MINOR RESEARCH PROJECT

Project title

"Isolation of active Principle for inhibition of Angiogenesis and PLA_2 from Bombax ceiba pentandra"
MRP(S)-1130/11-12/KAMY013/UGC-SWRO

Final report

Submitted To

The Joint secretary and Head
South Western Regional Office (SWRO)
University grants commission
P.K. Block, Gandhinagar
Palace Road
Bangalore – 560 009

Submitted by

Dr. Nagamani J E, M Sc., Ph.D, PGDND
Principal Investigator
Postgraduate department of Biochemistry

JSS COLLEGE OF ARTS, COMMERCE AND SCIENCE
Autonomous under University of Mysore
Reaccredited by **NAAC** with **'A'** Grade
Recognised by UGC as **'College with potential for excellence'**
Ooty road, Mysore – 570 025Phone: 0821-2548236, Fax: 0821 – 2548238
E-mail : jssautonomous@gmail.com Web : www.jsscacs.edu.in

JSS MAHAVIDYAPEETHA
JSS COLLEGE OF ARTS, COMMERCE & SCIENCE
(An Autonomous College of University of Mysore)
Re-accredited by NAAC with 'A' grade
Recognised by UGC as "College with Potential for Excellence"
OOTY ROAD, MYSORE - 570 025, KARNATAKA
ESTD - 1964
☎ 0821-2548236 & 2548380 Fax: 0821-2548238 E-mail: jssautonomous@gmail.com

DECLARATION AND CERTIFICATE

I hereby declare and certify that, the Minor Research Project entitled **"Isolation of active Principle for inhibition of Angiogenesis and PLA_2 from Bombax ceiba pentandra"** MRP(S)-1130/11-12/KAMY013/UGC-SWRO is a bonafide record of research work carried out by me during the year 2011-2013. Further certify that the work presented in the report is original and carried out according to the plan in the proposal and guidelines of the University Grants Commission.

Principal Investigator

CONTENTS

1. ANNUAL REPORT (ANNUAL BASIS) OF THE MINOR RESEARCH PROJECT

FIRST YEAR:

Bombax ceiba pentandra spike and fruit samples were collected from herbal garden maintained by JSS College, Mysore, Karnataka, India in the month of January and authenticated from the Department of Botany, University of Mysore, Karnataka, India.

The samples were dried at room temperature and powdered separately. Each of the sample was extracted separately with different solvents starting with non polar to polar solvents in the order of hexane, ethyl acetate, methanol and water. The crude residues were then resuspended in the respective solvents for further study.

The proximate composition of spikes and fruits extracts were carried out to determine the content of ascorbic acid, tannins, saponins, glycosides, proteins, total phenols, flavanoids as well as alkaloids.

Higher levels of flavanoids and polyphenols were present in the methanol extract of fruits and spikes in comparison to hexane, ethyl acetate and aqueous extracts.

The crude plant extract was screened for antioxidant activity namely DPPH radical scavenging assay, Nitric oxide radical scavenging assay, Hydroxyl radical scavenging assay and Superoxide radical scavenging assay. Methanol extract of fruit and spike exhibited significant antioxidant activity.

Thin layer chromatography was performed to partially purify the active principle from the solvent extracts. λmax was determined to check the purity of the isolated active principle scanning from 200 to 700ηm. Flavanoids and total phenols were estimated from the TLC fractions to determine their presence and concentration.

SECOND YEAR:

Indirect hemolytic assay was performed for secretory PLA_2 inhibition study using the crude extracts as well as partially purified samples.

Crude methanol extract of fruit as well as TLC fraction of the methanolic extract showed considerable PLA_2 inhibition activity.

Shell less CAM assay was carried out for antiangiogenesis study using fertilized eggs.

Crude acetone extract and TLC fraction of the fruit sample exhibited comparable antiangiogenesis activity.

2. INTRODUCTION

Angiogenesis is the process involving the growth of new blood vessels from pre-existing vessels. Physiological angiogenesis takes place mainly during wound healing and menstrual cycle in females. Pathological angiogenesis occurs in diseases such as cancer, chronic inflammatory disorders like rheumatoid arthritis (RA), psoriasis, endometriosis and diabetic retinopathy. An abnormal or excessive level of angiogenesis also contributes to vascular malformation, obesity, chronic inflammation, whereas insufficient angiogenesis is related to Alzheimer's disease, coronary artery disease, stroke, myocardial infarction and ulcer formation (Carmeliet and Jain).

