



Pharmacological and Phytochemical Evaluation of Ethonalic Extract of *Ixora brachiata* (Roxb) Leaves

Vairakkannu Sivasakthi¹, Kuppusamy Selvam^{1*}, Settu Loganathan¹ and Palanisamy Prakash¹

¹Department of Botany, Periyar University, PeriyarPalkalai Nagar, Salem 636 011, Tamil Nadu, India.

Authors' contributions

This work was carried out in collaboration among all authors. Author VS lab related work to bioassay test, preparation of the plant extract Author SL validation; investigation and author KS credit contribution not specified. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i32A31711

Editor(s):

(1) Dr. Syed A. A. Rizvi, Nova Southeastern University, USA.

Reviewers:

(1) Aline Oliveira Conceicao, State University of Santa Cruz, Brazil.

(2) Koffi Kouao Jean, Abrogoua University, Côte d'Ivoire.

(3) Sadia Sultan, University of Technology Mara, Malaysia.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/68621>

Original Research Article

Received 02 April 2021

Accepted 08 June 2021

Published 16 June 2021

ABSTRACT

Objective: Current scientific investigations have evidenced that there is increased attention in developing novel drugs from bio source for the human health concern. This study's main objective was to intervene phytochemical constituents present in the ethanol leaf extract of an aromatic plant, *Ixora brachiata* (Roxb) and to evaluate their ability on antioxidant, anticancer and larvicidal properties, using *invitro* models.

Methods: Antioxidant assay was performed by scavenging behaviour of DPPH, ABTS, H₂ O₂ and Reducing power screenings. The phytochemical composition was also screened using GC-MS technique. Cytotoxicity effect was determined by employing MTT Assay, the larvicidal potential was predicted by following the protocol described by WHO. In phytochemical screening higher concentration of alkaloids, flavonoids, phenolic and diterpenoids were detected in significant content. Geranyl linalool was the profound compound present, and it has been reported, used in various treatments for cancer ailments, similar to Taxol.

Results: The cytotoxicity values of ethanol extract were found to be 11.57 to 55.95% against the cancer cells (A549) and 12.36-63.8% range was observed against HeLa cancers cell line. Larvicidal effects of the extract showed minimum LC₅₀ value 14.69mg/ml against *Aedes aegypti* and 10.43mg/ml was recorded, against *Culex quinquefasciatus*, indicating, a promising capability of larvicidal action against mosquito larvae.

*Corresponding author: E-mail: selsaratbot@gmail.com, selsarat@yahoo.com;

Conclusions: This study envisaged that ethanol extracts of *I.brachiata* are a bright and capable therapeutic agent, to combat in the battle of life threatened diseases, and infectious emerging threats.

Keywords: *Ixora brachiata*; antioxidants; anti-cancer; larvicidal potential; phytochemicals.

1. INTRODUCTION

There is an increasing interest in conventional medicine usage from the plant in recent decades for varied health-related diseases worldwide. World Health Organization (WHO) underlined that traditional medicine from plant source full fills 80% the demand of primary Health care in the World. Use of plant source is a great promise, easily curable and effective to cure a wide spectrum of health-related disorders, specifically in tropical countries including India [1]. Plants are known to produce bioactive metabolites having their role in the treatment of diabetes, inflammation, microbial infections and cancer [2]. *Ixora* species belong to family Rubiaceae are important ethno medicinal plants and are reported to show various pharmacological effects such as antimicrobial, anti-inflammatory, diuretic, CNS depressant activities[3,4].Rubiaceae family with ethno medicinal uses in the treatment of skin diseases[5,6]. Antidematophytic, antioxidant and antimicrobial activities of *I.brachiata* was reported in earlier studies[6,7].

Plants are invested with numerous phytochemical constituents such as terpenoids, vitamins, tannins, flavonoids, quinone, alkaloids and amines abundant for antioxidant properties [8,9]. Several studies demonstrated anti-inflammatory, anti-mutagenic, antibacterial and anti-parasitic properties [10,11].Moreover, a limited survey of phytochemicals with the potential of anti-parasitic was described in published literature. Many investigations have told that Indian medicinal plants diversity and abundant, an extract of antioxidant-rich aromatic plant species may be useful for human's health care to cure various dreadful diseases, including cancer [12].

In public health concern, regulating the transmission of infected diseases is challenging, such as malaria, dengue, corona, etc. Current applications of bio-based products have shown to be considered as alternate for harmful chemical toxicants, eco-friendly, cost-effective and candidate weapon for combating in the battle against mosquitoes. Limited works were focused, targeting on the ethno botanical, pharmacological view in the recent past, therefore, in the present

study, an intervention on phytochemical constituents of leaf ethanol extracts of *Ixorabrachiata* (Roxb) and their potential of antioxidant, anticancer and larvicidal capabilities were evaluated.

2. MATERIALS AND METHODS

Ixora brachiata Roxb (Rubiaceae) leaves were collected from Boduvarayan malai of Kalvarayan Hills Eastern Ghats, Dharmapuri district, Tamil Nadu, India. The collected plant was authenticated by the Botanical Survey of India (BSI), Coimbatore, Tamil Nadu, India(voucher number is BSI/SRC/5/23/2020/Tech/523).

2.1 Extraction Preparation

Collected plant leaves were washed well, using deionised water and picked, fresh and infection-free, bright leaves and allowed to dry in the shade. Dried leaves were grinded, using mixy to get a usable analytical powder form. 25 g of plant materials were extracted with 100ml of ethanol by employing maceration measure and filtered, using what man No.1 filter paper to obtain filtrate of the extraction.

