Enzymatic and non-enzymatic antioxidant, anti-inflammatory and anticancer activities of Floscopa scandens Lour

Anto Suganya Regis, Jeya Jothi Gabriel

**Abstract**

**OBJECTIVE:** To explore the total phenolic and flavonoid content, enzymatic, non-enzymatic antioxidant properties, anti-inflammation and anticancer activities of hexane, ethyl acetate and methanol extracts of Floscopa scandens (F. scandens).

**METHODS:** Non-enzymatic antioxidant activity was examined by 2, 2-diphenyl-1-picrylhydrazyl assay, nitric oxide scavenging assay, hydroxyl radical scavenging assay, reducing power assay, hydrogen peroxide scavenging assay, superoxide scavenging assay and metal chelating assay. Enzymatic antioxidant ability was screened for the antioxidant enzymes such as ascorbate oxidase, peroxidase, catalase and polyphenol oxidase. The anti-inflammatory property was proved with the inhibition of protein denaturation and protease inhibitory assays. **In vitro** anticancer activity was assessed by cell viability assay.

**RESULTS:** Methanol extract contained high amount of phenols (198.41 mg catechol equivalent/gram extract) and flavonoids (101.70 mg quercetin equivalent/gram extract) showed higher activity than hexane and ethyl acetate extracts in all experiments. Fresh plant showed considerable enzymatic antioxidant activity.

**CONCLUSION:** The results revealed that the methanol extracts of F. scandens could be used as a potential source of antioxidant, anti-inflammatory and anticancer bioactive compounds.

**INTRODUCTION**

Man wants to live long and healthy. New lifestyle diseases such as cancer, diabetes, obesity, cardiovascular diseases, aging and inflammatory diseases are the main reasons for the early death of a man. Literature provides enough data to realize that many of the lifestyle diseases are due to oxidative stress which is created every day in our body. Free radicals are the initiators of oxidative stress. These free radicals seek stability through electron pairing with biological macromolecules and cause lipid peroxidation, DNA and protein damage if not eliminated. It leads to new lifestyle diseases and shortens human lifespan. Enzymes like superoxide dismutase (SOD), catalase and compounds like ascorbic acid, tocopherol and glutathione are the built-in protective mechanisms to protect against free radical damage in the human cell. Due to various pathological conditions and erratic lifestyle habits, these inbuilt protective mechanism gets disturbed and leads to the need for an external antioxidant supplement to protect against oxidative damage. Inflammation is an innate immune response of a host defensive mechanism. During the immune response, excess production of pro-inflammatory molecules such as tumor necrotic factor
(TNF)-α and nitric oxide (NO) are the primary sources of inflammation. These inflammatory molecules react with free radicals to cause cell death and tissue damage. For example, NO can react with peroxides to produce peroxynitrite, which causes irreversible cell membrane damage. Pharmaceutical companies have developed many synthetic antioxidants and anti-inflammatory agents against oxidative stress and inflammation diseases. The utmost impedes such as high cost, side effects and lack of availability persevere the alternative source for an effective antioxidant and anti-inflammatory agents. Plants are nature’s chemical factories. Antioxidant and anti-inflammatory agents from plants are preferable due to their availability, high productivity, safety and cost-effectiveness. Phenolics and flavonoids isolated from plants are excellent sources of antioxidant, anti-inflammatory and anticancer molecules. There are thousands of medicinal plants used as folk line treatment for many lifestyle diseases, but only a few of them were experimentally proved for their medicinal values. It provides the urge in search for new and inexpensive antioxidant, anti-inflammatory and anticancer agents from new or less studied plants.

Floscopa scandens (F. scandens) is a perennial herb belongs to the family Commelinaceae. It is a weed and traditionally used to treat broken bones, poisonous sting, sore eyes, abscesses, fever, pyoderma, and nephritis. This study is the first time report for the enzymatic, non-enzymatic antioxidant activities, anti-inflammatory and anticancer activities of various solvent extracts of F. scandens.

MATERIALS AND METHODS

Plant collection
Fresh F. scandens plants were harvested from the banana field of Pongumoodu, Thiruvananthapuram, Kerala, and authenticated by taxonomist Dr. D. Narasimhan, Centre for Floristic Research, Department of plant biology and biotechnology, Madras Christian College, Chennai. The herbarium specimen has been deposited in Loyola College, Chennai, Tamil Nadu, India, with the voucher number LCH 404. Plants were washed and dried under shade at room temperature. Dried plants were ground using an electric blender and filtered through sieves. The powdered drug was stored in airtight container at ambient temperature for further use.

