Investigation of anti-inflammatory and anti-cancer activity of *Justicia adathoda* metabolites

Susmitha Sudevan[1], Ranganayaki Parasivam[1], Shalini Sundar[1], Heema Velauthan[1] and Vijayaraghavan Ramasamy[2]*

[1]Deparment of Microbiology, Nehru Arts and Science College, Coimbatore, India
[2]Faculty of Biosciences, Nehru Arts and Science College, Coimbatore, India

Abstract: To analyse metabolic compounds of *Justicia adathoda* to evaluate against pathogens, inflammation and cervical cancer. The investigation exposed that the extracts of *Justicia adathoda* have potent metabolic to eradicate the human diseases. The antibacterial, tumorolytic and anti-inflammatory activity of ethyl acetate and aqueous extracts of *Justicia adathoda* (leaves) were assessed. *In vitro* anti-inflammatory activity was assessed by standard procedures. *Justicia adathoda* metabolic exhibit anticancer activity in human cervical cancer cell line (HeLa) (*in vitro*) analysis. Flavonoids, saponins, alkaloids, amino acids, tannins and terpenoids were present in both the extracts. The active components present in the extracts were found to be amino acids, alkaloids, lipids and triterpenoids which have antibacterial activity shows inhibition against *Salmonella* and *Escherichia coli*. *Justicia adathoda* possesses significant anti-inflammatory activity and it was confirmed by *in-vitro* analysis.The anticancer activity was found effective in human cervical cancer cell line (HeLa) (*in-vitro*) analysis. From the investigation could conclude that the metabolic compounds *Justicia adathoda* is effective against Anti-inflammation and ethyl acetate extract of *Justicia adathoda* are effective for Cancer.

Keywords: Alkaloids, hyaluronidase, HeLa, and *Justicia adathoda*.

INTRODUCTION

Predators such as insects, herbivorous mammals and fungi synthesized a broad range of chemical compounds from plants to preserve and carry out important biological functions. Less than 10% of the total compounds have been estimated so far from the 12, 000 compounds which are plant-mediated, for the predictable drugs synthesised by chemical compounds which are already in human usage for the indistinguishable process. Thus the plant mediates herbal drugs greatly helpful and conservative drugs in terms but at the same impending it may source detrimental side effects apart from the effective treatment.

Justicia adathoda, otherwise called as Malabar Nut, Adulsa, Adathoda, vasa or vasaka native to Asia, widely used in Ayurvedic and Unani systems of medicine. The plants are widely distributed in China, Nepal, India, Sri Lanka, Indonesia, Pakistan, and Malaysia. It grows like a shrubby four wide, leaves with lance-shaped. They are smooth-edged, arranged oppositely, and borne on short petioles. It appears dull brownish-green colour when it dry and bitter in taste. Oval stomata can be seen microscopically which is chloral hydrated. At the right angles to the ostiole, it has cells which appear in crescent-shaped. Warty hairs and small glandular hairs were bears in the epidermis. Beneath the epidermis, Cystoliths occur and its taxonomic position is as follows.

Justicia adathoda	
Scientific classification	
Kingdom:	Plantae
Division:	Angiosperms
Class:	Eudicots
Order:	Laminates
Family:	Acanthaceae
Genus:	Justicia
Species:	*J. adathoda*

Cardiovascular protection, abortifacient, antimicrobial, anti-tussive, anti-inflammatory, anticholinesterase and other important activities have been reported so far *J. adhatoda*. A small amount of active compounds present in the plant is responsible for the medicinal properties which can act as the human and animal beneficiary. Essential oil and quinazoline alkaloids are the important active compounds which were reported so far in *J. adathoda* for developing the best novel natural drug (Sandeep *et al.*, 2011).

In 1960-1980 most of the molecule work has been done and vasicine was isolated first isolated in 1924 *Adathoda vasica* (Acanthaceae) is the main source of this compound. In India, this plant has been utilized for more than 200 years for treating respiratory problems and because of its abortifacient activities. “Bromhexine”, is a derivative of this plant, now known for its molecular action and a plenty of work had been done on it.

**Corresponding author:* e-mail: metarrhizium@yahoo.co.in

Medicinal plants are the best source of drugs and it has been recognized by World Health Organization. To understand its efficacy, properties and safety it has to investigate accurately. A wide variety of secondary metabolites such as triterpenoids, tannins, flavonoids, etc are present in plants. To explore the metabolic compounds of *Justicia adathoda* and study its therapeutical applications the plant was subjected to investigation.