2.2 Qualitative Interpretation

The qualitative analysis on the extract of *Ixora brachiata* for detecting the occurrence of secondary metabolites such as alkaloids, saponin, tannins, flavonoids, glycosides, phenolic derivatives, steroids and carbohydrates by following specific tests.

2.2.1 Alkaloids

To identify the occurrence of alkaloids the leaf extract with a quantity of 0.2g mixed with HCL of 1% concentration and vibrated well for 2 min. The filtrate was further added with dragendroff's reagent, to form clean precipitation, indicated obviously, the occurrence of alkaloids.

2.2.2 Saponin

With a similar quantity of extract (0.2 g) wastested in a clean tube with 5ml of distilled water treated well, emergence of foam or fizz on heating was asymptomatic of presence of saponin.

2.2.3 Tannins

An amount of 0.2g leaf extract, blended with purified water wiped well and filtered the solution. The appearance of blue-black, green residual was a confirmation test of the presence of tannins.

2.2.4 Steroids

0.2 g of sample was added with 2ml of chloroform, in a test tube. Subsequently, concentrated H₂SO₄ was mixed in a manner of drop by drops to appear a bottom layer. Formation of reddish-brown colour at the middle phases, confirmed the occurrence of carotenoid, a type of steroids.

2.2.5 Flavonoids

3ml quantity of sample was added with a bit of magnesium salt, followed by one or two drops of HCL result a blood red colour specified the presence of flavonoids.

2.2.6 Phenolic Content

By liquefying Ferric chloride with 0.2g sample solution, offers a well delightful green colour precipitation, confirming the phenolic content presence.

2.2.7 Glycosides

In a test tube, with little quantity of sample, added with HCL acid, followed by NaOH for neutralizing. To this complex solution, one or two drops of Fehling's solution were put on to form reddish precipitations that justify the presence of glycoside in the extract.

2.3 Quantitative Estimation

2.3.1 Total alkaloid content (TAC)

Homogenized leaf extract 5mg was taken, with this 20ml of ethanol, and few drops of ammonia were poured. After 24hrs a little quantity of ethanol ammonia was mixed test sample was kept to evaporate by using flask evaporator. Obtained residue was added with in hydrochloric acid and was kept for spectacular.

2.3.2 Total phenolic content (TPC)

Determination for ethanolic leaf extract of *I. brachiata* was performed followed by the method

described by [13] 0.5ml of the test sample solution was taken with 2.25 ml of methanol. Further a little quantity of (0.22ml) folin reagent was mixed and vibrated well for 1min and leaf as such for a period of 8 min. Subsequently, the sodium carbonate solution of 2.0ml was blended and let it incubate for 120min with 25°C temperature. The absorbent value was measured at 756nm, against a blank ethanol solution as control.

2.3.3 Total flavonoids content (TFC)

The test solution leaf extract of *I. brachiata* was examined to detect the flavonoid content by following the protocol demonstrated previously by [14], estimation of flavonoid was carried out on the grounds of quercetin standard curve, made ready in 80% methanol and funding were expressed as mg quercetin/g dry weight.

2.3.4 Measurement of steroids

Steroid estimation was performed to determine the total steroid content in the examine leaf extract was employed by the protocol, revealed by [15], in cholesterol methodology.

2.4 Gas Chromatography-Mass Spectrometry GC-MS

GC-MS is a reliable technique, used to find out the chemical composition of the test samples in the present study, chemical nature of the leaf extract of *I. brachiata* was examined, by employing Hewlett Packard gas chromatography, annexed with HP-5 capillary column. Temperature of the injector was scheduled at 280°C with an increase of 1°C, per minute from 50°C to the optimum 280°C. Helium gas was applied as carrier with a rate of 1ml/min. The chemical constituents were determined by differentiating retention indices and data on mass spectra, made under similar tested conditions. Spotting of compounds was effected, according to the retention indices and mass spectra described in the Wiley and NIST data base and literature data as well, with assured samples [16].

2.5 Antioxidant Assay

2.5.1 2,2-Diphenyl-1-picrylhydrazyl assay (DPPH)

To evaluate the antioxidant activity of the leaf extract of *I. brachiata*, the DPPH free radical

scavenging assay was attempted. This confers the ability of free radical scavenging of the investigated samples extract. In the present study, antioxidant efficacy of *I. brachiata* was predicated by employing the methods used by previous investigations, performed by [7] and with slight alteration made suitable for our laboratory condition. The examined solutions contain diluted methanol extract with a concentration range of 0.1 to 1.0 mg/ml and 2.0ml of 0.16mM DPPH solution, stirred well, forcefully vibrated and incubated for half an hour and ability was measured using UV- vis spectrophotometer at 517nm, against blank solution used as control.

$$\text{DPPH scavenging effect (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

A_0 , absorbance of control; A_1 , absorbance of sample

2.5.2 Reducing power determination

The protocol was followed according to the [7] methods. Reducing power was measured based on the electron donation in the reduction of Fe^{3+} to Fe^{2+} . The addition of electrons from Fe^{3+} ions represented by formation of coloured complex with potassium ferric cyanide and ferric chloride.

2.5.3 Hydrogen peroxide scavenging measure

The determination of Hydrogen peroxide scavenging ability was performed by adapting the method described by investigator, [17] used as scavenging activity % = $[1 - (A_1 - A_2) / A_0] \times 100$

A_0 , absorbance of control, A_1 – absorbance of sample and A_2 – absorbance without sodium salicylate.

2.5.4 ABTS radical scavenging potential

This property of examined with leaf extract by followed the method demonstrated by [18] described in earlier published literature. As: $I = A_0 - A_1 / A_0 \times 100$,

A_0 is absorbance of control reaction, A_1 is absorbance of sample.