Angiogenesis is essential for tumor progression. When solid cancers are small, they are supplied with nutrients by diffusion from nearby blood vessels. In order to grow larger, they need their own blood vessels which they create by angiogenesis promoters such as VEGF, ACE, Endothelin etc.(Cao *et al.*). Tumors cannot grow larger than 2mm without angiogenesis. By stopping the growth of blood vessels, scientists hope to cut the means by which tumors can nourish themselves and thus become metastasis. Drugs that interrupt that process show promise in treating cancer.

As tumors are angiogenesis-dependent and that anti-angiogenic therapy might represent a good alternative for the treatment of solid tumors. An angiogenesis inhibitor is a substance that inhibits the growth of new blood vessels. Some angiogenesis inhibitors are a normal part of the body's control, some are administered as drugs, and some come from diet. The search for antiangiogenic agents has widened that includes compounds derived from natural agents.

Review of Research and Development in the Subject

Angiogenesis and lymphangiogenesis mediated by vascular endothelial growth factors (VEGFs) are main features of chronic inflammation and tumors. Secreted phospholipasesA_2 are overexpressed in human inflammatory lung diseases and cancer and they activate inflammatory cells by enzymatic and receptor-mediated mechanisms. The results support the involvement of sPLA_2 in angiogenesis (Granata *et al.*). It is proposed that sPLA_2

inhibitor introduces a novel approach in the control of cancer development (Chen *et al*.). In the mouse models of brain and lung cancer, Cytosolic PLA_2 and lysophospholipids have key regulatory roles in tumor angiogenesis. Hence $cPLA_2$ inhibition is a promising mode of antiangiogenic therapy (Amanda *et al*.).

There is considerable evidence to suggest that angiogenesis and chronic inflammation are codependent; recent studies have begun to reveal the nature of this link, which involves both augmentation of cellular infiltration and proliferation involving overlapping roles of regulatory growth factors and cytokines. There are potential benefits in targeting angiogenesis for the treatment of chronic inflammation which in turn affects angiogenesis (Jeffrey *et al*.).

Significance of the study

Many chemopreventive molecules that have powerful anti-inflammatory and antioxidant activity including sulforaphane, n-acetyl cystein, Curcumin, sibilin, xanthohumol are also known to inhibit angiogenesis. However there can be other critical molecular targets yet unidentified for angioprevention. As the herbal medicines have insignificant side effects and are less expensive in comparison to synthetic drugs. There is a continuing need for new angiopeventive molecules from natural herbs and medicinal plants (Nicola *et al*.).

Withaferin A, the active principal from *Withania somnifera* is a potent tumor inhibitor and antiangiogenic agent (Bargagna *et al*.). Solenopsin, an alkaloid from *Solenopsis invicta* is a naturally occurring inhibitor for angiogenesis (Arbiser *et al*.). The medicinal plants *Ailanthus malabarica*, *Tinospora crispa*, *Phyllanthus crinaria* methanolic extracts showed significant anti-angiogenic activity without acute cytotoxicity (Nq KW *et al*.).

Bombax ceiba pentandra, commonly known as red silk cotton tree is widely spread in Indian subcontinent and around the world. Ceiba pentandra belongs to the family malvaceae. It finds wide therapeutic applications in various tribal communities around the world. The tree is being exploited largely for medicinal and commercial purposes. Roots are used in the treatment of Leprosy, hypertension. Stem bark being used for relieving fever, asthma, edema and leaves powder for skin infections, sores, abscesses. Spike and young fruits being used in the treatment of snake bite and also in inflammatory diseases (Bhavan).

Research findings revealed that the stem bark extract has antimicrobial and antifungal activities (Sawhney *et al*.and Odama *et al*.). Ceiba pentandra leaves extract showed antipyretic activity as well as antihypertensive activity (Grosvenor *et al*.). Research on the toxicological studies proved that Ceiba pentandra has very low toxicity profile in all the tested animals and it is safe for oral medication (Sarkiyayi *et al*.).

3. METHODOLOGY

3.1 Solvent extraction:

Bombax ceiba pentandra young fruits and spikes were collected from herbal garden maintained by JSS College, Mysore, Karnataka, India in the month of January and authenticated from the Department of Botany, University of Mysore, Karnataka, India.

The samples were dried at room temperature were powdered separately. 100gm each of the samples were extracted separately with different solvents starting with non polar to polar solvents in the order of hexane, ethyl acetate, methanol and water. The crude residues of the extracts were then resuspended in the respective solvents for further study.

3.2 Proximate analysis:

The proximate composition of spikes and fruits extracts were carried out to determine the content of ascorbic acid, tannins, saponins, glycosides, proteins, total phenols, flavanoids as well as alkaloids.

The protein content was estimated by Bradford method. Total phenolic content was estimated using Folin–Ciocalteue reagent. Flavanoids were estimated following the method of Woiskey and Salatino. Ascorbic acid content was estimated using DNPH reagent.