MATERIALS AND METHODS

Collection and extraction justicia adathoda

The healthy leaves from were collected from the Tamil Nadu Agricultural University and it was authenticated as *Justicia adathoda* at a Botanical Survey of India, Coimbatore. Under running tap water the leaves were washed well. In distilled water and ethyl acetate, the trodden small pieces of fresh leaves were mixed in 1:10 ratio individually. By continuous grinding using homogenizer, the extractions were prepared and Whattman No.1 filter paper used to filter the residues. Using rotary evaporator the filtrates were vacuum dried and stored at 4°C.

Preparation of standard culture inoculums and antibacterial assay

Inappropriate solvents, the residues were redissolved and used the antibacterial assay. *Staphylococcus aureus, Aeromonas hydrophila, Enterococci, Escherichia coli* and *Salmonella typhi* were used for the study. As recommended by WHO(2009), in 2ml nutrient broth three or four isolated colonies were inoculated and incubated for Mac-Farland standard [0.5%] growth. On Muller Hinton Agar medium (Anon, 1996) the antibacterial activities of the aqueous and ethyl acetate extracts were determined by cup diffusion method. With the help of micropipette, the working suspension about 10, 20 and 40µl were filled in well, so that the final drug concentration was 10mg/well, 20mg/well and 40mg/well respectively and the same volume of control were filled in the wells. The Plates were incubated at 37°C for 24 hrs after few minutes till the extract diffuse in the medium. The inhibition zone (ZI) were measured and recorded after incubation.

Phytochemical analysis and estimation of protein

According to the method described by (Trease and Evan, 1989), Phytochemical analyses were carried for the analysis of phytochemicals like lipids, terpenoids, alkaloids, saponin, flavonoids tannins, and steroid. The crude extracts were used to estimate the protein content in the plant extracts by the standard method (Lowry *et al.*, 1951).

Fourier transform infrared spectroscopic analysis of protein secondary structures

The extract subjected to its structural confirmation by IR mass spectrometry. Using Perkin Elmer FTIR spectrometer (*v max* in cm^{-1}) IR (KBr) spectra were recorded on a Mass spectra using Agilent HP5937 spectrometer.

Analysis of Rf standards of metabolic compounds by TLC

The Silica gel plates were prepared nd dried in a hot air oven and broaden evenly about 2cm from the bottom as the origin, at the centre of each slide the aqueous extracts were loaded above from the edge.

With suitable solvent systems the tank was developed and saturated (Eskil Hultin, 1966).
Aminoacids : Butanol/Acetic acid/Water- 4:1:1
Alkaloids : Benzene/Methanol-80:20
Lipid : Chloroform/Methanol/Water-10:10:3
Flavonoids : Chloroform/Methanol-70:30
Triterpeniods : Acetic acid/Water-1:3

The solvent front was marked once it dry. 1% ethanolic solution of Aluminium chloride was used to visualize Flavonoids and viewed under 560nm UV light. UV light was used to visualize yellow and orange fluorescent spots of alkaloids and triterpenoids. Brown colour spots were observed for detection of lipids under Iodine vapour used. The purple colour was observed on plated for amino acids, which was sprayed by ninhydrin.

Repossession of the active compound from TLC

With the help of a clean and dry spatula, the dented the spots on the silica gel slides were collected in a beaker containing water (Bishnu Joshi, 2011) and left overnight. Stirred the content in the beaker and Whattman no.1 filter paper was used to filtrated. The active compounds filtered was used to determine the antimicrobial effect against *Salmonella typhi* and *Escherichia coli*.

