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EVALUATION OF ANTIOXIDANT, ANTIBACTERIAL, ANTI- INFLAMMATORY AND ANTI-ARTHRITIC ACTIVITY OF *JUSTICIA TRANQUEBARIENSIS*

D. Leema Rose Mary*[1], P. Marie Arockianathan[1], Priya Nagappan[1], V.Vinodini[2], R. Kanaga[2]

[1]Assistant Professor, Department of Biochemistry, St. Joseph's College of Arts and Science (Autonomous), Cuddalore – 607001.
[2]Research Scholar, Department of Biochemistry, St. Joseph's College of Arts and Science (Autonomous), Cuddalore – 607001.

***Corresponding Author: D. Leema Rose Mary**
Assistant Professor, Department of Biochemistry, St. Joseph's College of Arts and Science (Autonomous), Cuddalore – 607001.

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ABSTRACT
Plants give many important therapeutic products to improve our health system without any adverse effects. In the present study the aqueous, methanol and ethanol extracts of the *Justicia tranquebariensis* was prepared and tested for various phytochemical and biochemical analysis. The phytochemical analysis revealed the presence of Saponins, alkaloid, flavinoids, Phytosterols, gum and mucilage in all the extracts of plant. In biochemical analysis the anti-oxidant assay by DPPH scavenging method, anti-microbial activity by disc diffusion method, Invitro anti-inflammatory activity by HRBC stabilization method and in-vitro anti-arthritic activity by bovine serum albumin (BSA) denaturation method were analyzed. The results showed the methanolic and ethanolic extracts of plant exhibit maximum activity in all the parameters with respect to aqueous extract. The methanol extracts of *Justicia Tranquebariensis* was tested for anti-bacterial activity against control Ciprofloxacin shows maximum zone of inhibition. From these results, it was found methanolic and ethanolic extracts of plant shows good anti-oxidant, anti-inflammatory and anti-arthritic properties. So this plant extract can be exploding further to produce herbal active principles for therapeutic use.

KEYWORDS: Antioxidant activity, antibacterial activity, anti- inflammatory activity, anti-arthritic activity, *Justicia tranquebariensis.*

INTRODUCTION
Plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years and have served humans as with valuable components of seasonings, beverages, cosmetics dyes and medicines. Traditional medicine is the oldest method of curing diseases and infections and various plants been used in different parts of the world to treat human diseases and infections. Medicinal plants are known to owe their curative potentials to certain biological active substances, which exist in parts of the plants. The plant parts have also been used for various forms of diseases and infections.

Justicia tranquebariensis is belonging to the family of Acanthaceae. The local name is Thavasi murungai. Traditionally it called as **'Sivanarvembu'** which is used in the treatment of Leprosy, Cancer, Oedema, abscess, skin disorders.[1] It is a small shrub, which is widely distributed in southern parts of India. Roots are perennial, somewhat granulated. Stems are in young plants erect, in old ones uncertain Whitish grey and branches Stiff. Branches are annual, small, staggling, round, a little downy, jointed & swelled above the joints, from 1-2 ft. long. Leaves are short petiole, oval, round, entire, pretty smooth, obvate, simple, opposite and decussate. Stipules are absent. Flowers are white with pinkish-purple striate, bracts ovate-orbicular, shortely apiculate at apex, minutely pubescent, bracteoles elliptic-apatulate. Standing in the axills of the two rows of bracts that occupy forepart of the spikes; on the back part they are generally wanting.

Local people use this plant drug for inflammations. Leaf is used as expectorant, in Cold, Cough and nasal disorders.[2] Juices of leaves act as a cooling agent and aperients and also given to children in Small pox. Crushed leaves applied to contusions, diaphoretic, diuretic, rheumatism.[3] Paste made of the leaves applied externally on the swelling to reduce the pain. Root paste applied for tooth ache.[4] The leaf juice of *J.tranquebariensis* has been used to treat jaundice and leaf paste is applied over affected area to treat skin diseases.[5] Leaf juice, about15-20 ml, is administered orally for every one hour up to half of the day and

keeping of leaf paste externally on the sight of snake bite work as an antidote for Cobra bite.[6]

The alcoholic extract of the aerial part of the plant yielded phytochemical substances include phytosterols,brassicasterol,campesterol,7,22ergostadienol,stigmasterol,sitosterol,spinasterol,28-isofucosterol and betasitosterol-3-O-glucoside.[7] In this genus a lot 20 species have been chemically investigated and the major secondary metabolites isolated were lignans, flavinoids, steroids and terpenes.[8-11] The Asians species of justicia contain lignans, such as *J.tranquebariensis* in which arytetarline, (+)-laricireasionol, and (+)-medioresinol have been found.[12]

