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EVALUATION OF ANTICANCER AND ANTIOXIDANT PROPERTIES OF SELECTED *JUSTICIA TRANQUEBARIENSIS* PLANTUSED IN INDIAN TRADITIONAL MEDICATION

M. P. Jeyasekhar*[1], Jaslin Edward J[2], N. R. Livingston Raja[3]

[1*]Scott Christian College, Nagercoil, Kanyakumari, Tamil Nadu, India.

[2]Cape Bio Lab and Research Centre, No.27, CSI Complex, Marthandam, K.K Dist., Tamil Nadu, India.

[3]Department of Pharmacology, S A Raja Pharmacy College Vadakkanguam, Tamilnadu, India.

ABSTRACT

The current study was carried out to evaluate the anticancer and antioxidant, properties of the selected medicinal plant which is commonly used in Indian traditional therapy. The selected plant such as *Justicia tranquebariensis* was extracted in 70% ethanol solvent and evaluated for their *in vitro* anticancer and antioxidant activities. Antioxidant activities of the extract was determined by using DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay, reducing power assays with ascorbic acid such as Metal chelating assay, Superoxide scavenging assay and Hydroxyl radical scavenging assay. The results of the antioxidant study revealed that the selected *Justicia tranquebariensis* plant was found to be effective 1, 1-diphenyl-2-picrylhydrazyl (DPPH), hydroxyl (OH), and superoxide radical (SOR) scavenging agents. The results of anticancer study indicate that the extract has potent cytotoxic activity toward the selected In vitro Brine Shrimp Lethality Bioassay showed increase %Mortality of EJTB 10µg/ml (17.33±0.34) EJTB 100µg/ml (76.23±0.43) and 5-FU 10µg/ml (34.33±0.42) 5-FU 100µg/ml (95.33±0.53) various concentrations of the plant extract as well as the positive control (5-FU). The results of the present findings revealed that the selected plant has a possible resource for the finding of unique anticancer, and antioxidant agents.

KEYWORDS

Anticancer, Scavenging, Antioxidant, Lethality and Superoxide.

Author for Correspondence:
Jeyasekhar M P,
Scott Christian College,
Nagercoil, Kanyakumari, Tamil Nadu, India.
Email: liviverna2016@gmail.com

INTRODUCTION

Cancer is one of the most dangerous life-threatening diseases, with much more different type's cancer occurring due to some molecular changes within the cell cycle. It is the third leading cause of death worldwide following by

cardiovascular, respiratory and contagious diseases[1]. Cancer also has been a leading cause of death in the age group 45-64 years in developed countries due to changing standard of living, food habits and unavailability of curative treatment for many infectious diseases[2].

WHO, 2004 assessed that 12.5-13% of the peoples die due to cancer. The disease is extensively widespread, and in the West, almost a third of the population progresses cancer at some point of time during their life style modification. Even though the mortality due to cancer is high, many advances have been made both in terms of treatment and understanding the pathophysiology of the disease at the molecular level[3].

Furthermore, it is gradually being recognized that many of day today's illnesses are developed due to one among the reason is known as "oxidative stress" that results from an imbalance between the formation and neutralization of prooxidants in human biological system. Oxidative stress is originated by free radicals, which try to find stability through electron pairing activity with biological macromolecules such as proteins, lipids, and nucleic acids (DNA) in healthy human cells and it causes protein destruction and DNA damage along with lipid peroxidation. These changes are develops cancer, atherosclerosis, cardiovascular diseases, aging, and some inflammatory diseases[4]. Almost, every cells are exposed to oxidative stress, and therefore, oxidation and free radicals may be involve in carcinogenesis at multiple tumor sites of human system.

Antioxidants can be of both synthetic and natural origins. Natural antioxidants that are obtained from plants contain mainly phenolic compounds. Utilization of natural antioxidants from plants does not provoke adverse effects, while synthetic antioxidants are found to induce genotoxic effects[5]. Thus, free radicals and carcinogenesis are closely connected with one another rand drug therapy is considered as free radical scavenging medications may be supportive therapy for cancer disease. Due to neither absence of effective medicines, nor affordable cost of chemotherapeutic agents and side effects cancer drugs can be a cause of death. In this condition medicinal plants have a special place in the management of cancer and lack the adverse effects associated with the existing chemotherapeutic agents[6].