2.5.5 Superoxide radical scavenging assay

The potential of superoxide scavenging efficiency of examined solution was predicated by adhering

the method described by [18]. Super oxide radical scavenging activity (%) = $[(A_0 - A_1) / A_0 \times 100]$ Where A_0 is the absorbance of the control and A_1 is the absorbance of the standard sample.

2.6 Cytotoxicity of Human cell lines

To evaluate in-vitro cytotoxicity capability of the examined leaf extracts carried out by a well-known and reliable technique, MTT analysis, that was performed with two types of human cell lines such as A549 (lung cancer cell line) and HeLa Cell lines (Cancer cell lines). Both were obtained from National centre for cell science, Pune, India. Two were cultured in the medium of high Glucose; Dulbecco and modified Eagles medium culture medium augmented with FBS (10% V/V) with antibiotic. The cell lines were kept safely in the culture medium at the 37 °C and humidified CO_2 atmosphere.

2.6.1 MTT assay

The cytotoxic effects of the plant extract to be examined were carried out by employing an established MTT assay, as demonstrated in the literature cited in [19]. Monolayer cells were detached, using EDTA to obtain single-cell suspensions. Counting of viable cells was attempted, using a haemocytometer. Cell suspension was seeded into 96 well plates with a density of 10,000 cells/well and incubated with relative humidity. 5% CO_2 and 95% air, cells were treated with plant extract of examined species at varied dilution range (12.5 to 200 $\mu\text{g/ml}$) whereas DMSO was maintained as control, non-toxicant to the cells. A quantity of 20 μl of MTT was mixed, cultured and exposed for 24h incubation. At extended further the formazan crystals. The absorbance value was set up and measured at 590nm while control was measured at 620nm, in micro plate reader. A triplicate trails was performed and tested. The values expressed on mean and standard deviation. The rate of inhibition percentage can be computed, by adhering the formula given below.

Percentage of Inhibition = $(\text{Mean O value of Control cells} - \text{mean OD value of treated cells} / \text{Mean OD value of control cells})$

A linear graphical representation plotted for the comparison of viable fractions of tested cell lines, ethanol-treated leaf extract, at different concentration focusing on the survival curve. The curves were ideal linear, and IC_{50} value was derived.

2.7 Larvicidal Activity

2.7.1 Collection of mosquito larvae

Mosquitoes such as *Aedes aegypti* and *Culex quinquefasciatus* were gifted from ICMR-vector control Research centre, Madurai, to promote the investigation. The eggs and larvae were carefully maintained on soft trays with deionized water and exposed to room temperature ($25 \pm 2^\circ\text{C}$).

2.7.2 Dose response bioassay

With a slight alteration made in WHO proposed protocol and was adapted for conducting larvicidal assay [20]. The pattern of dose of leaf extract of *I. brachiata* against both mosquito species were determined as 0,5,10,20 and 25 mg/l put on the cup, where 20 numbers of selected 4thWister larva were introduced. Observation was made after 24hrs of exposure to predict toxicity effect of the extract, to the respective mosquito larvae an independent control was maintained without add on ethanol extract. After 24hrs of exposure, rate of mortality present was detected, by taking average values of triplicate trials. This method was used to derive LC_{50} and LC_{90} value, applying probity analysis [21].

2.8 Statistical Analysis

An analysis was carried out in triplicate ($n=3$). The data were presented as mean \pm SD which were subjected to One -way and Two - way analysis of variance, followed by Dunnett's multiple comparison tests ($P < .001$) using PRISM software version 5.2 (Graph Pad Software Inc, USA). The larval death was subjected to log Probit analysis after 24,48 h for calculating LC_{50} , LC_{90} values. The Chi-square values were calculated using SPSS ver. 20.0 (SPSS Inc., Chicago, IL, USA).

3. RESULTS AND DISCUSSION

3.1 Phytochemical Analysis

The outcome of the phytochemical investigation manifested that, a total of eleven bioactive molecules were identified and tabulated (Table-1) the pattern of observed peaks exhibited with highest quantity and profound alkaloids, total phenolic compounds, Flavonoids and steroids in ethanol leaf extract of examined species, than crude extract (Table-2) was recorded. A

comprehensive or broad spectrum of benefits from varied species of Genus *Ixora* extending its uses in the conventional medicinal system has received more attraction in searching for the chemical composition of *Ixorabrachiata*. Findings of the investigation revealed that a spectrum of classes of alkaloids, phenolic compounds, flavonoids tannin, saponin, and few steroid contents. It has been presumed that higher concentration of phenolic compounds, saponins and flavonoids were the responsible constituent's for various biological properties and antioxidants. It is noteworthy that several studies [22] reported that polyphenols from herbal source, known to be arrested the lipid peroxidation by extinguishing lipid peroxy radicals. It has been demonstrated [23] that the presence of glycosides in the leaf extracts of *I. brachiata* exerted and served as the predominant contributors of antioxidant capability, and was attributable to the fact of hydroxyl group.

3.2 Antioxidant assay of *Ixora brachiata*

Deciphered that antioxidant properties of the plant extract was directly associated with free radical scavenging, accomplishing in attenuating lipid peroxidation through terminating the chain reaction. In another study saponin and phenolic content were reportedly, accountable for effective antioxidant in *Acacia* species was detected [24]. In reducing antioxidant measure, by determining the transfer of electron in the reaction and from reducing the ability of ferric ions to ferrous in the examined leaf extract was a good indicator and associated with antioxidant capabilities. To Ability of radical scavenging, using DPPH measure, reveals that potential of inhibition concentration for ethanol leaf extract was found to be the value of 5% $3\mu\text{g/ml}$ at the highest concentration of 250ml^{-1} among tested. Interestingly, finding value of our investigation was known to be very close to the control value ($65.20\mu\text{g/ml}$), a favouring evident towards its scavenging efficiency (Fig. 1).

