EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 10
313-317
Year: 2017

A NOVEL INVESTIGATION OF *IN VITRO* ANTI-INFLAMMATORY AND ANTIOXIDANT ACTIVITY OF *FICUS KRISHNAE*

Amarvani P. Kanjikar, Aruna L.H., Ramesh L. Londonkar*

Department of Biotechnology, Biopharmaceutical and Nanobiotechnology Laboratory, Gulbarga University, Kalaburagi, India.

***Corresponding Author: Prof. Ramesh L. Londonkar**
Department of Biotechnology, Biopharmaceutical and Nanobiotechnology Laboratory, Gulbarga University, Kalaburagi, India.

Article Received on 06/08/2017 **Article Revised on 26/08/2017** **Article Accepted on 16/09/2017**

ABSTRACT
There is great importance of the genus *Ficus* among the living beings since time immemorial. Besides aesthetic and religious values, many species of this genus are of use in day to day life as food and in traditional medicine. *Ficus krishnae* belongs to the family Moraceae, which is one of the oldest plant native to India. The plant extract is used as traditional medicine for healing various diseases. The main aim of the present study was to investigate the in-vitro anti inflammation and antioxidant potential of petroleum ether and chloroform extracts of *Ficus krishnae* stem bark. *In vitro* anti-inflammatory studies were performed for the extracts of petroleum ether and chloroform using protein denaturation inhibition assay and antioxidant activity was screened by 2, 2 diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, ABTS, Phosphomolybdenum and reducing power assay. Finally percentage of inhibition of free radical and IC50 were calculated by the help of Statistical analysis. The results of present data has shown that the Chloroform extract of *Ficus krishnae* stem bark has contributed in causing high potential in-vitro anti inflammation and antioxidant activity fallowed by petroleum ether extract.

KEYWORDS: *Ficus krishnae*, Stem bark, Antioxidant, Anti-inflammatory, DPPH, ABTS, Phosphomolybdenum, Ferric reducing assay.

INTRODUCTION
Inflammation is a complex process of the body's nonspecific internal systems of defense, when cells in the body are damaged by microbes, physical agents or chemical agents; the injury is in the form stress. Which involves the increase of vascular permeability, increase of protein denaturation, membrane alteration and defensive response that is results in redness, pain, heat, swelling and loss of function that occurs depends on the site and extent of injury.[1] Injured tissue cells may release kinins, prostroglandins and histamine. These collectively cause increases of vasodilation (widening of blood capillaries) and permeability of the capillaries. The management of inflammation related diseases is a real issue in the rural community; the population in these areas uses many alternative drugs such as substances produced from medicinal plants.

Oxidation is reaction in which transfer of electrons from a substance to an oxidizing agent. This results in formation of free radicals, which start a chain reaction that leads to damage of living cells.[2] Oxygen is one of the major fundamental requirements for living organisms for its survival, it is highly reactive molecule which damages living organisms by producing reactive oxygen species.[3] The present day diseases are due to the shift in balance of pro-oxidant and antioxidant homeostasis in the body. These reactive oxygen species produced in the living cells include reactive oxygen species (ROS), such as superoxide radical (O^{-2}), hydrogen peroxide (H^2O^2) and the hydroxyl radical (^{-}OH) and these are well documented as cytotoxic intermediates.[4] The hydroxyl radical is unstable that reacts rapidly and non-specifically with biological molecules, these oxidants can damage the cells by starting chemical chain reactions through oxidizing DNA or proteins or lipid peroxidation.[5] Damage in DNA may possibly leads to cancer, it is not repaired by DNA repair mechanisms,damage to proteins result in enzyme inhibition, denaturation and protein degradation.[6]

