

PHYTOCHEMICAL SCREENING, AND EVALUATION OF ANTIBACTERIAL, ANTIOXIDANT AND CYTOTOXIC ACTIVITY OF *FICUS RACEMOSA* LINN.

JAY KAMBLI¹, ASHWINI PATIL¹, CHITHRASHREE², AND ROHINI KESHAVA^{3*}

¹Department of Biotechnology, Acharya's Bangalore B-School, Bangalore, Karnataka, India, 560 091, ²Biocontrol Research Laboratory, Department of Studies in Microbiology and Biotechnology, Bangalore University, Jnana Bharathi Campus, Bangalore, Karnataka, India, 560 056., ³Department of Life Sciences/Sericulture, Bangalore University, Jnana Bharathi Campus, Bangalore, Karnataka, India, 560 056.

Email: rohinikeshava@gmail.com

Received: 01 Mar 2014 Revised and Accepted: 15 Mar 2014

ABSTRACT

Objective: The medicinal plants are loaded with numerous effective antibacterial, antioxidants and anticancer agents which provide an alternative means of therapy to various infections caused by drug resistant bacteria, oxidative stress and dreadful diseases like cancer and other physiological disorders. The present study is designed to evaluate the biological properties of ethanolic extracts of bark, fruits and leaves of *Ficus racemosa* in terms of its antibacterial, antioxidant, cytotoxic activities and phytochemical analysis to find out the active compounds responsible for these activities.

Methods: Standard methods were adopted for the analysis of secondary metabolites and antioxidant activities. Antibacterial activity was determined by using standard agar well diffusion method. Anticancer activity of the extracts was assayed by XTT assay.

Results: Phytochemical investigation of ethanolic extract revealed the presence of phenols, flavonoids and alkaloids. Among all the extracts studied leaf extract showed good activity against all the test bacteria. In DPPH assay, the IC₅₀ value of crude ethanol bark extract was 52.04 µg/mL, whereas IC₅₀ value for reference ascorbic acid was 42.17 µg/mL. Cell viability assay on Calu6 cell line demonstrated that all the extracts at 160µg/ml were effective and the highest percentage of inhibition of 58.8% and 46.6 % was exhibited by hot ethanolic extracts of bark and fruits, respectively.

Conclusion: Detailed analysis of phytochemical properties of *F. racemosa* determines its significance as a rich source of molecules with high pharmaceutical value. In addition, this study has also explored the anticancer properties of these extracts against lung anaplastic carcinoma cell line Calu6, which has shown promising results. From this we can conclude the plant to be a natural source of antioxidants and phytochemicals with potent antimicrobial and anticancer properties.

Keywords: *Ficus racemosa*, Antioxidant activity, Antibacterial activity, Cytotoxic activity, DPPH, ABTS, XTT-assay.

INTRODUCTION

India is known for its rich diversity of medicinal plants and hence called botanical garden of the world [1]. Many of the natural products in plants of medicinal value offer us new sources of drugs which have been used effectively in traditional medicine. Most of the drugs today are obtained from natural sources or semi synthetic derivatives of natural products used in the traditional system of medicine [2]. A large number of medicinal plants are used in several formulations for the treatment of various diseases caused by microbes. Several plant species are used by many ethnic groups for the treatment of various ailments ranging from minor infections to dysentery, skin diseases, asthma, malaria etc. [3]. The drugs obtained from plants are less toxic; side effects are scanty and also cost effective.

Oxidative stress leads to many diseases, such as brain dysfunction, cancer, heart diseases, age related degenerative conditions, declination of the immune system, cancer, coronary arteriosclerosis, ageing processes, carcinogenesis, gastric ulcer and DNA damage [4, 5]. Phytochemicals are a rich source of antioxidants such as polyphenols and flavonoids, which can delay or inhibit the oxidation of biomolecules by regulation of oxidative chain reactions [6]. Polyphenols and flavonoids are used for the prevention and cure of various diseases which are mainly associated with free radicals [7]. Phenolic compounds have gained much attention, due to their antioxidant activities and free radical-scavenging abilities, which potentially have beneficial implications for human health [8]. Flavonoids are regarded as one of the most widespread groups of natural constituents found in plants [9], which are been reported to have radical scavenging effects through scavenging or chelating processes [10]. Several plant species rich in flavonoids are reported having disease preventive and therapeutic properties [11]. Antioxidant-based drug formulations are used for the prevention

and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer [12]. Therefore, in recent years, considerable attention has been directed towards the identification of plants with antioxidant ability.