In this investigation as depicted in the (Fig. 2), ferrous (Fe^{+3}) was transformed to Ferric (Fe^{+2}) in the treatment of leaf extract and the reducing potential value was observed as $53.83\mu\text{g/ml}$ and it was noticed that extract values was less than the standard value ($68.66\mu\text{g/ml}$). (Fig.3) shows that plant ethanol extract is moderately, effective scavenger of H_2O_2 by obtaining the Value of LC_{50} ($54.3\mu\text{g/ml}$), of the order of control value, ($60.55\mu\text{g/ml}$), vitamin C was used as standard. LC_{50} value of the extract was less than that of standard. ABTS radical scavenging ability of the

examined *I. brachiata* using varied concentration, resulting, and the value was found to be 52.60µg/ml at 250ml-1 concentration, that also about to the control value (61.45µg/ml) and was illustrated in (Fig.4). Superoxide radical's highest scavenging activity of ethanol leaf extract at the concentration of 250ml. The extract is close to that of standard value 72.20µg/ml; (Fig.5). In our study, antioxidants ability was shown to the high in measured four methods and may be due to the

occurrence of specific antioxidant potential phytoconstituents. The concentration reported in the present study was very close to the values of the standard employed[25] said that metabolites responsible for scavenging, and oxygen and free radicals associated with the quantity of the bioactive molecules. Superoxide anion radical ($O_2^{\cdot-}$) is formed by four-electron reduction of molecular oxygen into the water. In living organisms, $O_2^{\cdot-}$ is removed by the enzymes called superoxide dismutase (SOD) [20].

Table 1. Qualitative analysis of ethanol leaf extract

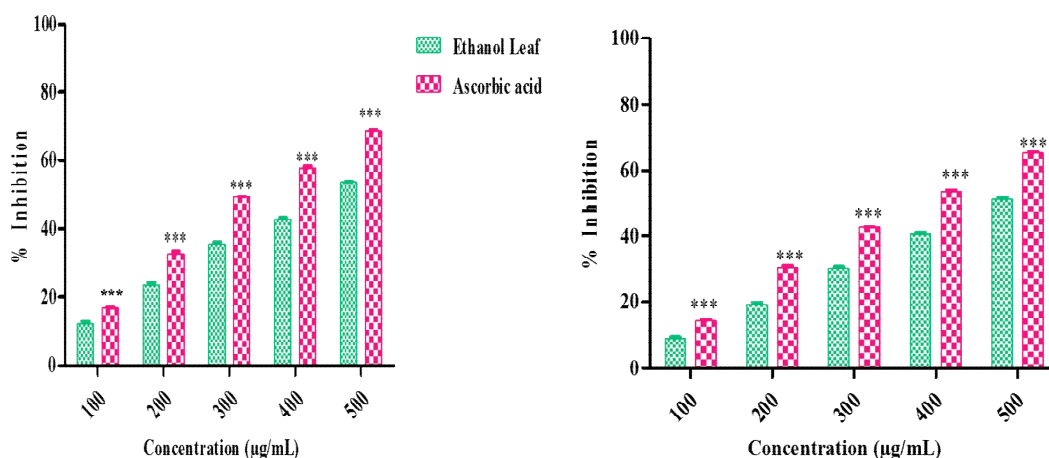
Phytochemicals	Ethanol
Alkaloids	+++
Phenols	+++
Flavonoids	+++
Tannins	+
Saponins	+
Terpenoids	+++
Steroids	+++
Carbohydrates	+
Glycosides	-
Amino acids	+
Proteins	+

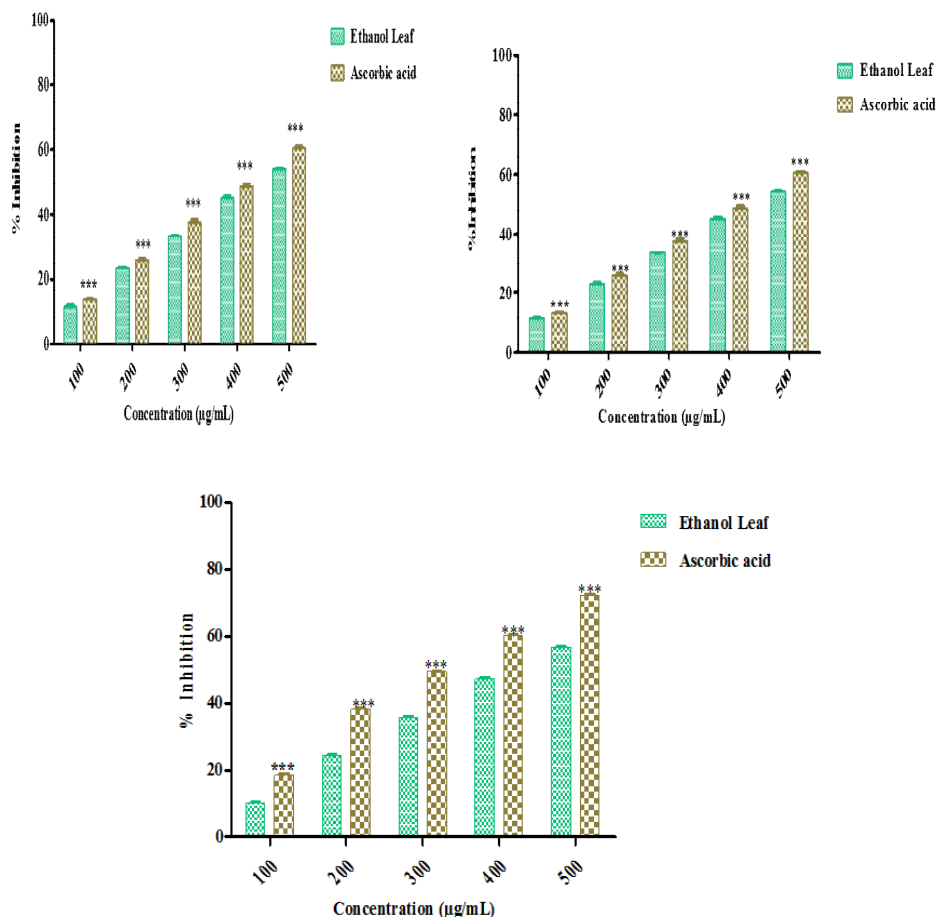
+ → present in small concentration; ++ → present in moderately high concentration; +++ → present in very high concentration; -- → absent.