Among the living beings the genus *Ficus* has attracts high importance since time immemorial; many species of this genus are being used as food and in traditional medicine. Various tribes, sub-tribes and races of the great Assamese society have valued several species of *Ficus* in their own way. As the genus is rich in diversity, this region possesses tremendous scope of exploitation of its members, as many species belonging to this genus have carried good properties for uses for the benefit of mankind.[7] *Ficus krishnae* belongs to the family Moraceae is known by different names Krishna fig,

Krishna's butter cup (in English) & Makkhan Katori (in Hindi) and natine to India. It is large, fast growing, evergreen tree grow up to 30 m tall, with spreading branches and aerial roots.[8,9] The unique structure of the tree is that the leaves have a pocket-like fold at the base. All parts of this plant are useful in treatment of ulcers, vomiting, vaginal complaints, fever, inflammations and leprosy. It has also been proved that it has an anti-diabetic and antihyperlipidemic activity.[10] The *Ficus krishnae* plant extract shows the good sources of bioactive compounds and antimicrobial activity.[11] However; no antioxidant and anti-inflammatory works of stem bark of this species has been reported. Therefore study will prove to be useful in documentation of this plant and provide guidelines for further study on *Ficus krishnae*.

MATERIALS AND METHODS

Inhibition of albumin denaturation

The anti-inflammatory activity of stem bark of *Ficus krishnae* was subjected through isolation from non polar solvents like petroleum ether and chloroform extract was studied by using inhibition of egg albumin denaturation technique which was carried out according to G. Leela prakash et al.[12] and Sakat et al.[13] followed with minor modifications.The reaction mixture (5 ml) consisted of 0.2 ml of egg albumin(from hen's egg), 2.8 ml of phosphate buffered saline(PBS, pH 6.4) and 2 ml of varying concentrations of petroleum ether and chloroform extract of *Ficus krishnae* stem bark to meet the final concentration of 62.5, 125, 250, 500, 1000µg/ml respectively. Similar volume of double distilled water was served as control.The sample extracts were incubated at 37±2 °C for 15 mins and then heated at 70 °C for 5 mins. After cooling, their absorbance of the turbidity is measured at 660nm (UV-Visible Spectrophotometer). The experiment was performed in triplicate. The Percentage inhibition of protein denaturation was calculated as follows:

Percentage inhibition = (Abs Control –Abs Sample) X 100/ Abs control

DPPH Radical-Scavenging Activity

The free radical-scavenging activity of the extracts (non polar) of stem bark of *Ficus krishnae* was measured using the DPPH method [14] with slight modification. About 1mL methanolic solution of 0.1mM DPPH was added to 3mL of the extract solution at different concentrations (5, 10, 20, 40, 80,100µg/mL). The resultant mixture was incubated in the dark for 10 min at room temperature. The absorbance was measured at 517 nm in UV-visible spectrophotometer.Ethanol (95%), DPPH solution and ascorbic acid (AA) were used as blank, control and reference standard respectively. Radical-scavenging activity was expressed as the inhibition percentage of free radicals by the sample and it is calculated using the following formula:

%Inhibition = [(Ac − At)/Ac] ×100

Where Ac is the absorbance of the control (blank, without sample) and At is the absorbance of the test sample. All tests were performed in triplicate and the graph was plotted with the mean value.

ABTS Radical Scavenging Assay

The ability of the extract to scavenge for the ABTS radical was determined by the method [15] with modified. Accordingly the working solution was prepared by mixing two stock solutions i.e, 7mM ABTS and 2.4mM potassium per sulphate and kept it in dark at room temperature for 16 hours. It was further diluted with 80% ethanol to obtain an absorbance value of 700±0.005 nm. The extract at different concentrations (5, 10, 20, 40, 80, 100µL) were incubated with 2.7mL of ABTS mixture at 30˚C for 15 mins and the absorbance was recorded at 734nm. BHT at the same concentration is used as a reference. The radical scavenging activity was calculated as fallows:

Scavenging rate = ((As − Ai)/As) ×100,

Where As is the absorbance of pure ABTS mixture and Ai is the absorbance of ABTS mixture in the presence of extract.

