



PHYTOCHEMICAL AND CHEMOPREVENTIVE INVESTIGATION ON THE LEAVES OF *Justicia tranquebariensis* L.

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ABSTRACT

Present investigation deals with the phytochemical and chemo preventive studies of *Justicia tranquebariensis* L belonging to the family Acanthaceae, a medicinally important herb. Phytochemical studies confirmed the presence of flavanoid, phenolic, sterols, alkaloids, steroidal glycosides, carbohydrates, proteins, tannins, terpenoids and proteins. The total phenolic content of leaves was 143.08 and the total flavanoid content was 27.14. The medicinal properties of this plant nicely correlate with its phytochemical content and Chemo preventive activities and this study may bring to light a lead molecule for treatment of cancer and inflammation in future. This may help the future researchers to carry out further studies in detail.

Keywords :- *Justicia*, Phytochemical, Chemo preventive, Flavanoid.

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INTRODUCTION

The medicinal plants find application in pharmaceutical, cosmetic, agricultural and food industry[1]. The scientists isolated active constituents of the medicinal herbs and after testing some were found to be therapeutically active aconitine, atisine nicotine, strychnine, digoxin, atropine, morphine are some examples[2]. Recently research has supported biological activities of some medicinal herbs to treat cancer is such a segment where researches are expecting new molecules from herbs that can provide us with tools for fighting this dreaded disease. *Allamanda cathartica* (allamandin), *elphatopus elatus* (elephantpoin), *helium autumnale* (helenalin) *vernonia hymenlepis*, *heliotropium indicum* (indicine-n-oxide) and *daphne mezereum* (merzerien) are medicinal plants that have shown significant tumour inhibiting effect.[3]

Justicia tranquebariensis L obtained from Acanthaceae family. Regional names are pindi in

Sanskrit, Sivanarvembu, Tvashoomoorunghi in tamil, pinikonda in telugu, Shiva naaruballi in kannada.[4,5]. Leaves are in orbicular or obovate, puberulous or pubescent bracts or ovate. This leaves are used in skin disease, cooling, aperients action, cut wounds eye infections and stomach problem[6]. Chemo preventive are beginning to think that perhaps the best way to catch cancer is to target inflammation. Inflammations (Latin inflammare to set on fire) is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells or irritants.[7]. Inflammation is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process.

Cancer is the second common cause of death in developed countries. This insidious disease occurs due to anomaly in mitotic cell cycle. Cancer is the general term of series of neoplastic disease which are characterized by

changes in a cell leading to their abnormal proliferation. If the process is not arrested it may progress until it causes the death of the organism.[8] . Biological response modifier, which alter tumour host metabolic and immunological relationships .Gene therapy, chemo preventive agents' cancer vaccine. Now days due to availability of improvised scientific modern instruments new chemical compounds can be easily isolated, identify and their pharmacological action can be evaluated.

Advanced studies can be conducted in cases of new and therapeutically active important compounds by their clinical use after a careful thorough investigation of their efficacy and toxicity studies. [9,10,11].

Some chemo preventive drugs can have severe side effects in some patients, which is an issue when considering long term administration of a drug to healthy people who may or may not develop cancer. For this reason, most chemo preventive drug recommended soldly for people at high risk of developing cancer because they are most likely to benefit from treatment [12].

MATERIAL AND METHODS:

Preparation of extracts

The leaves were extracted with various solvent such as Petroleum ether, chloroform ethyle acetate, ethanol and aqueous.

Preliminary Phytochemical analysis

The petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extract of *J. tranquebariensis* subjected to following test for the identification of its various phytoconstituents by standard method. Alkaloids were identified by Dragendroff's test, flavonoids were identified by Shinodha test, carbohydrates were identified by Fehling's test, proteins were identified by Million's test, Glycosides were identified by Borntrager's test, phenols were identified by Liebermann's test and tannins were identified by Ferric chloride test [13,14,15,16].

Quantitative phytochemical analysis

Estimation of total phenolic content

1ml (1mg/1ml) of various extracts of *J. tranquebariensis* (ethanol, chloroform, aqueous) were taken in separate test tube. Add 0.5 ml of Folin Ciocalteu reagent (1N) and allowed to stand for 15 minutes. Then 1 ml of 10% sodium carbonate solution was added. Finally the mixture were mixed with distilled water and made up of 10 ml, allowed to stand for 30 mins at room temperature and total phenols were determined by spectrophotometrically at 760nm [17,18,19].

Estimation of total Flavanoid content

An adequate quantity of quercetin was dissolved in ethanol to get a stock solution of 1mg 1ml. Further dissolution were made to get concentration ranging from 5-50 mg per ml. 1ml of the above standard solution were

taken in different volumetric flask 0.1 ml of aluminium chloride solution, 0.1 ml potassium acetate solution and 2.8 ml of ethanol were added and final volume was then made upto 5ml with distilled water. After 20 mins the absorbance was measured at 415 nm [20].

Pharmacological screening

A chemoprevention agent that blocks the very first step .But most researchers would consider a drug successful if it could stop the disease progressing from any stage to the next.

Many in chemoprevention are beginning to think that perhaps the best way to catch cancer is it target inflammation. Chronic inflammation appears to encourage tumours by prompting the growth of new blood vessels and a remodelling of the extra cellular matrix creating a prime setting for normal cell growth to turn malignant.