Table 2. Quantitative analysis in ethanol leaf extract of *Ixorabrachiata*

Secondary metabolites	Ethanol extract
Alkaloids (mg/g)	4.50 ±0.80
Total phenols (mg/g)	4.20 ±0.50
Flavonoids (mg/g)	3.30 ±0.30
Steroids (mg/g)	2.50 ±0.40

Values are expressed as mean±SD (n=3).





Figs. 1-5. In-vitro antioxidant activity of using ethanol leaf extract of *Ixorabrachiatra* 1) DPPH radical scavenging activity, 2) Reducing power assay 3) Hydrogen peroxide scavenging activity, 4) ABTS radical scavenging activity, 5) Superoxide radical scavenging activity

3.3 GC-MS Detector

Ethanol leaf extract of *I. brachiata* was examined using GC-MS technique to find the composition of the chemical compounds in the extract and obtained spectrum as depicted in (Table-3& Fig. 6). Identification was performed based on the peak area and values, retention time taken in the chamber. Results deciphered that, two predominant molecules such as 1, 6, 10, 14-hexadecatetraen-3-01, 3, 7, 11, 15-tetramethyl-(E,E) and Beta-d-glucopyranose, 4,0-beta-d-galactopyranosyl. In our present study, interestingly, we detected the presence of geranyl linalool (1, 6, 10, 14-Hexa decatetraen-3-01 and 3, 7, 11, 15-tetramethyl-EE) a diterpenoid, in the leaf extract of *I. brachiata*, using GC-MS analysis. This chemical compound is a scented, aromatic compound, often used in the treatment of cancer drugs. They have described that ROS covering superoxide radical

and Hydrogen peroxide, have shown to close affinity with nucleic acids, protein and fat, causing a loss of integrity of the architecture of the cells and its functionality. Similar to this study, we observed, the ethanolic leaf extract of *I. brachiata* treated viable cells showed more alteration in the structural integrity, maybe due to the initiation of ROS and presence of diterpenoid compounds, clinically used for broad spectrum of biological properties including cytotoxicity, anticancer and antioxidants and analgesic were reported [26].

3.4 Cytotoxicity Assessment

In the present investigation, we found, the viability of cancer cells screening, using MTT was carried out. Resulting of MTT measure, ethanol extract of examined plant species showed more

Table 3. Identification of GC-MS compound in ethanol leaf extract of *Ixorabrachiat*

S.No	Compound Name	% of Peak Area	Molecular formula	Uses
1.	1,3-Propanediol,2 hydroxymethyl- 2-nitro triacetate	10.83	C ₁₀ H ₁₅ O ₈	Biological application
2.	Propanoic acid, 2-(Aminoxy)-	8.86	C ₃ H ₇ O ₃ N	Infection diseases
3.	Beta.-D-glucopyranose, 4-o-.beta.-d-galactopyranosyl	12.66	C ₁₂ H ₂₂ O ₁₁	Nutrient intake activity
4.	Isoxazolidine, 5-ethyl-2,4-dimethyl-, trans	8.79	C ₃ H ₇ O ₃ N	Anti fungal properties
5.	Neopentane-,1-diol diacetate	9.39	C ₉ H ₁₆ O ₄	Antibacterial
6.	2,6-Pyrazinediamine	7.99	C ₄ H ₆ N ₄	Hair dye (causes skin irritation)
7.	9,12-octadecadienoic acid (Z,Z)-, trimethylsilyester	4.48	C ₂₁ H ₄₀ O ₂ Si	Analgesic, purgative
8.	4-Heptanol, 2-methyl	1.95	C ₈ H ₁₈ O	Biological application
9.	2-[1,2-Dihydroxyethyl]-9 [.beta.ribofuranosyl]hypoxanthine	1.94	C ₁₂ H ₁₆ O ₇ N ₄	Antibiotic
10.	1,6,10,14-hexadecatetraen-3-ol, 3,7,11,15-tetramethyl-, (E,E)-	13.78	C ₂₀ H ₃₄ O	Biological application
11.	Sulfurous acid, decyl 2-propyl ester	2.32	C ₁₃ H ₂₆ O ₃ S	Malaria, cold, cough, cancer, diabetes, hypertension
12.	Eicosane, 9-octyla	2.62	C ₁₃ H ₂₈ O ₃ S	Biological application
13.	Nonadecane, 1-Bromo	5.21	C ₁₉ H ₃₉ Br	Biological application
14.	1Monolinoleoylglycerol trimethylsilyl ether	3.13	C ₂₇ H ₅₄ O ₄ Si ₂	Antimicrobial, Antioxidant, Antiinflammatory, Diuritic
15.	Di-N-decylsulfone	2.29	C ₂₀ H ₄₂ O ₂ S	Antihelminntic, Antioxidant, Anti fungal, antimicrobial
16.	Guanidine, methyl	1.93	C ₅ H ₁₁ O ₃ N	Treatment of Myasthenia
17.	Octadecane, 9-ethyl-9-heptyl	1.76	C ₂₀ H ₄₂ O ₂ S	Antimicrobi al