Phosphomolybdenum Assay

The antioxidant activity of petroleum ether and chloroform extract of stem bark of *Ficus krishnae* was evaluated by the green phosphomolybdenum complex according to the method of Prieto et al [16]. An aliquot of 5, 10, 20, 40, 80, 100µL of extract solutions were mixed with 3mL of reagent solution (0.6 M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate) in a 5mL vial. The vials were capped and incubated in a water bath at 95 ◦C for 90 mins. The extracts were cooled at room temperature and the absorbance of the mixture was measured at 695 nm against a blank. The total antioxidant activity was expressed as the absorbance of the sample at 695 nm. The higher absorbance value is indicated higher antioxidant activity.

Ferric Reducing Power

The reducing power of the non-polar solvent extracts of stem bark of *Ficus krishnae was* determined by the method of Jungmin et al.[17] with minor modifications. An aliquot (5, 10, 20, 40, 80, 100µl) of sample, 250µl of sodium phosphate buffer (200 mM, pH 6.6), and 250µl of potassium ferricyanide (1%) were mixed and incubated in a water bath at 50 °C for 20 mins. The reaction was derminated by adding 250µl of trichloroethanoic acid solution (10%, w/v). Take 750µl of the mixture and then add equal volume of distilled water and 75µl of ferric chloride solution (0.1%, w/v) then incubate it for 10 mins at room temperature. Methanol was used as blank. The intensity of the Prussian blue color was measured at 700 nm using a UV-Visible spectrophotometer. Results are expressed as the mean absorbance value.

RESULTS AND DISCUSSION

Inhibition of albumin denaturation

Protein Denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. Denaturation of proteins is a well-documented cause of inflammation. As part of the investigation on the mechanism of the anti-inflammation activity, ability of plant extract to inhibit protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation. Maximum inhibitions of petroleum ether and chloroform extracts have shown 54%, 70% and their IC_{50} values were 620µg/ml and 120µg/ml respectively. Diclofenac sodium, a standard anti-inflammation drug has showed the maximum inhibition 63% at the IC50 value concentration of 90µg/ml compared with control Figur 1.

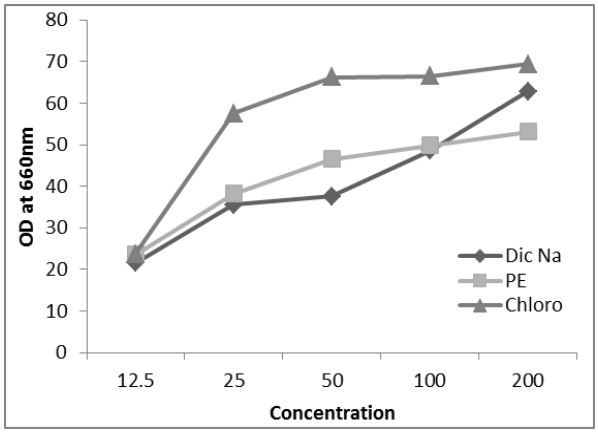

Fig. 1: Anti inflammatory activity by albumin denaturation method of *Ficus krishnae* stem bark extract of non polar solvents.

DPPH Radical Scavenging Activity

DPPH is stable free radical contains purple color, it can be reduced completely when DPPH radicals react with reducing agents and then electrons become paired-off and the solution loses the color from purple to colorless stoichimetrically with the number of electrons taken up (Chidambara Murthy *et al*., 2003)[18]. From figure 2 it is evident that the radical scavenging activity of petroleum ether and chloroform extracts of *F. krishnae* stem bark extracts ranged from 16.87% to 60.50% and 22.98% to 61.04% respectively, where as Ascorbic acid and BHT have showed 86.35% to 98.20% and 70.37% to 93.35% respectively.