MATERIALS AND METHODS

Extraction of *J. tranquebariensis:* The leaves of *Justicia tranquebariensis* were washed thoroughly under running tap water and shade dried for three weeks at room temperature. After that dried leaves ground into a powder and then extracted by water, ethanol and methanol extracts were prepared using Soxhlet apparatus. Then the extract filtered by using filter paper. The filtrate is placed in china disc and evaporates the filter. Finally collected the crude extract and calculated the % of yield.

Table No. 1: Percentage of Extracts.

S. No.	Extracts	% Yield
1)	Water	1.95 %
2)	Methanol	1.40%
3)	Ethanol	2.60%

METHODS FOR PHYTOCHEMICAL INVESTIGATION

The various test were carried out to screen the phytochemicals in the water, ethanol and methanol extract of *J. traquebariensis.*[14,15]

METHOD FOR ANTIBACTERIAL ACTIVITY

COLD EXTRACTION: 10gm of *J. tranquebariensis* was suspended in 80 ml of respective solvent (methanol, ethanol and aqueous) and mixed well and kept still for 24 hours at room temperature and filtered and the filtrates was collected and allowed to dry .The total extract value was noted gravimetrically.

ANTIBACTERIAL ACTIVITY BY DISC DIFFUSION METHOD

The target strains used for screening antibacterial activity were obtained from National Collection of Industrial Microorganism (NCIM), Pondicherry. The bacterial strains used were *Bacillus subtilis NCIM 2920, Staphylococcus aureus NCIM 2079, Pseudomonas aeruginosa NCIM 2053, Salmonella abony NCIM 2257, Escherichia coli NCIM 2065.*Test pathogens were spread on the test plates with Mueller Hinton Agar-MHA (kept in auto clave at 121^{o}c for 15minutes at 15psi, and kept at incubator for 30minutes). The antibiotic discs and one positive bacteria sterile disc in 6 mm diameter were kept and impregnated in the agar and the medicament (methanol extract of *J. tranquebariensis* in the concentration of 10mg/ml was loaded in each disc with various concentrations (0.5mg, 1.0mg, 2.0mg and 10µg-positive control) in all plates. The test plates were incubated for 24hrs. The zone of inhibition (mm in diameter) were read and taken as the activity against the test pathogen.[13] The whole process was done inside the laminar air flow chamber. Positive control–Ciprofloxacin (30mcg), Kanamycin (30 mcg). Whattman filter paper (No.1) was used to prepare discs approximately 6 mm in diameter which are placed in a Petri dish and sterilized in a hot air oven.

METHOD FOR ANTIOXIDANT ACTIVITY

Determination of DPPH scavenging assay

DPPH radical scavenging activity of extract was determine according to the method reported by Blois (1958). An aliquot of 0.5 ml of sample solution in methanol was mixed with 2.5 ml of 0.5 Mm methanolic solution of DPPH. The mixture was shaken vigorously and incubated for 30 min in the dark at room temperature. The absorbance was measured at 517 nm using UV spectrophotometer. Ascorbic acid was used as a positive control. DPPH free radical scavenging ability (%) was calculated by using the formula. % of inhibition = absorbance of control (Ao) – absorbance of sample (As) / absorbance of control ×100.[14,15]

$$\text{Scavenging effect \%} = \frac{Ao - As}{Ao} \times 100$$

METHOD FOR IN-VITRO ANTI-INFLAMMATORY ACTIVITY

The human red blood cell (HRBC) membrane Stabilization method

The blood was collected from healthy human volunteer who had not taken any NSAIDs (Non steroidal anti-inflammatory drug) for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) and centrifuged at 3,000 rpm for 20 min and packed cells were washed three times with isosaline (0.85%, pH 7.2). The volume of the blood was measured and reconstituted as 10% v/v suspension with isosaline. The principle involved here is stabilization of human red blood cell membrane by hypo tonicity induced membrane lysis. The assay mixture contains 1ml phosphate buffer [pH 7.4, 0.15 M], 2 ml hypo saline [0.36 %], 0.5 ml HRBC suspension [10 % v/v] with 0.2ml of plant extracts and standard drug diclofenac sodium of various concentrations (100 and 200 µg/ml) and control (distilled water instead of hypo saline to produce 100 % hemolysis) were incubated at 37^{o}C for 30 min and centrifuged respectively. The hemoglobin content in the suspension was estimated using spectrophotometer at 560 nm.[16,20]