Preparation of plant extracts
Plant extracts were prepared by serial extraction method which involved successive extraction with solvents of increasing polarity from a low polar (Hexane) to high polar solvents (Ethyl acetate and Methanol) to ensure that a wide polarity range of compounds could be extracted. About 50 g of dried powder was soaked in 800 mL hexane for 72 h with intermittent shaking at 120 rpm in a shaker. The extract was filtered through Whatman No. 1 filter paper and the solvent in the filtrate was removed completely to get a constant dry weight of the extract. The remaining plant residue from hexane extract was dried completely at room temperature and soaked in 800 mL of ethyl acetate and then in methanol successively as above mentioned and the extracts were collected. The concentrated extracts were stored at 4 °C for further use.

Determination of total phenolic content
The total phenolic content of F. scandens hexane, ethyl acetate and methanol extracts was assessed according to the Folin-Ciocalteau method with some modifications. The test was performed in triplicate. The calibration curve for catechol was obtained with six data points. The total phenol content of F. scandens was calculated by comparing the results with the catechol calibration curve and expressed as mg of quercetin equivalents per gram of extract.

Determination of total flavonoid content
The total flavonoid content was determined with aluminium chloride (AlCl₃) according to a known method using quercetin as a standard. The test was performed in triplicate. The calibration curve for quercetin was obtained. The total flavonoid content of F. scandens was calculated by comparing the results with quercetin calibration curve and expressed as mg of quercetin equivalents per gram of extract.

DPPH radical scavenging assay
DPPH quenching ability of F. scandens hexane, ethyl acetate and methanol extracts were measured using 0.15% methanol DPPH solution. The ability of F. scandens to scavenge the DPPH radical was calculated using the following equation:

\[ \text{DPPH scavenging effect (\%)} = \left( \frac{A_0 - A_1}{A_0} \right) \times 100 \]  \text{[Equation 1]}  

Where \( A_0 \) is the absorbance of the control, and \( A_1 \) is the absorbance of the sample. Ascorbic acid was used as positive control. All samples were tested in triplicate.

Nitric oxide radical inhibition assay
At physiological pH, in an aqueous solution sodium nitroprusside spontaneously generates nitric oxide. When it interacts with oxygen to produce nitrite ions, it can be estimated by the use of Griess Illosvoy reaction. In the present investigation, naphthylethylenediaminedihydrochloride (0.1% w/v) was used for the preparation of Griess Illosvoy reagent instead of 1-aphthylamine (5%). Ascorbic acid was used as positive control. The NO scavenging activity of F. scandens was calculated using the formula in equation 1.

Reducing ability assay
The reducing ability of F. scandens hexane, ethyl acetate and methanol extracts were evaluated according to
the method of Oyaizu.\textsuperscript{15} Butylated hydroxyltoluene (BHT) was used as positive control.

**Hydroxyl radical scavenging assay**
The method described by Kunchandy and Rao with minor modifications was performed for the hydroxyl radical scavenging assay.\textsuperscript{16} Ascorbic acid was used as positive control. The hydroxyl radical scavenging activity of F. scandens was calculated using the formula in equation 1.

**Total antioxidant capacity (Phosphomolybdate assay)**
The total antioxidant capacity of the F. scandens was estimated by phosphomolybdate method using ascorbic acid as standard.\textsuperscript{17} The antioxidant capacity was estimated using the formula in equation 1.

**Hydrogen peroxide scavenging activity**
The ability of plant extracts to scavenge hydrogen peroxide can be estimated according to the method of Ruch \textit{et al.}\textsuperscript{18} Ascorbic acid was used as positive control. The percentage of hydrogen peroxide scavenging by F. scandens was calculated using the formula in equation 1.

**Superoxide radical scavenging activity**
The photoreduction of riboflavin generated Superoxide radical (O$_2^•$) and was detected by nitro blue tetrazolium (NBT) reduction method described by Winterbourne \textit{et al.}\textsuperscript{19} Ascorbic acid was used as the reference compound. Percentage inhibition was calculated using the formula in equation 1.

