

Antimicrobial, anti-inflammatory, and anticancer activities of leaves extracts of *Filicium decipiens*

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ABSTRACT

Filicium decipiens (fern-tree) belongs to the family Sapindaceae. It grows in Asia and Africa region and is traditionally used for diabetes treatment in India. The plant has been documented to have molluscicidal, diabetic nephropathy, and anti-inflammatory properties. In the present study, experimental data on different leaves extracts of *F. decipiens* were collected for their preliminary phytochemical investigations along with antimicrobial, anti-inflammatory, and anticancer activities using *in-vitro* assays. The phytochemical investigations display the existence of alkaloids, tannins, flavonoids, saponins, glycosides, steroids, triterpenoids, and carbohydrates in the extracts prepared using three different solvents. Antimicrobial property was established against six bacterial and fungal strains. Petroleum ether extract exhibited higher antimicrobial activity as compared to chloroform and water extract. Anti-inflammatory assay was performed on RAW264.7 murine macrophage cells and petroleum ether extract exhibited reduced nitrite oxide production having IC₅₀ value 493.7 µg/ml. Cytotoxicity was determined against MCF-7 (Breast) and A-549 (Lung) cancer cells and paclitaxel was used as a standard drug, whereas cytocompatibility was assessed against mouse fibroblast cells (L929). Different sensitivities were observed against different study cell lines in a dose-dependent manner. These study findings may provide biological evidence for the application of *Filicium decipiens* extract.

1. INTRODUCTION

Plants have been used as a source of raw material for remedies since ancient practice and several of them are globally recognized as medicine [1]. They contain biologically active constituents to protect human beings and such constituents act as protective agents against several human ailments steps such as mortal carcinogenesis, performing origination, development phases, or terminating the DNA destructing cell mutations. These medicinal plant sources could probably be used to offer more efficacious anticancer agents [2]. Such properties are well co-related previously to the existence of certain phytochemicals. Hence, medicinal plants play an active role in cancer prophylaxis and management [3].

The identification and assessment of biologically active plant extract/s have been of abundant importance to biomedical scientists in the exploration of newer and safer drug toward the disease management. Cancer is a second most deadly disease in the world and currently many clinically effective regimens are in practice. The plant-based drug discovery and development have displayed

an essential role in cancer management and indeed over the last half-century many therapeutic plant/s along with their secondary metabolites have been employed [4]. Rationale use of medicinal plants is an effective approach in the chemotherapeutic management of cancer and is one of the major parts of treatment for several years as many medicinal plants with anticancer activity are previously documented in literature [5]. As the attention in simple and organic lifestyle develops, the awareness in plant-based drugs also rises. In addition to this, side effects and interaction possibilities are boundaries in synthetically developed anticancer medicines. Hence, plant/s has been studied globally to explore them as possible sources of anticancer agents [6].

Filicium decipiens is a tropical fern-like leaf tree which grows in Asia and Africa. The plant is usually found in high altitude regions up to 1000 m [7]. It is an evergreen tree found abundantly in peninsular India. The leaves are compound and very large. Each leaf consists of 12–16 leaflets, each leaflet is 4–6 inches in length and relatively narrow [8]. The flowers are very small and white in color and the tree produces both male and female flowers. The fruits are olive size, drupe and dark in color, and form in clusters. The stem bark is black in color [9]. Ethanolic extract of *F. decipiens* has been previously documented for anti-inflammatory property and sitosterol has been recognized from the methanolic extract of *F. decipiens* leaves. Furthermore, four

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new saponins were identified and isolated from stem bark of *F. decipiens* [10]. In view of the above effects, this study aimed to evaluate preliminary phytochemical investigations and *in-vitro* efficacy for antimicrobial, anti-inflammatory, and anticancer potential of different leaf extracts of *F. decipiens*.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

The solvents used for extraction were obtained from Merck Limited, Mumbai, India. Dulbecco's Modified Eagle's Medium (DMEM), lipopolysaccharide (LPS), aminoguanidine (AG), 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT), brain heart infusion (BHI) agar, nutrient broth, fetal bovine serum (FBS), penicillin, and streptomycin were obtained from HiMedia Pvt. Ltd., Mumbai, India.