The percentage of hemolysis of HRBC membrane can be calculated as follows:

% Hemolysis = (Optical density of Test sample / Optical density of Control) X 100

The percentage of HRBC membrane stabilization can be calculated as follows:

% Protection = 100 – [(Optical density of Test sample/Optical density of Control) X 100]

METHOD FOR ANTIARTHRITIC ACTIVITY

Invitro anti-arthritic activity by inhibition of protein denaturation method

Samples containing Bovine Serum albumin (5% W/V Ethanol solution) and test solution (25mg/ml) which is made up to 1ml. Similarly 2 duplicates were prepared and made up to 1ml.Test control contains Bovine serum albumin (5% W/V Ethanol solution)and distilled water which is made up to 1ml using distilled water. Product control consists of distilled water and test solution which is again made up to 1ml. A standard solution (1ml) consists of 0.9ml of Bovine serum albumin (5%W/V Ethanol solution) and 0.1ml of Aspirin (25mg/ml). All the above solutions were adjusted to pH 6.3 using 1N HCl. The samples were incubated at 37ºC for 20 minutes and the temperature was increased to keep the samples at 57ºC for 3 minutes. After cooling, add 2.5ml of phosphate buffer to the above solutions. The absorbance was measured using UV-visible spectrophotometer at 416nm.[21] The control represents 100% protein denaturation. The results were compared with Aspirin (25mg/ml). The percentage inhibition of protein denaturation can be calculated as,

% inhibition = [100 - (optical density of test solution - optical density of product control) ÷ (Optical density of test control)] × 100

RESULTS

PHYTOCHEMICAL SCREENING

Table No. 2: Qualitative analysis of carbohydrate with various extracts of *J. tranquebariensis.*

S. NO	TEST NAME	METHANOL	ETHANOL	WATER
a)	Molisch's test	+	+	+
b)	Fehling's test	+	+	+
c)	Benedict's test	+	+	+
d)	Barfoed's test	+	+	+
e)	Starch test	_	_	_

+ indicate presence; - indicate absence

The results of qualitative phytochemical screening of the plants extracts, shows the presence of Carbohydrates (reducing compounds) in ethanol, methanol and water extract.

Table No. 3: Qualitative analysis of Amino acid with various extracts of *Justicia tranquebariensis.*

S. NO	TEST NAME	METHANOL	ETHANOL	WATER
a)	Ninhydrin test	+	+	+
b)	Millon's test	+	+	+
c)	Biuret test	+	+	+

+ indicate presence; - indicate absence

The results of qualitative phytochemical screening of the plants extracts, shows the presence of Protein and free amino acid in ethanol, methanol and water extract.

Table No. 4: Qualitative phytochemical screening of various extract of *J. tranquebariensis.*

S. No	TESTS	METHANOL	ETHANOL	WATER
1)	Oils and Fats	+	+	+
2)	Alkaloids	+	+	+
3)	Phytosterols	+	+	+
4)	Flavonoids	+	+	+
5)	Tannins	_	_	_
6)	Saponins	+	+	+
7)	Gums and mucilage	+	+	+

+ indicate presence; - indicate absence

The results of qualitative phytochemical screening of the plants extracts, showed the presence of alkaloids, flavinoids, Saponins, oil and fat, gum and mucilage, Phytosterols and absence of tannin respectively.

ANTIOXIDANT ACTIVITY

Antioxidant analysis in different extracts using DPPH

The DPPH scavenging assay was done for the various extract of the plant ***J. tranquebariensis*** with four different concentrations (10, 50, 100,500 µg/well) and compared with control (ascorbic acid). All the three extracts exhibited potential antioxidant activity. The scavenging activity of ethanol extract reached high % compared with methanol and water extract.