Chemo prevention aims to prevent premalignant cells from completing the process of carcinogenesis. These agents have clear cellular targets and are intended to treat people at high risk –those with a family history of disease or a known genetic mutation or who are already known to have pre cancerous cell [12].

Osmotic fragility test

The fragility of RBC was determined by placing the cells in graded series of hypotonic saline solutions buffered at pH 7.4 with 150 mM phosphate. Concentration ranging from 0.2% to 0.9% NaCl were made up to in a final volume of 10ml water and blood sample .A 10 µl sample washed RBC washed to 1990 µl of each hypotonic saline solution and immediately mixed by inverting several times .The tubes were allowed to stand for 150 min at room temperature .To determine the effect of the aqueous extracts 10µl of extract(30mg /ml)were added to 1980 µl of each hypotonic saline ,then 10 µl of RBC added and the mixture treated as described earlier. The number of RBC not lysed at each saline concentration was determined using a labomate 2000 microscope model and a haemocytometer (Neuberger's cell).Haemolysis was calculated using the following equation.

$$\text{Percentage of haemolysis} = \frac{\text{OD of test}}{\text{OD of control}} \times 100$$

$$\text{Percentage Of protection} = \frac{100 - \text{OD of test}}{\text{OD of control}} \times 100$$

The mean corpuscular fragility (determined from the concentration of saline causing 50% haemolysis of RBC) was obtained from a plot of lysis (%) versus NaCl concentration [21,22].

RESULT

The qualitative phytochemical analysis of *J. tranquebarensis* showed the presence of sterols, alkaloids, steroidal glycosides, carbohydrates, proteins, tannins, terpenoids and proteins (Table No: 1).

The mean corpuscular fragility (determined from the concentration of saline causing 50% haemolysis of RBC) was obtained from a plot of lysis (%) Vs NaCl concentration. 50% chemoprevention occurs at 100.95 microgram of aqueous extract of *J. tranquebarensis* (Table No: 4).

Pharmacological activity: Effect on chemo preventive activity of *J. tranquebarensis*

Table 1. Qualitative phytochemical analysis of *J. tranquebarensis*

S.No.	Tests	Pet.ether Extract	Chloroform Extract	Ethyl Acetate Extract	Ethanol Extract	Aqueous Extract
1	Sterol	+	+	+	+	-
2	Carbohydrate	-	-	-	+	+
3	Proteins	-	+	+	+	+
4	Alkaloids	-	-	-	+	-
5	Glycosides	-	-	-	-	-
6	Tannins	-	+	+	+	+
7	Flavonoids	+	-	+	+	-
8	Terpenoids	+	+	+	+	-

'+' - Presence, '-' - Absence

Table 2. Quantitative analysis of *J. tranquebarensis* total Phenolic content

Standard		Absorbance (nm)	Test		Absorbance(nm)			Total phenolic content
Sample	Conc. in $\mu\text{g/ml}$		Sample	Conc. in $\mu\text{g/ml}$	I	II	III	mg GAE/gm of extract
Gallic acid	2	0.229	Methanol	50	0.97	0.772	0.895	152.24
	4	0.452		100	1.753	1.365	1.532	133.92
	6	0.695						
	8	0.918						
	10	1.162						143.08

Total phenolic content of *J. tranquebarensis* was 143.08

Table 3. Quantitative analysis of *J. tranquebarensis* total Flavanoid content

Standard		Absorbance	Test		Absorbance			Total flavonoid content
Sample	Conc. in $\mu\text{g/ml}$		Sample	Conc. in $\mu\text{g/ml}$	I	II	III	mg of GAE/gm of extract
Quercetin	0	0	Me OH Extract of <i>J.tranqueb ariensis</i>	0.5	0.623	0.633	0.585	18.66
	5	0.099						
	10	0.215						
	15	0.371						
	20	0.532		1	1.31	1.41	1.135	35.620
	25	0.701						
	30	0.839						
	35	0.982						
	40	1.154						
	45	1.277						
	50	1.437						
								27.14

Total Flavanoid content of *J. tranquebarensis* was 27.14

Table 4. Osmotic fragility is measured using UV spectrophotometry at 540 nm

S.no.	Blood+plant extract + saline(A1) O.D	Blank O.D(A2)	Difference (A1-A2)O.D	Percentage Protection (%)
1	0.152	0.054	0.098	79
2	0.278	0.201	0.077	84
3	0.254	0.222	0.032	93
4	0.268	0.259	0.009	98
5	0.361	0.290	0.071	85

CONCLUSION

Finally, the present study suggested that, among the plant of leaves of *Justicia tranqubariensis*: family: Acanthaceae such as qualitative analysis, quantitative analysis which have been studied and presented. The chemo preventive activity has been evaluated using osmotic fragility method. Chemo preventive agents can be used in combination with chemotherapeutic agents to enhance the effect at lower doses and thus minimise

chemotherapy induced toxicity. Because cancer is primarily a disease of old age, less toxic therapy is a major priority. The phytochemical screening may serve to identify the phytoconstituents that are responsible for the chemo preventive action and this study may bring to light a lead molecule for treatment of cancer and inflammation in future. This is may help the future researchers to carry out further studies in detail.

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