from 100-1000 µg/mL and the results were given in terms of mg/mL of extract.

By applying the method of Lukowski et al. (2022), total terpenoid content was estimated. Each extract (200 µL) was mixed with 1500 µL of chloroform and 200 µL of concentrated sulphuric acid. The mixture was incubated for 30 minutes and the upper layer was removed, and lower layer was dissolved in 1.5 mL of methanol. Absorbance was read at 540 nm against the blank containing water instead of extract. The standard curve of linalool ranging from 100-1000 µg/mL was used and the concentration of terpenoids was calculated.

Based on the method of Saptarini et al. (2016), total phytosterol content was estimated. To 500 µL of each extract, 1500 µL of chloroform was added and the top layer was pipetted out. Then 1500 µL of the ferric chloride-acetic acid mixture was added followed by the addition of 1000 µL of concentrated sulphuric acid. Absorbance was measured at 540 nm against the blank containing water instead of extract and concentration was calculated using the standard curve of β-sitosterol.

Total phenol content was quantified using the method of Iqbal et al. (2015). To 100 µL of each extract, 250 µL of Folin-Ciocalteu reagent was added followed by the addition of 1000 µL of 5% sodium carbonate and incubated for 10 minutes. The absorbance was measured at 720 nm using spectrophotometer against the blank containing water instead of extract. The concentration of phenols was calculated with the standard curve of gallic acid ranging from 100-1000 µg/mL.

Total tannin content was determined by employing the method of Rebaya et al. (2015). To 200 µL of each extract, 1200 µL of vanillin (4.0%) and 600 µL of concentrated hydrochloric acid were added and mixed well. The mixture was incubated for 10 minutes and absorbance was read at 520 nm using spectrophotometer against the blank. The concentration was calculated using the standard curve of catechin.

2.5. LC-MS analysis

An UHPLC-QTOF-MS instrument (Waters, XEVO-G2-XS QTOF) with electro spray ion source was utilised to capture high-resolution mass spectra. The system is combined with a Waters Acquity UHPLC which includes an Acquity auto sampler, sample manager, LC pump, column component oven and PDA detector. Chromatographic separation was performed using BEH C18 reverse phase column (2.1 x 50 mm, 1.7 mm particle size). Mobile phase A (HPLC grade water) and solvent B (Acetonitrile with 0.1% formic acid) and a linear gradient elution program was applied as follows: 0-1.0 min (20% A & 80% B); 1.1-2.0 min (40% A & 60% B); 2..1-3.5 min (85% A & 15% B); 3.6-5.0 min (90% A & 10% B); 5.1-7.0 min (50% A & 50% B); 7.1-8.0 (20% A & 80% B) by given total run time of 8.1 min. The flow rate was maintained at 0.3 mL/min and the column temperature was set at 30 °C. Injection volume was set up in 10 µL and the pressure limit was 15,000 psi. Mass determination of phytochemicals from each sample was performed using the following MS conditions. The instrument was operated with ESI source in negative ion mode. The ionization was optimized in -ve mode at source conditions: Capillary voltage 3.0 kV; cone voltage 35 kV; source temperature 120 °C and de-solvation temperature, 350 °C. Nitrogen gas was supplied from an in-house generator and gas flow was set at 50 and 800 L/h for cone and de-solvation, respectively. High-purity argon (99.9%) was used as a collision gas at a flow rate of 1.5 mL/min. The collision energy was set at 6.0 eV and mass-spectra were obtained in the continuum mode across the range from 150 to 1000 *m/z*.

2.6. Column chromatography

The selected methanolic extract based on phytochemical analysis and quantification was subjected to column chromatography. For column chromatography, a stationary phase made up of silica gel (60-120 mesh, Merck, India) was packed with hexane in a glass column (38 cm long x 2.2 cm wide) without the presence of air bubbles. For saturation, 200 mL of hexane was used to wash the column. The silica column was loaded with the dry slurry of methanolic extract of *F. racemosa*. Hexane, ethyl acetate and methanol were used in different combinations (hexane + ethyl acetate and ethyl acetate + methanol) for the elution process. A total of 30 fractions (F) each with 25 mL volume were eluted, including F1-F2 (100% hexane), F3-F9 (50% ethyl acetate in hexane), F10-F16 (100% ethyl acetate), F17-F23 (50% ethyl acetate in methanol) and F24-F30 (100% methanol). The total phenolic concentration (TPC) analysis was performed for all the fractions and the higher TPC containing fraction (F18) was further subjected to column chromatography for eluting the sub-fractions (Supplementary Fig. 4). Different solvent combinations (hexane + ethyl acetate & ethyl acetate + methanol) were used to elute 23 sub-fractions like SF1-SF3 (100% hexane), SF4-SF6 (50% ethyl acetate in hexane), SF7-SF9 (100% ethyl acetate), SF10-SF13 (75% ethyl acetate in methanol), SF14-SF17 (50% ethyl acetate in methanol), SF18-SF20 (25% ethyl acetate in methanol) and SF21-SF23 (100% methanol). All these sub-fractions (SF1-SF23) were analyzed for TPC and the selected fractions SF11, 12 15, 19 & 21 were screened for phytochemicals, of which only SF15 contained

high TPC and devoid of all other phytochemicals (tests as mentioned in subsection 2.3) was selected for characterization through direct MS analysis.

2.7. Direct mass spectrometry

Mass determination of phytochemicals in selected fraction SF15 was performed using the negative ion mode mass spectrometry analysis. The instrument was operated with electron spray ionization (ESI) source in the negative ion mode. The ionization was optimized in negative volts mode at given source conditions (capillary voltage 3.0 kV; cone voltage 35 kV; source temperature 120 °C and de-solvation temperature 350 °C). Nitrogen gas was supplied with the flow set at 50 and 800 L/h for cone and de-solvation, respectively. High-purity argon (99.9%) was used as a collision gas at a flow rate of 1.5 mL/min. The collision energy was set at 6.0 eV and mass spectra were obtained in the continuum mode across the range from 400 to 800 *m/z*.

2.8. Superoxide radical scavenging

Superoxide radical scavenging activity of *Ficus* fraction SF-15 was determined by the method of Zhishen et al. (1999). In the reaction mixture, there were 100 µL of different concentrations (10-0.125 mg/mL) of fraction SF15, 1 mL of riboflavin (0.003 mM), 1 mL of methionine (10 mM) and 1 mL of NBT (0.1 mM), which was held under a fluorescent lamp (15 W) for 15 minutes. A formazan with a purple color was generated because of the reaction between superoxide radicals with NBT. The absorbance of illuminated samples was noted at 560 nm and the percentage inhibition of radical scavenging activity was calculated using the formula:
Radical scavenging activity (%) = ((Control absorbance - test absorbance)/Control absorbance) x 100 (Eqn. 1)
On the basis of the concentration versus scavenging activity curve, the IC_{50} value was determined.

2.9. Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity was measured by following the methodology of Buyukbalci and Nehir (2008). Series of dilutions were prepared for the SF15 fraction (10.125, 0.250, 0.500, 1.00 and 10.0 mg/mL) using methanol. A reaction mixture was prepared by adding 0.1 mL of the SF15 fraction, 0.1 mL of H_2O_2 solution (0.5%), 1 mL of potassium iodide solution (1.0%), 1 mL of HCl (3.0%) and mixed well. This was followed by the addition of 1.5 mL of toluidine blue solution (0.1% *w/v*), 1 mL of sodium acetate and mixed well. Control group was prepared by adding 1 mL of H_2O instead of SF15 fraction sample. Absorbance was measured at 600 nm spectrometrically using spectrophotometer and gallic acid was used as the standard for determining the hydrogen peroxide scavenging activity**.**

2.10. Carbonyl assay

Determination of protein oxidation inhibition was performed by following the procedure of Frijhoff et al. (2015). The total protein-bound carbonyl content was calculated by derivatizing the protein carbonyl adducts with dinitrophenyl hydrazine (DNPH). This results in a stable dinitrophenyl hydrazone product, which was then measured spectrophotometrically using spectrophotometer at 540 nm. The goat skin tissue, which collected from local slauther-house, was homogenized with 10 mL of TCA (10%) and collected in a separating funnel and defatted with 10 mL of hexane. The protein-containing bottom layer was taken for the carbonyl test. Tissue lysate of goat skin (100 µL) was combined with *Ficus* fraction (100 µL), 500 µL of $FeSO_4$ (20 mM) and H_2O_2 (5.0%) and incubated for 1 hour at 37 °C in the dark. Then, 500 µL of the DNPH solution (5 mM in 2.5 M HCl) was added. After centrifuging the mixture at 2000 rpm for 5 minutes, the pellet was collected and extracted four times with 1 mL of a solution of ethyl acetate and ethanol (1:1, *v/v*) to get rid of the DNPH. Following the extraction, the pellet was diluted in 1 mL of guanidine hydrochloride (6 M) in order to measure the absorbance at 540 nm against the blank. Gallic acid was used as standard and the inhibition of protein oxidation level was calculated and expressed on percentage basis.

2.11. TBARS assay

Inhibition of lipid peroxidation in goat skin model was estimated using TBARS assay (Nivedha et al., 2020). The amount of malonaldehyde (MDA), a by-product of fatty acid peroxidation that turns pink when it reacts with 2-thiobarbituric acid (TBA), which was measured to determine the extent of lipid peroxidation. The goat skin tissue (0.5 g) was homogenized with 10 mL of TCA (10.0%) and collected in a separating funnel and 10 mL of hexane was added and mixed well with a closed lid and rested for 5 min. The lipids were separated as top layer (100 µL) and mixed with *Ficus* fraction (100 µL) and combined with 500 µL of $FeSO_4$ (20 mM) and H_2O_2 (5.0%). Then, the mixture was incubated for 1 h at 50 °C and after cooling, 1 mL of trichloroacetic acid, (TCA, 10%) and thiobarbituric acid (TBA, 0.8% at pH 4) were added. The mixture was further incubated at 90 °C for 30 minutes and the absorbance was determined at 520 nm against the blank (without tissue lysate) and standard (with gallic acid instead of the fraction).