Table No. 5: DPPH scavenging activity of *Justicia tranquebariensis.*

EXTRACTS	10 µg /ml		50 µg /ml		100 µg /ml		500 µg /ml	
ETHANOL	37.738090	37.738090	60.515870	67.757930	88.690480	83.333340	114.444400	106.587300
METHANOL	41.944440	42.420630	61.865080	70.000000	85.793600	85.496000	88.750000	88.452400
WATER	42.162700	47.222220	72.123020	72.420630	79.166660	89.761900	101.011900	104.484100
A.ACID	39.345600	42.350000	64.970000	69.790000	78.137000	82.460000	96.328000	99.378000

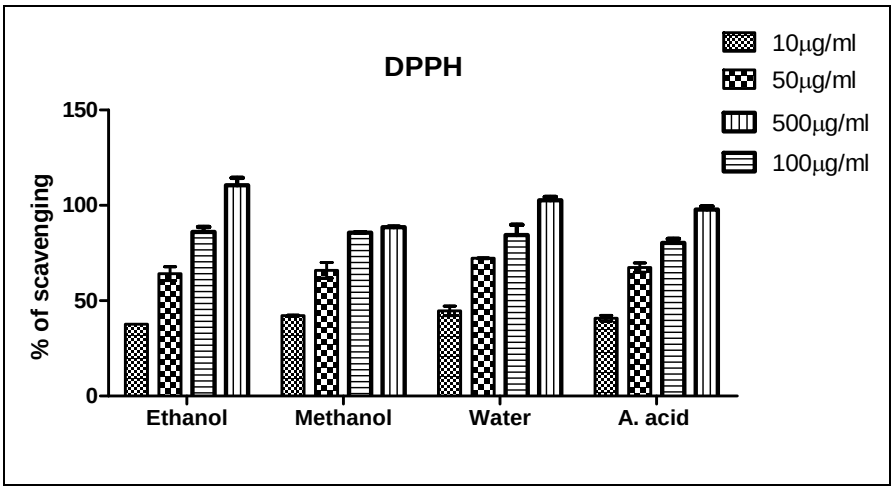


Figure No. 1: DPPH scavenging activity of various extracts.

ANTIBACTERIAL ACTIVITY

Table No. 6: The Amount of sample obtained from the cold extraction of *J. tranquebariensis* with the different extracts of water, Methanol, Ethanol respectively.

Solvent used	Weight of the sample obtained (g/10g)	% of sample obtained	Volume (µl) of sample taken to make 500 mcg
Water	**1.2109**	**12**	**1.23**
Methanol	**0.6934**	**6.9634**	**3.6**
Ethanol	**0.36**	**3.6**	**5.5**

Note: The extract was resuspended, 5ml for water; 5 ml for methanol and 3 ml for ethanol.

Among the extracts of methanol and ethanol extract shows the anti-bacterial zone formation and water extracts showed no inhibitory effect with the control Kanamycin in all the tested bacteria was shown in table no:7, figure no:3.

Figure No. 2: Cold extract of *J. tranquebariensis.*

Figure No. 3: Inhibitory Effect of *J. tranquebariensis* with Control Kanamycin.

Table No. 7: Effect of Antibacterial activity of *J. tranquebariensis* and control Kanamycin.

Name of the organism	Control	Zone of inhibition		
	Kanamycin -30mcg	Water extract	Methanol extract	Ethanol extract
Bacillus subtilis	22	0	*	*
Staphylococcus aureus	21	0	*	*
Pseudomonas aeruginosa	13	0	*	*
Salmonella abony	19	0	*	*
Escherichia coli	20	0	*	*

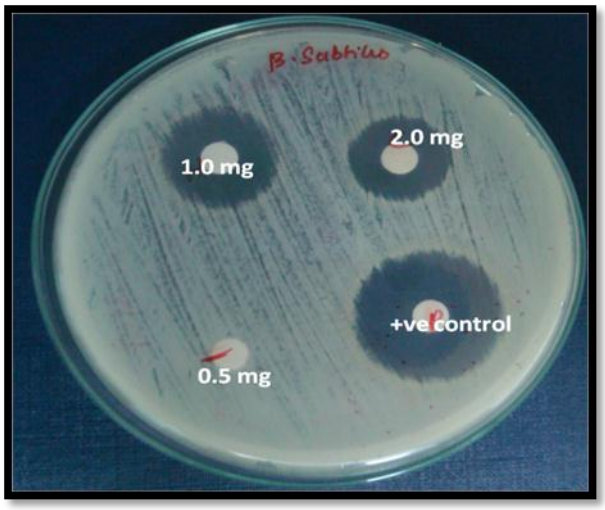
0-no growth; * -zone of inhibition

The methanolic extract of *Justicia traquebariensis* shows the maximum zone of inhibition in their positive control.