**Metal chelating activity**
The chelating of ferrous ions by F. scandens hexane, ethyl acetate and methanol extracts were estimated by the method of Dinis \textit{et al.}\textsuperscript{20} with 2 mM FeCl$_3$ and 5 mM ferrozine. Ethylenediaminetetraacetic acid (EDTA) was used as positive control. The percentage inhibition of ferrozine-Fe$^{2+}$ complex formation was calculated by using the formula in equation 1.

**Preparation of enzyme extracts**
Extraction of enzymes from fresh plants of F. scandens was carried out using the methods of Nayyar and Gupta,\textsuperscript{9} Hakiman and Maziah.\textsuperscript{20} Fresh whole plant of F. scandens (0.5 g) wasground with 8 mL solution containing 50 mM potassium phosphate buffer (pH 7.0) and 1% polyvinylpyrrolidone. The homogenate was centrifuged at 15 000 rpm for 30 min and the supernatant was collected for the following antioxidant enzymes assays.

**Ascorbate oxidase activity**
Ascorbate oxidase activity in the fresh plant extract of F. scandens was measured by the method of Dialinas \textit{et al.}\textsuperscript{21} The decrease in absorbance was observed at 265 nm due to ascorbate oxidation and calculated using extinction coefficient, mM$^{-1}$cm$^{-1}$.

**Peroxidase activity**
The peroxidase activity in the fresh plant extract of F. scandens was measured by the method of Reddy \textit{et al.}\textsuperscript{22} using 0.05 M buffered pyrogallol. The peroxidase activity was calculated using an extinction coefficient of oxidized pyrogallol (4.5 L/M).

**Catalase activity**
Catalase activity of the fresh plant extract of F. scandens was determined by the methods of Aebi and Luck.\textsuperscript{23,24} The reaction mixture (1 mL) contains potassium phosphate buffer (pH 7.0), 250 mL of enzyme extract of F. scandens and 60 mM H$_2$O$_2$ to initiate the reaction. The reaction was measured at 240 nm for 3 min and H$_2$O$_2$ consumption was calculated using extinction coefficient 39.4 mM$^{-1}$cm$^{-1}$.

**Polyphenol oxidase (PPO) activity**
The activity of polyphenol oxidase which comprises of catechol oxidase and laccase can be simultaneously determined by the method of Esterbauer.\textsuperscript{25} Fresh whole plants of F. scandens (0.5 g) were homogenized in about 10 mL medium containing 50 mM Tris-HCl, 0.4 M sorbitol and 10 mM NaCl at pH 7.2. The homogenate was centrifuged at 2000 rpm for 10 min and the supernatant was used for the assay. One unit of either catechol oxidase or laccase is defined as the amount of enzyme that transforms 1 umole of dihydroxyphenol to 1 umole of quinine per minute under the assay conditions. The activity of PPO was calculated using the following formula:

\[
K \times \Delta A/\min
\]

where \(K\) for catechol = 0.272 and \(K\) for laccase = 0.242

**Inhibition of albumin denaturation**
Inhibition of albumin denaturation by F. scandens was evaluated according to the protocol of Ullah \textit{et al.}\textsuperscript{26} with diclofenac sodium as standard. The percentage inhibition of protein denaturation was calculated by using the formula in equation 1.

**Proteinase inhibitory activity**
Proteinase inhibitory activity of F. scandens was evaluated according to Oyedepo \textit{et al.}\textsuperscript{27} with diclofenac sodium as standard drug. The percentage inhibition of proteinase inhibitory activity of F. scandens was determined using the formula in equation 1.

**HepG2 cell maintenance**
Human hepatoma cell line (HepG2) was purchased from National Center for Cell Science, Department of Biotechnology, Pune, India. Monolayers of cells were grown in RPMI-1640 medium and supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), antibiotics (penicillin 100 U/mL, streptomycin 10 µg/mL) and 1 mmol/L sodium pyruvate under standard conditions (37 °C) in a controlled humidified atmosphere containing 50 mL/L CO$_2$.
Drug preparation
F. scandens extract was prepared as a stock solution in dimethyl sulfoxide (DMSO) and stored at −20 °C. For each experiment, F. scandens extract was diluted with cell culture medium to the concentration indicated with a final DMSO concentration of 1% (v/v). Two culture medium with or without 1% DMSO were added in all experiments. There was no difference between these controls in all experiments; thus, we employed culture medium with 1% DMSO as the control for comparison.