2.12. Statistical analysis

All the experiments carried out with five replicates (n = 5). All the data were expressed as mean ± standard deviation and statistical analysis was performed by using Student's t-test using excel programme (MS office) to determine significance difference between the control and treated samples. The asterisk symbol on the bars indicate that the values are significantly different (*p < 0.05) when compared to untreated control.

3. Results and Discussion

3.1. Qualitative results of phytochemicals

There are different class of phytochemicals such as alkaloids, flavanoids, terpenoids, phytosterols,

saponins, phenols and tannins present in *Ficus racemosa* bark extracts (Supplementary Table 1). Among these photochemicals, alkaloids generally have tertiary amine, which reacts with Dragendroff's reagent (potassium bismuth iodide) formed an orange-red complex in only water extract of *F. racemosa* with low intensity. Flavonoids react with sodium hydroxide and gave yellow-colored compound in methanolic extract with high-intensity, low intensity in water extract and absent in other extracts (Supplementary Table 1). Terpenoids react with concentrated sulphuric acid and formed carbocation which imparts dark red color in all extracts of *F. racemosa* with high intensity in methanolic extract. Phytosterols react with chloroform, acetic acid, and concentrated sulphuric acid and formed a red colored bi-sterol complex in the lower layer in all extracts of *F. racemosa* with high intensity in methanolic extract and medium intensity in water extract. Saponins did not react with distilled water to align themselves vertically on the surface with a hydrophobic end oriented away from the water and does not results in prolonged foam showing the absence in all the extract of *F. racemosa* bark (Supplementary Table 1). Phenols were present with high intensity in methanolic extract and medium intensity in water extract, which react with ferric chloride to form ferric phenoxide which gaves dark blue color. Tannins on reaction with lead acetate formed creamy precipitate (lead tannates) in methanolic extract of *F. racemosa* with high intensity, low intensity in water extract and absent in other extracts. Thus, the overall qualitative analysis shows methanolic extract has different class of phytochemicals like flavonoids, terpenoids, phytosterols, phenols and tannins in high amounts. In agreement to our results, a previous literature revealed that ethanolic extract of *F. racemosa* bark had the presence of flavanoids, terpenoids, phytosterols, phenols, tannins and absence of alkaloids and saponins (Veerapur et al., 2009).

3.2. Quantitative results of phytochemicals

The total phenols in the *F. racemosa* extract react with phosphomolybdic-phosphotungstic acid of Folin-Ciocalteu reagent and gave blue chromophore in alkaline medium (Blainski et al., 2013). Methanolic extract of *F. racemosa* bark has significantly ($p < 0.05$) higher concentration of phenols (29.93 mg/mL) than the other extracts (Fig. 1A). Phenols have antioxidant property (Kumar and Goel, 2019). Similar to our results, a previous report showed that the total phenols content was high in *F. racemosa* (12.36 mg GAE/100 g) (Sulaiman and Balachandran, 2012). Tannin concentration was significantly ($p < 0.05$) higher in methanolic extract of *F. racemosa* (8.98 mg/mL) compared to other extracts (Fig. 1A). The reaction was binding of aldehyde group of vanillin to sixth carbon of tannins present in the extract to form red chromophore (Schofield et al., 2001). Pharmacological activities of tannins include antioxidant (Skrovankova et al., 2015) and anti-microbial (Marin et al., 2015). Ellagic acid is a tannin compound isolated from *F. racemose* bark (Ahmed and Urooj, 2010b).

The extract containing flavonoids with the C-4 keto group and C-3 or C-5 hydroxyl group reacts with aluminium chloride in an alkaline medium and forms acid-stable reddish color complexes. High amount of flavonoids 0.23 ± 0.08 mg/mL was present in chloroform extract of *F. racemosa* bark extract followed by water extract (0.20 ± 0.04 mg/mL) (Fig. 1B). Pharmacological properties of flavonoids includes anticancer, antioxidant, anti-inflammatory and antiviral properties (Ullah et al., 2020). Bergenin (flavonoid) was the major flavonoid component reported in *F. racemosa* bark (Yadav et al., 2015). The alkaloids in the extract was precipitated as alkoloid-bismuth iodide complex when Dragendroff's reagent was added and in the presence of thiourea, it produces the yellow colur (Sreevidya and Mehrotra, 2003). The water extract of *F. racemosa* bark was quantified to have a significantly ($p < 0.05$) higher alkaloid concentration of 0.18 ± 0.01 mg/mL than the other extracts (Fig. 1B). Alkaloids have antioxidant, anti-inflammatory and anti-cancer activities (Heinrich et al., 2021). No alkaloidal compound reported previously in *F. racemosa* bark.

Terpenoids in the extract react with chloroform to give derivatives of alcohol, which reacts with concentrated sulphuric acid to give a reddish-brown complex (Das et al., 2014). The maximum concentration of terpenoids (0.73 ± 0.01 mg/mL) was found in methanolic extract of *F. racemosa* bark followed by water extract (0.27 ± 0.01 mg/mL) and chloroform extract (0.24 ± 0.01 mg/mL) (Fig. 1C). Wound healing (James and Dubery, 2009) and anti-inflammatory (Vasas and Hohmann, 2014) properties are known for terpenoids. Lupenol is a terpenoid compound present in *F. racemosa* bark (Shiksharthi and Mittal, 2011). Significantly ($p < 0.05$) maximum phytosterol content of 3.28 ± 0.03 mg/mL was present in methanolic extract of *F. racemosa* bark extract, which is followed by water extract (1.23 ± 0.02 mg/mL) (Fig. 1C). Phytosterol in the extract reacts with ferric-chloride-acetic acid and concentrated sulphuric acid mixture to gives a pinkish red color complex (Saptarini et al., 2016). Anticancer (Ali et al., 2015), anti-inflammatory (Kariuki et al., 2012) and anti-diabetic (Nualkaew et al., 2015) are the pharmacological activities reported for phytosterols. Beta-sitosterol and stigmasterol were isolated from *F. racemosa* bark (Shiksharthi and Mittal, 2011).

This quantitative analysis shows that methanolic extract of *F. racemosa* bark has high content of flavonoids, terpenoids, phytosterols, phenols and tannins and hence, it was chosen for further investigation. Traditionally, water extract of *F. racemosa* bark has been used for several medicinal purposes. In this study, the qualitative and quantitative analysis provides that methanolic extract had significantly higher content of phytochemicals than water extract. Therefore, methanolic extract of *F. racemosa* bark can be used for medicinal uses for higher efficiency.

3.3. Comparison of different *Ficus* species barks

The phytochemical screening of methanolic extract of bark material of different *Ficus* species such as *Ficus racemosa*, *F. religiosa*, *F. benghalensis* and *F. hispida* were compared and the results are shown in Fig. 2 and Supplementary Table 2.

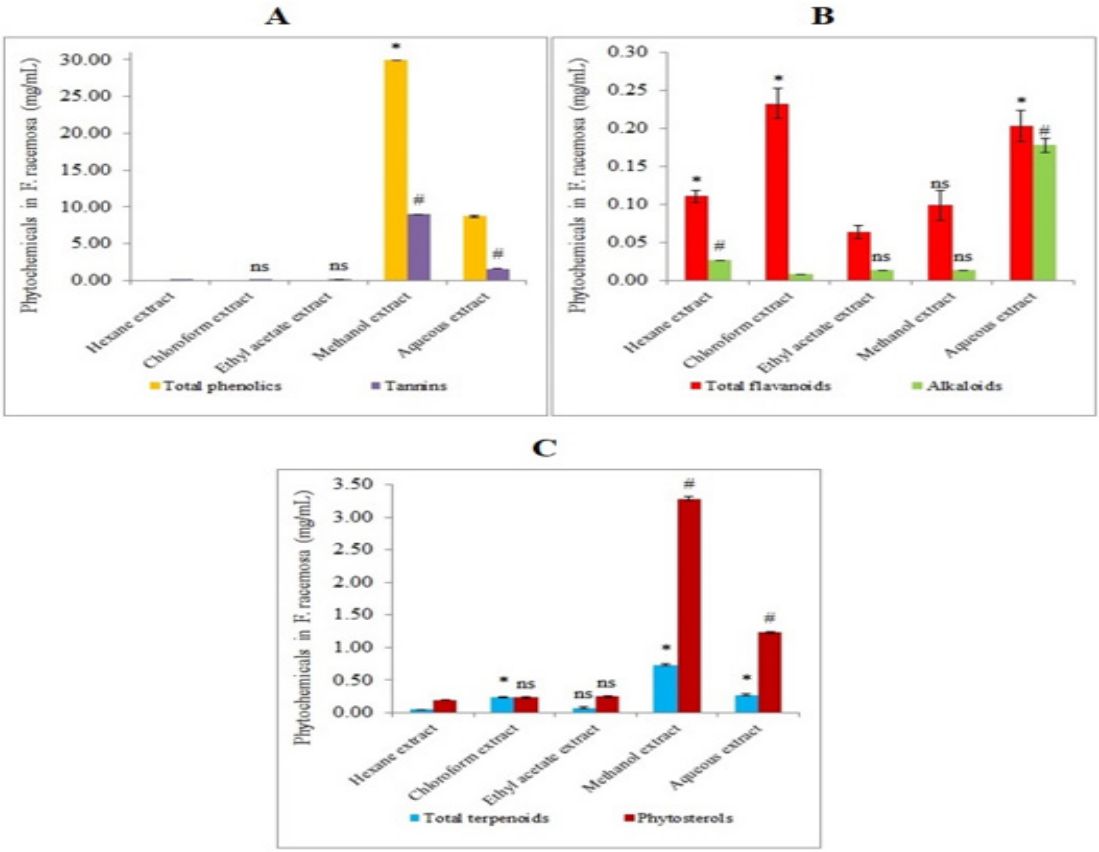

Fig. 1. Quantitative results on various phytochemicals including total phenols and tannins (**A**), flavonoids and alkaloids (**B**) and terpenoids & phytosterols (**C**) in different solvent extracts of *Ficus racemosa* bark. According to Student's t-test, * symbol on each bar indicates statistical significance ($p < 0.05$) of phytochemical content in each extract compared to the respective phytochemical content of hexane extract. The symbol 'ns' on the bar indicates that the value is not significant compared to the hexane extract.