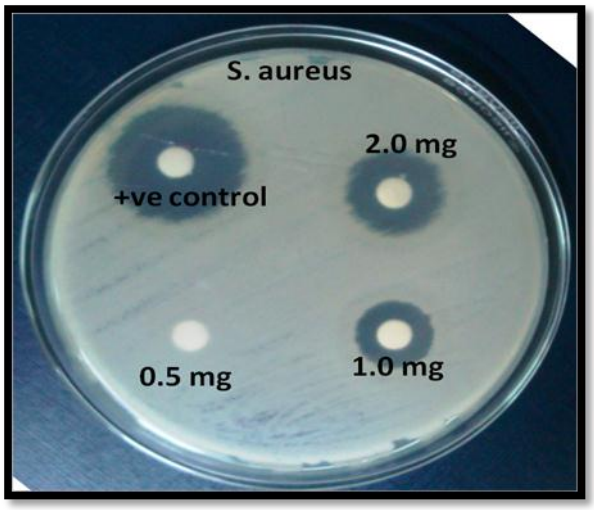
Table No. 8: Effect of Antibacterial activity of *J. tranquebariensis* and control Ciprofloxacin.

Name of the organism	Zone of inhibition			Control (Ciprofloxacin)
	0.5 mg/disc	1.0 mg/disc	2.0 mg/disc	10 ug/disc
Staphylococcus aureus	0.0	11	13.6	29.1
Bacillus subtilis	0	13.3	13.5	30.0
Escherichia coli	0	0	0	14.2
Pseudomonas aeruginosa	0	0	0	10.5

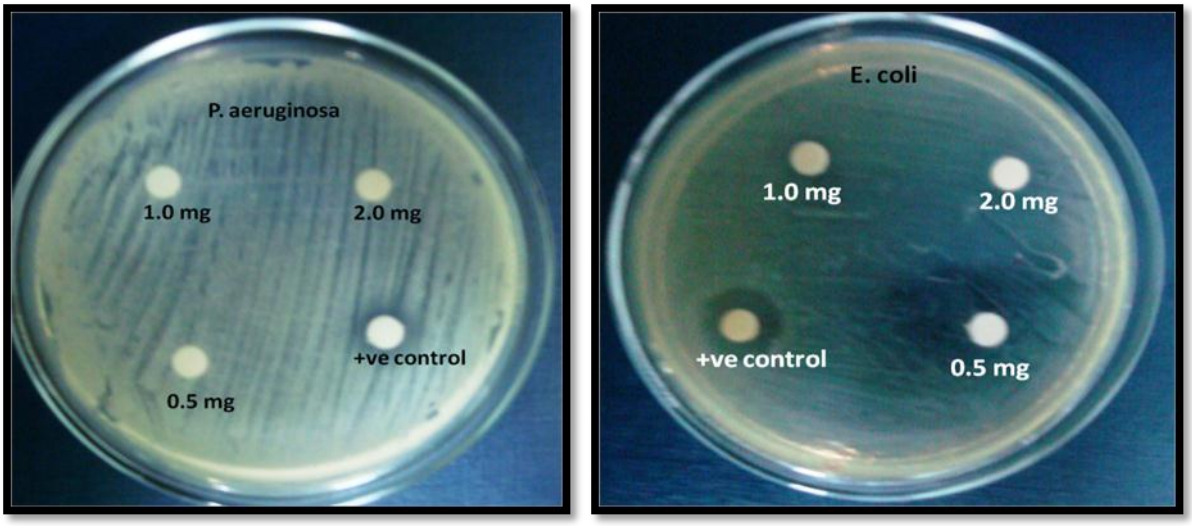
The different pathogenic strains show different results with various concentration of the sample. It reveals that the sample with highest concentration, produce maximum results and the sample with low concentration, gives low results.



STAPHYLOCOCCUS AUREUS



BACILLUS SUBTILIS



ESCHERICHIA COLI **PSEUDOMONAS AEROGINOSA**

Figure No. 4: Antibacterial activity of *J. tranquebariensis* and control Ciprofloxacin.

ANTI-INFLAMMATORY ACTIVITY

The HRBC Membrane stabilization method was used for the in-vitro anti- inflammatory activity of the methanol, ethanol and water extracts of *Justicia tranquebariensis.* The HRBC Membrane stabilization activity of *Justicia tranquebariensis* showed stabilization and hemolysis in hypotonic solution while compared with standard Diclofenac. The HRBC Membrane stabilization activity/protection increased while the membrane hemolysis was decreased (table no:10, figure no: 5 & 6).