The evaluation and the discovery of new anticancer agents is long-term process that encompasses many steps. The goal of screening medicinal plant is to search for excellent anticancer agent avertable to human malignancies. In defiance of astonishing advances in modern medicine, such as surgery, radiotherapy, chemotherapy, and hormone therapy, cancer remains a worldwide health problem. Newmann and Cragg [13] reported in their analysis that sources of many new drugs in recent years are derived directly from natural products. Alkaloids, the naturally occurring, pharmacologically active organic compounds have made major impact on plant medicine because of its vast application. In addition to possessing various medicinal properties [14, 15, 16], alkaloids have been shown to be potent anti-cancer agents [17]. These facts are in favor with the new call for medicinal plant identification namely local plants, in conjunction with anticancer properties.

Several members of the genus *Ficus* (Family: *Moraceae*) are being used traditionally in a wide variety of ethnomedical remedies. One of them is *Ficus racemosa* syn. *Ficus glomerata* (Gular; Udumbara) [18], a moderate sized avenue tree widely distributed throughout India, northern Australia, and other parts of Asia. *Ficus racemosa* Linn. Commonly known as 'cluster fig', is used widely in Indian folk medicine for the treatment of various diseases, including jaundice, dysentery, diabetes, diarrhea and inflammatory conditions [19]. Apart from the usage in traditional medicine, scientific studies indicate that *F. racemosa* possesses various biological effects such as hepatoprotective [20], chemopreventive [21], anti-diabetic [22], anti-inflammatory [23], antipyretic [24], and antidiuretic [25]. The bark has also been evaluated for cytotoxic effects using 1BR3, Hep

G2, HL-60 cell lines and found to be safe and less toxic than aspirin, a commonly consumed anti-inflammatory drug [27]. The present study aims to explore the phytochemical properties, antibacterial, antioxidant and cytotoxic activities of cold and hot (Soxhlet) ethanol extracts of *Ficus racemosa*. The phytochemical and antioxidant screening of these plants is a prerequisite for verification and utilization as new sources of herbal drugs. Although several studies are available on various *Ficus* spp., less work has been done on *F. racemosa*. In this study we have performed a detailed analysis of all the phytochemical properties of *F. racemosa* which determine its significance as a rich source of molecules with high pharmaceutical value. In addition, this study has also explored the anticancer properties of these extracts against lung anaplastic carcinoma cell line Calu6.

MATERIALS AND METHODS

Processing of plant materials and extraction

The different parts of *Ficus racemosa* viz. leaves, fruits and bark were collected from their natural habitat. They were washed with distilled water and were shade dried and powdered. Cold and hot (Soxhlet) ethanolic extracts of the powdered plant material were prepared. For cold extraction twenty five grams of powdered sample was soaked in 250 ml of ethanol, agitated manually, and allowed to extract for 48 hours. Extracts were then filtered using Whatmann No 1 Filter paper and the filtrates were evaporated. The extracts were stored at 4°C until further processing. For hot (Soxhlet) extraction, twenty five grams of dried powder of plant material was extracted with ethanol. The ethanol extracts were then distilled, evaporated and vacuum dried. The crude extracts thus obtained were used directly for phytochemical screening and also for assay of antibacterial, antioxidant and cytotoxic potential.

Phytochemical screening

Determination of total phenolic contents

The plant extracts were analysed for total phenolic, flavonoid and alkaloid contents. The total phenolic content of plant extracts were determined using Folin-Ciocalteu reagent [28]. Plant extracts (100 µl) were mixed with 0.5 mL of Folin-Ciocalteu reagent and 1.5 mL of 20% Na₂CO₃ solution. The mixture was shaken well and made up to 5 ml using distilled water. The reaction was allowed to stand for 2 hour and then the absorbance was measured at 765 nm against a reagent blank. The data obtained were used to estimate the phenolic contents using a standard graph obtained from various concentrations of gallic acid. The total phenolic content is expressed as µg of gallic acid equivalents (GAE) per mL of the plant extracts.

Total flavonoid content was determined as described by Jia et al., [28]. Five hundred micro liter of extract was diluted with 2 mL of distilled water and subsequently with 150 µl of 5% NaNO₂ solution. After 6 min 200 µl of 10% AlCl₃.H₂O was added and mixed. Immediately the volume is brought to 5 mL using distilled water and allowed to stand at RT for 15 min. The absorbance of the mixture was determined at 510 nm. Quercetin was used as standard and the results were expressed as µg of quercetin equivalents (QE) per mL of the extract.