F. racemosa contains significantly ($p < 0.05$) higher level of total phenolic compounds (29.93 mg/mL), tannins (8.98 mg/mL) and phytosterols (3.28 mg/mL) when compared to other *Ficus* species (1.57-19.06 mg/mL of total phenols; 0.30-5.57 mg/mL of tannins and 0.29-1.15 mg/mL of phytosterols). On the other hand, flavonoid level is significantly lower in *F. racemosa* (0.10 mg/mL) when compared to other *Ficus* species (4.10-8.95 mg/mL) (Fig. 2A). *F. religiosa* (0.24 ± 0.01 mg/mL) and *F. benghalensis* (0.07 ± 0.01 mg/mL) possess significantly ($p < 0.05$) higher level of alkaloids in methanolic extract while it is absent in *F. racemosa* (Fig. 2B). The previous studies also suggested that different class of phytochemicals show variation among species (*F. religiosa*, *F. glomerata*, *F. retusa* and *F. carica*) and total phenols and tannin content was found maximum in *F. religiosa* (Rawat et al., 2012). In another study, *Pinus sylvestris* had rich polyphenols and tannins while terpenoids are more in the related species *Pinus nigra* (Nisca et al., 2021). Among the different species, *F. racemosa* has high amount of different classes of phytochemicals and if other *Ficus* species barks are used instead of *F. racemosa* due to increased market demand, the medicinal efficiency could change because of the fact that the amount of the phytochemicals in other *Ficus* species is lesser compared to the *F. racemosa* bark. Also, the phytochemical variability detected in the present work among *Ficus* species would be helpful to identify the *F. racemosa* bark and also to discriminate other *Ficus* bark materials.

3.4. Comparison of trunk and branch barks

The phytochemical profile of methanolic extract of *F. racemosa* trunk bark was compared with branch bark in Fig. 3A-B and Supplementary Table 3. Alkaloids and flavonoids content are comparable between branch bark and trunk bark of *F. racemosa*. However, phytosterols (0.20 mg/mL), phenols (0.90 mg/mL) and tannins (0.42 mg/mL) are present significantly ($p < 0.05$) lower amounts in branch bark compared to trunk bark. This shows that trunk bark of *F. racemosa* has high amount of phytochemical than branch bark. The trunk bark of *F. racemosa* is the authentic material that is being used as a drug in Siddha and Ayurveda systems of Indian medicine. Adulteration may happen with branch bark due to increase in market demand for *F. racemosa* trunk bark, which may leads to lower

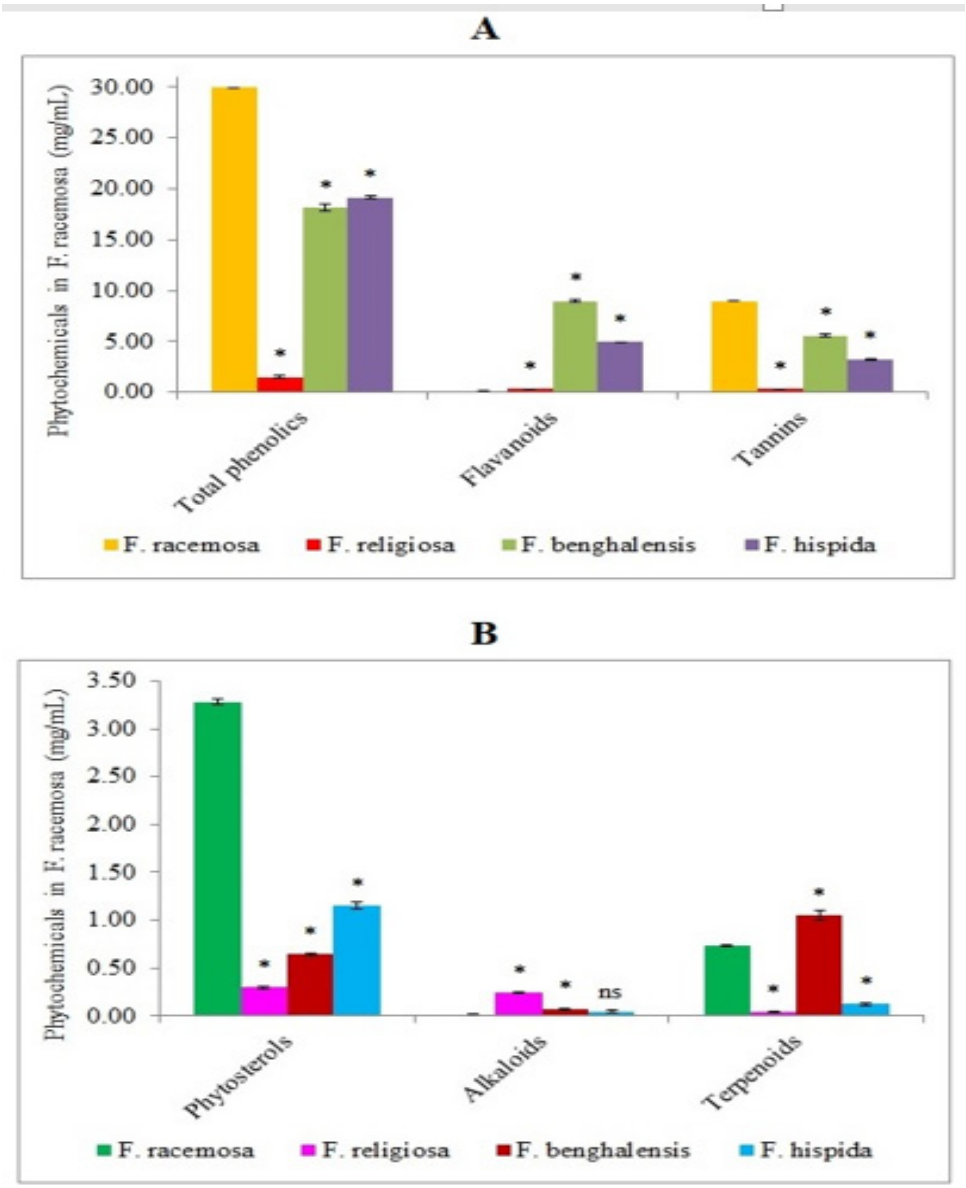

Fig. 2. Variation in the content of phytochemicals including total phenols, flavonoids & tannins (**A**) and phytosterols, alkaloids & terpenoids (**B**) in methanolic extract of bark material of *F. racemosa* and other *Ficus* species. According to Student's t-test, * symbol on each bar indicates statistical significance ($p < 0.05$) of phytochemical content of other *Ficus* species bark compared to the bark sample of *F. racemosa*. The symbol 'ns' on the bar indicates that the value is not significant compared to the *F. racemosa* bark.

the medicinal efficiency. Similar to our results, the phenolic content was reported as higher in trunk bark compared to branch bark in Silver fir (Vek et al., 2022) and Srivastava et al. (2016) also depicted that content of total phenols and flavonoids were lesser in branch bark than trunk bark of *Myrica esculenta*.

3.4. Comparison of trunk and branch barks

The phytochemical profile of methanolic extract of *F. racemosa* trunk bark was compared with branch bark in Fig. 3A-B and Supplementary Table 3. Alkaloids and flavonoids content are comparable between branch bark and trunk bark of *F. racemosa*. However, phytosterols (0.20 mg/mL), phenols (0.90 mg/mL) and tannins (0.42 mg/mL) are present significantly ($p < 0.05$) lower amounts in branch bark compared to trunk bark. This shows that trunk bark of *F. racemosa* has high amount of phytochemical than branch bark. The trunk bark of *F. racemosa* is the authentic material that is being used as a drug in Siddha and Ayurveda systems of Indian medicine. Adulteration may happen with branch bark due to increase in market demand for *F. racemosa* trunk bark, which may leads to lower the medicinal efficiency. Similar to our results, the phenolic content was reported as higher in trunk bark

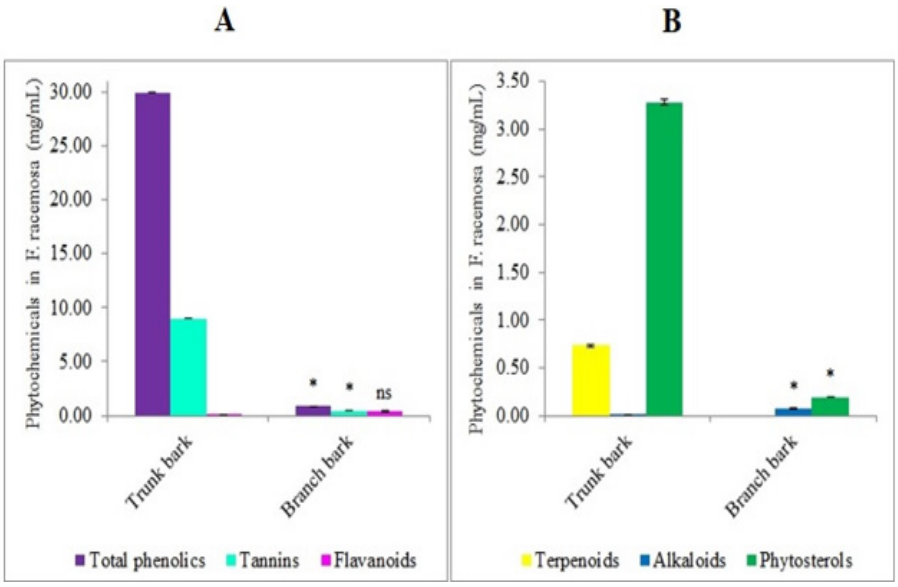

Fig. 3. Comparison of phytochemicals including total phenols, tannins & flavonoids (**A**) and terpenoids, alkaloids & phytosterols (**B**) between methanolic extracts of trunk and branch barks of *F. racemosa*. According to Student's t-test, * symbol on each bar indicates statistical significance ($p < 0.05$) of phytochemical content of branch bark compared to the trunk bark of *F. racemosa*. The symbol 'ns' on the bar indicates that the value of branch bark is not significant compared to the trunk bark of *F. racemosa*.

compared to branch bark in Silver fir (Vek et al., 2022) and Srivastava et al. (2016) also depicted that content of total phenols and flavonoids were lesser in branch bark than trunk bark of *Myrica esculenta*.