Table No. 9: In vitro anti-inflammatory activity of *J. tranquebariensis* by HRBC Membrane Stabilization.

S. No	Type of extract	Concentration (µg/ml)	% Inhibition of denaturation
1.	Water	200	2.646 ± 0.9523
2.	Water	400	2.753 ± 1.239
3.	Methanol	200	1.941 ± 2.117
4.	Methanol	400	2.093 ± 1.767
5.	Ethanol	200	2.086 ± 2.000
6.	Ethanol	400	2.182 ± 1.972
7.	Diclofenac	100	0.038 ± 0.345
8.	Diclofenac	200	0.295 ± 1.518

Table No. 10: Effect of *J. tranquebariensis* on HRBC membrane stabilization and hemolysis.

S. No	Type of extarct	Concentration (µg/ml)	% stabilisation	% hemolysis
1.	Water	200	-272.47	372.47
2.	Water	400	-124.65	224.6
3.	Methanol	200	47.23	52.76
4.	Methanol	400	61.22	38.78
5.	Ethanol	200	10.36	89.69
6.	Ethanol	400	58.21	41.79
7.	Diclofenac	100	76.53	23.47
8.	Diclofenac	200	81.32	18.68

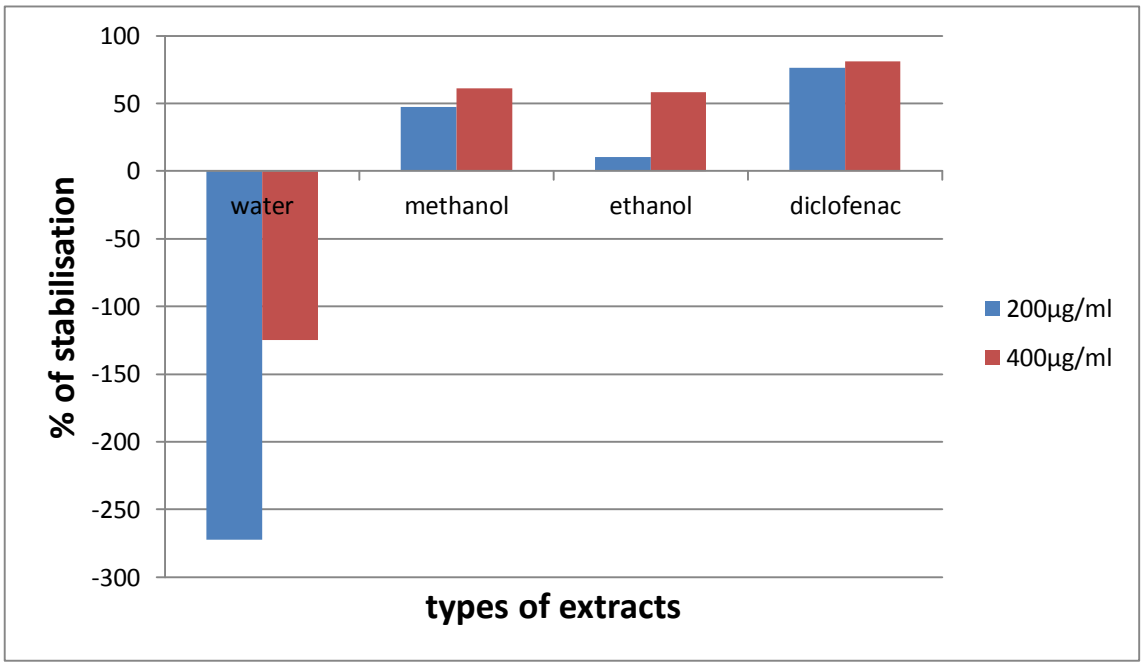


Figure No. 5: Effect of *J. tranquebariensis*on HRBC membrane stabilization.