Total alkaloid content was measured by Dragendorff's method [29]. It is based on the formation of yellow bismuth complex in nitric acid medium with thiourea. 5 mL of extract was dissolved in dilute HCL and 2 drops of Dragon drop's was added, a crystalline precipitate indicates presence of alkaloid. After centrifugation, the supernatant was decanted completely and the precipitate was further washed with alcohol. After centrifugation, filtrate was discarded and the residue was then treated with 2 mL of 1% disodium sulfide solution. The brownish black precipitate formed was then centrifuged. Completion of precipitation was checked by adding 2 drops of disodium sulfide. The residue was dissolved in 2 mL concentrated nitric acid and diluted to 10 mL in a standard flask with distilled water. 1 mL was then pipetted out and 5 mL of 3% thiourea solution was added to it and absorbance was measured at 435 nm against the blank containing nitric acid and thiourea. The amount of bismuth present in the solution was calculated by multiplying the absorbance values with the factor, taking suitable dilution factor in to

consideration. The factor is obtained from the standard curve, which is a constant for different concentrations.

$$\text{Factor} = \frac{\text{Concentration}}{\text{Absorbance}}$$

The calibration curve was obtained with Bismuth nitrate pentahydrate stock solution. Series dilutions of the stock solution were made by pipetting out 1, 2, 3, 4, 5, 6, 7, 8, and 9 mL stock solution into separate 10 mL standard flasks and diluting to volume with distilled water. 1 mL of this solution was taken, and 5 mL thiourea solution was added to it. The absorbance value of the yellow solution was measured at 435 nm against colorless reagent blanks.

Assay of antibacterial activity of plant extracts

Antibacterial activity was assayed with the standard agar well diffusion method [30]. Different concentrations of the extracts (100, 200, 300 µg/ml) were prepared by using 2% DMSO (dimethylsulphoxide). 10µl of 24h test cultures such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis* were seeded onto respective Mueller-Hinton agar medium by spread plate method. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well. Antibacterial activity was also assayed with the standard disc diffusion method. The filter paper discs (5mm in diameter) impregnated with the extracts is placed on test organisms. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the disc. The DMSO was used as a negative control.

Evaluation of total Antioxidant Capacity (TAC) by Phosphomolybdenum method

The total antioxidant capacity of the plant extracts was determined by phosphomolybdate method using ascorbic acid as a standard [31]. The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate / Mo (V) complex at acid pH. An aliquot of 0.1 ml of sample solution was mixed with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 765 nm against a blank. A typical blank contained 1 ml of the reagent solution and the appropriate volume of the solvent and incubated under the same conditions. The antioxidant activity is expressed as the number of gram equivalent of ascorbic acid. Ascorbic acid equivalents were calculated using standard graph of ascorbic acid. The experiment was conducted in triplicates and values are expressed as equivalents of ascorbic acid in µg / mg of extract [32].

Assay of antioxidant activity by DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging activity

The free radical scavenging capacity of the extracts was determined using DPPH [33]. The DPPH solution (0.006% w/v) was prepared in 95% ethanol. Different concentrations of the test sample which is to be examined for antioxidant activity is prepared (10-50µg/ml). 3 ml of different concentration of test sample of *F. racemosa* extracts were mixed with 1 ml of DPPH solution in dark. Ascorbic acid which is strong antioxidizing agent is taken as standard. 3 ml of different concentration of standard solution of ascorbic acid was mixed with 1 ml of DPPH solution in dark.

The prepared solution of ascorbic acid and plant extracts samples was incubated for half an hour and then absorbance is taken with the help of U.V. Spectrophotometer at 517 nm. Ethanol serves as a blank and the experiment was expressed as the inhibition percentage of free radical by the sample and was calculated using the following formula;

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

Assay of antioxidant activity by ABTS (2, 2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity

ABTS assay was performed according to the protocol Re et al. [34]. The stock solution was prepared by mixing equal volumes of 7 mM ABTS solution and 2.45 mM potassium persulfate solution followed by incubation for 12 h at room temperature in the dark to yield a dark-colored solution containing ABTS radicals. Working solution was prepared freshly before each assay by diluting the stock solution by mixing of stock solution to 50% methanol for an initial absorbance of about 0.700 (± 0.02) at 745 nm, with temperature control set at 30°C. Varying concentrations (10-50 μ g/ml) of the plant extracts were allowed to react with 3 ml of the ABTS solution and the absorbance readings were recorded at 734 nm [34]. Ascorbic acid was used as positive controls. The scavenging activity was estimated based on the percentage of ABTS radicals scavenged by the following formula:

$$\text{ABTS radical scavenging activity (\%)} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