3.5. Comparison of bark and wood

Methanolic extract of bark of *F. racemosa* was compared with wood, in which except flavonoids (0.49 ± 0.04 mg/mL), all other phytochemicals like alkaloids (0.06 ± 0.01 mg/mL), terpenoids (0.11 ± 0.01 mg/mL), phytosterols (0.09 mg/mL), phenols (1.01 ± 0.05 mg/mL) and tannins (0.32 ± 0.03 mg/mL) are present in significantly ($p < 0.05$) lower amount in wood compared to *F. racemosa* bark (Fig. 4A-B and Supplementary Table 4). Based on qualitative analysis, saponins was found absent in both bark and wood of *F. racemosa*. This evaluation depicts that wood has very low amount of phytochemicals compared to trunk bark of *F. racemosa*. In agreement to our findings, previous study revealed the presence of more number of phytochemicals (32 compounds) in the bark compared to wood (28 compounds) of *Sonneratia caseolaaris* (Ghalib et al., 2011). This gives clear statement that wood can not be used as an alternative for *F. racemosa* bark even if market demand rises and if used, the therapeutic efficacy could be reduced.

3.6. Effect of storage of bark material

The methanolic extract of freash bark of *F. racemosa* was compared with stored bark, in which all classes of phytochemicals such as flavonoids (4.10 ± 0.04 mg/mL), terpenoids (0.72 ± 0.01 mg/mL), phytosterols (3.76 ± 0.03 mg/mL), phenols (9.87 ± 0.08 mg/mL) and tannins (2.29 ± 0.03 mg/mL) were recorded in stored bark (Fig. 5A-B and Supplementary Table 5). Both phenols and tannin levels of stored bark are significantly ($p < 0.05$) reduced when compared to fresh bark. Alkaloids and saponins are absent in stored bark of *F. racemosa*. Significant increase in phytosterols (3.76 ± 0.03 mg/mL) and non-significant increase in flavonoid contents (4.10 ± 0.04 mg/mL) were noticed during storage compared to fresh bark (3.23 ± 0.03 mg/mL of phytosterols and 0.10 ± 0.02 mg/mL of flavonoids). This shows that the storage for 1 month can change in the amount of phytochemicals in bark of *F. racemosa* and hence it is advisable to use fresh bark of *F. racemosa* to obtain maximal medicinal benefits. Literature also sugested that *Chichona* bark stored for long period results in decreased alkaloid content (Canales et al., 2020).

3.7. UPLC-QTOF-MS results

UPLC-QTOF-MS results for methanolic bark extracts of *F. racemosa*, *F. religiosa*, *F. benghalensis* and *F. hispida* are given in the Fig. 6 and Supplementary Table 6. Out of eight compounds detected, four are present in methanolic bark extract of *F. racemosa*, which includes kaempferol-3-*O*-beta-D-6-malonyl-glucoside, *iso*-orientin, sinomenine and procyanidin C1. The compound kaempferol-3-*O*-beta-D-6-malonyl-glucoside was detected as a first peak at a retention

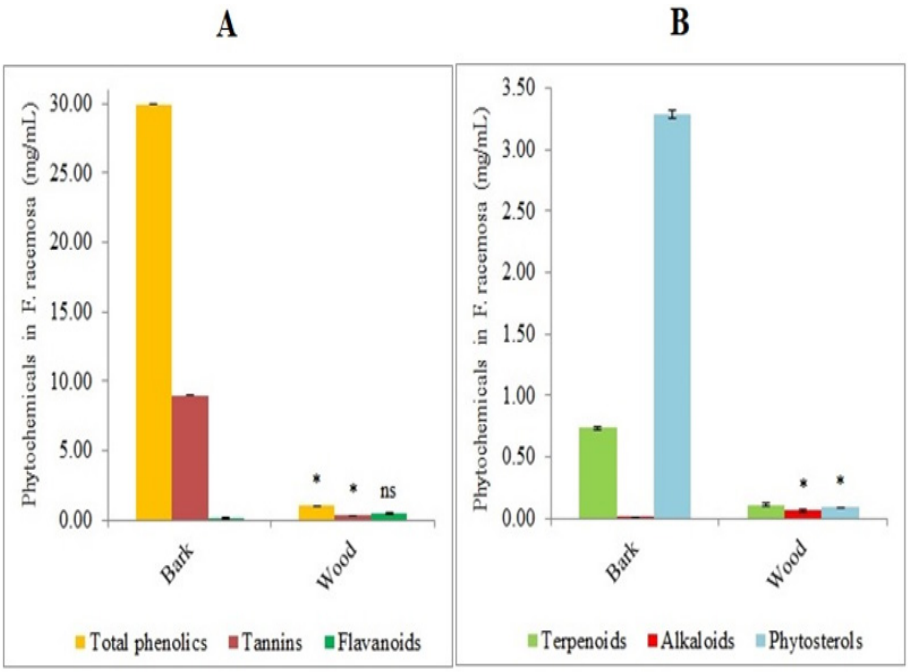

Fig. 4. Differences in the phytochemicals including total phenols, tannins & flavonoids (**A**) and terpenoids, alkaloids & phytosterols (**B**) content of methanolic extract of bark and wood materials of *F. racemosa*. According to Student's t-test, * symbol on each bar indicates statistical significance ($p < 0.05$) of phytochemicals content of wood compared to the trunk bark of *F. racemosa*. The symbol 'ns' on the bar indicates that the value of wood extract is not significant compared to bark extracts of *F. racemosa*.

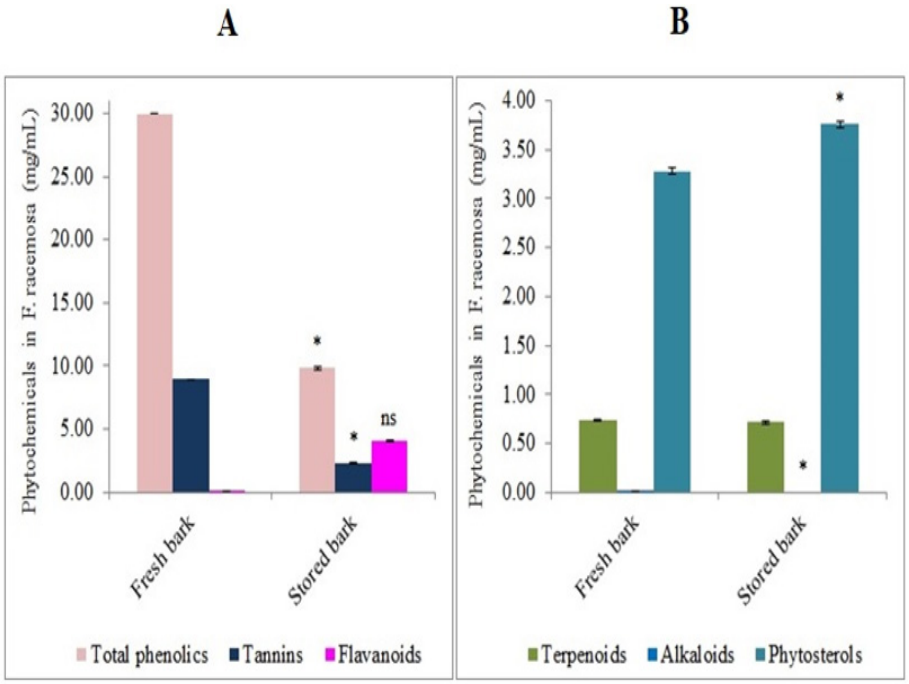

Fig. 5. Variability in the phytochemicals including total phenols, tannins & flavonoids (**A**) and terpenoids, alkaloids & phytosterols (**B**) contents of methanolic extract of fresh and stored bark samples of *F. racemosa*. According to Student's t-test, * symbol on each bar indicates statistical significance ($p < 0.05$) of phytochemicals content of stored bark sample compared to fresh bark of *F. racemosa*. The symbol 'ns' on the bar indicates that the value of stored sample is not significant compared to fresh bark of *F. racemosa*.

time of 1.223 min with parent mass of 533 *m/z* and daughter ions mass of 341 and 191 *m/z*. As the fifth peak, the *iso*-orientin compound was detected at the retention time of 4.450 min and the mass of the parent ion 447 *m/z*. The sinomenine was detected as an seventh peak at a retention time of 4.707 min with the mass of 328 *m/z* parent ion and daughter ion mass of 327 *m/z*. The procyanidin C1 was detected as the eighth peak at the retention time of 4.960 min with 865 *m/z* parent mass and 417 and 289 *m/z* daughter ions mass. Kaempferol-3-*O*-β-D-glucoside (also known as Astragalin) is a flavonoid that had anti-inflammatory and antioxidant properties (Soromou et al., 2012). Iso-orientin is a *C*-glucosyl flavone with robust antioxidant, anti-inflammatory and ameliorative properties against hyperglycemia, hyperlipidemia and insulin resistance (Ziqubu et al., 2020). Sinomenine is an alkaloid derived compound, which has anti-inflammatory effects, analgesic properties and antioxidant properties (Jiang et al., 2020). Procyanidin C1 is a flavonoid, having potent pharmacological properties, such as antioxidant, antibacterial, anti-inflammatory and anti-tumor activities (Chen et al., 2022).

Meranzin is the only compound in methanolic bark extract of *F. religiosa* detected at retention time of 5.453 min and parent mass was 260 *m/z*. It has the ability to reverses psychosocial stress-induced mood disorders, gastrointestinal dysfunction and cardiac disease (Liu et al., 2021). In methanolic bark extract of *F. benghalensis* there are two compounds quinic acid and loganic acid. Quinic acid was detected at retention time of 1.240 min with parent mass 191 *m/z*. Radioprotection, anti-neuroinflammatory and antioxidant are pharmacological activities of quinic acid (Jang et al., 2017). At the retention time of 4.977 min, loganic acid was detected with parent mass 375 *m/z* and daughter ions mass of 353, 351, 215 *m/z*. Methanolic bark extract of *F. hispida* was found to have one compound, oleanane. Oleanane was detected at retention time of 1.223 min with parent mass 440 *m/z* and daughter ions mass of 341, 197 and 195 *m/z*. It suppresses inflammation and oxidative stress (Liby et al., 2012).