In vitro anti-inflammatory activity

Hyaluronidase inhibition assay

3-5U hyaluronidase in 100µl in 20mM sodium phosphate buffer (pH7) with 77mM $NaCl_2$, 0.1% BSA were pre-incubated with different concentrations of the active compound and aqueous extract of *Justicia adathoda* for 15mins at 37°C. 100µl of hyaluronic acid (0.03% in 300 mM Sodium Phosphate (pH 5.35) was added to the incubation mixture and incubated for further 45mins at 37°C. With 1ml of acid albumin solution made up of 0.1% BSA in 24mM Sodium acetate and 79mM acetic acid (pH 3.75), the undigested hyaluronic acid was precipitated. The absorbance of the reaction mixture was measured at 600 nm in Colorimeter after the mixture was kept at room temperature for 10mins and Absorbance in the absence of enzyme was used as a reference value of the maximum of inhibition. The reference standard was Aspirin.

$$\% \text{ inhibition} = \frac{\text{Abs control - Abs sample}}{\text{Abs control}} \times 100$$

Inhibition of albumin denaturation

1% aqueous solution of bovine albumin fraction and plant extract mixed together and pH was adjusted. It was incubated at 37ºC for 20 min and then heated to 51ºC for 20 min and at 660nm the absorbance was measured spectrophotometrically (Sakat *et al.,* 2010). Protein denaturetion was calculated as Percent inhibition:

$$\% \text{ inhibition} = \frac{\text{Abs control - Abs sample}}{\text{Abs control}} \times 100$$

Membrane stabilization test

Preparation of red blood cells (RBCs) suspension

In a centrifuge tube fresh whole human blood (10 ml) was transferred. The tubes were subjected to centrifugation for about 3000 rpm for 10min and were washed with an equal volume of normal saline three times. The volume of blood was measured as 10% v/v and reconstituted suspension with normal saline (Sakat *et al.,* 2010, Sadique *et al.,* 1986).

Heat-induced hemolytic

1ml of test sample solution and 1ml of 10% RBCs suspension, the only saline was added to the control test tube kept in water bath at 56ºC for 30min. The tubes were washed under running tap water after incubation. The reaction combination was centrifuged at 2500 rpm for 5 min and the supernatants were observed at 560nm absorbance. Membrane stabilization activity was calculated as mentioned above.

Protein inhibitory action

0.06mg trypsin, 1ml of 20mM Tris HCl buffer (pH 7.4) and a 1ml test sample of different concentrations were mixed together. The content was incubated at 37ºC for 5 min and then casein about 1ml of 0.8% (w/v) was added. An additional 20min was given for inhibition and to terminate the reaction 2ml of 70% perchloric acid was added. The buffer was used as blank and the supernatant was read at 210nm. The proteinase inhibitory activity was calculated as a percentage of inhibition (Sakat *et al.,* 2010, Oyedapa *et al.,* 1999).

Anticancer activity in HeLa cell line

Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS) was used to grow the cell line was obtained from National Centre for Cell Science (NCCS), Pune. Temperature about 37°C, 5% CO_2, 95% air, and 100% relative humidity was maintained to maintain the cell cultures. The culture medium was passaged weekly and changed twice a week.

Cell line treatment

To give a final density of $1x10^5$cells/ml, were the viable cells were counted using hemocytometer, the monolayer cells were detached with trypsin-ethylene diamine tetraacetic acid (EDTA) and diluted with medium containing 5% FBS to make single cell suspensions. into 96-well plates, one hundred micro litres per well of cell suspension were seeded at a plating density of 10,000 cells/well and incubated. The plates were to allow for cell attachment at 100% relative humidity 5% CO_2, 95% air and 37°C. The ethyl acetate extracts *Justicia adathoda* was diluted to twice the desired final maximum test concentration and dissolved in neat dimethylsulfoxide (DMSO) with serum-free medium. The cells were treated with serial concentrations of the ethyl acetate extract of *Justicia adathoda* after 24hrs. 100µl of these different sample dilutions were added to the appropriate wells already containing 100µl of the medium. The plates were incubated following the sample addition, for an additional 48 h at 37°C, 5% CO_2, 95% air and 100% relative humidity. Triplicate was maintained for all concentrations and the medium without samples was act as a control (Mosmann, 1983).

MTT assay

Succinate-dehydrogenase, a mitochondrial enzyme in living cells, cleaves the tetrazolium ring, converting the MTT (3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide, a yellow water-soluble tetrazolium salt) to an insoluble purple formazan. Therefore, the number of viable cells is directly proportional to the amount of formazan produced (Monks *et al.,* 1991).