Justicia tranquebarensis is a Sub shrub belongs to the family *Acanthaceae* and juices of leaves act as a cooling agent and aperients and also given to children in Small pox. Crushed leaves applied to contusions. Paste made of the leaves applied externally on the swelling to reduce the pain. Root paste applied for tooth ache[7]. On the other hand, no more scientific diuretic studies carried out with *J. tranquebarensis* in order to confirm its predictable beneficial possessions of the selected plant. Taking these deliberations of the above facts, the present study has been made to evaluate the anticancer, and antioxidant activities of selective medicinal plants used in Indian traditional medicine system.

MATERIAL AND METHODS

Drugs and Chemicals

5-Flouro uracil (Flouracil, Cadila), ethanol were used in this study. All substances were prepared immediately before use and the reagents were used as analytical grade.

Plant Materials

The leaves of *Justicia tranquebariensis* were collected from waste land of Thiruvananthapuram, Kerala.India). The plant was authenticated by Mr. Chelladurai, Research Botanist (Rtd), CCRAS Tirunelveli, Tamil Nadu.

Extract Preparation

The freshly collected *Justicia tranquebariensis* leaves were shade dried and coarsely powdered. The powdered materials were extracted with ethanol. The last traces of the solvent were removed and concentrated to dryness under vacuum using a rotary evaporator. The dried extract was weighed and then kept at 4ºC until ready for use. The yield of the extract was 5.65% (w/w). In each experiment, the extract was diluted with water to desired concentration.

Phytochemical Screening

A Preliminary phytochemical screening of *Justicia tranquebariensis* was conducted to determine the presence or absence of alkaloids, tannins, phenols, saponins, volatile oil, ascorbic acid, carbohydrates and glycosides by Wagner test, Braemer's test, Frothing test, Molisch's test and Borntrager's test[8].

ANTI-CANCER ACTIVITY

***In Vitro* Brine Shrimp Lethality Bioassay**[9]

Brine shrimp lethality bioassay is widely used in the bioassay for the bioactive compounds. Here cytotoxicity screening of the samples were carried against a simple zoological organism, brine shrimp nauplii. *Artemiasalina* leaches (brine shrimp eggs) were placed in a small tank containing 3.8% noniodized NaCl solution (sea water) for two days to hatch the shrimp and to be matured as nauplii. After hatching active naupli free from egg shells were collected from brighter portion of hatching chamber and used for assay. The nauplii were drawn through a glass capillary and placed in each vial containing brine solution and maintained at room temperature for 24 hour under light and surviving larvae were counted. Experiments were conducted along with control (vehicle treated), different concentrations of test substances and standard drug. The percentage lethality was determined by comparing the mean surviving larvae of test and control tubes. LC_{5O} were obtained from the best fit plotted concentration verses percentage lethality[10]. The percentage mortality (%M) was also calculated by dividing the number of dead nauplii by the total number, and then multiplied by 100%. This is to ensure that the death (mortality) of the nauplii is attributed to the bioactive compounds present in the plant extract[11].

***In vitro* antioxidant activity**

Dpph (1, 1-diphenyl –2-picrylhydrazyl[12]

4.3 mg of DPPH (1, 1-Diphenyl –2-picrylhydrazyl) was dissolved in 3.3ml methanol; it was protected from light by covering the test tubes with aluminum foil. 150µl DPPH solution was added to 3ml methanol and absorbance was taken immediately at 517nm for control reading. Various concentrations of extracts as well as standard compound (Ascorbic acid) were taken and the volume was made uniformly to 150µl using methanol. Each of the samples was then further diluted with methanol up to 3ml and to each 150 µl DPPH was added. Absorbance was taken after 15 min. At 517nm using methanol as blank on UV-visible spectrometer. The IC_{50} values for each drug compounds as well as standard preparation were calculated. The DPPH free radical scavenging activity was calculated using the following formula:

% scavenging = [Absorbance of control - Absorbance of test sample/Absorbance of control] X 100

SUPER OXIDE FREE RADICAL SCAVENGING ACTIVITY[13]

The reaction mixture contained 1ml of NBT solution (0.3mM prepared in phosphate buffer, pH-7.4), 1ml of NADH solution (0.936mM prepared in phosphate buffer, pH-7.4) ethanol extract of *J. tranquebariensis* in different concentration (1.25, 2.5, 5, 10, 20µg/ml) were added. Finally, reaction were accelerated by adding 100µL PMS solution (120µM prepared in phosphate buffer, pH-7.4) to the mixture. The reaction mixture was incubated at 250C for 5min and absorbance at 560nm was measured against methanol as control. Percentage inhibition was calculated as follows.