The comparison among the UPLC-QTOF-MS results for methanolic bark extracts of *F. racemosa*, *F. religiosa*, *F. benghalensis* and *F. hispida* was found to have no similarity in matched compounds. This shows that there were difference in phytpchemical compounds present in different species belonging to same genus. Also, it highlights that if other *Ficus* species are used in place of *F. racemosa* the efficacy may decrease due to difference in compound. This is supported by the literature (Singh et al., 2016), in which bark extract of six *Terminalia* species was found to have different compounds. The variation in the phytochemical profile registered in UPLC-QTOF-MS study could be employed as species-specific chemical markers to identify and authenticate actual genuine herbal material (*F. racemosa*) and also to discriminate the other morphologically similar *Ficus* species bark samples.

3.8. Purification of *F. racemosa* extract

There were 30 fractions collected from column chromatographic separation of methanolic extract of *F. racemosa* bark. Among the 30 fractions F18 fraction had high total phenolic content of 14.29 mg/mL (Supplementary Fig. 4), which was subjected for further purification by column chromatography and 23 sub-fractions were collected. Among the collected sub-fractions, SF11, SF12, SF15, SF19 and SF21 showed higher TPC of 4.82, 2.97, 4.04, 3.14 and 3.48 mg/mL, respectively. Among these sub-fractions, SF11 and SF12 had other phytochemicals like terpenoids and phytosterols in addition to phenols and tannins, so they were considered mixture of several compounds and hence not selected for further analysis (Supplementary Table 6). In SF15, 19 and 21, only phenols and tannins were detected and based on TPC value, SF15 was selected for direct MS analysis and also subjected to *in vitro* antioxidant assays. Direct MS (negative ion mode) result of fraction SF15 was given in Supplementary Fig. 5. In direct MS, the X-axis represented in mass to charge ratio (*m/z*) and Y-axis in percentage of mass signal abundance (%). The mass peak signals detected at 533, 341 and 191 *m/z* were matched with mass bank library and identified as kaempferol-3-*O*-β-D-glucoside. Medicinal value of this compound includes anti-microbial effects (Rigano et al., 2007), neuroprotective effects (Kim et al., 2017) and cardioprotective effect (Zhou et al., 2019). Hence, presence of kaempferol-3-*O*-β-D-glucoside might play a vital role in the medicinal properties exhibited by *F. racemosa* bark.

3.9. Superoxide scavenging activity

Superoxide is a reactive oxygen species (ROS) containing a negatively charged dioxygen group. Its dynamic changes can provide broad implications in physiological and pathological conditions. Superoxide anion is produced from molecular oxygen by single electron addition and it serves as a precursor for free radicals such as hydrogen peroxide, hydroxyl and nitric oxide (Costa et al., 2021). Both superoxide and its daughter radicals have the potential to react with biological macromolecules and thereby induce tissue damages. In this assay, photo-induced reduction of riboflavin leads to superoxide radical formation from surrounding oxygen in presence of methionine and the generated superoxide radical, which reduces the NBT into purple color formazan that can be measured at 560 nm (Liang et al., 2015). Superoxide radical was found to be scavenged moderately (55.56 ± 3.14% at 10 mg/mL) by fraction SF15 with IC_{50} value of 0.92 mg/mL, which was significantly ($p < 0.05$) lower than that of gallic acid (75 ± 2.07% at 10 mg/mL, IC_{50} = 0.62 mg/mL) (Fig. 7A). However, the *Ficus* fraction exhibits comparable scavenging activity to that of gallic acid at low concentrations of 1 mg/mL (54.44 ± 1.57%), 0.250 mg/mL (35.56 ± 3.14%) and 0.125 mg/mL (22.22 ± 3.14%). The scavenging activity can be increased by increasing the concentration of fraction of *F. racemosa* bark extract. Such superoxide scavenging can reduces oxidative stress in cells and thereby promoting overall cellular health (Lim et al., 2021).

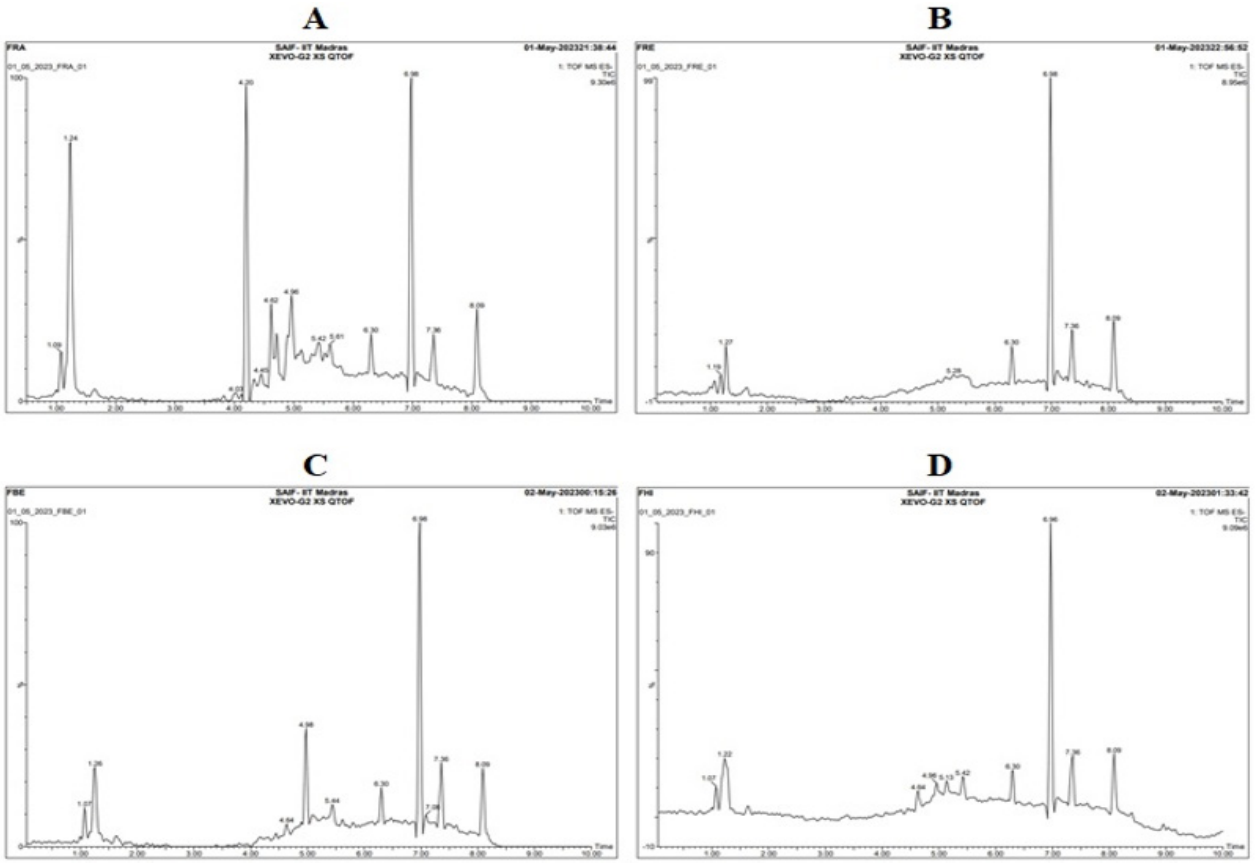

Fig. 6. Phytochemical profile of *Ficus racemosa* (**A**), *F. religiosa* (**B**), *F. benghalensis* (**C**) and *F. hispida* (**D**) analyzed through UPLC-QTOF-MS technique.

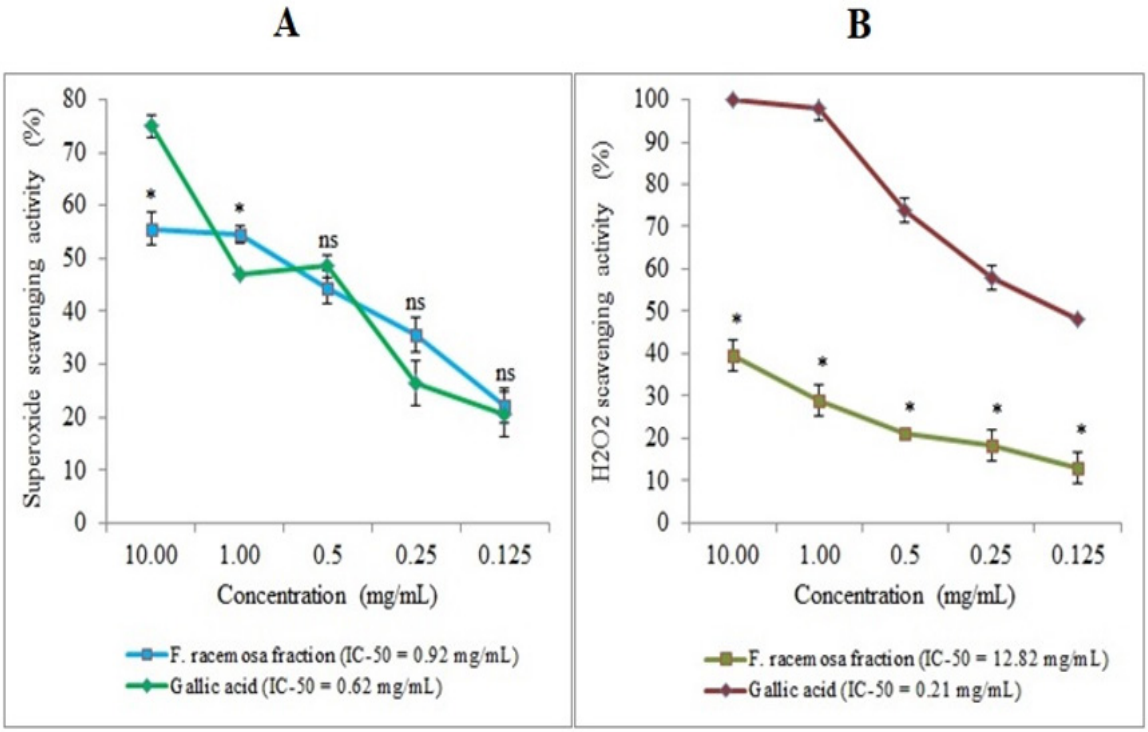

Fig. 7. Free radical scavenging activity of *F. racemosa* fraction against superoxide (**A**) and hydrogen peroxide radicals (**B**). According to Student's t-test, * symbol on each point indicates statistical significance ($p < 0.05$) of *F. racemosa* fraction compared to the standard gallic acid. The symbol 'ns' on each point indicates that *F. racemosa* fraction is not significant compared to gallic acid.