15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added after 48 h of incubation, to each well and incubated at 37°C for 4h. The medium contains MTT was then flicked off and the formed formazan crystals. They were measured for the absorbance at 570 nm using micro plate reader after solubilised in 100µl of DMSO. The percentage cell viability was calculated as follows:

% Cell viability = [A] Test / [A]control x 100

RESULTS

Phytochemical analysis

The *Justicia adathoda* plant extracts obtained by aqueous extract and ethyl acetate contain metabolic compounds which are mentioned in table 1.

Antibacterial assay

The maximum zone of inhibition in *Aeromonas hydrophila* observed in both the extracts of *J. Adathoda* showed followed by *Salmonella typhi* and minimum zone of inhibition found in *Escherichia coli* (table 2).

Spectral metabolic inference and separation of active compounds

In full agreement with the proposed structure the spectral data (IR) of the synthesized compound. The functional groups of IR (KBr) *v* cm-1: 3377.23 (OH), 2926.93 (C-H), 1625.7 (C=O), 1075.6 and 933.378 (S=S) fig. 1. 170 mg/g amount of protein content is present in *Justicia adathoda*. The R_f value of 0.87, 0.86, 0.91, 0.43 and 0.88 of the aqueous extract denotes amino acids, alkaloids,

flavonoids, lipids and triterpenoids. The organisms such as *Salmonella* and *Escherichia coli* were found effective tested against the separated active compounds amino acids, alkaloid, flavonoids, lipids and triterpenoids. The active compound lipid produces maximum zone of inhibition found in *Escherichia coli* and alkaloid produce a minimum zone of inhibition (table 3 and fig. 1).

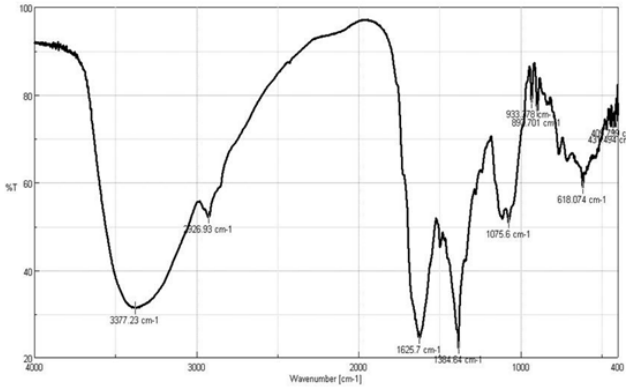

Fig. 1: FTIR spectral data of *J. adathoda*

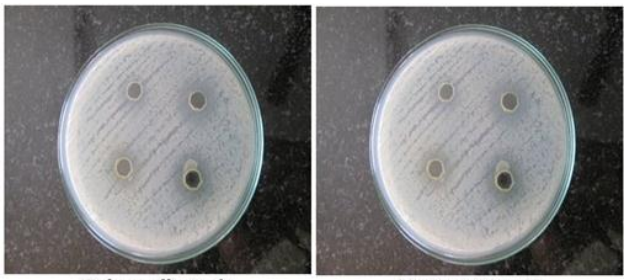

Fig. 2: Anti microbial activity of active compounds

In-vitro anti-inflammatory activity by Hyaluronidase inhibition assay

For an *in-vitro* anti-inflammatory activity, the percentage of inhibition was tabulated in table 4. The cause inflammation was well documented as denaturation of proteins. The mechanism of the anti-inflammation activity was investigated by the ability of extract protein denaturation. In the analysis of heat-induced albumin denaturation found as effective. The maximum inhibition 56.4% at the concentration of 200µg/ml in the standard anti-inflammation drug. The mechanism of anti-inflammatory action of aqueous extract was studied by the stabilization of RBCs membrane. In heat-induced hemolysis procedure the extracts were found effectively inhibiting. An additional mechanism of anti-inflammatory effect for membrane stabilization was provided by these results. At the site of inflammation because of this effect the release of the lysosomal content of neutrophils may occur.

In-vitro anti cancer activity

The parameters like inhibition of growth, cell viability and morphological changes were compared for MTT assay with control (untreated), after treatment with various concentrations (18.75µg, 37.5µg, 75µg, 150µg, 300µg) of ethyl acetate extraction of *Justicia adathoda*. The metabolic of *Justicia adathoda* of decreased the viability the cell viability. Significant decreases in cell viability were observed in the concentrations 75µg/ml, and 150µg/ml, with respect to different concentrations of the extracts (fig. 2). The percentage of viability was less significant than the growth inhibitory activity. The IC_{50} was obtained in the value of 176µg/ml. The percentage of growth inhibition of the treated cells with different doses of *Justicia adathoda* was seen in fig 3a, fig 3b and table 5.