Table 4. Larvicidal activity on ethanol leaf extracts of *Ixorabrachiat*

Time exposure	Concentration (mg/l)	Mortality Percent	LC ₅₀ (mg/l) (LCL-UCL)	LC ₉₀ (mg/l) (LCL-UCL)	χ ²
24 hours	25	71.66±2.87	14.69	36.53	0.85
	20	63.33±0.50	(11.97-17.32)	(30.43-49.07)	
	15	51.66±2.87			
	10	38.33±2.87			
	5	28.33±1.66			
24 hours	0	00.00±0.00			1.24
	25	81.66±2.87	10.43	30.72	
	20	73.33±0.50	(6.97-12.85)	(26.06-39.74)	
	15	61.66±2.87			
	10	48.33±2.87			
	5	36.66±1.66			
	0	00.00±0.00			

Larvicidal activity of ethanol leaf extracts of *Ixorabrachiat* against *Aedes aegypti* and *Culex quinquefasciatus* LC₅₀-Lethal concentration kills 50% of the exposed larvae, LC₉₀-Lethal concentration kills 90% of the exposed larvae. LCL - Lower confidence limit, UCL - Upper confidence limit

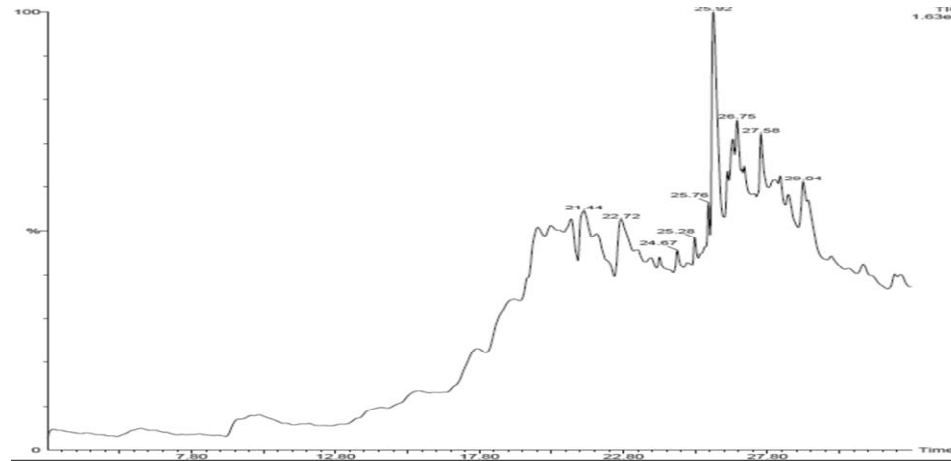
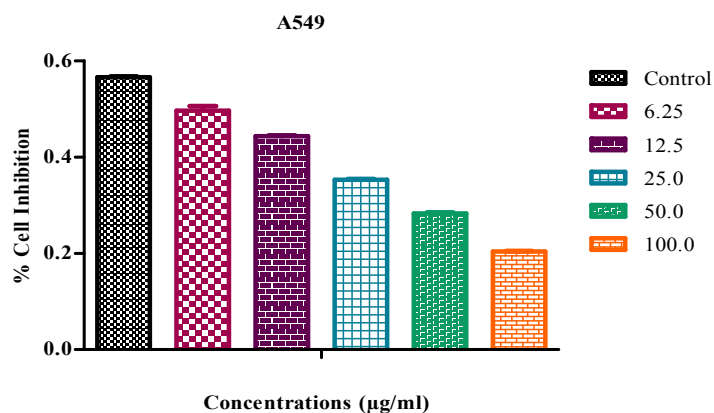


Fig. 6. GC-MS analysis of Ethanolic Leaves Extract of *Ixorabrachiat* Roxb



S

Figs 7&8. In vitro cytotoxicity effect of ethanol leaf extract *Ixorabrachiatra* at various concentration of 6.25µg/ml to 100µg/ml against A549 and Hela cancer cell lines the value are presented as mean ±SD(n=3)

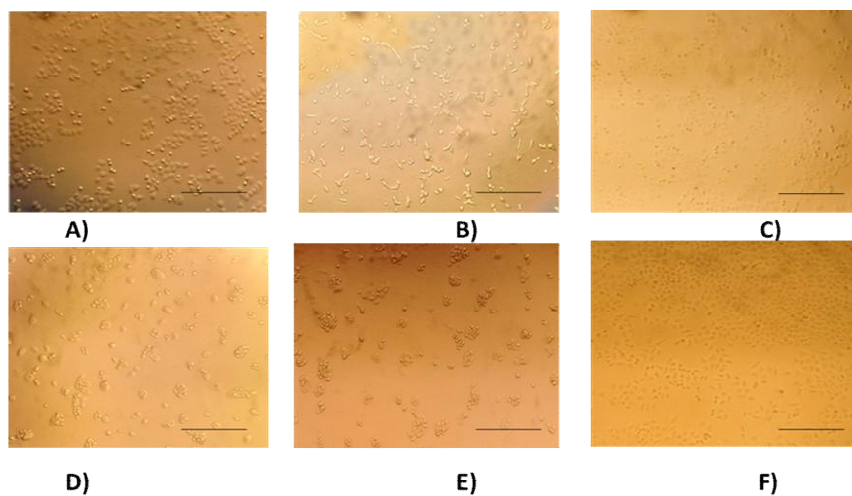


Fig. 9. Cytotoxicity of *Ixorabrachiatra* robs ethanol leaf extracts and control on A549 cancer cell line at different concentrations at a) control b)6.25µg/ml c) 12.5 µg/m d) 25 µg/ml e) 50 µg/ml f) 100 µg/ml