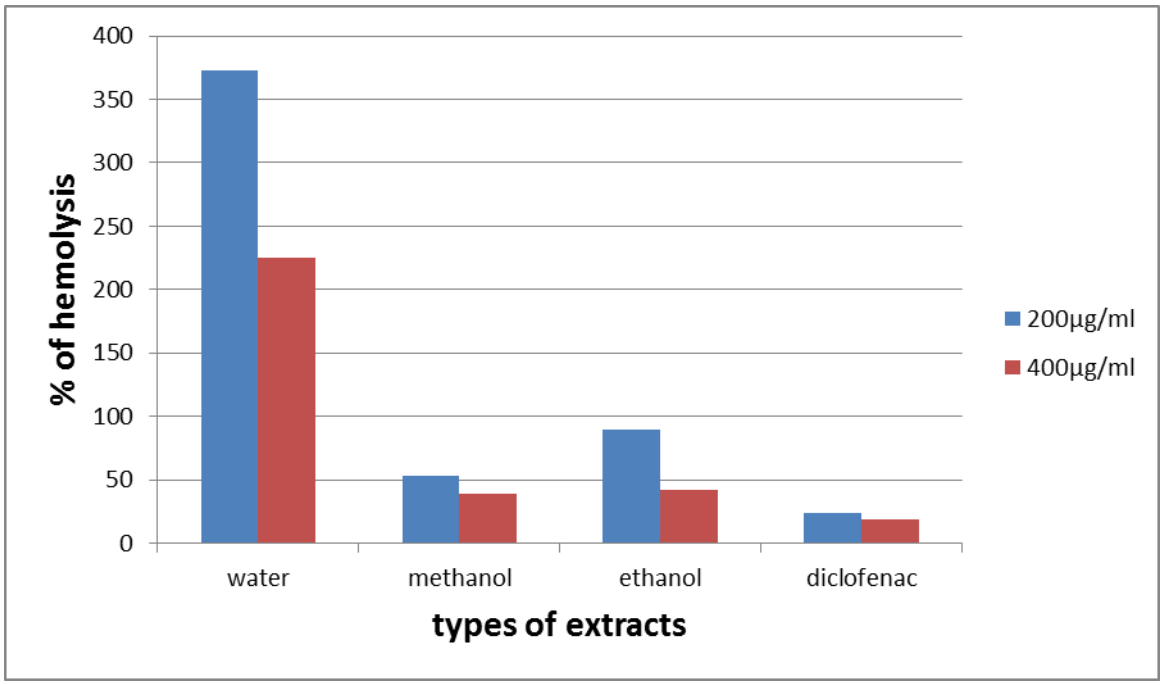


Figure No. 6: Effect of *J. tranquebariensis*on HRBC membrane Hemolysis.

ANTI-ARTHRITIC ACTIVITY

The invitro anti-arthritic activity of *J. tranquebariensis* by protein denaturation method is shown in Table no: 11, Figure no: 7.

Table No. 11: Invitro anti-arthritic activity of *J.tranquebariensis* by protein denaturation.

Concentration(mg/ml)	*J. tranquebariensis*			Diclofenac sodium
25	Water	Methanol	Ethanol	76.83
	59.7	55.3	45.4	

From the result of the present study, it can be stated that all the extracts of leaves is capable of controlling the production of auto antigen and there by it inhibit the denaturation of proteins and its effect was compared with the standard drug diclofenac sodium. The percentage protection was found to be 59.7% (water), 55.3%

(methanol), 45.4% (ethanol) and 76.83% (Diclofenac sodium).All the extracts showed dose dependant response. This effect may be due to the presence of steroids, alkaloids and flavonoid present in various fractions.