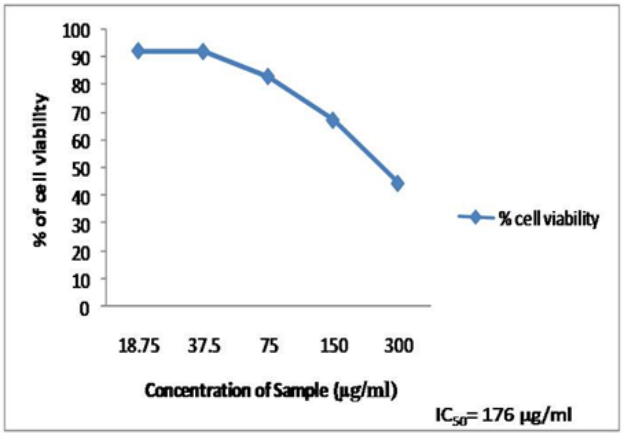

Fig. 3: Inhibitory level of *Justicia adathoda* at different concentration in Hela cell line

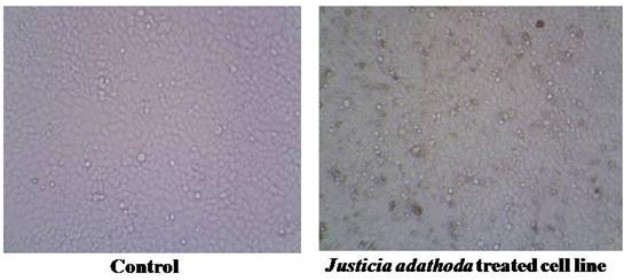

Fig. 4: Hela cell line- cell viability

DISCUSSION

Some of the important bioactive compounds such as essential oil and quinazoline alkaloids are reported from *J. adhatoda* and it acts as an essential device for developing novel drugs for herbal and natural remedy (Ganatra *et al.*, 2011).

In various solvent extract, the metabolic compounds such as saponins, cardinolites, phenols, flavonoids, terpenoids tannins, steroids, and quinones were observed (Rashmi and Mathew, 2012). Possible combinations of solvents were used in chromatography for evaluating the plant leaves extracts on thin layer chromatography (TLC) which showed different R_f values and for separation of phytochemicals.

Table 1: Phytochemical analysis of aqueous and Ethyl acetate extract of *Justicia adathoda*

Phytochemical constituents	Observation	
	Aqueous extract	Ethyl acetate extract
Alkaloids	+	+
Steriods	+	+
Saponin	-	-
Lipids	+	+
Terpenoids	+	+
Flavonoids	+	+

'+' – Present '-' – Absent

Table 2: Antibacterial activity of *Justicia adathoda*

Test organisms	Aqueous extract Zoneofinhibition (in diameter)				Ethyl acetate extract Zoneofinhibition (in diameter)			
	10µl	20µl	30µl	control	10µl	20µl	30µl	control
Salmonella typhi	1mm	2mm	3mm	-	2mm	5mm	8mm	1mm
Bacillus sp.	2mm	3mm	4mm	-	3mm	6mm	11mm	2mm
Escherichia coli	1mm	2mm	3mm	-	2mm	5mm	9mm	1mm
Enterococci sp.	1mm	2mm	3mm	-	2mm	4mm	7mm	1mm
Staphylococcus aureus	1mm	2mm	3mm	-	2mm	4mm	8mm	1mm

Table 3: Antimicrobial activity of active compounds

Active compounds	Aqueous extraction (in diameter)	
	Test organism	
	Salmonella	*E. coli*
Lipid	1mm	1mm
Flavonoids	Nil	Nil
Alkaloids	Nil	1mm
Amino acids	Nil	Nil

Table 4: Anti inflammatory activity of *Justicia adathoda*

S. No	Sample	Hyaluronidase inhibition assay (%)	Albumin denaturation (%)	Membrane stabilization (%)	Proteinase inhibition (%)
1	*J.adathoda*aqueous extract	36	32.2	38.7	39.7
2	Lipids	2.1	2.3	2.1	1.9
3	Flavonoids	3.2	3.5	3.1	3.1
4	Alkaloids	2.5	1.9	2.1	1.1
5	Alkaloids	2.4	1.7	2.3	1.9
Standard drug	Aspirin (200 µg/ml)	56.7	54.6	57.6	58.2

Biologically active compounds were under discussion of *Justicia adhathoda* which can be used for pharmacological studies. The alkaloids vasicine and vasicinone play an important role in that. The plant extract could be recognized by the alkaloid (Sunitha Bansod and Mahendra Rai, 2008).