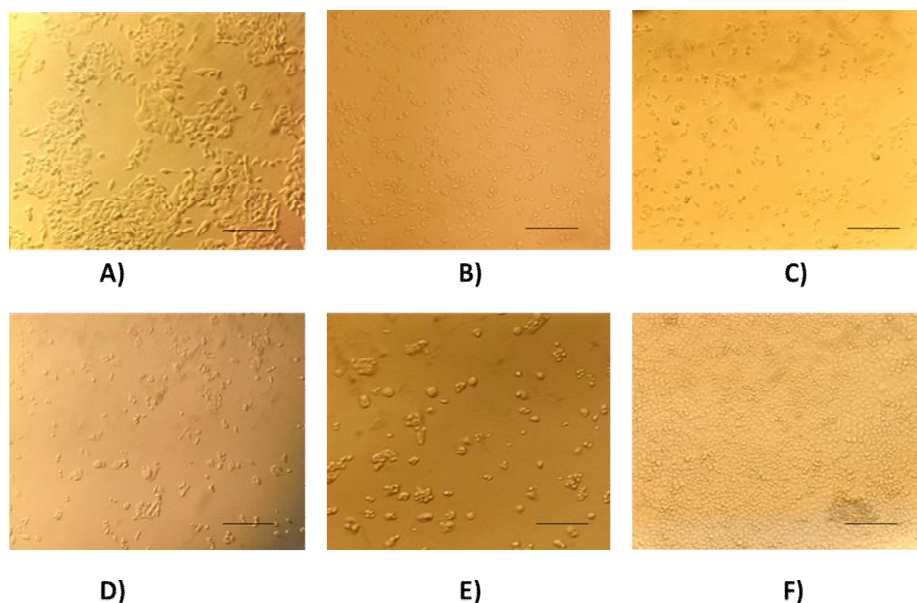


Fig. 10. Cytotoxicity of *Ixorabrachiata robs* ethanol leaf extracts and control on HeLa cancer cell lines at different concentrations at a) control b) 6.25 µg/ml c) 12.5 µg/ml d) 25 µg/ml e) 50 µg/ml f) 100 µg/ml

effective in the cytotoxicity against cancer cell line A549 and HeLa cells. The observations made based on the alteration in the morphological integrity of the cells noticed in viable cells, by increasing concentration of the extract, on dose dependant manner. The Cytotoxicity effects of ethanol leaf extracts was recorded and depicted in (Figs.7, 8) the morphological alteration in the integrity of cell architecture was shown to the in (Figs.9, 10). The optimum effectiveness was detected in the toxicity to cancer cells and Helacells were found to be in the range of 11.57 – 55.97 µg/ml and 12.36 – 68.95 µg/ml to the respective cell lines. Thus, the presence of terpenoids in the *I. brachiata* contributed, significant anticancer actions in the cancer cell lines such as A549 and HeLa cell, and it was proved in the MTT assay. Moreover, [22] reported that the presence of alkaloids in the plant extract has made a remarkable constituent in anticancer effects. Clinically a large number of spectrums of alkaloids are in use for treating many types of cancer like Taxol.

3.5 Larvicidal Bioassay

Determination of larvicidal potential of leaf extract of *I. brachiata* with varied concentrations such as 0, 5, 10, 20, 25 mg/ml was evaluated and deionized water used a standard. The results

exhibited that LC₅₀ and LC₉₀ values due to the extract effects were found to be 14.69 and 36.53 mg/ml against examined larvae of *A. aegypti*. Similarly, larval mortality was observed with LC₅₀ and LC₉₀ values such as 10.43 and 30.72 mg/ml attributing to the extract against *C. quinquefasciatus*. Detected values are an indicator of profound potential of larvicidal capabilities over dreadful malaria disease causative agents. The results showed that the ethanol crude extracts were effective against the mosquito species *A. aegypti* and *C. quinquefasciatus*. Previously, the most highest larval mortality was found in ethanolic extracts presence in the extracts can be the cause of their Larvicidal activity. Preparations using aqueous leaf extract of *Carmona retusa* showed high toxicity of three major mosquito species, such as *A. aegypti*, *A. stephensi* and *C. quinquefasciatus* [27-33].

Observation of the present experiment showed and concluded that higher concentration of alkaloids, flavonoids; diterpenoids in the extract were the predominant contributors of anticancer effects. Besides, the potential of leaf extract of *I. brachiata*, exhibited a pronounced larvicidal effect against 4th instar larvae of *A. aegyptis* and *C. quinquefasciatus* was evaluated. Obtained results of LC₅₀ values were 14.69 and 36.53 mg/ml, to the individual species were indications,

promising ability and evident for the occurrence of the constituents in the leaf extract.