3.10. Scavenging effect on hydrogen peroxide

Hydrogen peroxide is produced when oxygen reacts with certain metal ions, such as iron or copper, or with enzymes called peroxidases. Hydrogen peroxide is highly reactive and damages various biomolecules in cells, including proteins, lipids and DNA, leads to oxidative stress. Hydrogen peroxide scavenging activity refers to the ability of certain substances or compounds to remove hydrogen peroxide from biological systems and prevent its harmful effects (Phaniendra et al., 2015). In this experiment, hydrogen peroxide reacts with potassium iodide in acid medium liberating iodine. The liberated iodine bleaches the blue color of toluidine blue in the presence of sodium acetate to a colorless species and is measured at 600 nm (Pasha et al., 2016). The hydrogen peroxide scavenging activity of fraction SF15 of *F. racemosa* bark extract was significantly ($p < 0.05$) less effective in all the concentrations (39.47 ± 3.72% at 10 mg/mL) with IC_{50} value 12.82 mg/mL compared to the standard gallic acid (100% at 10 mg/mL) which has IC_{50} value of 0.21 mg/mL (Fig. 7B). Generally hydrogen peroxide is involved in the development and progression of cancer by promoting DNA damage and cellular mutations (Vilema-Enríquez et al., 2016). Scavenging hydrogen peroxide may help to prevent the development of diseases like cancer. Also, excessive amounts of hydrogen peroxide can delay wound healing and hence scavenging excess hydrogen peroxide can promote faster healing of wounds (Loo et al., 2012).

3.11. Inhibition of lipid peroxidation

Free radicals like superoxide, hydrogen peroxide and hydroxyl radicals attack lipids that have carbon-carbon double bonds during a process known as lipid peroxidation, which worsens the integrity of biological membranes (Ayala et al., 2014). Lipid peroxidation refers to the oxidative degradation of lipids, particularly unsaturated fatty acids by ROS. Goat skin's fatty material was added to the sample in this case along with ferrous and hydrogen peroxide (Fenton reaction). As a result, aldehydes are produced, malondialdehyde (MDA) being one of them, which is a significant indicator of oxidative damage. The TBA assay (Thiobarbituric acid reactive substances, TBARS) is a commonly used method to measure the levels of lipid peroxidation in biological samples (Khoubnasabjafari et al., 2015). The TBARS assay is based on the reaction between malondialdehyde (MDA, a byproduct of lipid peroxidation), and TBA, which forms a pink-colored complex that can be measured spectrophotometrically. The lipid peroxidation inhibition capacity of fraction SF15 was 74.44 ± 1.57% which is nearly same (not significant) as standard gallic acid (81.11 ± 2.71%) at 10 mg/mL concentration (Fig. 8A). The uses of inhibition of lipid peroxidation include protection against oxidative stress, inflammation, aging and prevention of chronic diseases.

3.12. Inhibition of protein oxidation

Protein oxidation is the process through which ROS including superoxide, hydrogen peroxide and hydroxyl radicals can alter the proteins. Numerous oxidative modifications, such as carbonylation, sulfonation, and nitration may arise as a result of protein oxidation (Gonos et al., 2018). The carbonyl test is a widely used method to measure the levels of protein carbonylation, which is a marker of oxidative damage to proteins (Song et al., 2020). It provides valuable information about the

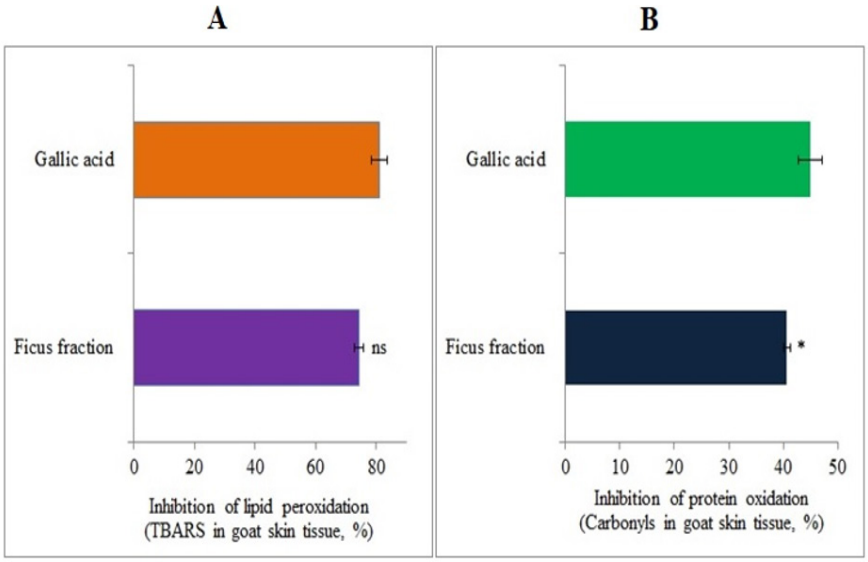

Fig. 8. Inhibition capacity of *F. racemosa* fraction against lipid peroxidation (**A**) and protein oxidation (**B**) in goat skin tissue model. According to Student's t-test, * symbol on each bar indicates statistical significance ($p < 0.05$) of *F. racemosa* fraction-treated sample compared to the standard gallic acid-treated sample. The symbol 'ns' on each point indicates that *F. racemosa* fraction is not significant compared to gallic acid.

efficacy of interventions aimed at preventing or reducing protein oxidation. Protein carbonylation can occur when proteins are exposed to ROS and other oxidative agents, leading to the formation of carbonyl groups on specific amino acid residues. Here, hydrogen peroxide and ferrous are introduced to proteins of goat skin, which oxidized the proteins and led to the formation of carbonyls. The DNPH reacts with the carbonyl group in the proteins to form dinitrophenylhydrazone, which can be measured spectrophotometrically (Apriceno et al., 2018). The protein oxidation inhibition capacity of fraction SF15 (40.65 ± 2.15%) was found significantly ($p < 0.05$) lower than that of standard gallic acid (45 ± 2.12%) (Fig. 8B). It shows that the capacity of *Ficus* fraction SF15 was found moderately effective to inhibit the oxidation of proteins. Inhibition of protein oxidation is an important target to mitigate oxidative stress-related diseases. As protein oxidation is closely associated with the aging process, inhibition of protein oxidation can prevent age-related diseases (Reeg and Grune, 2015).

4. Concluding remarks

The study concludes that the methanolic extract of *F. racemosa* bark contains higher amount of phytochemicals than aqueous extract, so it could be used to replace aqueous extract, which is traditionally used in Siddha and Ayurveda. Among the different *Ficus* species, methanolic extract of *F. racemosa* bark was found to possess higher content of phytochemicals than *F. religiosa*, *F. benghalensis* and *F. hispida* and therefore, usage of other *Ficus* species bark instead of *F. racemosa* bark could reduce the medicinal efficiency. Trunk bark of *F. racemosa* was quantified to have high amount of phytochemicals than branch bark. Also, bark was found to contain higher concentration of different phytochemicals than *F. racemosa* wood. Stored bark of *F. racemosa* was found to exhibit lesser amount of phytochemicals than fresh bark. These data suggests that trunk bark of *F. racemosa*, which was traditionally used in Indian system of medicine has high content of phytochemicals. So, if other materials such as wood, branch bark or stored bark are used in place of trunk bark, the efficacy may be reduced. The UPLC-QTOF-MS data reveals there are no similar compounds among the *Ficus* species and hence genuine herbal drug F. racemosa bark could be precisely identified by using phytochemical markers. Fraction SF15 purified from methanol extract was identified as kaempferol-3-*O*-β-D-glucoside. *In vitro* analysis of SF15 fraction exhibits moderate superoxide and hydrogen peroxide scavenging activities, high inhibition of lipid peroxidation and moderate inhibition of protein oxidation. As the fraction SF15 exhibited good antioxidant property in skin model, it can be used for the manufacturing various cosmetic products, so that it can protect the cells from oxidative damages caused by free radicals and thus prevents skin disease and aging process.

Supplementary file

Supplementary file includes Supplementary Fig. 1-5 and Supplementary Table 1-7.

Author contribution statement

All authors contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.

Conflict of interest

The authors declare that there is no conflict of interests.

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References

Ahmed, F., Sharanappa, P., Urooj, A., 2010. Antibacterial activities of various sequential extracts of *Ficus racemosa* stem bark. Pharmacog. J. 2, 203-206.

Ahmed, F., Iqbal, M., 2018. Antioxidant activity of *Ricinus communis*. Org. Med. Chem. Int. J. 5(4), 107-112.

Ahmed, F., Urooj, A., 2011. Pharmacognostical studies on *Ficus racemosa* stem bark. Pharmacog. J. 3(19), 19-24.

Ahmed, F., Urooj, A., 2010. *In vitro* studies on the hypoglycemic potential of *Ficus racemosa* stem bark. J. Sci. Food Agric. 90, 397-401.

Ahmed, F., Urooj, A., 2010. Traditional uses, medicinal properties, and phytopharmacology of *Ficus racemosa*: A review. Pharm. Biol. 48(6), 672-681.