3.3 Antioxidant assay

3.3.1 DPPH radical scavenging assay:

1, 1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity was determined as per the method of Habila. 0.1mL of the solvent extracts were taken in different test tubes and volume in each of the test tube was made up to 100µL using methanol. 3mL of 0.1mM DPPH in methanol was added to each of the test tube and the mixture was shaken vigorously and allowed to stand for 20 minutes. Absorbance of the solutions were measured at 517ηm using spectrophotometer (Shimadzu UV-2550). Ascorbic acid (0.1mg/mL) was used as control for the assay.

3.3.2 Nitric oxide radical scavenging assay

Nitric oxide radical scavenging assay was conducted as per the method of Marcocci. 0.1mL of the plant extracts were added to the reaction mixture containing 2.5mL of sodium nitroprusside (10mM), 0.5mL of phosphate buffered saline (pH-7.4). The reaction mixture was incubated at room temperature for 150 minutes and 1mL of Griess reagent was added to all the test tubes. The reaction mixture was allowed to stand for 30 minutes at room temperature and the absorbance of the chromophore formed was read at 546ηm. BHT (0.1mg/mL) was used as positive control for the assay.

3.3.3 Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity of the extracts was estimated as per the method of Shang17. 0.1mL each of the plant extract was added to the reaction mixture containing 0.1mL of Deoxyribose (3mM), 0.5mL of FeCl3(0.1mM), 0.5mL of EDTA (0.1mM), 0.5mL of Ascorbic acid (0.1mM), 0.5mL of H2O2 (1mM) and 0.8mL of Phosphate buffer (20mM pH 7.4). The reaction mixture was incubated at 37oC for 1hour. Then 1mL of Thiobarbutiric acid (TBA) as well as 1mL of 2.8% Trichloro acetic acid (TCA) were added and incubated at 100^{o}C for 20 minutes. Thiobarbutiric acid reactive substances formed were measured after cooling the mixture and measuring the absorbance at 532ηm.

3.3.4 Superoxide radical scavenging assay

The assay was done following the method of Khanna. 0.1mL each of the plant extract was added to the reaction mixture containing 50mM phosphate buffer (pH-7.6), 20µg/ml riboflavin, 12mM EDTA and 0.1mM NBT. The reaction was initiated by illuminating the reaction mixture for 5 minutes and the absorbance was measured at 590ηm. Inhibition of blue formazone formation was considered for scavenging activity. Quercitin (0.1mg/mL) was used as positive control.

3.4 Partial purification by TLC

The solvent system was standardized for the separation of active principle from the methanol extracts of spike and fruit that exhibited significant antihemolytic property. The solvent system used was – Ethyl acetate: hexane: methanol: acetic acid::2:2:1:0.5.

Isolated fractions were once again subjected to 2D – TLC and preparatory TLC for further purification of the active principle. Scrapped matter was isolated, redissolved in methanol and supernatant was collected. UV absorption spectrum was taken for the TLC fraction and λ_{max} was determined to check the purity of the isolated active principle scanning from 200 to 700ηm.

Flavanoids and total phenols were estimated from the TLC fractions to determine their presence and concentration.

3.5 Secretory PLA_2 inhibition study

Indirect hemolytic assay

The assay was carried out according to the method of *Boman and Kelleta*. The substrate for indirect hemolytic activity was prepared by suspending 1ml of fresh human red blood cells and 1ml of fresh hen's egg yolk in 8ml of phosphate buffered saline (pH – 7.4). 1ml of suspension was incubated with 20μg of bovine pancreas PLA_2(P8913) for 30 minutes at 37^oC and the reaction was stopped by the addition of 9ml of ice cold PBS. The suspension was centrifuged at 2000rpm for 20 minutes and the released hemoglobin was read at 540ηm.

Similarly PLA_2 was incubated with different solvent extract of the plant for 15 minutes and then 1ml of the substrate was added to each of the tubes and incubated for 30 minutes at 37^oC and the reaction was stopped by the addition of 9ml of ice cold PBS. The suspension was centrifuged at 2000rpm for 20 minutes and the released hemoglobin was read at 540ηm.

3.6 Anti-angiogenesis assay

Shell less Chorioallantoic Membrane

Fertilized hens eggs were procured from Indian Veterinary Research Institute (IVRI), Bangalore, and the surface was sterilized using 70% alcohol. The eggs were incubated in

fan assisted humidified incubator at 35-37^{o}C. On the 4th day in the laminar flow cabinet, the eggs were wiped with 70% alcohol and shell was cracked out into thin film of the hammock, Egg preparation was covered with sterile glass plate and returned to the incubator.

