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IN-VITRO PHARMACOLOGICAL ACTIVITIES OF *JASMINUM MALABARICUM WIGHT*

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ABSTRACT

Jasminum Malabaricum is a species of flowering plant in the family Oleacceae, native to southern parts of India and Sri Lanka. *Jasminum Malabricum* is used for the treatment of cataract, as a blood purifier and in cosmetic and detergent industries. It is known for its ethno medicinal importance like antibacterial, antioxidant, blood purifier, antitumor properties. It is also used for eye infections and leaves of this plant are crushed and are applied externally on wounds. In the present study the antioxidant activity and anticoagulant activity of aqueous extract and anticancer activity of methanol extract of the plant in study was investigated. The antioxidant activity was carried out by using hydrogen peroxide scavenging assay. The anticoagulant activity was carried out using Prothrombin Time test method. The aqueous extracts of *Jasminum Malabaricum*, was tested for blood coagulation effects in normal human blood plasma and was found to significantly prolong the Prothrombin Time (PT) of normal human blood plasma. The results were compared with standard Heparin. The anticancer activity was evaluated against human breast cancer cell line (MDA MB 231) by MTT assay method. The anticancer activity was dose dependent. IC_{50} value of anticancer activity against MDA MB 231 cell line was found to be 50µg/ml. Our results confirm that the methanol extract of *Jasminum Malabaricum* exhibited significant effect against MDA MB 231 cell lines and aqueous extract of the same had significant anticoagulant activity and antioxidant activity.

INTRODUCTION:

Medicinal plants- a powerful health and scientific interest in medicinal plants has burgeoned due to increased efficiency of new plant derived drugs, growing interest in natural products and rising concerns about the side effects of conventional medicine. Herbal medicine is also called botanical medicine or phytomedicine and is defined as the use of whole plants or part of plants to prevent or treat illness. Plant parts used include seeds, roots, leaves, bark or flowers. The use of various herbal remedies and preparations are described there out human history representing the origin of modern medicines. Many conventional drugs are plant based such as aspirin derived from bark of willow, digonin derived from fonglove, quinine derived from the bark of cinchona and many more. Based on

current research and financial investments into medicinal plants, it seems that they will continue to play important roles in human health [1]. *Jasminum Malabaricum* is used for the treatment of cataract, as a blood purifier and in cosmetic and detergent industries. It is known for its ethno medicinal importance like antibacterial, antioxidant, blood purifier, antitumor properties. The leaves and stems segments were used as explants for callus growth [2]. The liquid which comes out of the freshly cut stem of the plant is used to treat eye infections [3]. The plant is used in combination with other plants for the treatment for herpes [4]. The flowers of the plant are used for mental disorders [5]. The leaves of these plants are crushed in lime juice and are applied externally on wounds [6].

Experimental Section

Plant collection

The leaves, roots and bark of *Jasminum malabaricum wight* was collected from the North Districts of Goa in the month of July. The plant *Jasminum malabaricum wight* was identified and confirmed by botanist Dr. M. K. Janarthanam, Head, Department of Botany, Goa University, Goa, India.

Macroscopic Characters

The macroscopic characteristic evaluation was carried out for shape, size, color and fracture of the drug.

Extract preparation

The leaves, roots and bark of *Jasminum malabaricum* were shade-dried and pulverized to a powder in a mechanical grinder. The powder of the plant (200mg each) was extracted by maceration with solvent methanol and water separately. The methanol and water extract of *Jasminum malabaricum* were used for further studies.

Determination of Physico-Chemical Constant

Physico-chemical constants such as the percentage of Total ash value, Acid insoluble ash, Water soluble ash and Loss on drying were calculated based upon standard procedures prescribed in Indian Pharmacopoeia.

Preliminary Phytochemical tests

The methanol and aqueous extract of *Jasminum malabaricum wight* were screened for preliminary phytochemical constituents.

Antioxidant Activity

Hydrogen peroxide scavenging assay.