Determination of Cytotoxicity of the extracts

Cell lines and culture medium

The Lung anaplastic carcinoma Calu6 (ATCC NO. HTB-56) were obtained from the American Tissue Culture Collection (ATCC, USA). The Calu6 cells were grown and maintained in Dulbecco's Modified Eagle's Medium (DMEM, Gibco), supplemented with 10% fetal bovine serum (FBS), 100 μ g/ml penicillin and 50 μ g/ml of amphotericin B. The cells were cultured in a 5% CO₂ incubator at 37°C in a humidified atmosphere. The culture was sub-cultured and maintained by changing media every two to three days and routinely checked under an inverted microscope for any contamination.

XTT assay for cell viability

Assay of anticancer activity of the extracts

Cells were grown in T₂₅ tissue culture flask to the 60-70 % confluency. The cells are trypsinized, counted in haemocytometer and diluted with DMEM media. 50,000 cells were seeded in 96 well microtitre plates in 100 μ l of DMEM media and incubated for 24 hrs at 37°C in 5% CO₂ for cell adherence.

The diluted ranges of extracts were added to each well and the final concentrations of the test extracts were 10, 20, 40, 80, and 160 μ g/ml. Untreated cells were used as reference. After treatment, the

plates are incubated for 24 hrs at 37°C in 5% CO₂. After 24 hrs of incubation, 50 μ l of XTT-PMS solute was added to each well and incubated in a humidified atmosphere for another 2-4 hrs for color development. The absorbance of the color was measured on 96-well microplate reader Tecan micro plate reader (Magellan™ data analysis software) at 450nm wave length. Percentage viability of the cells was calculated at corresponding concentrations of the sample with reference to untreated cells.

Experiments were carried out in triplicate wells, repeated atleast three times. Values are presented as the mean % cell viability \pm SD.

$$\% \text{ Anticancer Activity} = \left(\frac{\text{Ac} - \text{As}}{\text{Ac}} \right) \times 100\%$$

Where Ac and As referred to the absorbance of control and the sample, respectively.

Statistical analysis

All tests were conducted in triplicate. The results are expressed as means \pm SD. Analysis of variance and significant differences among the means were tested by the one-way ANOVA, using SPSS (Version 16.0).

RESULTS AND DISCUSSION

Qualitative and Quantitative and Estimations of Phytochemicals

The results of quantitative screening of phytochemicals are presented in Table 1. The ethanolic extracts of *F. racemosa* fruits, leaves and bark were evaluated for the presence of total phenolics. The total phenolic content was found to be higher in the cold extracts of bark (1042 \pm 15.04 μ g mL⁻¹) followed by cold extract of leaf (1012 \pm 26.4 μ g mL⁻¹). A positive correlation was observed between phenolic content and free-radical scavenging activity of the extracts and these observations corroborate with results from other studies [35, 36, 37, 38 and 39]. Among the studied samples, the flavonoid content was ranging from 21.3 \pm 4.04 μ g mL⁻¹ (hot extraction of leaves) to 43.7 \pm 6.65 μ g mL⁻¹ in hot extraction of fruits (Table 2). Flavonoids have been shown to be highly effective scavengers of most oxidizing molecules, including singlet oxygen, and various free radicals [40] implicated in several diseases. The results of total alkaloids extracted from different parts of *Ficus racemosa* are presented in Table 1.

The results show that the bark contains relatively high percentage of alkaloids compared to leaves and fruits. According to the studies conducted by Garba and Okeniyi [41], alkaloids extracted from *C. papaya*, *C. procera*, *M. indica* and *P. guajava* were effective against most of the test microbes indicating a broad spectrum of activity.

Table 1: Phytochemical screening of cold and soxhlet [hot] ethanolic extracts of bark, leaves and fruits of *F. racemosa* (concentration of phytochemicals expressed as μ g mL⁻¹).