The IC_{50} values of petroleum ether, chloroform, ascorbic acid and BHT are 1.88±2.35 µg/ml, 82.5±0.6 µg/ml, 3.5±1.2 µg/ml and 4.5±2.4 µg/ml respectively.

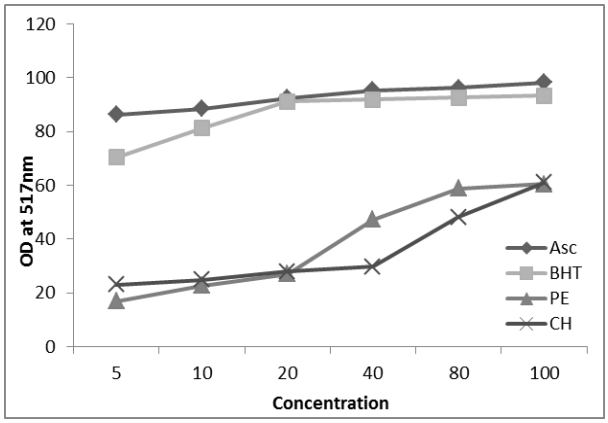

Figure 2: Shows the in DPPH antioxidant activity of petroleum ether, chloroform extract of *Ficus krishnae,* ascorbic acid and BHT standards.

ABTS radical scavenging assay

Proton radical scavenging is an important attribute of antioxidants. ABTS a protonated radical has characteristic absorbance maxima at 734 nm which decreases with the scavenging of the proton radicals [19]. It was noted in figure 5 that Compound 3 at 50µg/ml recorded (65.19%) fast and effective scavenger of the ABTS radical and this activity was comparable to that of BHT at the same concentration inhibition percent was 88.68 %. On the other hand, Moderate levels of antioxidant activity were presented with compound 1, 4 and total extract , They found to be 48.59%, 42.05% and 45.24% respectively. A sample possessing ABTS free radical-scavenging activity has indicated that it acts as a hydrogen donor and terminated the oxidation process by converting free radicals to more stable products, whereas a compound exhibiting a positive result in the FRAP assay was an electron donor and it terminated the oxidation chain reaction by reducing the oxidized intermediates into the stable form [20]. On the other hand some compounds which have ABTS+ scavenging activity did not show DPPH scavenging activity.

The IC_{50} values of petroleum ether, chloroform, ascorbic acid and BHT are 4.23±1.3 µg/ml, 4.8±1.6 µg/ml, 4.0±1.5 µg/ml and 5.3±1.24 µg/ml respectively.

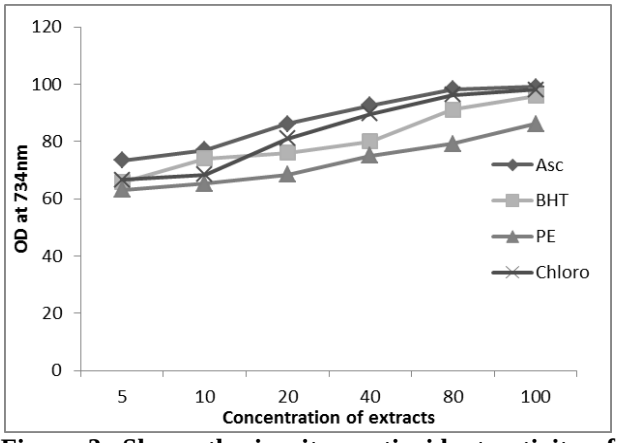

Figure 3: Shows the in vitro antioxidant activity of petroleum ether, chloroform extract of *Ficus krishnae,* ascorbic acid and BHT standards.