2.2. Plant Material

The plant was identified and authenticated from ICMR-National Institute of Traditional Medicine. The plant specimen was preserved in the herbarium (RMRC-1388). *F. decipiens* (Sapindaceae) leaves were collected from the campus of Sahyadri Science College Shimoga (Karnataka). The collected leaves were washed with the help of water and were shade dried at room temperature ($32 \pm 2^\circ\text{C}$). The leaves were grinded and the powdered mass was stored for the subsequent extraction procedure.

2.2.1. Extraction

The leaves powder (200 g) was extracted individually using petroleum ether, chloroform, and water (1500 ml) in a Soxhlet assembly for 48 h followed by filtration and evaporation process using a rotary evaporator at $35\text{--}40^\circ\text{C}$ to obtain petroleum ether (FDP), chloroform (FDC), and water (FDH) extracts separately.

2.3. Phytochemical Analysis

The leaves powder of *F. decipiens* was extracted with petroleum ether, chloroform, and water as solvents to get polar and non-polar components. Different phytochemical tests have been assessed for alkaloids, flavonoids, glycosides, phenol, saponins, steroids tannins, proteins, and carbohydrates by following the previously reported procedure of Debela. [11].

2.4. Antimicrobial Activity

2.4.1. Micro-organisms sources and maintenance

Gram-positive bacteria, namely (*Staphylococcus aureus* MTCC 96, *Staphylococcus mutans* MTCC 497, and *Enterococcus faecalis* MTCC 439) were selected for anti-microbial study. While, gram-negative bacteria (*Escherichia coli* MTCC 443, *Klebsiella pneumonia* MTCC 109, and *Pseudomonas aeruginosa* MTCC 741) and a fungal strain *Candida albicans* were experimentally maintained in BHI medium at KAHER's Dr. Prabhakar Kore Basic Science Research Centre, Belagavi. The subculturing of selected organisms was performed at regular intervals.

2.4.2. Determination of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and minimum fungicidal concentration (MFC)

In present study, broth dilution method was used and the extracts were diluted to give final concentration of 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, and 0.39 mg/ml. A hundred microliters of each micro-organism were added in tubes with the same volume of the nutrient

broth and the extracts of *F. decipiens*. The tubes were incubated aerobically at 37°C for 24–48 h [12]. The least concentration (higher dilution) of the extract does not produce any growth (no turbidity) in the 24 h. They are compared with control tubes and are recorded as MIC.

The value of MBC was decided by sub-culturing test dilution (MIC) against newly prepared BHI agar plates. The plates were incubated for 24 h at 37°C . The maximum dilution displayed no single bacterial colony on the BHI agar plates which were recorded as MBC. While the minimum concentration that stops fungal growth after aerobic incubation was recorded as MFC.

2.5. Anti-inflammatory Activity

2.5.1. Cell culture of RAW 264.7

RAW 264.7 (Murine macrophage cells) was cultured in DMEM with 2 mM L-glutamine having 10% HI-FBS, 100 IU/ml penicillin, and 100 $\mu\text{g/ml}$ streptomycin in a humidified atmosphere at 37°C in the presence of 5% CO_2 [13].

2.5.2. Cell viability – MTT assay

The murine macrophage cells were grown at a density of 2×10^5 cells/ml in 96 well plates with the extracts of *F. decipiens* (25–800 $\mu\text{l/ml}$). 1 $\mu\text{g/ml}$ LPS of cells was incubated. Two hundred micrometer AG along with 1 $\mu\text{g/ml}$ LPS was considered as control of decrease in nitrite oxide (NO) formation. For nitrite quantity, the culture supernatant has been removed. Five microliter of MTT solution was added and the plates were incubated at 37°C for 4 h. To thaw the formazan crystal, the solution of MTT was detached and dimethyl sulfoxide (DMSO) was added to the plate. Incubation for 10 min at room temperature absorbance was recorded at 540 nm using ELISA plate reader [14,15].

2.5.3. Nitrite oxide determination

NO was determined by evaluating the nitrite with the help of Griess reagent. With the equal volume of cell culture was mixed with Griess reagent [16] followed by incubation for 10 min, diazo dye was formed. Absorption of the formed diazo dye was assessed at 540 nm. For the evaluation of nitrite concentration, it was determined by comparing with a sodium nitrite standard calibration curve.