Phytochemicals (μ g mL ⁻¹)	Bark		Leaves		Fruits	
	cold	hot	cold	hot	cold	hot
Phenols	1042 \pm 15.04	953 \pm 10.87	1012 \pm 26.4	802 \pm 30.6	199 \pm 13.5	174 \pm 7.93
Flavonoids	39.03 \pm 3.6	35.6 \pm 5.73	23.4 \pm 3.64	21.3 \pm 4.04	29.5 \pm 4.33	43.7 \pm 6.65
Alkaloids	355 \pm 22.9	392 \pm 12.3	51.3 \pm 6.02	29.7 \pm 4.5	80.6 \pm 8.96	90.3 \pm 8.43

Values are the means \pm SD of data from three independent experiments.

Table 2: Antimicrobial activity of bark, leaf and fruit extracts of *Ficus racemosa* (concentration of extracts 100 μ g mL⁻¹)

Test Microorganisms	Diameter of zone of inhibition (mm)					
	Bark		Leaves		Fruits	
	cold	hot	cold	hot	cold	hot
<i>E. coli</i>	8.8 \pm 0.76	10.3 \pm 0.58	11 \pm 1.0	8.6 \pm 0.63	14.5 \pm 1.32	8.6 \pm 0.7
<i>S. aureus</i>	8.3 \pm 0.64	7.23 \pm 0.68	9 \pm 1.0	14.7 \pm 0.58	9.5 \pm 0.5	14.7 \pm 0.58
<i>B. subtilis</i>	16.6 \pm 1.52	9.7 \pm 0.58	10.7 \pm 0.58	9.7 \pm 0.57	-	9.7 \pm 0.57
<i>P. aeruginosa</i>	11.13 \pm 1.02	-	12.2 \pm 0.68	10.3 \pm 0.64	-	10.4 \pm 0.64

Values are the means \pm SD of data from three independent experiments.

Total Antioxidant Capacity Phosphomolybdenum assay

The result of total antioxidant activity (TAC) is shown in Figure 1. Among all the extracts tested hot ethanolic extracts of bark ($78.8 \mu\text{g mL}^{-1}$) was found to be more effective in reduction of Mo (VI) to Mo (V) while the lowest effects was shown by cold ethanolic extracts of fruits ($20.3 \mu\text{g mL}^{-1}$).

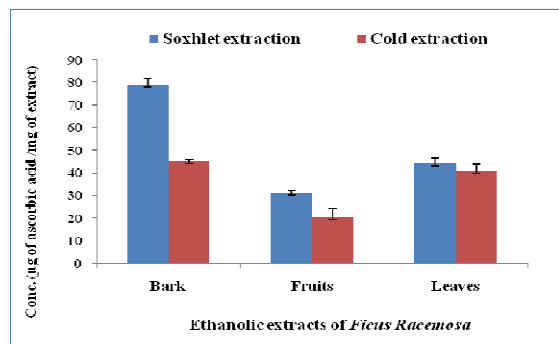


Fig. 1: Total antioxidant capacity of soxhlet and cold ethanolic extraction of bark, leaves and fruits of *Ficus racemosa* by Phosphomolybdenum method.

DPPH and ABTS radical scavenging activity

Table 3 shows the significant decrease in the concentration of DPPH radical due to scavenging ability of the *Ficus racemosa*. The results of the present study showed that cold extract of bark had high (IC_{50} $52.04 \mu\text{g/ml}$) DPPH radical scavenging activity than leaves and fruit extracts. The lowest activity (IC_{50} $91.23 \mu\text{g/ml}$) was seen in hot extracts of leaves (the concentration of the plant extract used for assay was $50 \mu\text{g/ml}$).

This indicates that bark of *Ficus racemosa* can be good source of natural antioxidants. The suppressive effect on ABTS cation radical was assayed at $50 \mu\text{g/ml}$ for all the extracts (Table 3). The maximum scavenging activity of 35.73% was observed in soxhlet extraction of bark followed by leaves extract (34.15%). This finding demonstrates that bark, leaf and fruit extracts of *Ficus racemosa* are capable of non-enzymatically inhibiting the free radical, produced in biological systems, which is a precursor of many ROS and is shown to be harmful for various cellular components [42].

Oyedemi *et al.*, [43] attributed the antioxidant activity observed in the aqueous stem bark extract of *S. henningsii* to the presence of flavonoids, flavonols, phenols and proanthocyanidins. Hence scavenging activity of ABTS and DPPH radicals by the *Ficus racemosa* plant extracts was found to be considerable; which implies that it may be useful for treating radical related pathological damage especially at higher concentrations.