Cell viability assay
The methyl thiazol tetrazolium bromide (MTT) assay by Mosmann was followed to assess the cell viability. In a 96-well microtiter plate HepG2 cells were plated at a density of 1 × 10⁴ cells per well in a final volume of 100 µL modified Eagle medium (MEM). The cells were treated with F. scandens methanol extract (2.5, 5, 10, 20, and 40 µL/mL) for 24, 48, and 72 h. At the end of the 72 h incubation period, the reaction was terminated by adding 20 µL of 5 mg/mL stock MTT to each well. The reaction was allowed to proceed for 2 h at 37 °C after that culture medium was removed. After the addition of 0.1 mL of DMSO at 37 °C for 1 h in the dark the formazan crystals were dissolved. Cyclophosphamide was used as positive control. The intensity of the colour was developed, reflecting the number of live cells, was measured at a wavelength of 595 nm in a microplate reader (BIO-RAD Model 3550). All the values were compared to the corresponding controls.

Statistical analysis
Results were analysed and expressed as mean ± standard error of mean. Using GraphPad Prism software the IC₅₀ values were calculated. The P values were calculated by students t-test using GraphPad Prism software version 7.01 (GraphPad Software Inc., La Jolla, CA, USA).

RESULTS

Total phenols
Phenolic compounds of F. scandens extracts reacted with the phosphomolybdic acid in Folin- Ciocalteau reagent to produce a blue colored complex in alkaline medium, which can be measured at 650 nm. The amount of total phenolic compounds present in extracts of F. scandens was determined from linear regression equation of calibration curve, (y = 0.001x – 0.0084, R² = 0.977) and expressed a catechol equivalent in mg/ml of extracts. The total phenolic content of hexane, ethyl acetate and methanol extracts of F. scandens recorded as 95.43, 132.86 and 198.41 (mg catechol equivalent/gram extract) respectively.

Total flavonoid estimation
Flavonoids present in F. scandens extracts bound with aluminium chloride to form a yellow colored complex and measured at 510 nm. Total flavonoids present in extracts was determined from linear regression equation of calibration curve, (y = 0.0113x – 0.2191, R² = 0.9923) and expressed a quercetin equivalent in mg/ml of extracts. The total flavonoid content of hexane, ethyl acetate and methanol extracts of F. scandens expressed as 52.06, 76.42 and 101.70 (mg quercetin equivalent/gram extract) respectively.

DPPH scavenging activity
DPPH scavenging activity was observed by the bleaching of the purple colour reaction mixture to yellow. Decreased in absorption is directly proportional to the increase of scavenging activity by the extract. The methanol extract of F. scandens showed dose-dependent scavenging activity than hexane and ethyl acetate extract. The DPPH scavenging activity was given in Figure 1. The IC₅₀ values of hexane and ethyl acetate extract of F. scandens were >1000. Figure 11 depicted the IC₅₀ value of methanol extract of F. scandens.

Nitric oxide scavenging activity
F. scandens extracts were excellent scavengers of nitrite. The methanol extract of F. scandens showed better activity than hexane and ethyl acetate extracts. IC₅₀ value for F. scandens methanol extract was (583.70 ± 0.14) µg/mL and ascorbic acid showed (355.40 ± 0.11) µg/mL. Nitric oxide scavenging efficiency of F. scandens extracts was shown in Figure 2. The IC₅₀ values of hexane and ethyl acetate extract of F. scandens for nitric oxide scavenging activity were >1000. The IC₅₀ value of methanol extract of F. scandens was showed in Figure 11.
Hydroxyl radical scavenging

Hydroxyl radical scavenging capacity of hexane, ethyl acetate and methanol extracts of F. scandens was measured by the decrease in the intensity of red colour produced by the molybdate formed by the reaction between radicals and 2-deoxyribose. The methanol extract of F. scandens showed higher activity than hexane and ethyl acetate extracts. Hydroxyl radical scavenging activity of F. scandens was depicted in Figure 3. Hexane and ethyl acetate extract of F. scandens showed >1000 IC_{50} values. The IC_{50} values of methanol extract of F. scandens and standard were depicted in Figure 11.

Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity of F. scandens extracts was given in Figure 4. When compared to the standard ascorbic acid, the extracts of F. scandens showed less activity. It showed the maximum inhibition of 40.2%) ± 1.5%. Standard ascorbic acid exhibited 92.1% ± 1.4% of inhibition. The IC_{50} values of hexane and ethyl acetate extracts were >1000. Figure 11 described the IC_{50} value of methanol extract of F. scandens.

Total antioxidant capacity

Total antioxidant assay measure both water soluble and acid soluble antioxidants in the extracts. In this assay, the reduction of Mo (VI) to Mo (V) by the extracts of F. scandens was measured at 695 nm. The total antioxidant capacity of F. scandens was compared with the standard ascorbic acid. Total antioxidant capacity of F. scandens was increased in the order of H < EA < M. The phosphomolybdate scavenging activity of F. scandens was given in Figure 5. Figure 11 depicted the IC_{50} value of methanol extract of F. scandens.

Superoxide scavenging activity

In this assay, F. scandens extracts inhibited the production of nitroblue tetrazolium formazan, which can be measured by the decrease in the intensity of red colour produced by the molybdate formed by the reaction between radicals and 2-deoxyribose. The methanol extract of F. scandens showed higher activity than hexane and ethyl acetate extracts. Superoxide scavenging activity of F. scandens was depicted in Figure 5. Figure 11 depicted the IC_{50} value of methanol extract of F. scandens.

**Figure 2** Nitric oxide scavenging activity of F. scandens extracts and ascorbic acid in different concentrations (200, 400, 600, 800, 1000 µg/mL)

All values are represented as mean ± standard error of mean. "P < 0.05 and "P < 0.01, compared to negative control group.

**Figure 3** Hydroxyl radical scavenging activity of F. scandens extracts and ascorbic acid in different concentrations (200, 400, 600, 800, 1000 µg/mL)

All values are represented as mean ± standard error of mean. "P < 0.05 and "P < 0.01, compared to negative control group.

**Figure 4** Hydrogen peroxide scavenging activity of Floscopa scandens extracts and ascorbic acid in different concentrations (200, 400, 600, 800, 1000 µg/mL)

All values are represented as mean ± standard error of mean. "P < 0.05 and "P < 0.01, compared to negative control group.
measured at 560 nm. The conception of superoxide anion by the F. scandens extracts was indicated by the decrease of absorption. The methanol extract of F. scandens showed the concentration-dependent inhibition activity against superoxide anion. Figure 6 showed the scavenging ability of F. scandens against superoxide anion. The IC_{50} values of methanol extract of F. scandens and standard were presented in Figure 11.

**Reducing powder**

In reducing power assay, the colour of the test solution changed from yellow to green depending on the reducing power of F. scandens extract and the standard. An increase in the reduction of Fe^{3+} to Fe^{2+} was indicated by the increase in absorption values. Reducing power of the F. scandens extracts were compared with the standard BHT and was depicted in Figure 7. The extracts showed reducing power in the order of H < EA < M.
Metal chelation

Formation of Fe$^{2+}$-ferrozine complex produced red colour in the presence of extract, which was measured at 562 nm. 50% of metal chelation was done at the concentration of 782.2 and 355.4 µg/mL. Figure 8 showed the metal chelation activity of F. scandens extracts. The IC$_{50}$ values of hexane and ethyl acetate extract of F. scandens were $>$1000. IC$_{50}$ value of methanol extract and standard were illustrated in Figure 11.

Enzymatic antioxidant activity

The level of antioxidant enzymes such as ascorbate oxidase, catalase, peroxidase, polyphenol oxidase presented in the fresh plant of F. scandens was estimated. In comparison to all these enzymes, the fresh plant of F. scandens had a high amount of peroxidase [(175.48±7.30308) U/g of fresh weight] followed by ascorbate oxidase [(63.17460 ± 0.83992) U/g of fresh weight]. Catalase presented in less quantity.
([0.59109 ± 0.11062] U/g of fresh weight] and polyphenol oxidase [catechol oxidase (0.009228 ± 0.00021) U/g of fresh weight] presented in very less quantity.