2.6. Cytotoxic Screening

2.6.1. Cell culture and dilution of extracts

The breast adenocarcinoma cells (MCF-7), lung carcinoma cells (A-549), and mouse fibroblast (L929) were purchased from the National Centre for Cell Sciences (NCCS) Pune, Maharashtra. The cells were subcultured in DMEM media with FBS of 10%, penicillin-streptomycin, and non-essential amino acids (1%) followed by CO_2 incubation. The extract (FDH) was dissolved in distilled water. While, FDP and FDC were dissolved in DMSO at the concentration of 1 mg/ml.

2.6.2. Cytotoxic assay

In a 96-well plate, the cells were seeded at a density of 2×10^3 at 37°C in 5% CO_2 . Several concentrations (1000 $\mu\text{g/mL}$, 500 $\mu\text{g/mL}$, 250 $\mu\text{g/mL}$, 125 $\mu\text{g/mL}$, 62.5 $\mu\text{g/mL}$, and 31.25 $\mu\text{g/mL}$) of each extracts were assessed against selected cells. The treated cells were incubated for 48 h. The result of the extract on cells was compared to standard drug-treated cells. Same treatment regimen was monitored for the L929 cell line. After 48 h, the medium of the treated cells was removed (100 μL) and was incubated with 50 μL MTT solution [17].

One hundred microliter of DMSO was added to solubilize the formazan crystal which was obtained after the incubation process. Absorbance was measured at 540 nm by an ELISA reader. The assay was performed in triplicate.

3. RESULTS AND DISCUSSION

3.1. Phytochemical

The phytochemical investigation of the plant was carried out according to the earlier reported procedure [Table 1]. It revealed the existence of phytochemicals such as steroids, alkaloids, flavonoids, tannins, saponins, glycosides, triterpenoids, and carbohydrates in different extracts (FDP, FDC, and FDH) of leaves of *F. decipiens*. These phytoconstituents are known to exhibit medicinal as well as physiological activities [18,19].

3.2. Antimicrobial

The antimicrobial activity would be of most importance in therapeutic treatment with the help of plant extracts [20]. In the current study of three extracts (FDP, FDC, and FDH) were assessed for their antimicrobial activity against certain Gram-positive micro-organisms (*S. aureus*, *S. mutans*, and *E. faecalis*), Gram negative micro-organisms (*E. coli*, *K. pneumonia*, and *P. aeruginosa*), and fungus (*C. albicans*). By the broth dilution method, the susceptibility of extracts was tested. MIC, MBC, and MFC values obtained for each extract against the tested micro-organisms are summarized in Table 2. Standard antimicrobial agent ciprofloxacin and fluconazole were used. The petroleum ether extract depicted a

significant antimicrobial activity. The FDP extract was more active as compared to FDC and FDH.

FDP resulted in significant antimicrobial activity as compared with chloroform and water extract. Although it is unclear about the mechanistic pathway of plant components works, however, it is clear that the efficiency of the extract depends on the kind of the solvent used. When compared to aqueous extract, the organic extract showed more potent antimicrobial activity. This study observation indicates the existence of non-polar residues in the extract which have higher both bactericidal and bacteriostatic capacities.

3.3. Anti-inflammatory

The MTT assay of extracts (FDP, FDC, and FDH) was carried out with murine macrophages cells (RAW 264.7). Six different concentrations of each extract were used. Cells were stimulated by 1 µg/mL LPS. During a co-incubation period of 24 h, the extract effect was determined by NO formation. Assay validity was assessed by observing untreated cells as a negative control. The LPS-treated cells were considered as a positive control [21]. The extracts decreased the stimulated NO formation in tested concentration. IC₅₀ values and percentage of NO production are tabulated in Table 3. Inflammation is a natural defense process in the human body which is responsible for damaging external stimuli such as chemical toxins and microbial infections. Inflammation activated macrophage produced iNOS leading to huge production of NO, greater inflammatory mediator. To assess the effect of *F. decipiens* was measured by iNOS expression using RAW 264.6 cells.