The classification of antimicrobial activity was done earlier as strong, medium or weak as per the activity of plants (Riaz Ullah *et al.,* 2013). From *A. vasica* leaves essential oil was extracted and tested against bacterial strains. Low zone of inhibition was observed in *B. Cereus* and maximum zone of inhibition was obtained in *E. coli* which indicated the essential oil could be used for the human purpose (Zaika, 1988). *A. fumigates* and *A. niger* was evaluated for the antifungal activity by the oils extracted from *A. vasica*. While compared with the control maximum zone of antifungal activity was observed in *A. niger* and *A. fumigatus*. Maximum activity was obtained by the mixed oils. From the investigation, it was clear that the plant oils can be used to cure mycotic infections. It has various pharmacological aspects.

To evaluate the quantitative and qualitative variations in secondary metabolites different solvents and visualization technique was employed to for each extract. The procedure for the analysis of the metabolic compounds in plant extracts of different solvent is similar. The compounds profile which is related to medicinal use biological compounds gives the data of information (Cristiane, 2009).

Table 5: Anti cancer effect of ethyl acetate *J. adathoda* effect on HeLa cell line for % of viability

Conc. (µg/ml)	% Cell viability
18.75	92.15
37.5	91.88
75	82.97
150	67.32
300	44.75

Using modified hens egg chorioallantoic membrane the anti-inflammatory activity of methanolic extracts of (non-alkaloid fractions, saponins and alkaloids) were evaluated. It showed by showing potent activity at a dose of 50µg/pellet equivalent to that of hydrocortisone. Lesser activity was observed in the methanolic extract (Chakraborthy and Brantner, 2011).

To manage and control the cancer control there should be readily applicable, inexpensive, accessible and acceptable approach to chemoprevention obtainable. The plant phenolic compounds are natural products of secondary metabolism. The range of valuable activities was observed in phenolic compounds, which inhibit the carcinogenesis potently. Miscellaneous bioactivities were observed in flavourings.Individually able to scavenge a wide range of reactive species and higher antioxidant potent flavonoids are known to be anti-cancer agents. Antiulcer, antibacterial, anti-inflammatory, antimutagenic and antileishmanial were observed in tannins and it also attributes for wide attention for cancer treatment which exhibits strong, signal transduction pathways blocking, an enzyme regulating, and apoptotic activities. (Huang *et al.*, 2010).

Justicia beddomei plant extract reported for the cytotoxic potential of the A549 lung adenocarcinoma cell lines and the Inhibitory Concentration is 22.73µg/ml. The cytotoxicity potential attributed by the metabolic compounds such as phenolics, flavonoids, tannins in the extracts (Prabavathy and Nachiyar, 2013).

CONCLUSION

From the investigation, we could conclude the *Justicia adathoda* exposed to have potent phytochemical and antimicrobial activity. The metabolic of *Justicia adathoda* were originate to be more or less active against almost all tested pathogenic strains. Hence, *Justicia adathoda* can source of natural antimicrobials that can serve as a substitute for conventional medicines. The qualitative analysis of the extracts from the leaf sample of *Justicia adathoda* showed the presence of tannins, saponin, flavonoids, steroid, lipids, amino acids and terpenoids. The active compounds such as amino acids, flavonoids, alkaloids, lipids have anti-inflammatory activity and can be considered as a resource for a potential anticancer agent.

ACKNOWLEDGEMENTS

We acknowledge our profound gratitude to the Department of Microbiology, Nehru Arts and Science College, TM Palayam, Coimbatore for providing the facilities for research work. We are highly indebted to Dr. Anirudhan (Principal) Dr. J Rathinamala, Dr. T Balasaravanan and Dr. Meenatchisundaram [Associate Professors] Nehru Arts and Science College, TM Palayam, Coimbatore for their valuable help to complete this work.