On the 5th day the samples were applied on to the filter paper discs, placed over the blood vessel and eggs were returned to the incubator. Results were observed next day.

4. RESULT

4.1 Proximate analysis

Proximate analysis of B. ceiba pentandra spike and fruit extracts reported considerable amounts of protein and ascorbic acid in the aqueous extract of the fruits as compared to that of spikes.

Higher levels of flavanoids, tannins and polyphenols were present in the methanol extract of fruits and spikes in comparison to hexane, ethyl acetate, chloroform and aqueous extracts (Table – 1).

Extract	**Proteins**	**Phenols**	**Flavanoids**	**Tannins**	**Alkaloids**	**Saponins**	**Ascorbic acid**	**Glycosides**
Aqueous extract (spike)	++	++	++	+	+	+	+	+
Aqueous extract (fruit)	++	++	++	+	+	+	+	+
Methanol extract (spike)	-	+++	+++	++	-	-	-	-
Methanol extract (fruit)	-	+++	+++	++	-	-	-	-
Ethyl acetate extract (spike)	-	+	+	-	-	-	-	-
Ethyl acetate extract (Fruit)	-	+	+	-	-	-	-	-
Chloroform extract (spike)	-	+	-	-	-	-	-	-
Chloroform extract (fruit)	-	+	-	-	-	-	-	-
Hexane extract (spike)	-	-	-	-	-	-	-	-
Hexane extract (fruit)	-	-	-	-	-	-	-	-

Table 1: Proximate analysis

4.2 Antioxidant assay

4.2.1 DPPH radical scavenging assay:

Table 2A, refers to DPPH radical scavenging assay. DPPH, a nitrogen centered free radical was reduced in the presence of different solvent extracts of fruit and spike.

Ascorbic acid used as positive control showed 92.91% scavenging activity which is followed by methanol extract of fruit showing 82.95% activity. Methanol extract of spike

as well as aqueous extract of fruits exhibited considerable scavenging activity of 76.25% and 70.01% respectively.

Table 2A: DPPH radical scavenging assay

Sample	Aqueous extract	Methanol extract	Ethyl acetate extract	Chloroform extract	Hexane extract
Ascorbic acid (Positive control)	92.91+0.120[a]	82.27+0.087[a]	86.03+0.067[a]	86.43+0.092[a]	84.01+0.071[a]
Spike	67.26+0.038[a]	76.25+0.056[a]	46.72+0.045[a]	64.5+0.098[a]	38.87+0.075
Fruit	70.01+0.090[a]	82.95+0.105[a]	54.13+0.087[a]	67.21+0.069[a]	68.03+0.064[a]

4.2.2 Nitric oxide radical scavenging assay:

Table 2B, refers to Nitric oxide radical scavenging activity. Nitric oxide generated from sodium nitroprusside interacts with oxygen to form nitric ions whose concentration was estimated using Griess reagent.

Methanol extract of fruits and spike showed maximum scavenging activity of 90.04% and 84.48% respectively. BHT in water showed a scavenging activity of 92.4%.

Sample	Aqueous extract	Methanol extract	Ethyl acetate extract	Chloroform extract	Hexane extract
BHT (Positive control)	92.4+0.164[a]	88.27+0.096[a]	86.04+0.088[a]	87.08+0.396[a]	82.21+0.152[a]
Spike	77.14+0.111[a]	84.48+0.095[a]	58.55+0.110[a]	63.98+0.091[a]	48.44+0.094[a]
Fruit	69.31+0.085[a]	90.04+0.059[a]	67.14+0.068[a]	81.51+0.127[a]	65.52+0.129[a]

Table 2B: Nitric oxide radical scavenging assay

4.2.3 Hydroxyl radical scavenging assay:

Table 2C, refers to Hydroxyl radical scavenging assay. Hydroxyl radicals were generated by the Fenton reaction and the TBA reacting substance was estimated photo metrically.

Aqueous and methanol extracts of fruits showed higher scavenging activities of 80.42% and 80.14% when compared to other extracts.

Sample	Aqueous extract	Methanol extract	Ethyl acetate extract	Chloroform extract	Hexane extract

Quercitin (Positive control)	82.22+0.090[a]	82.92+0.104[a]	74.53+0.126[a]	77.87+0.124[a]	71.00+0.057[a]
Spike	69.21+0.075[a]	72.05+0.056[a]	36.12+0.092	47.98+0.095[a]	53.21+0.065[a]
Fruit	80.42+0.111[a]	80.14+0.068[a]	65.05+0.071[a]	68.25+0.083[a]	69.56+0.103[a]

Table 2C: Hydroxyl radical scavenging assay

4.2.4 Superoxide radical scavenging activity

Table 2D, refers to Superoxide radical scavenging activity. The superoxide radical generated from Riboflavin and NBT in the presence of light were scavenged by the different solvent extracts of spike and fruits.