% Inhibition = [Absorbance of control - Absorbance of test sample/Absorbance of control] X 100

Hydroxyl Radical Scavenging Activity[14]

The scavenging activity for hydroxyl radicals was measured with Fenton reaction. Reaction mixture contained 60µL of 1.0mM FeCl2 , 90µl of 1mM 1, 10-phenanthroline, 2.4ml of 0.2 M phosphate buffer (pH 7.8), 150µL of 0.17 M H_2O_2, and 1.0ml of *J. tranquebariensis* extract at various concentrations (1.25, 2.5, 5, 10, 20µg/ml). Adding H_2O_2 started the reaction. After incubation at room temperature for 5 min, the absorbance of the mixture at 560nm was measured with UV visible spectrometer. The percentage inhibition of hydroxyl scavenging activity was calculated using the following formula

% scavenging/Inhibition = [Absorbance of control - Absorbance of test
sample/Absorbance of control] X 100

Metal Chelating Assay[15]

50µl of 2mM $FeCl_2$ was added to 1 ml of different concentrations of the *J. tranquebariensis* extract (1.25, 2.5, 5, 10, 20µg/ml). The reaction was initiated by the addition of 0.2ml of 5Mm ferrozine solution. The mixture was vigorously shaken and left to stand at room temperature for 10 min. The absorbance of the solution was thereafter measured at 562nm and percentage inhibition was calculated.

% Inhibition = [Absorbance of control - Absorbance of test
sample/Absorbance of control] X 100]

RESULTS

Preliminary Photochemical Screening

J. tranquebariensis leaves extracts were subjected to qualitative chemical tests for the detection of various phytoconstituents such as alkaloids, carbohydrates, proteins and amino acids, glycosides, flavonoids, tannins, phenolic compounds, saponins. The phytochemical screening results are shown in Table No.1.

In Vitro Brine Shrimp Lethality Bioassay

Bioassay is performed by using Brine shrimp eggs. The mortality rate of Brine shrimp is calculated and LC50 value also determined. The *J. tranquebariensis* extract showed positive results that are increased percentage of mortality of brine shrimps indicating that the test samples are biologically active as compared to 5-Flouro Uracil (5-FU) is taken as positive control. Table No.2 represents the %Mortality of various concentrations of the plant extract as well as the positive control (5-FU).

The data's EJTB (Ethanolic Extract of *J. tranquebariensis)* were expressed as Mean± S.D as compared with standard.

In vitro Antioxidant Activity

Various concentrations (1.25, 2.5, 5, 10, 20) µg/ml of methanol extract of *J. tranquebariensis* were tested for antioxidant activity like DPPH, Metal chelating activity, Reducing power assay, Superoxide scavenging assay, Hydroxyl radical scavenging assay. Ascorbic acid was taken as standard. The studies were performed in a triplicate manner, and then the %inhibition was found for each concentration of extract. The IC_{50} was calculated for the extract as well as the ascorbic acid (standard) and summarized in Table.

The data were expressed as Mean± S.D .The study was performed in triplicate manner.

DPPH (1, 1-Diphenyl 2, picryl hydrazyl) EJTB Ethanolic extract of *J. tranquebariensis*

The percentage of DPPH radical scavenging activity of ethanol extract of *J. tranquebariensis* is presented in Table No.3. The ethanolic extract of *J. tranquebariensis* was found to be more effective. The DPPH radical scavenging activity of the extract increases with increasing concentration. The IC_{50} of the ethanolic extract of *J. tranquebariensis* and ascorbic acid were found to be 320µg/ml and 470µg/ml respectively.

Metal Chelating Activity

The data were expressed as Mean ± S.D .The study was performed in triplicate manner. EJTB – ethanolic extract of *J. tranquebariensis*.

Metal binding capacity of ethanolic extract of *J. tranquebariensis* at various concentrations (1.25, 2.5, 5, 10, 20µg/ml) were examined and the values were presented in Table No.4. The ethanolic extract of *J. tranquebariensis* was found to have more effective chelating activity. The IC_{50} of the ethanolic extract of *J. tranquebariensis* and EDTA were found to be 450µg/ml and 415µg/ml respectively.

Reducing Power Assay

The data were expressed as Mean± S.D. The study was performed in triplicate manner. EJTB - Ethanolic extract of *J. tranquebariensis*.

The percentage inhibition of reducing power assay was increased with an increase in concentration of EJTB presented in Table No.5. The percentage inhibition of EJTB was found to be 61.86±0.43, 70.23±0.54 at a concentration of 1.25µg/ml, 20µg/ml respectively. The standard drug ascorbate was found to be 68.65±0.65, 75.68±0.62 at a concentration of 1.25µg/ml, 20µg/ml respectively.