Akshaya, M., Swetha, K.S., Keerthana, D., Vadivel, V., 2013. *In vitro* studies on wound healing mechanisms of leaves of *Ipomoea carnea*, an Indian ethnomedicine. S. Afr. J. Bot. 154, 239-250.

Ali, H., Dixit, S., Ali, D., Alqahtani, S.M., Alkahtani, S., Alarifi, S., 2015. Isolation and evaluation of anticancer efficacy of stigmasterol in a mouse model of DMBA-induced skin carcinoma. Drug Des. Devel. Ther. 28(9), 2793-2800.

Apriceno, A., Girelli, A.M., Scuto, F.R., 2018. Design of a heterogeneous enzymatic catalyst on chitosan: Investigation of the role of conjugation chemistry in the catalytic activity of a Laccase from *Trametes versicolor*. J. Chem. Technol. Biotechnol. 93(5), 1413-1420.

Ayala, A., Muñoz, M.F., Argüelles, S., 2014. Lipid peroxidation: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid. Med. Cell. Longev. 2014, 360438.

Bhalerao, S.A., Sharma, A.S., 2014. Ethenomedicinal, phytochemical and pharmacological profile of *Ficus religiosa* Roxb. Int. J. Curr. Microbiol. App. Sci. 3(11), 528-538.

Bhaskara Rao, R., Murugesan, T., Pal, M., Saha, B.P.,

Mandal, S.C., 2003. Antitussive potential of methanol extract of stem bark of *Ficus racemosa* Linn. Phytother. Res. 17(9), 1117-1118.
Bhaskara Rao, B.R., Anipama, K., Swaroop, A., Murugesan, T., Pal, M., Mandal, S.C., 2002. Evaluation of anti-pyretic potential of *Ficus racemosa bark*. Phytomedicine 9, 731-733.
Blainski, A., Lopes, G.C., de Mello, J.C., 2013. Application and analysis of the Folin Ciocalteu method for the determination of the total phenolic content from *Limonium brasiliense* L. Molecules 18(6), 6852-6865.
Buyukbalci, A., Nehir, E.I.S., 2008. Determination of *in vitro* antidiabetic effects, antioxidant activities and phenol contents of some herbal teas. Plant Foods Hum. Nutr. 63(1), 27-33.
Canales, N.A., Gress Hansen, T.N., Cornett, C., Walker, K., Driver, F., Antonelli, A., Maldonado, C., Nesbitt, M., Barnes, C.J., Rønsted, N., 2020. Historical chemical annotations of *Cinchona* bark collections are comparable to results from current day high-pressure liquid chromatography technologies. J. Ethnopharmacol. 1, 249.
Chandrashekhar, C.H., Latha, K.P., Vagdevi, H.M., Vaidya, V.P., 2008. Anthelmintic activity of the crude extracts of *Ficus racemosa*. Int. J. Green Pharm. 2, 100-103.
Chen, H., Wang, W., Yu, S., Wang, H., Tian, Z., Zhu, S., 2022. Procyanidins and their therapeutic potential against oral diseases. Molecules 27(9), 2932.
Costa, T.J., Barros, P.R., Arce, C., Santos, J.D., da Silva-Neto, J., Egea, G., Dantas, A.P., Tostes, R.C., Jimenez-Altayo, F., 2021. The homeostatic role of hydrogen peroxide, superoxide anion and nitric oxide in the vasculature. Free Radic. Biol. Med. 162, 615-635.
Das, B.K., Al-Amin, M.M., Russel, S.M., Kabir, S., Bhattacherjee, R., Hannan, J.M., 2014. Phytochemical screening and evaluation of analgesic activity of *Oroxylum indicum*. Indian J. Pharm. Sci. 76(6), 571-575.
Deyab, M., Elkatony, T., Ward, F., 2016. Qualitative and quantitative analysis of phytochemical studies on brown seaweed, *Dictyota dichotoma*. Int. J. Eng. Dev. Res. 4, 674-678.
Dharmadeva, S., Galgamuwa, L.S., Prasadinie, C., Kumarasinghe, N., 2018. *In vitro* anti-inflammatory activity of *Ficus racemosa* L. bark using albumin denaturation method. Ayush 39(4), 239-242.
Frijhoff, J., Winyard, P.G., Zarkovic, N., Davies, S.S., Stocker, R., Cheng, D., Knight, A.R., Taylor, E.L., Oettrich, J., Ruskovska, T., Gasparovic, A.C., 2015. Clinical relevance of biomarkers of oxidative stress. Antioxid. Redox. Signal. 23(14), 1144-1170.
Ghalib, R.M., Hashim, R., Sulaiman, O., Awalludin, M.F.B., Mehdi, S.H., Kawamura, F., 2011. Fingerprint chemotaxonomic GC-TOFMS profile of wood and bark of mangrove tree *Sonneratia caseolaris* (L.) Engl. J. Saudi Chem. Soc. 15(3), 229-237.
Gonos, E.S., Kapetanou, M., Sereikaite, J., Bartosz, G., Naparło, K., Grzesik, M., Sadowska-Bartosz, I., 2018. Origin and pathophysiology of protein carbonylation, nitration and chlorination in age-related brain diseases and aging. Aging 10(5), 868.
Heinrich, M., Mah, J., Amirkia, V., 2021. Alkaloids used as medicines: Structural phytochemistry meets biodiversity - An update and forward look. Molecules 26(7), 1836.
Iqbal, E., Salim, K.A., Lim, L.B.L., 2015. Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of *Goniothalamus velutinus* (Airy Shaw) from Brunei Darussalam. J. King Saud Univ. Sci. 27(3), 224-232.
James, J.T., Dubery, I.A., 2009. Pentacyclic triterpenoids from the medicinal herb, *Centella asiatica* (L.) Urban. *Molecules* 14(10), 3922-3941.
Jang, S.A., Park, D.W., Kwon, J.E., Song, H.S., Park, B., Jeon, H., Sohn, E.H., Koo, H.J., Kang, S.C., 2017. Quinic acid inhibits vascular inflammation in TNF-α-stimulated vascular smooth muscle cells. Biomed. Pharmacother. 96, 563-571.
Jiang, W., Fan, W., Gao, T., Li, T., Yin, Z., Guo, H., Wang, L., Yanxing, H., Jian-Dong, J., 2020. Analgesic mechanism of sinomenine against chronic pain. Pain Res. Manag. Article No. 1876862.
Joy, P.P., Thomas, J., Mathew, S., Skaria, B.P., 2001. Medicinal plants. Trop. Agric. 2, 123-125.
Kariuki, D.K., Kanui, T.I., Mbugua, P.M., Githinji, C.G., 2012. Analgesic and anti-inflammatory activities of 9-hexacosene and stigmasterol isolated from *Mondia whytei*. Phytopharmacology 2, 212-223.
Keshari, A.K., Kumar, G., Kushwaha, P.S., Bhardwaj, M., Kumar, P., Rawat, A., Kumar, D., Prakash, A., Ghosh, B., Saha, S., 2016. Isolated flavonoids from *Ficus racemosa* stem bark possess antidiabetic, hypolipidemic and protective effects in albino Wistar rats. J. Ethnopharmacol. 181, 252-262.
Khoubnasabjafari, M., Ansarin, K., Jouyban, A., 2015. Reliability of malondialdehyde as a biomarker of oxidative stress in psychological disorders. Bioimpacts: BI 5(3), 123.
Kim, K.M., Im, A.R., Lee, S., Chae, S., 2017. Dual protective effects of flavonoids from *Petasites japonicus* against UVB-induced apoptosis mediated via HSF-1 activated heat shock proteins and Nrf2-activated heme oxygenase-1 pathways. Biol. Pharm. Bull. 40(6), 765-773.
Kobmoo, N., Hossaert-Mckey, M., Rasplus, J.Y., Kjellberg, R. 2010. *Ficus racemosa* is pollinated by a single population of a single agaonid wasp species in continental South-East Asia. Mol. Ecol. 19, 2700-2712.
Kumar, N., Goel, N., 2019. Phenolic acids: Natural versatile molecules with promising therapeutic applications. Biotechnol. Rep. (Amst.) 24, 00370.
Liang, J.Y., Cheng, C.W., Yu, C.H., Chen, L.Y., 2015. Investigations of blue light-induced reactive oxygen species from flavin mononucleotide on inactivation of *E. coli*. J. Photochem. Photobiol. B: Biol. 143, 82-88.
Liby, K.T., Sporn, M.B., 2012. Synthetic oleanane triterpenoids: Multifunctional drugs with a broad range of applications for prevention and treatment of chronic disease. Pharmacol. Rev. 64(4), 972-1003.
Lim, S.H., Lee, H.S., Lee, C.H., Choi, C.I., 2021. Pharmacological activity of *Garcinia indica* (Kokum), An updated review. Pharmaceuticals 14(12), 1338.
Liu, X.F., Zhou, J., Tian Zhang, T., Ken Chen, K., Min Xu, M., Lei Wu, L., Jin Liu, J., Yun Ke Huang. Y., Bin Bin Nie, B., Xu Shen, X., Ping Ren, P., Huang, X., 2021. Meranzin hydrate elicits antidepressant effects and restores reward circuitry. Behav. Brain Res. 398, 112898.
Loo, A.E., Wong, Y.T., Ho, R., Wasser, M., Du, T., Ng, W.T., Halliwell, B., 2012. Effects of hydrogen peroxide on wound healing in mice in relation to oxidative damage.