With respect to the positive control Ascorbic acid (82.7%), methanol extract of spike (77.83%) and fruit (75.40%) exhibited considerable scavenging activities.

Sample	Aqueous extract	Methanol extract	Ethyl acetate extract	Chloroform extract	Hexane extract
Ascorbic acid (Positive control)	80.46+0.116[a]	82.70+0.098[a]	72.92+0.069[a]	75.54+0.130[a]	73.50+0.125[a]
Spike	70.53+0.098[a]	77.83+0.147[a]	69.65+0.114[a]	50.23+0.141[a]	42.71+0.095[a]
Fruit	64.46+0.045[a]	75.40+0.094[a]	52.71+0.086[a]	60.23+0.087[a]	49.62+0.127[a]

Table 2D: Superoxide radical scavenging assay

4.3 Partial purification by TLC

Table 3, refers to the concentration of flavanoids as well as polyphenols in the partially purified methanol extract of fruit and spike. UV spectra of the TLC fraction (Fig 2 and 3) indicates maximum absorption at 226ηm and 280ηm confirming the presence of phenolics and flavanoids.

Also quantitative estimation revealed higher concentration of phenols as well as flavanoids in the methanol extract of fruit and considerable amount of flavanoids in the methanol extract of spike.

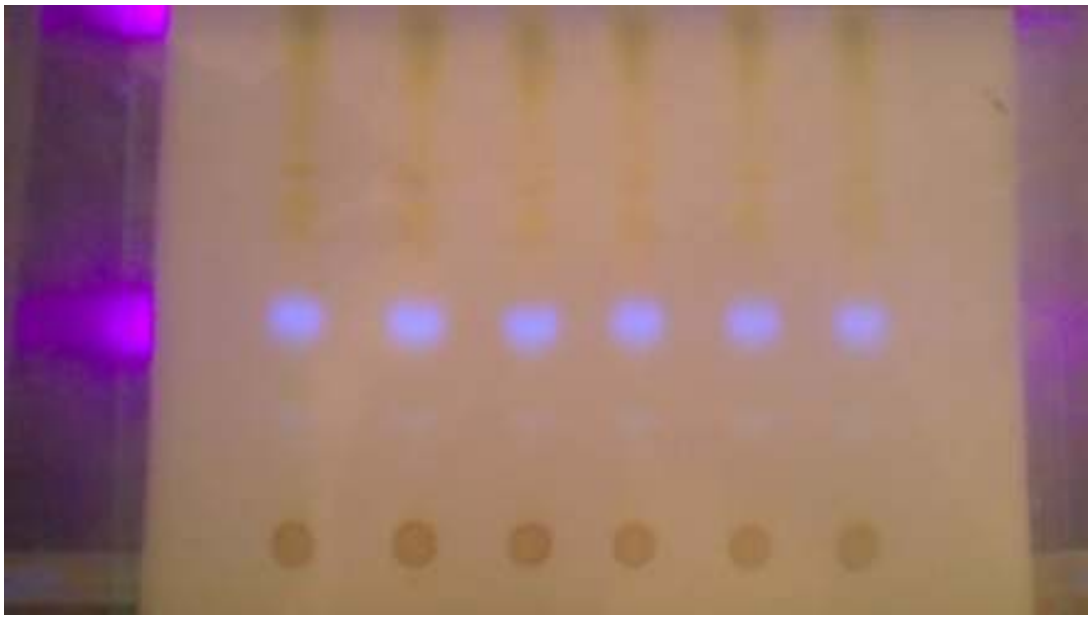
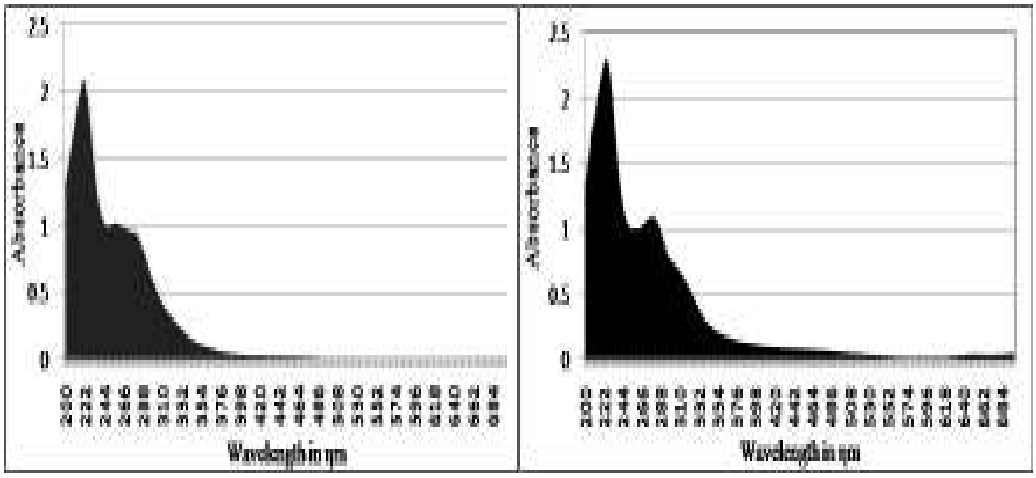