**Inhibition of protein denaturation**
In anti-protein denaturation assay efficiency of F. scandens extracts to inhibit the heat-induced egg album denaturation was measured at 660 nm against the standard drug diclofenac sodium. The methanol extract of F. scandens showed greater activity than hexane and ethyl acetate extracts. Fifty percentage inhibition of protein denaturation observed in methanol extract at the concentration of 479.6 µg/mL and diclofenac sodium showed 423.8 µg/mL. The results were shown in Figure 9. Hexane and ethyl acetate extracts showed IC₅₀ values more than 1000. Figure 11 illustrated the IC₅₀ values of methanol extract of F. scandens and standard.

**Proteinase inhibitory activity**
In this study, the efficiency of F. scandens extracts to inhibit the degradative activity of trypsin against casein was observed at 210 nm. Diclofenac sodium was used as a standard. The Methanol extract of F. scandens showed higher activity than hexane and ethyl acetate extracts. Proteinase inhibitory activity was depicted in Figure 10. The IC₅₀ values of hexane and ethyl acetate extract of F. scandens were > 1000. The IC₅₀ values of methanol extract of F. scandens and standard shown in Figure 11.

**Cell viability assay**
To estimate the optimal dose for F. scandens methanol extract treatment on HepG2 cells, the cell growth was determined in the presence of various doses of F. scandens methanol extract (Figure 12). As the dose of F. scandens methanol extract increased, the cell growth was inhibited in the cell line. The growth inhibition by F. scandens methanol extract in HepG2 cells was observed for 24, 48, and 72 h, and the maximum growth inhibitory effects with F. scandens methanol extract were observed at 72 h. F. scandens methanol extract presented strong cytotoxic effects at concentrations above 10 µg/mL. The resulting growth curves showed that the inhibition was concentration and time-dependent.

**DISCUSSION**
Weeds are considered as unwanted and troublesome. F. scandens is a weed in the banana fields and lands near water. This plant does not gain much importance as medicinal due to the lack of scientific evidence to support its traditional uses as an anti-inflammatory agent. The present investigation made an attempt to study its antioxidant, anti-inflammatory and anticancer properties and to support its medicinal uses by scientific evidence. Ethical issues and the lack of rationale for animal are certain problems in using animals for experimental pharmacological research. Hence we have performed easy and cost-effective *in vitro* methods to study the antioxidant, anti-inflammatory and anticancer activity of F. scandens. Moreover, it is insufficient to conclude the versatility of antioxidants in crude extracts by doing a single antioxidant assay. Therefore we performed various non-enzymatic assays in crude extracts and enzymatic antioxidant assays in fresh plant extract.