PLOS ONE 7(11), 49215.
Lukowski, A., Jagiełło, R., Robakowski, P., Adamczyk, D., Karolewski, P., 2022. Adaptation of a simple method to determine the total terpenoid content in needles of coniferous trees. Plant Sci. 314, 111090.
Mandal, S.C., Maity, T.K., Das, J., Pal, M., Saha, B.P., 1999. Hepatoprotective activity of *Ficus racemosa* leaf extract on liver damage caused by carbon tetrachloride in rats. Phytother. Res. 13, 430-432.
Marin, L., Miguélez, E.M., Villar, C.J., Lombó, F., 2015. Bioavailability of dietary polyphenols and gut microbiota metabolism: Antimicrobial properties. Biomed Res. Int. 2015, 905215.
Mukherjee, P.K., Saha, K., Murugesan, T., Mandal, S.C., Pal, M., Saha, B.P., 1998. Screening of anti-diarrhoeal profile of some plant extracts of a specific region of West Bengal, India. J. Ethnopharmacol. 60(1), 85-89.
Murti, K., Kumar, U., Lambole, V., 2010. Pharmacological properties of *Ficus racemosa*-A review. Pharmacologyonline 2, 802-807.
Nisca, A., Ștefănescu, R., Stegăruș, D.I., Mare, A.D., Farczadi, L., Tanase, C., 2021. Comparative study regarding the chemical composition and biological activity of pine (*Pinus nigra* and *P. sylvestris*) bark extracts. Antioxidants (Basel) 10(2), 327.
Nivedha, K., Sivasakthi, S., Prakash, A., Devipriya, N., Vadivel, V., 2020. *In vitro* studies on antioxidant and cyto-protective activities of polyphenol-rich fraction isolated from *Mangifera indica* leaf. S. Afr. J. Bot. 130, 396-406.
Nualkaew, S., Padee, P., Talubmook, C., 2015. Hypoglycemic activity in diabetic rats of stigmasterol and sitosterol-3-*O*--D-glucopyranoside isolated from *Pseuderanthemum palatiferum* (Nees) Radlk. leaf extract. J. Med. Plants Res. 9, 629-635.
Paarakh, P.M., 2009. *Ficus racemosa* Linn.-An overview. Nat. Prod. Rad. 8, 84-90.
Pant, D.R., Pant, N.D., Saru, D.B., Yadav, U.N., Khanal, D.P., 2017. Phytochemical screening and study of antioxidant, antimicrobial, antidiabetic, anti-inflammatory and analgesic activities of extracts from stem wood of *Pterocarpus marsupium* Roxburgh. J. Intercult. Ethnopharmacol. 6(2), 170-176.
Pasha, C., Narayana, B., Stancheva, K., 2016. Determination of selenium in environmental samples using toluidine blue and safranine O as new reagents. Ind. Technol. 3(1), 102-107.
Phaniendra, A., Jestadi, D.B., Periyasamy, L., 2015. Free radicals: Properties, sources, targets, and their implication in various diseases. Indian J. Clin. Biochem. 30(1), 11-26.
Rao, C.V., Verma, A.R., Vijayakumar, M., Rastogi, S., 2008. Gastroprotective effect of standardized extract of *Ficus glomerata* fruit on experimental gastric ulcers in rats. J. Ethnopharmacol. 115(2), 323-326.
Ratnasooriya, W.D., Jayakody, J.R., Nadarajah, T., 2003. Antidiuretic activity of aqueous bark extract of Sri Lankan *Ficus racemosa* in rats. Acta Biol. Hung. 54(3), 357-363.
Rawat, A.K.S., Tiwari, S.S., Srivastava, A., 2012. Comparative botanical and phytochemical evaluation of medicinally important stem bark of *Ficus* species. Asian Pac. J. Trop. Dis. 2, 33-37.
Rebaya, A., Belghith, S.I., Baghdikian, B., Leddet, V.M., Mabrouki, F., Olivier, E., kalthoum Cherif, J., Ayadi, M.T., 2015. Total phenolic, total flavonoid, tannin content, and antioxidant capacity of *Halimium halimifolium* (Cistaceae). J. App. Pharm. Sci. 5(1), 52-57.
Reeg, S., Grune, T., 2015. Protein oxidation in aging: Does it play a role in aging progression? Antioxid. Redox Signal. 23(3), 239-255.
Rigano, D., Formisano, C., Basile, A., Lavitola, A., Senatore, F., Rosselli, S., Bruno, M., 2007. Antibacterial activity of flavonoids and phenylpropanoids from *Marrubium globosum* ssp. *libanoticum*. Phytother. Res. 21(4), 395-397.
Santhi, K., Sengottuvel, R., 2016. Qualitative and quantitative phytochemical analysis of *Moringa concanensis* Nimmo. Int. J. Curr. Microbiol. App. Sci. 5(1), 633-640.
Saptarini, N.M., Indriyati, W., Shalihat, A., 2016. Colorimetric method for total phytosterols content analysis in soybean (*Glycine max*), soymilk, and soy yoghurt. J. Chem. Pharm. Res. 8(4), 1458-1464.
Schofield, P., Mbugua, D.M., Pell, A.N., 2001. Analysis of condensed tannins: A review. Anim. Feed Sci. Technol. 91(2), 21-40.
Sekar, M., Nur Fatin, Z., Nuraina, A., Nur Afiqah, A.A., Abdin Shakirin, M.N., Hafizati Izuani, M., Nurul Syuhada, S., Mohd Syafiq, A., 2016. Comparative antioxidant properties of methanolic extract of red and white dragon fruits. Int. J. Curr. Pharm. Res. 8(3), 56-58.
Shaikh, J.R., Patil, M., 2020. Qualitative tests for preliminary phytochemical screening: An overview. Int. J. Chem. Stud. 8(2), 603-608.
Shiksharthi, A.R., Mittal, S., 2011. *Ficus racemosa*: Phytochemistry, traditional uses and pharmacological properties: A review. J. Adv. Pharm. Res. 4, 6-15.
Singh, A., Bajpai, V., Kumar, S., Kumar, B., Srivastava, M., Rameshkumar, K.B., 2016. Comparative profiling of phenolic compounds from different plant parts of six *Terminalia* species by liquid chromatography-tandem mass spectrometry with chemometric analysis. Ind. Crops Prod. 87, 236-246.
Skrovankova, S., Sumczynski, D., Mlcek, J., Jurikova, T., Sochor, J., 2015. Bioactive compounds and antioxidant activity in different types of berries. Int. J. Mol. Sci. 16(10), 24673-24706.
Song, Y.R., Kim, J.K., Lee, H.S., 2020. Serum levels of protein carbonyl, a marker of oxidative stress, are associated with overhydration, sarcopenia and mortality in hemodialysis patients. BMC Nephrol. 21, 281.
Soromou, L.W., Chen, N., Jiang, L., 2012. Astragalin attenuates lipopolysaccharide-induced inflammatory responses by down-regulating NF-kappa-B signaling pathway. Biochem. Biophys. Res. Commun. 419, 256-261.
SPI, Siddha Pharmacopoeia of India-Siddha Pharmacopoeial Publications, Volume 1, 2008 https://pcimh.gov.in/WriteReadData/RTF1984/1536744071.pdf
Sreevidya, N., Mehrotra, S., 2003. Spectrophotometric method for estimation of alkaloids precipitable with Dragendorff's reagent in plant materials. J. AOAC Int. 86(6), 1124-1127.
Srivastava, B., Sharma, V.C., Pant, P., Pandey, N.K., Jadhav, A.D., 2016. Evaluation for substitution of stem bark with

small branches of *Myrica esculenta* for medicinal use - A comparative phytochemical study. J. Ayurveda Integr. Med. 7(4), 218-223.
Sulaiman, C.T., Balachandran, I., 2012. Total phenolics and total flavonoids in selected Indian medicinal plants. Indian J. Pharm. Sci. 74(3), 258-260.
Tripathi, R., Kumar, A., Kumar, S., Prakash, S. Singh, A.K., 2015. *Ficus benghalensis* Linn.: A tribal medicine with vast commercial potential. Indian J. Agric. Sci. 1, 95-102.
Ukoha, P., Cemaluk, E.A.C., Nnamdi, O.L., Ejikeme, P.M., 2011. Tannins and other phytochemical of the *Samanaea saman* pods and their antimicrobial activities. Afr. J. Pure Appl. Chem. 5, 237-244.
Ullah, A., Munir, S., Badshah, S.L., Khan, N., Ghani, L., Poulson, B.G., Emwas, A.H., Jaremko, M., 2020. Important flavonoids and their role as a therapeutic agent. Molecules 25(22), 5243.
Vasas, A., Hohmann, J., 2014. *Euphorbia* diterpenes: Isolation, structure, biological activity and synthesis (2008-2012). Chem. Rev. 114(17), 8579-8612.
Veerapur, V.P., Prabhakar, K.R., Parihar, V.K., Kandadi, M.R., Ramakrishana, S., Mishra, B., Satish Rao, B.S., Srinivasan, K.K., Priyadarsini, K.I., Unnikrishnan, M.K., 2009. *Ficus racemosa* stem bark extract: A potent antioxidant and a probable natural radioprotector. Evid. Based Complement. Alternat. Med. 6(3), 317-324.
Vek, V., Šmidovnik, T., Humar, M., Poljanšek, I., Oven, P., 2022. Comparison of the content of extractives in the bark of the trunk and the bark of the branches of silver fir (*Abies alba* Mill.). Molecules 28(1), 225.
Vilema-Enríquez, G., Arroyo, A., Grijalva, M., Amador-Zafra, R.I., Camacho, J., 2016. Molecular and cellular effects of hydrogen peroxide on human lung cancer cells: Potential therapeutic implications. Oxid. Med. Cell. Longev. 2016, Article No. 1908164, doi: 10.1155/2016/1908164.
Yadav, R.K., Nandy, B.C., Maity, S., Sarkar, S., Saha, S., 2015. Phytochemistry, pharmacology, toxicology, and clinical trial of *Ficus racemosa*. Pharmacogn. Rev. 9(17), 73-80.
Zhishen, J., Mengcheng, T., Jianming, W., 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 64(4), 555-559.
Zhou, Z., Li, N., Zhang, H.F., Wang, Q.Q., Yu, Q., Wang, F., Dai, Y.H., Wang, D., Liu, D.C., 2019. Simultaneous quantitative analysis of 11 flavonoid derivatives with a single marker in persimmon leaf extraction and evaluation of their myocardium protection activity. J. Nat. Med. 73, 404-418.
Ziqubu, K., Dludla, P.V., Joubert, E., Muller, C.J.F., Louw, J., Tiano, L., Nkambule, B.B., Kappo, A.P., Mazibuko-Mbeje, S.E., 2020. Isoorientin: A dietary flavone with the potential to ameliorate diverse metabolic complications. Pharmacol. Res. 158, 104867.