Phosphor-molybdenum radical scavenging activity:
This assay is based on the reduction of antioxidant compounds by the subsequent formation of a green phosphate Mo (V) complex from Mo (VI) to Mo (V) at acidic pH, which is measured at 695nm. The reducing activity of petroleum ether extract, chloroform extract and BHT are presented in Figure-4 it represents a dose dependent curve. Phosphomolybdenum reduction assay increases with increase in concentration of extract of *Pterocarpus marsupium* bark, in accordance with our results.[21]

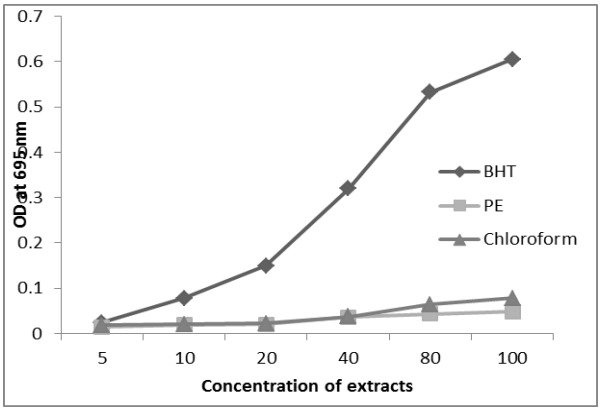

Figure 4: Shows the Phosphomolybdenum Assay of petroleum and chloroform extracts of Ficus krishnae stem bark extract.

Reducing power assay
The reducing ability of different solvent extracts of *Ficus krishnae* stem bark, which shows potential antioxidant activity, was evaluated by using potassium ferricyanide reduction method. The main principal of this method is presence of reductants in the extracts causes the reduction of Fe+/ ferricyanide complex to the ferrous form; it is monitored by measuring the formation of Prussian blue at 700nm. All the extracts will have exhibitrd some degree of reducing power (Figure 5). The higher absorbance value was indicated with the chloroform which has shown high antioxidant activity when compared with standards BHT and ascorbiac acid fallowed by petroleum ether.

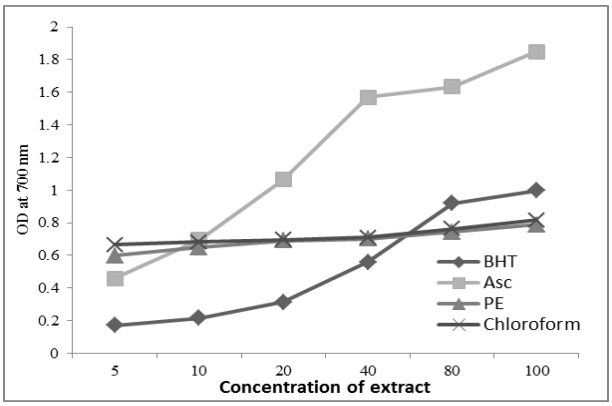

Figure 5: Shows the Ferric Reducing Power assay for petroleum ether and chloroform extract of *Ficus krishnae.*

CONCLUSION

The overall finding of the results of present investigation is concluded that the chloroform extract of *F. krishnae* stem bark possesses a strong antioxidant and anti-inflammatory potentials due to the presence of bioactive compounds. These findings supports that this plant is having a potential sources of natural pharmacological agents which might be helpful in preventing the progress of various chronic diseases. Further, investigations on the isolation and characterization of individual phenolic compounds will be required to elucidate their different antioxidant mechanism and existence of possible synergisms among the compounds.

ACKNOWLEDGMENT

The author's thanks the University Grant Commission (UGC), New Delhi for providing financial support to carry out this research work under Rajiv Gandhi national fellowship (RGNF) scheme.