Fig. 1: THIN LAYER CHROMATOGRAPHY OF METHANOL EXTRACT

Fig. 2: UV absorbance of TLC fraction from Spike

Fig. 3: UV absorbance of TLC fraction from Fruit



4.4 Secretory PLA_2 inhibition study

Indirect hemolytic assay:

Table 4, explains the antihemolytic activity of various extracts of spike and fruits. The lysis of erythrocytes in the presence of $sPLA_2$ in the absence of plant extract was considered as 100% hemolytic activity.

Among all the extracts, methanolic extracts of fruit exhibited considerable antihemolytic activity and lesser amount of MDA. In presence of aqueous, ethyl acetate, chloroform and hexane extracts, erythrocytes were partially hemolysed.

Partially purified bioactive molecules from TLC fraction of methanol extract of fruit established high antihemolytic activity.

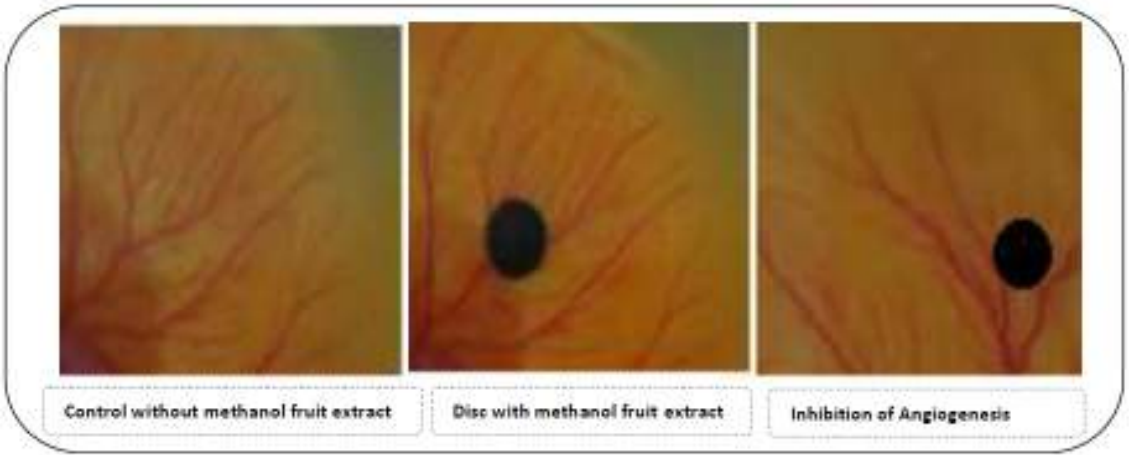
Sample	% Hemolysis		Malondialdehyde (µgm/ml)	
	Fruit	Spike	Fruit	Spike
$sPLA_2$ + Substrate(Negative control)	100	100	2.330	2.330
Ascorbic acid + $sPLA_2$ + Substrate (Positive control)	6.38	6.38	0.148	0.148
Aqueous extract + Substrate	11.56	10.83	0.269	0.252
Methanol extract + Substrate	10.29	9.27	0.239	0.215
Ethylacetate extract + Substrate	13.34	11.76	0.310	0.274
Chloroform extract + Substrate	12.13	10.96	0.282	0.255
Hexane extract + Substrate	15.54	13.13	0.362	0.305
Aqueous extract + $sPLA_2$ + Substrate	37.45	32.76	0.872	0.763
Methanol extract + $sPLA_2$ + Substrate	19.14	14.57	0.786	0.456
Ethylacetate extract + $sPLA_2$ + Substrate	44.71	39.69	1.041	0.924
Chloroform extract + $sPLA_2$ + Substrate	72.32	70.87	1.685	1.651
Hexane extract + $sPLA_2$ + Substrate	69.18	61.43	1.611	1.431
TLC fraction of Methanol extract + $sPLA_2$ + Substrate	11.51	9.21	0.268	0.214

Table 4: Indirect hemolytic assay

4.5 Anti-angiogenesis assay

Anti-angiogenesis activity of crude extract as well as TLC purified samples were tested using *in-vivo* CAM model (Fig 4 and Fig 5). The 6^{th} day old embryo after treatment with plant extract was examined for the number of vessels and their reduction in the area surrounding the applied disc. 100 µg of TLC fraction from Methanol extract of the fruit

sample exhibited greater inhibition of blood vessels in comparision to other solvent extract.