4. CONCLUSION

The conclusion of the study was a maximum number of bioactive compounds present in the leaf extracts. GC-MS analysis revealed the presence of 17 bioactive compounds. The leaf extract exhibited strong antioxidant activities. Further, the extracts showed significant anticancer activity against the HeLa cell line were using MTT assay. Moreover, the extract was comparatively more toxic to *Cx. quinquefasciatus* followed by *Ae. aegypti*. Further the ethanol extract could be used for further pharmaceutical applications. The various nanoparticles (Ag, Au, ZnO, CuO, TiO₂, etc.) will be synthesis from *I. brachiata* leaf extracts in future studies.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENT

The authors Acknowledge to Periyar University, Salem to providing University Research Fellowship (Ref. No. PU/AD-3/URF/2016). The authors also thank to Department of Botany, School of Life Sciences, Periyar University, Salem, Tamil Nadu-636 011, India, for providing infrastructural facility.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

- Kalpna Devi Rajesh, SubramaniVanitha, NakulanVatsala Rajesh, AnnamalaiPannerselvam. Qualitative and Quantitative Physicochemical Analysis in Four Pteridophytes. *Int J Pharm Sci Reve Res.* 2014;27(2):405-412.
- Carocho MI, C.F.R. Ferreira. The role phenolic compounds in the fight against cancer- a review, *Anti-cancer Agents. MedChem.* 2013;(13):1236-1258.
- Khart AR, Nambiar VV, Tarkasband YS, Pujari RR. A review on phytochemical and pharmacological activity of genus *Ixora*. *IJRPC.* 2013;3(3):628-635.
- Usha M, Appavoo MR, ImmanuelG. *Ixora*, an overview: *EJPMR* 2016;3(2):146-154.
- Pandyana S, Ashalatha M, Rao R, Phamocognosy of an ethno medicine: *Ixorabrachiataroxb.* *IRJP.* 2011;2(10):62-63.
- Poojari M, Padyana S, Rao BR, Evaluation of antioxidant and antimicrobial properties of *IxorabrachiataRoxb.* *E J Chem.* 2009 ;6(3):625-628.
- SadeghiNejad B, Deokule SS. Antidematophytic activities of *Ixorabrachiata* Roxb. *J Bio chem Res.* 2009;3(10):344-348.
- Zheng W, Wang Sy. Antioxidant activity and Phenolic compounds in selected herbs. *J Agri Food Chem.* 2001;49(11) :5165-5170.
- Cai YZ, Sun M, Corke H. Antioxidant activity of betalains from plants of Amaranthaceae. *J Agri Food Chem.* 2003;51(8):2288-2294.
- Sala A, Recio MD, Giner RM, Manez S, Tournier H, Schinella G, Rios JL, Anti-inflammatory and antioxidant properties of *Helichrysumitalicum.* *J Pharm Pharmacol.* 2002;54(3):365-371.
- Rice-Evans CA, Miller JN, Bolwell PG, Bramley PM, Pridham JB. The relative activities of plant-derived polyphenolic flavonoids. *Free radical Res.* 1995;22: 375-383.
- Sharma BD, Yyas MS, Ethnobotanical studies on the fern and fern allies of Rajasthan. *Bull of Bot Survey of India.* 1985;27:90-91.
- Lim YY, Tee JJ. Antioxidant properties of several tropical fruits: A comparative study. *Food Chem.* 2017;103:1003-1008.
- Chang C, Yang M, Wen HJ. Estimation of total flavonoid content in profiles by two complementary colorimetric methods. *J Food Drug Anal.* 2002;10:178-182.
- Zak B, Cholesterol methodologies: A review. *ClinChem*1977; 23:1201.
- Nabavi SM, Ebrahimzadeh MA, Abavi SF, Jafari M. Free radical scavenging activity and antioxidant capacity of *Eryngiumcancasicum* Trantv and *froripia sub piñata*. *Pharmacol.* 2008; 3:19-25.
- Vijaybasker P, Shiyamala V. Antioxidant properties of seaweed polyphenol from *Turbinaria ornate* (Turner). *J Agardh Asian pac J Trop Biomed.* 2012;(2):S90-S98.

18. Kono Y. Generation of Superoxide radical during antioxidant of hydroxylamine and an assay for superoxide dismutase. Arch Biochem Biophys. 1978;186:189-95.
19. About WS, A method of Computing the Effectiveness of an Insecticide. J Econ Entomol. 1925; 18(2): 265-267. DOI:10.1093/18.2.265a.
20. Kim DH, Kim JH, Back SH, Seo JH, Kho YH, Oh TK, Lee CH. Enhancement of tyrosinase inhibition of the extract of *veratrumpatulum* using cellulase. *BiotechnolBioeng*2004;87:849-854.
21. Wang H, Nair MG, Strasburg GM, Chang YC, Booren AM, Gray JL, Dewitt DL. Antioxidant and anti-inflammatory activities of anthcyanins and their aglycon, cyanidin, from tart cherries. J Nat Prod .1999;62:294-296.
22. Ganie SA, Jan A, Muzaffar S, Zargar BA, Hamid R, Zargar MA. Radical scavenging and antibacterial activity of *Arnebia benthamii* methanol extract. Asia. Pac. J. Trop. Med. 2012;5(10):766–72.
23. Yemilov V, Rubio J, Becchi M, Friesen MD, Pignatelli B, Oshima H. Formation of nitroguanine by the reaction of guanine with peroxonitrite *In vitro* Carcinogenesis. 1995;16:2045-2050.
24. Ramzi A, Mothana Ulrike Lindequist, Ranate, Guenert, Patrick J, Bednarski. Studies of the anticancer, antimicrobial and antioxidant potential of selected Yemeni Medicinal plants from the island Soqotra.BMC complementary and Alternative medicine.2009;9:7.
25. Kenichi Iwasaki. Anticancer effect of Linalool via cancer specific hydroxyl radical generation in human colon cancer. World J Gastroenterol. 2016;22(44):9765-9774.
26. Oberlies NH, Kroll AJ. Camptothecin and faxol Historic achievements in Natural products research. J Nat Prod. 2004;67:129-135.

© 2021 Sivasakthi et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:
<http://www.sdiarticle4.com/review-history/68621>