REFERENCES

1. Gerard J Tortora, Sandra Reynolds, eds. Principles of Anatomy and Physiology.
2. Sies.H. Oxidative stress: Oxidants and antioxidants, Exp-Physiol, 1997; 82(2): 291-295.
3. Davies.K. Oxidative stress: the paradox of aerobic life, Biochem Soc Symp, 1995; 61: 1-35.
4. Valko.M., Leibfritzn.D., Moncol.J., Cronin.M., Mazur.M , Telser.J., Free radicals and Antioxidants in normal physiological functions and human disease, Int J Biochem Cell Biol, 2007; 39(1): 44-48.
5. Stohs.S., Bagchi. D., Oxidative mechanisms in the toxicity of metal ions, Free RadicBiolMed, 1995: 18(2): 321–336.
6. Nakabeppu..Y., Sakumi.K., Sakamoto.K., Tsuchimoto.D., Tsuzuki.T., Nakatsu.Y., Mutagenesis and carcinogenesis caused by the oxidation of nucleic acids, BiolChem, 2006; 266(1-2): 37-56.
7. Sajida Begum, I. C. Barua, Ficus Species and its Significance. International Journal of Computer Applications in Engineering Sciences, 2012; 2: 2231-4946.
8. Anonymous. Wealth of India: Raw Materials Vol. IV. New Delhi: CSIR, 1956; 25.
9. Biswas K. Observations on the systematic position of *Ficuskrishnae. Curr Sci*, 1934; 3(8): 424-7.
10. Kirtikar KR, Basu BD. Indian Medicinal Plants Vol. III. Dehradun: International Book distributors, 2005; 2312.
11. Amarvani P Kanjikar, Ramesh L Londonkar. Pharmacognostic Evaluation, Phytochemical Screening and Antimicrobial Activity of Stem Bark of *Ficus Krishnae* International Journal of Pharmacognosy and Phytochemical Research, 2017; 9(5): 733-738.
12. G. Leelaprakash, S. Mohan Dass"Invitro Anti-Inflammatory activity of Methanol extract of EnicostemmaAxillare", Int. J. Drug Dev. & Res, 2011; 3(3): 189-196.

13. Sakat S, Juvekar AR, Gambhire MN. In vitro antioxidant and anti-inflammatory activity of methanol extract of Oxalis corniculata Linn. International Journal of Pharma and Pharmacological Sciences, 2010; 2(1): 146-155.
14. Das Moonjit, MalipeddiHimaja, N. ArunaiNambiraj and Reshma Rajan Phytochemical analysis, Antioxidant activity and *inVitro* growth inhibition of struvite crystals by *Ipomoea eriocarpa* leaf extracts Journal of Food Biochemistry, 2015; 1745–4514.
15. Huang, H.B. Ke, J.S.He, X.Q.Ban, H.Zeng, Y.W.Wang. Extracts of *Halenia elliptica* exhibit antioxidant properties in vitro and in vivo. Food and Chemical Toxicology, 49(2011): 185–190.
16. P.Prieto, M.Pineda, M.Aguilar. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal. Biochem, 269(1999): 337–341.
17. Jungmin Oh, Heonjoo Jo, Ah Reum Cho, Sung-Jin Kim, Jaejoon Han. Antioxidant and antimicrobial activities of various leafy herbal teas. Food Control, 31(2013): 403-409.
18. Chidambara Murthy, K.N., Vanitha, A., Mahadeva Swamy, M. and Ravishankar, G.A. Antioxidant and Antimicrobial Activity of *Cissus quadrangularis* L. J Med Food, 2003; 6(2): 99-105.
19. Sindhu Mathew, T. Emilia Abraham. In vitro antioxidant activity and scavenging effects of Cinnamomum verum leaf extract assayed by different methodologies. Food and Chemical Toxicology, 44(2006); 198–206.
20. Suganya Tachakittirungrod, Fumio Ikegami and Siriporn Okonogi. Antioxidant Active Principles Isolated from Psidium guajava Grown in Thailand. Sci Pharm, 2007; 75: 179–193.
21. Ramesh L Londonkar, Aruna L H, Amarvani P Kanjikar,.Potential Investigation of *In Vitro* Antioxidant, Anti-Inflammatory and Anti-Haemolytic Activities from Polar Solvent Extracts of *Pterocarpus marsupium.* International Journal of Pharmacognosy and Phytochemical Research, 2017; 9(1); 100-107.