The IC_{50} values were found to be 150µg/ml, 80µg/ml, respectively**.**

Superoxide Scavenging Assay

The data were expressed as Mean± S.D. The study was performed in triplicate manner. EJTB - Ethanolic extract of *J. tranquebariensis*

The percentage scavenging of superoxide anion examined at different concentrations of ethanolic extracts of *J. tranquebariensis* (1.25, 2.5, 5, 10, 20µg/ml) were presented in Table No.6. The IC_{50} values of ethanolic extract of *J. tranquebariensis* were found to have strong superoxide radical scavenging activity when compared to that of standard ascorbate. The IC_{50} of the ethanolic extract of *J. tranquebariensis* and Ascorbate were found to be 410µg/ml and 60µg/ml respectively.

Hydroxyl Radical Scavenging Activity

The data were expressed as Mean± S.D. The study was performed in triplicate manner. EJTB - Ethanolic extract of *J. tranquebariensis*

The percentage inhibition of hydroxyl radical scavenging assay was increased with an increase in concentration of EJTB presented in Table No.7. The percentage inhibition of EJTB was found to be 61.34±0.48, 71.56±0.52 at a concentration of 1.25µg/ml, 20µg/ml respectively. The IC_{50} values were found to be 44.12µg/ml, 33.55µg/ml, 24.19µg/ml, 14.08µg/ml respectively.

The reducing power assay and Hydroxyl radical scavenging activity of EJTB showed similar effect with that of Standard ascorbic acid. Thus from the *Invitro* antioxidant study, it was found that EJTB possess more activity by scavenging the free radicals with less IC_{50} value.

DISCUSSION

For a long period of time, herbal plant and its metabolites have been a valuable source of natural products for maintaining human health conditions, especially in the last period, with more intensive studies for natural therapies. The use of plant compounds for pharmaceutical purposes has gradually increased. Nearly 80-85% of the global population still depends upon the herbal drugs for their health care[16]. In the ancient few years, cancer has remained a major cause of death and the number of individuals living with cancer is continuing to expand. Hence, a major portion of the current pharmacological research is devoted to anticancer drug design customized to fit new molecular targets. Due to the enormous tendency of plants, which synthesize a variety of structurally diverse bioactive compounds, the plant kingdom is a potential source of chemical constituents with antitumor and cytotoxic activities[17].

In this present study we have tested of phytoconstituents studies, *in vitro* anticancer and antioxidant activity on leaf ethanol extract of *J. tranquebariensis*. The results were exhibited as the presence of certain phytoconstituents such as flavonoids, tannins, saponins, phytosterols, alkaloids and carbohydrates, potent anticancer, antioxidant activity with less IC_{50} values in the scavenging of DPPH, Superoxide free radical, Hydroxyl radical and in the metal chelating activity and Reducing power assay. The potent anticancer and antioxidant activity of the alcoholic extract may be due to the presence of phytosterols, phenolic compounds such as tannins and flavonoids[18].

The Ethanol extract of the plant *J. tranquebariensis* subjected to the *in vitro* Brine shrimp lethality bioassay. This method is widely used in the bioassay for the bioactive compound of anticancer studies. Based on the results, the brine shrimp lethality of the plant extract was found to be concentration-dependent manner activity. The lethality of the extract to brine shrimps indicated the presence of potent cytotoxic and probably antitumor components of the *J. tranquebariensis* plant[10].

Free radicals are generated in the body by various metabolic reactions may cause DNA strand breaks and chromosome deletions and rearrangements and it plays an important role in tumor promotion and progression. The ability of Free radical scavenging compounds to ameliorate diseased conditions is appreciated. Thus the human body is protected by antioxidants against damage by the free radicals. For these reasons, the search for antioxidants as cancer chemo-preventive agents is a continued process[19].

DPPH is widely used to evaluate the free radical scavenging effect of natural antioxidant. DPPH is a stable free radical at room temperature. The bleaching of DPPH molecules can be correlated with the number of available hydroxyl groups. We can infer that, the activity of the extract may be probably due to the presence of substance with an available hydroxyl group. The extracts are able to reduce the stable radical DPPH to the yellow coloured diphenyl picrylhydrazine[20]. Super oxides are produced from molecular oxygen due to oxidative enzymes of body as well as by non-oxidative reactions by auto catecholamine. Overproduction of super oxide anion radical contributes to redox imbalance and associated with harmful physiological consequences. Super oxide anion are generated in PMS-NADH system by the oxidation of NADH and assayed by the reduction of NBT resulting in the formation of blue formazan[13].