Table 3: Antioxidant activity (% of scavenging) and IC_{50} value of cold and soxhlet [hot] ethanolic extract of bark, leaves and fruits of *Ficus racemosa* by DPPH and ABTS method (concentration of plant extracts $50 \mu\text{g mL}^{-1}$).

Extracts		DPPH % of scavenging	ABTS % of scavenging	DPPH IC_{50} ($\mu\text{g mL}^{-1}$)	ABTS IC_{50} ($\mu\text{g mL}^{-1}$)
Standard		74.86 ± 1.4	84.86 ± 0.1	42.17	32.14
Cold	Bark	52.86 ± 0.37	30.94 ± 0.39	52.04	81.03
	Fruit	41.47 ± 0.1	26.33 ± 0.62	59.41	114.41
	Leaves	32.44 ± 0.53	26.11 ± 0.31	74.87	116.86
Soxhlet	Bark	45.14 ± 0.14	35.73 ± 0.25	59.91	69.31
	Fruit	39.39 ± 0.2	19.35 ± 0.36	60.81	154.08
	Leaves	30.22 ± 0.05	34.15 ± 0.26	91.23	95.62

Values are the means \pm SD of data from three independent experiments.

Determination of Cytotoxic Activity by XTT assay

The crude hot ethanolic extracts of bark, fruits and leaves of *Ficus racemosa* were evaluated in vitro for their inhibitory ability against the growth of Calu-6 cell lines using the XTT colorimetric assay (Figure 2).

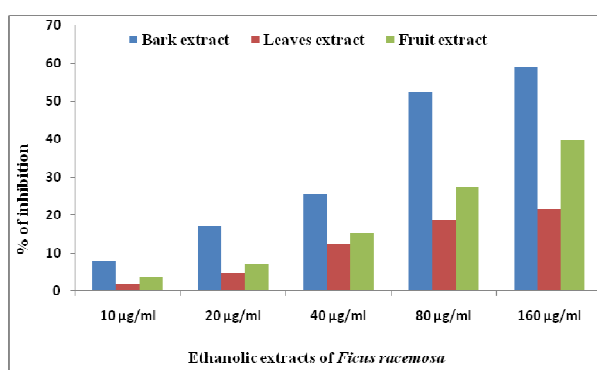


Fig. 2: XTT assay. Effect of different concentrations of hot ethanolic extracts of *Ficus racemosa* bark, leaves and fruits on calu-6 cell lines under invitro conditions. Values are the means of data from three independent experiments.

The results of our study showed that all the extracts in varied concentrations possessed cytotoxic activity on Calu-6 cells in a concentration dependent manner.

It was found that all the extracts showed a significant inhibition at the higher concentrations of $160 \mu\text{g/ml}$. The highest percentage of inhibition of 58.8 and 39.6% was exhibited by hot ethanolic extracts of bark and fruits, respectively. The cytotoxic effect of plants is principally contributed by the presence of secondary metabolites like alkaloid, glycoside, steroid, tannin, terpenoid and flavonoid in their extract [44]. This is also consistent with our observation because the phytochemical group analysis of the extract showed the presence of alkaloid, phenols and flavonoids in significant higher concentration.

CONCLUSION

The present research work concludes that *Ficus racemosa* is important medicinal plant with varied pharmacological spectrum. The phytochemical screening revealed chemical constituents that form the foundation of their pharmacological activity. The cold and Soxhlet (hot) extracts of *Ficus racemosa* has good efficacy against gram positive and gram negative bacteria in higher concentration. The ABTS and DPPH in vitro assays indicate that these plant extracts are a significant source of natural antioxidant, which might be helpful in preventing the various diseases associated with oxidative stresses. The cytotoxicity exerted against cancer cell lines suggests bioactive principles in the plant. This shows that the plant could be useful as antitumor, anticancer and as antimicrobial agent.

ACKNOWLEDGEMENT

The authors are thankful to Dr. Yogish, Director and Dr. Ramesh Thylur, Senior scientist, Skanda Life sciences, Bangalore, for giving the facility of doing cytotoxic activity.