3.4. Anticancer

The extracts of *F. decipiens* have been investigated for cytotoxic effect on MCF-7, A-549, and L929 cells. The IC₅₀ values of extracts are tabulated in Table 4. The study finding displayed reduced cell viability and cell development inhibition in a dose-dependent manner. Six different concentrations were used to determine the percentage of cell viability. For MCF-7 and A-549, FDP and FDC extract having more cytotoxicity. FDP, FDC, and FDH extracts presented no cytotoxic effect on noncancerous normal mouse fibroblast (L929). The result obtained for each extract can be compared with the standard drug (paclitaxel).

Extracts of the plant showed different properties on various cell lines. The choice of the cell line due to the sensitivity of the active compounds in the extract [22,23]. The extracts provide active inhibition ranges from 32% at 1000 µg/mL to 95% at 31.2 µg/mL for MCF-7 cell line. While, 32% at 1000 µg/mL to 94% at 31.2 µg/mL for

Table 1: Phytochemical analysis of *Filicium decipiens* extracts.

Sr. no.	Tests	Pet. ether (FDP)	Chloroform (FDC)	Water (FDH)
1.	Carbohydrates	-	-	+
2.	Glycosides	-	-	+
3.	Flavonoids	-	+	+
4.	Tannins	-	+	+
5.	Saponins	-	+	+
6.	Alkaloids	-	+	+
7.	Triterpenoids	+	+	+
8.	Steroids	+	+	+
9.	Proteins	-	-	-

(+) present (-) absent, FDP: *Filicium decipiens* petroleum ether extract; FDC: *Filicium decipiens* chloroform extract; FDH: *Filicium decipiens* water extract

Table 2: Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and minimum fungicidal concentration (MFC) (mg/ml) of the extracts.

Extracts		Micro-organisms							Test	<i>Candida albicans</i>
		<i>Staphylococcus aureus</i>	<i>Staphylococcus mutans</i>	<i>Enterococcus faecalis</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Klebsiella pneumonia</i>			
FDP	MIC	6.25	3.12	6.25	6.25	3.12	6.25	MIC	1.56	
	MBC	12.5	6.25	12.5	12.5	6.25	6.25	MFC	6.25	
FDC	MIC	12.5	25	6.25	25	6.25	6.25	MIC	6.25	
	MBC	25	50	25	50	12.5	12.5	MFC	12.5	
FDH	MIC	1.56	3.12	6.25	6.25	3.12	6.25	MIC	3.12	
	MBC	25	25	50	12.5	25	25	MFC	6.25	
Std. (µg/ml)	MIC/MBC	2	2	2	1	< 4	1	MIC/MFC	16	

FDP: *Filicium decipiens* petroleum ether extract; FDC: *Filicium decipiens* chloroform extract; FDH: *Filicium decipiens* water extract

Table 3: IC₅₀ values of plant extracts on RAW264.7 cells.

(a)			
Extracts	IC ₅₀ (µg/ml)		
FDP	493.70		
FDC	1295.00		
FDH	753.40		
(b)			
% of NO inhibition			
Concentration (µl/ml)	FDP	FDC	FDH
800.00	42.48	61.44	52.66
400.00	53.29	68.97	58.78
200.00	66.30	75.55	65.36
100.00	75.86	86.68	72.88
50.00	87.62	95.61	86.36
25.00	96.39	97.65	94.83

a: IC₅₀ values, b: % NO inhibition. FDP: *Filicium decipiens* petroleum ether extract; FDC: *Filicium decipiens* chloroform extract; FDH: *Filicium decipiens* water extract

Table 4: IC₅₀ value of extracts in µg/ml

Extracts/drug	Cancer cell lines	
	MCF-7	A-549
FDP	210.900	252.300
FDC	252.800	315.400
FDH	295.800	321.200
Standard paclitaxel	298.867	273.250

the A-549 cell line. The result exhibited that FDP extract significantly inhibited the MCF-7 and A-549 and was the most effective extract with IC₅₀ value of 210 µg/ml for MCF-7 and 252 µg/ml for A-549. The FDP extract was more active while comparing with FDC and FDH observations.

4. CONCLUSION

The crude extracts of leaves of *F. decipiens* could be newer source of development of plant-based therapy for disease management. The extracts have been tested with different activities such as antimicrobial, anti-inflammatory, and anticancer. The findings from these studies indicated that petroleum ether extract has the most significant results for all tested activities. It may be effective to use in the management of cancer and inflammation.

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6. CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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