Hydroxyl radical scavenging activity is measured as the percentage of inhibition of hydroxyl radical generated in the Fenton's reaction mixture. Among the oxygen radicals, hydroxyl radical is the most reactive and induces severe damage to adjacent biomolecules such as protein, DNA and lipids; cause's lipids peroxidation. The Fenton reaction generates hydroxyl radicals (OH) which degrade DNA deoxyribose, using Fe2+ salts as an important catalytic component. Oxygen radicals may attack DNA either at the sugar or the base, giving rise to a large number of products[12]. From the above findings the study was revealed that the ethanol extract of the *J. tranquebariensis* plant having potent cytotoxic and antioxidant property.

Table No.1: Preliminary phytochemical screening of extracts of *J. tranquebariensis*

S.No.	Phytoconstituents	*J. tranquebariensis*
1	Glycoside	+
2	Carbohydrates	+
3	Flavonoids	-
4	Protein	-
5	Alkaloids	+
6	Tannins	+
7	Saponin	+
8.	Phenolic compounds	+

+ indicates presence and - indicates Absence

Table No.2: Determination of % Mortality of *J. tranquebariensis* extract

S.No	Concentration In (µg/ml)	% Mortality	
		EJTB	5-Flourouracil
1	0	-	-
2	10	17.33±0.34	34.33±0.42
3	20	21.34±0.87	47.67±0.65
4	40	35.67±0.45	57.33±0.47
5	60	42±0.53	65.33±0.43
6	80	63.33±0.27	81.33±0.35
7	100	76.23±0.43	95.33±0.53

Table No.3: DPPH assay of *J. tranquebariensis* extract and Ascorbic acid

S.No	Concentration (µg/ml)	% Inhibition	
		EJTB	Ascorbic acid
1	1.25	40.48±0.42	31.48±0.20
2	2.5	41.38±0.36	36.76±0.09
3	5	42.57±0.32	40.12±0.06
4	10	42.76±0.64	42.56±0.58
5	20	45.58±0.04	48.54±0.43
6	IC_{50} Value	320µg/ml	470µg/ml

Table No.4: Metal chelating activity of *J. tranquebariensis* extract and Ascorbic acid

S.No	Concentration (µg/ml)	% Inhibition	
		EJTB	Ascorbic acid
1	1.25	36.84±0.44	25.64±0.65
2	2.5	38.42±0.09	30.13±0.49
3	5	40.12±0.87	36.14±0.63
4	10	44.21±0.56	42.48±0.97
5	20	46.47±0.48	47.54±0.21
6	IC_{50} Value	450µg/ml	415µg/ml

Table No.5: Reducing power assay of *J. tranquebariensis* extract and Ascorbic acid

S.No	Concentration (µg/ml)	% Inhibition	
		EJTB	Ascorbic acid
1	1.25	61.86±0.43	68.65±0.65
2	2.5	65.37±0.28	70.08±0.76
3	5	66.43±0.65	71.95±0.45
4	10	67.46±0.37	73.54±0.42
5	20	70.23±0.54	75.68±0.62
6	IC_{50} Value	150µg/ml	80µg/ml

Table No.6: Superoxide scavenging assay of *J. tranquebariensis* extract and Ascorbic acid

S.No	Concentration (µg/ml)	% Inhibition	
		EJTB	Ascorbic acid
1	1.25	56.45±0.76	61.24±0.64
2	2.5	57.64±0.65	63.29±0.57
3	5	61.10±0.38	66.58±0.66
4	10	63.28±0.54	68.56±0.47
5	20	64.38±0.73	72.64±0.53
6	IC_{50} Value	415µg/ml	80µg/ml

Table No.7: Hydroxyl radical scavenging activity of *J. tranquebariensis* extract and Ascorbic acid

S.No	Concentration (µg/ml)	% Inhibition	
		EJTB	Ascorbic acid
1	1.25	61.34±0.48	66.18±0.56
2	2.5	63.27±0.62	68.56±0.64
3	5	66.43±0.54	71.45±0.37
4	10	68.25±0.83	73.54±0.28
5	20	71.56±0.52	74.41±0.41
6	IC_{50} Value	415µg/ml	80µg/ml

CONCLUSION

It can be concluded that the plant selected in the present study having significance in traditional medicine can be measured as a source for the isolation, identification, and progress of novel and active anticancer and antioxidant agents. However, the research data of the present findings may serve as a guideline for the standardization and validation of natural drugs having the selected medicinal plant as components. This study may also be useful to the other researchers to take forward the references for further scientific evaluation of anticancer and anti-oxidant activity.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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