REFERENCES

- Vedavathy S, Mrudula V, Sudhakar A. A Tribal Medicine in Chittoor district, Andhra Pradesh, India. Vedams E Books (P) Ltd. New Delhi, 1997.
- Sukanya SL, Sudisha J, Hariprasad P, Niranjana SR, Prakash HP and Fathima SK. Antimicrobial activity of leaf extracts of Indian medicinal plants against clinical and phytopathogenic bacteria. Afr. J. Biotechnol 2009; 8(23): 6677-82.
- Prashanth Kumar V, Neelam SC, Padh H and Rajni M. Search for Antibacterial and Antifungal Agents from Selected Indian Medicinal Plants. Journal of Ethnopharmacology 2006; 107: 182-88.
- Singh S, Carg V, Yadav D, Beg MN, Sharma N. In-vitro antioxidative and antibacterial activities of various parts of *Stevia rebaudiana* (bertoni). Int J Pharmacy and Pharm Sci 2012; 4(3): 468-73.
- Venkata SP, Raju K, Murali MC, Hara SS, Praneth DVS. Antioxidant activity and phytochemical analysis of *Scolopia crenata* (flacourtiaceae) stem bark. Int J Pharmacy and Pharm Sci 2012; 4(3): 315-19.
- Arya V, Yadav S. Comparative assessment of relative antioxidant activity of sequential leaf extracts of *Cassia occidentalis* and *C. tora*. Pharmacologyonline 2011; 1: 529-43.
- Choudhary S, Verma SK, Rahega G, Kaur P, Joshi K, Gill, KD. The L-type channel blocker nimodipine mitigates cytoskeletal proteins phosphorylation in dichlorvos-induced delayed neurotoxicity in rats. Basic Clin. Pharmacol. Toxicol 2006; 98: 447-55.
- Govindarajan R, Singh DP, and Rawat AK. High-performance liquid chromatographic method for the quantification of phenolics in Chyavanprash a potent Ayurvedic drug. J. Pharm. Biomed. Anal 2007; 43: 527-32.
- Choudhary S, Babeet Singh, Tanwer Rekha Vijayvergia and Tribhuwan Singh. Preliminary phytochemical screening and primary metabolites of *Melothria maderaspatana* (linn.) cong. International Journal of Biological & Pharmaceutical Research 2013; 4(3): 168-71.
- Cook NC, Samman S. Flavonoids-chemistry, metabolism, cardioprotective effects, and dietary sources. Nuts. Biochem 1996; 7: 66-76.
- Ramos S. Effects of dietary flavonoids on apoptotic pathways related to cancer chemoprevention. Journal of Nutritional Biochemistry 2007; 18: 427-42.
- Devasagayam TPA, Tilak JC, Bololo KK, Sane KS, Ghaskadbi SS, Lele RD. Free radicals and antioxidants in human health: Current status and future prospects. Journal of Association of Physicians of India 2004; 52: 794-804.
- Newman DJ, Cragg GM. Natural products as sources of new drugs over the last 25 years. 2007; 70(3): 461-77.
- Yu HH, Kim KJ, Cha JD, et al. Antimicrobial activity of Berberine alone and in combination with ampicillin or oxacillin against methicillin-resistant *Staphylococcus aureus*. Journal of Medicinal Food 2005; 8: 454-61.
- Han J, Lin H, and Huang W. Modulating gut microbiota as an anti-diabetic mechanism of berberine. Medical Science Monitor 2011; 17: 164-67.
- Ji YB. Active Ingredients of Traditional Chinese Medicine: Pharmacology and Application, People's Medical Publishing House Cp., LTD, 2011.
- Gueritte F, Fahy J. The vinca alkaloids. In Cragg GM, Kingston DGI, Newman DJ (eds) Anticancer Agents from Natural Products, Taylor and Francis Group, Florida, 2005, pp. 123-36.
- Kulkarni PH, Shahida A. The Ayurvedic Plants. Delhi: Satguru publications, xvi, 2004; 334.
- The wealth of India, Raw Materials. Publication and Information Directorate, CSIR, New Delhi, 1952; vol 4: 35-36.
- Mandal SC, Tapan K, Maity J, Das M, Pal M, Saha BP. Hepatoprotective activity of *Ficus racemosa* leaf extract on liver damage caused by carbon tetrachloride in rats. Phytother. Res. 2003; 13: 430-32.
- Khan N, Sultana S. Chemomodulatory effect of *Ficus racemosa* extract against chemically induced renal carcinogenesis and oxidative damage response in Wistar rats. Life Sci 2005; 29: 1194-1210.