5. DISCUSSION

In recent years the use of herbs in traditional medicine has gained attention as they are being proven as the promising sources of various bioactive molecules ranging from simple molecules like phenolic acids, phenyl propanoids, flavanoids to highly polymerized compounds namely lignins, melanins and tannins. Polyphenols exhibit a wide range of pluripharmacological effects including antimicrobial, anti-inflammatory, hepatoprotective and anticarcinogenic actions. Flavanoids are the most common and widely distributed subgroups.

In the present study proximate analysis as well as TLC fraction showed higher concentrations of phenols and flavanoids in the methanol extract of spike and fruit. The significant antioxidant activities including DPPH, Hydroxyl, Superoxide as well as Nitric oxide radical scavenging activities of methanol extract of spikes and fruits may be endorsed for the presence of higher amounts of the above mentioned plant secondary metabolites.

Significant relationship between elevated antioxidant activities with high amount of total phenolics content has been extensively discussed. Generally, the antioxidant activity of phenolics is greatly contributed by their structures, stressing on the presence of hydrogen-donating hydroxyl groups, and those with more hydroxyl groups possess greater antioxidant capacity (*Cai Y et al*.).

Many investigations have proven that varieties of flavonoid molecules possess anti-inflammatory activity on various animal models of inflammation. Especially, some flavonoids were found to inhibit chronic inflammation of several experimental animal models. They could regulate cellular activities of the inflammation-related cells: mast cells, macrophages, lymphocytes, and neutrophils. Flavonoids inhibit histamine release from mast cells and others inhibit T-cell proliferation (*Yang C S et al*.).

In addition, certain flavonoids modulate the enzyme activities of arachidonic acid (AA) metabolizing enzymes such as phospholipase A_2 (PLA_2), cyclooxygenase (COX), and lipoxygenase (LOX) and the nitric oxide (NO) producing enzyme, nitric oxide synthase (NOS). An inhibition of these enzymes by flavonoids reduces the production of AA, prostaglandins (PG), leukotrienes (LT), and NO, crucial mediators of inflammation. Thus,

the inhibition of these enzymes exerted by flavonoids is definitely one of the important cellular mechanisms of anti-inflammation.

In the current study Methanol extract of the fruit sample including the crude as well as TLC fraction exhibited significant antihemolytic activity, the consequence of $sPLA_2$ inhibition. Inhibition of the secretory Phospholipase A_2 enzyme by the plant extract inhibits the release of arachidonic acid there by protecting the lysis of erythrocytes. Lesser the cell lysis lesser will be the production of Malondialdehyde.

Angiogenesis is essential in tumour growth and metastasis as the process provides necessary oxygen and nutrition for the growing tumour. Antiangiogenic effect of *Bombax ceiba pentandra* fruit extract was performed by *in vivo*, CAM assay. Treatment of the CAMs with TLC fraction of methanolic fruit extract changed the vascularisation pattern; the extract inhibited the new blood vessels formation in the treated CAMs as well as distortion of existing vasculature. Analysis of TLC fraction indicated the presence of significant quantities of Flavanoids and phenols.

Among the known angiogenesis inhibitors, flavonoids seem to play an important role. However, the mechanism behind the antiangiogenetic effect of flavonoids could be inhibition of protein kinases. These enzymes are implicated to play an important role in signal transduction and are known for their effects on angiogenesis (*Oikawa et al*.).

CONCLUSION

Presence of considerable amounts of flavonoids, phenols as well as tannins in the methanolic extract of *Bombax ceiba pentandra* fruit could be ascribed for significant antioxidant, $sPLA_2$ inhibition as well as antiangiogenic properties.
Further purification and characterization of the active principle has to be undertaken

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Annexure -III

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002.

Final Report of the work done on the Minor Research Project.