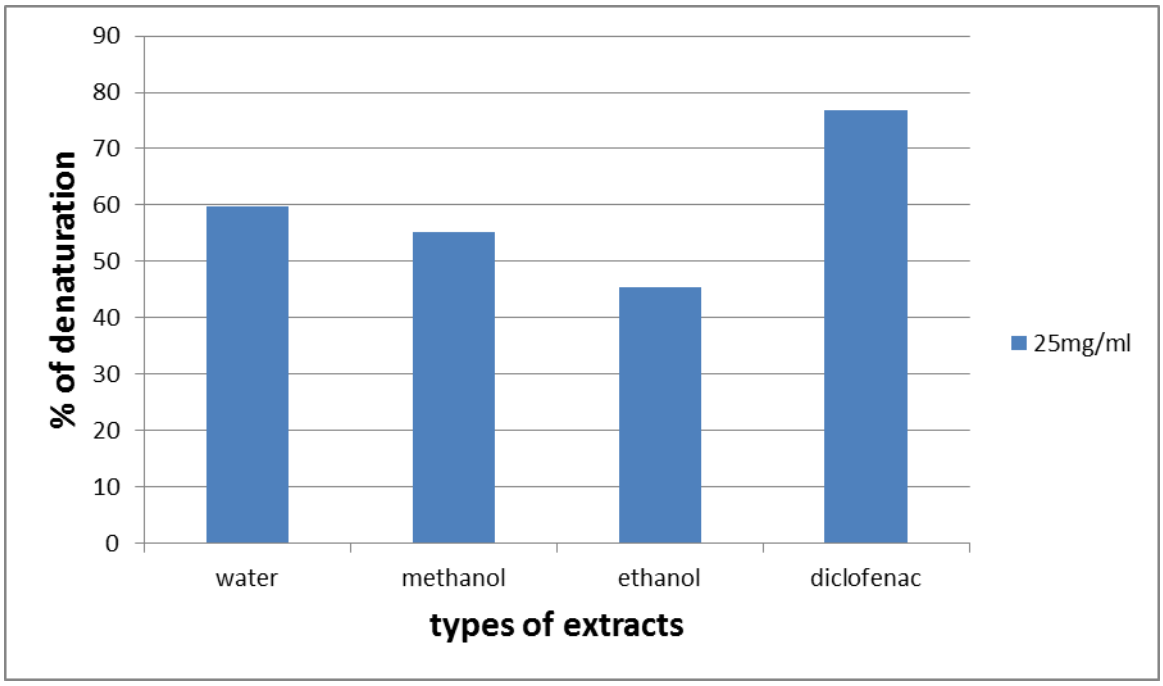


Figure No: 7. Effect of *J. tranquebariensis* on the Anti-arthritic Activity.

DISCUSSION

Phytochemical analysis are used to check the genuine nature of the crude drug, thus it plays an important role in preventing the possible steps of adulteration. Based on the reported traditional use in the literature, in the present investigation different extract of *J. tranquebariensis* was taken. Theplant extracts revealed the presence of various biochemical compounds such as flavonoid, saponins, alkaloid, phytosterols, oils and glycoside.[22] So the phytochemical tests justify their therapeutic potential. These phyto constituents have been reported to have multiple biological effects.

The antimicrobial activities of extracts of *J. tranquebariensis* were assessed. Likewise ethanol extract, methanol extract, and water extract of plant showed no anti bacterial activity compared to kanamycin antibiotic. But the methanol extract against Ciprofloxacin antibiotic shows the maximum zone of inhibition. Therefore, the anti bacterial activity of plant extracts depends on the species of plant, the type of solvent and the type of tested microorganism.[23]

Antioxidants may offer resistance against oxidative stress by scavenging the free radicals, inhibiting lipid peroxidation etc. The DPPH radical scavenging Antioxidant properties of *J. tranquebariensis* by Non enzymatic Analysis activity has been extensively used for screening antioxidants from the extracts. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in colour (from deep-violet to light- yellow) where measured at 517nm wavelength. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The ethanol extract of *J. tranquebariensis* exhibited maximum antioxidant activity.

In-vitro anti-inflammatory studies of *J. tranquebariensis* demonstrated the suppression of both inflammation and arthritis. One of the causes of rheumatoid arthritis is denaturation of proteins and inhibition denaturation is one of the in vitro tests to screen anti-inflammatory drugs. The *J. tranquebariensis* fabricates significant activity by inhibition of protein denaturation method and its effect was compared with the standard drug Diclofenac sodium. The production of auto antigen in certain arthritic disease may be due to denaturation of protein.

Stabilization of the HRBCs membrane by hypo tonicity induced membrane lysis was studied to establish the mechanism of anti-inflammatory action of *J. tranquebariensis.* Therefore, our present in vitro studies on *J. tranquebariensis* extracts demonstrate the depression of inflammation. Hence, it can be used as a potent anti-inflammatory agent. Some species of the genus *Justicia* have been used in the traditional system of medicine for the treatment of fever, pain, inflammation.[24-26]

From the results of present study it can be stated that water extract is capable of controlling the production of auto antigen and inhibits denaturation of protein in

rheumatic disease. Hence proper isolation of the active constituents might help in the findings of new compounds in the fields of anti-inflammatory drug research. Studies related to active constituent enzyme expression (COX2, lipoxygenase) are necessary to understand the mechanism of action in relation to the observed anti-inflammatory activity.

CONCLUSION

The *in-vitro* studies on leaves of *J. tranquebariensis* showed the presence of significant anti-inflammatory and anti- arthritic activity. The Activity may be due to the presence of terpenoids, steroids, alkaloids, flavonoids and lignins. Our future aim is to isolate the chemical constituents responsible for the above activities and also to carry out the *in-vivo* investigation.

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