- Rao BR, Murugesan T, Sinha S, Saha BP, Pal M, Mandal SC. Glucose lowering efficacy of *Ficus racemosa* bark extract in normal and alloxan diabetic rats. Phytother. Res. 2002; 16: 590-92.
- Mandal SC, Maity TK, Das J, Saha BP, Pal M. Anti-inflammatory evaluation of *Ficus racemosa* Linn. leaf extract. J. Ethnopharmacol 2000; 72: 87-92.
- Rao BR, Anupama K, Swaroop KR, Murugesan T, Pal M, Mandal SC. Evaluation of anti-pyretic potential of *Ficus racemosa* bark. Phytomedicine 2002; 9: 731-33.
- Ratnasooriya WD, Jayakody JR, Nadarajah T. Antidiuretic activity of aqueous bark extract of Sri Lankan *Ficus racemosa* in rats. Acta Biol Hung 2003; 54(3-4): 357-63.
- Li RW, Leach DN, Myers SP, Lin GD, Leach GJ, Waterman PG. A new anti-inflammatory glucoside from *Ficus racemosa* L. Planta Med 2004; 70: 421-26.
- Yu J, Ahmedna M and Goktepe I. Effects of processing methods and extraction solvents on concentration and antioxidant activity of peanut skin phenolics. Food Chemistry 2005; 90: 199-206.
- Jia Z, Mengcheng T, and Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem 1999; 64: 555-59.
- Sreevidya N and Mehrotra S. Spectrophotometric method for estimation of alkaloids precipitable with Dragendorff's reagent in plant materials. J. AOAC Int 2003; 86: 1124-27.
- NCCLS. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard, 2000; 5th ed. NCCLS document M7-A5. NCCLS, Wayne, Pa.
- Umamaheswari M, Chatterjee TK. Invitro antioxidant activities of the fractions of *Cocinnia grandis*. African Journal of Traditional Complementary and Alternative Medicine 2008; 5(1): 61-73.
- Aderogba MA, Okoh EK and Idowu TO. Evaluation of the antioxidant activity of the secondary metabolites from *Piliostigma reticulatum* (DC.) hochst. J. Biol. Sci 2005; 5: 239-42.
- Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakrin J Sci. Technol 2004; 26: 211-16.
- Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 1999; 26: 1231-37.
- Oki T, Masuda M, Furuta S, Nishibia Y, Terahara N, Suda I. Involvement of anthocyanins and other phenolic compounds in radical-scavenging activity of purple-fleshed sweet potato cultivars. Food Chem Toxicol 2002; 67: 1752-56.
- Singh BN, Singh BR, Singh RL, Prakash D, Sarma BK, Singh HB. Antioxidant and antitumor sensing activities of green pod of *Acacia nilotica* L. Food Chem Toxicol 2009; 47: 778-86.
- Igbinosa OO, Igbinosa HI, Chigor VN, Uzunugbe OE, Oyedemi SO, Odjadjare EE, et al. Polyphenolic contents and antioxidant potential of stem bark extracts from *Jatropha curcas* (Linn). Int J Mol Sci 2011; 12: 2958-71.
- Melinda KP, Rathinam X, Marimuthu K, Diwakar A, Ramanathan S, Kathiresan S, et al. A comparative study on the antioxidant activity of methanolic leaf extracts of *Ficus religiosa* L, *Chromolaena odorata* (L.) King & Robinson, *Cynodon dactylon* (L.) Pers. and *Tridax procumbens* L. Asian Pac J Trop Med 2011; 3(5): 348-50.
- Oyedemi SO, Bradley G, Afolayan AJ. *In vitro* and *in vivo* antioxidant activities of aqueous extract of *Strychnos henningsii* Gilg. Afr J Pharm Pharmacol 2010; 4(2): 70-78.
- Bravo L. Polyphenols: chemistry, dietary sources, metabolism and nutritional significance. *Nutr Reviews* 1998; 56: 317-33.
- Garba S and Okeniyi SO. Antimicrobial activities of total alkaloids extracted from some Nigerian medicinal plants. Journal of Microbiology and Antimicrobials 2012; Vol. 4(3), pp. 60-63.
- Shirwaier A, Shirwaikar A, Punitha ISR. Antioxidant studies on the methanol stem extract of *Coscinium fenestratum*. Nat. Prod. Sci 2007; 13: 40-45.
- Oyedemi SO, Bradley G, Afolayan AJ. *In vitro* and *in vivo* antioxidant activities of aqueous extract of *Strychnos henningsii* Gilg. Afr J Pharm Pharmacol 2010; 4(2): 70-78.
- Ozc elik B, Kartal M, Orhan I. Cytotoxicity, antiviral and antimicrobial activities of alkaloids, flavonoids, and phenolic acids. Pharm. Biol 2011; 49: 396-402.