1	Project report No. 1st/2nd/3rd/Final	:	Final Report
2	UGC Reference No.:	:	MRP (S)-1129 /11-12/KAMY013/UGC-SWRO
3	Period of report	:	27.12.11 to 30-12-2014
4	Title of research project	:	Isolation of active principle for inhibition of angiogenesis and sPLA2 from *Bombax ceiba pentandra*
5	(a) Name of the Principal Investigator	:	Dr. Nagamani J E
	(b) Dept. and University/College where work has progressed	:	JSS College of Arts, Commerce and Science, Ooty Road, Mysore-570025
6	Effective date of starting of the project	:	27.12.2011
7	Grant approved and expenditure incurred during the period of the report		
	a. Total amount approved	:	Rs. 1,00,000-00 Amount released as first installment (100% Recurring and 50% Non-recurring)
	b. Total expenditure	:	**Rs. 100130.00**
8	Report of the work done		**Report enclosed**
	(i). Brief objective of the project	:	1. Isolation and partial purification of the Bioactive molecule involved antioxidant $sPLA_2$ activity 2. Isolation of Bioactive principle using Thin layer Chromatographic techniques 3. Antiangiogenic activity studies of methanol extracts by Shell less CAM assay.
	(ii). Work done so far and results achieved and publications, if any, resulting from the work (Give details of the papers and names of the journals in which it has been published or accepted for publication)	:	Enclosed as separate attachment (Work done) 1. Antioxidant and antihemolytic activities of *Bombax ceiba pentandra* spike and fruit extracts 2. A study on the inhibition of $sPLA_2$ and angiosuppressive activities of *Bombax ceiba pentandra* fruit extract 3. Oral presentation on antioxidant and sPLA2 inhibition of *Bombax ceiba pentandra* spike and fruit extracts at

			National conference on 'Biotechnology and society' organised by Kuvempu university sponsored by UGC
	(iii).Has the progress been according to original plan of work and towards achieving the Objective, if not, state reasons	:	The progress of the project work is according to the original plan.
	(iv). Please indicate the difficulties, if any, experienced in implementing the project	:	The main objectives of the project has been successfully completed accordingly
	(v). If project has not been completed, please indicate the approximate time by which it is likely to be completed. A summary of the work done for the period (Annual basis) may please be sent to the Commission on a separate sheet.	:	Completed
	(vi). If the project has been completed, please enclose a summary of the findings of the study.Two bound copies of the final report of work done may also be sent to the Commission	:	Status of the project- completed Copies of the final report of the work done has been enclosed Two bound copies of the final report of work done is enclosed
	(vii). Any other information which would help in evaluation of work done on the project. At the completion of the project, the first report should indicate the output, such as (a) Manpower trained (b) Ph. D. awarded (c) Publication of results (d) other impact, if any	:	Two papers Published, one oral presentation was done.

PRINCIPAL INVESTIGATOR
Dr. Nagamani J E

PRINCIPAL

Annexure VIII

Final Report of the work done on the Minor Research Project.

1	Title of research project	:	"Isolation of active Principle for inhibition of Angiogenesis and PLA2 from Bombax ceiba pentandra"
2	(a) Name and address of the Principal Investigator	:	Dr. Nagamani J E, Assistant Prof. JSS College of Arts Commerce and Science Ooty Road Mysore
3	(a) Name and address of the Institution	:	JSS College of Arts Commerce and Science Ooty Road Mysore
4	UGC approval letter no and date	:	MRP(S)-1130/11-12/KAMY013/UGC-SWRO
5	Date of implementation	:	27.12.2011
6	Tenure of the project	:	21 Months (18 + 3)
7	Total grant allocated	:	Rs 1,00,000.00
8	Total grant received		Rs 95,000
9	Final Expenditure	:	Rs 1,00130.00
10	Title of the project	:	"Isolation of active Principle for inhibition of Angiogenesis and PLA2 from Bombax ceiba pentandra"
11	Brief objective of the project	:	1. Isolation and partial purification of the Bioactive molecule involved in antimicrobial and antioxidant activity using Chromatographic technique. 2. Antiangiogenic activity studies of methanol and ethyl acetate solvent extracts by Shell less CAM assay. 3. Isolation of Bioactive principle using Thin layer Chromatographic techniques
12	Whether objectives were achieved		The objectives of the study has been successfully completed
13	Achievements from the project		Two paper has been published and one oral presentation was done in a National Conference.

14	Summary of the findings		The results of phytochemical analysis, antioxidant and antiangiogenic activities of this plant has laid the foundation for further investigation. The anti inflammatory activity of this plant extract have shown promising results.
15	Contribution to the society		The active ingredients of this plant is to be identified and invivo studies using animal models should be done to ascertain the therapeutic use of these bioactive principles for drug preparation.
16	Whether any Ph.D. enrolled/ produced out of the project		No

Dr. Nagamani J E
PRINCIPAL INVESTIGATOR

PRINCIPAL