REFERENCES

Anonymous (1996). The Indian pharmacopoeia. The government of India New Delhi. Ministry of Health and family welfare.

Bishnu Joshi (2011). Phytochemical extraction and antimicrobial properties of different medicinal plants. *J. Microbiol Antimicrob*. **3**(1): 1-7.

Chakraborthy A and Brantner AH (2011). Study of Alkaloids from *Adathoda vasica Nees* on their ant-inflammatory activity. *Phytother Res.* **15**(6): 532-534.

Cristiane P (2009). Victório1 Flavonoid extraction from Alpinia zerumbet (Pers.) Ecl. Quím., *São Paulo.*, **34**(1): 19-24.

Eskil Hultin, (1966). Thin layer chromatography of plant extracts. *Acta Chemical Scandinavica* **20**(6): 1588-1592.

Huang WY, Cai, YZ and Zhang Y (2010). Natural phenolic compounds from medicinal herbs and dietary plants: Potential use for cancer prevention. *Nutr. Cancer.* **62**(1): 1-20.

Lowry OH, Rosebrough NJ, Farr AL and Randall RJ (1951). Protein measurement with Folin phenol reagent. *J. Biol. Chem*. **193**(1): 265.

Monks A, Scudiero D, Skehan P and Shoemaker, Kenneth P, David Vistica C, Hose John, Langley P, Cronise A, Vaigro W, Marcia G, Goodrich H, Campbell J Mayo and Michael B (1991). Feasibility of high flux anticancer drug screen using a diverse panel of cultured human tumour cell lines. *J. Natl. Cancer Inst.*, **83**(11): 757-766.

Mosmann T (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and

cytotoxicity assays. *J. Immunol. Methods,* **65**(1-2): 55-63.

Oyedapo OO and Famurewa AJ (1995). Antiprotease and membrane stabilizing activities of extracts of *Fagara Zanthoxyloides, Olax subscorpioides* and *Tetrapleura tetraptera*. *Int. J. Pharmacogn*., **33**(1): 65-69.

Prabavathy D and Valli Nachiyar C (2013). Cytotoxic and Phytochemical analysis of *Justicia beddomei* and its endophytic *Aspergillus* sp. *Asian J. Pharm. Clin. Res.*, **6**(5): 159-161.

Rashmi Pa and Linu Mathew (2012). Antimicrobial activity of leave extracts of *Justicia adhathoda* L. in comparison with vasicine. *Asian Pac. J. Trop. Biomed*., **2**(3): 155-156.

Riaz Ullah, Jameel A, Khader, Naser M, AbdEIslam, Farman Ullah, Muhammad Ullah, Kamin Khan, Sultan Ayaz (2013). Antioxidant activities of different crude fractions of *Sonchus eruca*. *Life Sci. J*., **10**(2): 835-837.

Sadique J, Al-Rqobahs WA, Bughaithand EI-Gindi Ar (1989). The bioactivity of certain medicinal plants on the stabilization of RBS membrane system. *Fitoterapia,* **60**(1): 525-532.

Sakat S, Juvekar AR and Gambhire MN (2010). *In vitro* antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. *I. J. Pharm. Pharm. Sci.,* **2**(1): 146-155.

Sandeep Dhankhar, Ramanjeet Kaur, Ruhil S, Balhara M, Seema Dhankhar and Chhillar AK (2011). A review on *Justicia adhatoda*: A potential source of natural medicine. *Afr. J. Plant Sci*. **11**(5): 620-627.

Sunil H, Ganatra, Shweta P, Durge and Patil SU (2011). Preliminary phytochemicals investigation and TLC analysis of *Ficus racemosa* leaves. *I. Chem. Pharm. Res.,* **4**(5): 2380-2384

Sunita Bansod and Mahendra Rai (2008). Antifungal activity of essential oils from indian medicinal plants against human pathogenic *Aspergillus fumigatus* and *A. niger*. *World J. Med. Sci.*, **3**(2): 81-88.

Trease GE and Evans WC (1989). A text book of Pharmacognosy. Bailliere Tindall., 13th edition, London, UK, pp.176-180.

Zaika LL (1988). Spices and herbs: Their antibacterial activity and its determination. *J. Food Saf.,* **23**(9): 97-118.