Phenolic compounds and flavonoids are the essential part of human diet due to its antioxidant, anti-inflammatory, anticancer and anti-diabetic activities.77 The methanol extract of F. scandens showed a considerable amount of phenols and flavonoids than hexane and ethyl acetate extracts. Literature has proved methanol extracts of plants from Commelinaceae family exhibited potential antioxidant, anti-inflammation and anticancer activity. The methanol extract of Cyanotis fasciculata showed 70.21 % of total antioxidant capacity with respect to its high total phenol and flavonoid content. It also exhibited high activity against DPPH and hydroxyl radicals also showed high reducing power.30,31 The methanol extract of Commelina clavata flowers exhibited activity for hydrogen peroxide, reducing power and total antioxidant capacity.31 The methanol extract of Commelina benghalensis possessed anti-inflammatory activity by inhibiting lipoxygenase, which directly proportional to its flavonoid content.32 In the same way in our present study methanol extract of F. scandens showed potential non-enzymatic antioxidant, anti-inflammatory and anticancer activity than hexane and ethyl acetate extracts. In DPPH assay the methanol extracts of F. scandens proved its antioxidant ability by donating hydrogen to DPPH and reduced its colour from purple to yellow in a dose-dependent manner. In nitric oxide scavenging activity, the methanol extract of F. scandens showed its nitrite scavenging activity by competing with oxygen and inhibited the formation of nitrite and showed by means of reduction of colour in a concentration-dependent manner. In hydroxyl scavenging assay, F. scandens methanol extract proved its scavenging activity by reacting with molybdate and reduced the colour of the reaction mixture in a dose-dependent manner. The methanol extract of F. scandens showed negligible activity than the standard for H₂O₂ scavenging assay. It might be due to the presence of compounds responsible for H₂O₂ scavenging in fewer amounts or the antagonistic effect of other phytochemicals in the crude extract.33 In total antioxidant capacity, the methanol extract of F. scandens showed its ability to form green colour phosphate/Mo (V) complex in concentration dependent manner. In superoxide anion scavenging assay, the methanol extract of F. scandens proved its scavenging ability and in reducing power assay it exhibited good reducing ability by terminating free radical chain reaction *via* donating hydrogen atom and proved its antioxidant ability. F. scandens metha-
nol extracts showed a considerable amount of metal chelators that disturbed the Fe$^{2+}$-Ferrozine complex and reduced the intensity of red colour. Traditionally fresh plants of F. scandens are used for its medicinal uses. Therefore in this current study, we have investigated the antioxidant enzymes present in the fresh whole plant extract of F. scandens. It has antioxidant enzymes such as peroxidase, ascorbate oxidase and catalase in considerable amount. Presence of these antioxidant enzymes substantiates its usefulness as a fresh plant for traditional uses. F. scandens is commonly used to treat inflammatory conditions; hence it is important to validate its traditional use scientifically. Anti denaturation assay is the convenient method to test the anti-inflammatory activity of plant extract in vitro condition. Heat-denatured proteins are proved to be as effective as native proteins which are responsible for delayed hypersensitivity. Conventional Nonsteroidal Anti-inflammatory Drugs like phenylbutazone and indomethacine prevent denaturation of protein along with the inhibition of endogenous prostaglandins production. The methanol extract of F. scandens proved its high anti-inflammatory activity by preventing the heat-induced denaturation of egg albumin. The integrity of the cell needs the balance between proteases and antiproteases. During inflammation, leukocytes release serine proteinases which can cause the degradation of tissues. It is necessary to inhibit the proteinases activity by antiproteinases. For example, pancreatic leukocyte proteinase inhibitors protect the tissue by inhibiting proteinases secreted from neutrophils (cathepsin G, elastase and trypsin), pancreatic acinar cells (chymotrypsin and trypsin) and mast cells (chymase and tryptase). The methanol extracts of F. scandens showed high protease inhibitory activity thus proved its anti-inflammatory ability. There are no traditional uses or folk line information of F. scandens as a candidate for the treatment of cancer. In our present study, we examined the efficacy of the methanol extract of F. scandens as an anticancer agent. Low concentration of F. scandens strongly inhibited human HepG2 cells growth in vitro. F. scandens may exhibit anti-proliferative activity via the regulation of cell cycle. It might disrupt the progression of cell cycle by increasing the number of cancer cells, hence preventing cell entering next cycle. To estimate the optimal dose for F. scandens on HepG2 cells, the cells were cultured with various doses of F. scandens. As the dose of F. scandens increased, the cell growth was inhibited in the cell line. The strong cytotoxic effect of F. scandens was seen at concentrations above 10 µg/mL. The maximum growth inhibitory effects were observed at 72 h. The resulting growth curves revealed that the inhibition cell growth by extracts of F. scandens was concentration and time-dependent. In the present study, it is clear that these antioxidant, anti-inflammatory and anticancer bioactive compounds and enzymes present in F. scandens are responsible for its traditional uses as a candidate for inflammation (sore eye, broken bones, febrifuge, pyoderma, abscess, acute nephritis, ophthalmia) and to fight against various new lifestyle diseases. Methanol extract showed high phenolic and flavonoid concentration. Plants rich in phenols and flavonoids showed high antioxidant, anti-inflammatory and anticancer ability. It is clear that a part of this antioxidant, anti-inflammatory and anticancer activity of methanol extract of F. scandens is due to the presence of phenolic compounds and flavonoids. In addition, the antioxidant and anti-inflammatory activity might also due to the synergistic effect of more than two compounds present in the extract. F. scandens is used as a food supplement in New Guinea, from our findings, this plant could also be used as a better source for food industries as well pharmaceutical companies for its effective bioactive principle compounds. This study will help to understand the multifaced antioxidant, anti-inflammatory and anticancer ability of F. scandens. Bioassay-guided phytochemical and in vitro pharmacological studies are needed in an attempt to isolate and characterize the active antioxidant, anti-inflammatory and anticancer compounds from the extract, as a source for future novel bioactive compounds for food and pharmaceutical formulations.

REFERENCES


Gell PGH, Benacerraf B. Studies on hypersensitivity-II delayed hypersensitivity to denatured proteins in guinea pig. Immunology 1959; 2(1): 64-70.