Hydrogen peroxide scavenging activity of aqueous extract of *J. malabaricum* was estimated by replacement titration. Aliquot of 1.0mL of 0.1mM of H_2O_2 and 1.0mL of various concentrations of aqueous extract of *J. malabaricum* i.e. 500 µg/ml, 1000 µg/ml, 1500 µg/ml and 2000 µg/ml, followed by 2 drops of 3% ammonium molybdate, 10.0ml of 2M of H_2SO_4 and 7.0ml of 1.8M of KI. The mixed solution was titrated with 5.09 mM oh $Na_2S_2O_3$ until yellow color disappeared. Ascorbic acid (200µg/ml) was used as standard [7]. Percentage of scavenging of hydrogen peroxide was calculated as:

$$\% \text{ inhibition} = V_o - V_1 / Vo \times 100$$

Where,

V_o was volume of $Na_2S_2O_3$ solution used to titrate the control sample in the presence of hydrogen peroxides (without aqueous extract of *J. Malabricum*). V_1 was the volume of $Na_2S_2O_3$ solution used in the presence of aqueous extract of *J. Malabricum.*

Anticoagulant Activity

a. Blood collection and plasma sample preparations:

Blood samples were drawn via venepuncture from healthy individuals, from the right arm of each individual and were placed separately in containers containing sodium citrate, (1 part citrate: 9 parts of blood sample) to prevent the clotting process. The blood samples were centrifuged for 15 minutes (3000rpm) to separate the pure platelet plasma from the red blood cells. The pure plasma was stored at room temperature in separate tubes until the Prothrombin test was carried out.

b. Anticoagulant assay:

The plasma 0.2ml was placed in a series of test tubes and incubated for 4 minutes at 37^0C in an incubator. To this, 0.2ml of aqueous extract of *Jasminum malabaricum* was added in different concentrations i.e. 200, 300, 400, 500µg/ml. To this mixture, 0.3ml of 25mM calcium chloride was added and immediately stopwatch was started. The tubes were shaken or tilted after every 5 seconds until the clot was formed which is indicated by the formation of highly viscous solution. The stopwatch was stopped immediately and time was noted down. This is the Prothrombin Time of plasma sample. The same procedure was followed to find the coagulation time using normal saline as a control and heparin as a standard, which is an anticoagulant available in the market [8].

Anticancer activity

MDA MB 231 cell lines were procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in DMEM and MEM respectively supplemented with 10% inactivated Fetal Bovine Serum (FBS), Penicillin (100IU/ml), Streptomycin (100µg/ml) and Amphotericin B (5µg/ml) in a humidified atmosphere of 5% CO_2 at 37^OC until confluent. The cells were dissociated with TPGV solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in $25cm^2$ culture flask and all experiments were carried out in 96 microtiter plates (Trasons India Pvt. Ltd., Kolkata, India). Anticancer studies were performed as per Francis *et al*[9],each weighed test drugs were separately dissolved in distilled DMSO and volume was made up with DMEM and MEM respectively supplemented with 2% inactivated FBS to obtain a stock solution of 1mg/ml concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out anticancer studies. The monolayer cell culture was trypsinized as and the cell count was adjusted to 1.0 × 105 cells/ml using DMEM and MEM respectively containing 10% FBS. To each well of the 96 well microtiter plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100µl of different test concentrations of test drugs were added on to the partial monolayer in microtire plates. The plates were then incubated at 37^OC for 3 days in 5% CO_2 atmosphere, and microscopic examinations were carried out and observations were noted every 24h intervals. After 72h, the drug solutions in the wells were gently shaken and incubated for 3h at 37^OC in 5% CO_2 atmosphere. The supernatant was removed and 100µl 0f propanol was added and the plates were gently shaken to solubilize the formed formazen. The absorbance was measured using microplate reader at a wavelength of 540nm. The percentage growth inhibition was

calculated using the following formula and half maximal inhibitory concentration (50% IC, or IC_{50}) values was determined.

% Growth inhibition = 100 – Mean OD of individual test group / OD of Control group X 100

RESULTS AND DISCUSSION

India is one of the most promising regions for discovering novel biologically-active substances from its flora. More efforts are needed to explore potent anticancer and insecticidal plants from the mother earth and save humans around the world.

Macroscopic Characters

The macroscopic characters of *Jasminum malabaricum wight* root, stem and bark can serve as diagnostic parameters for the correct identification of the plant.

Physico-Chemical Characters

Ash value represents the purity of the drug. The percentage of Total ash value, Acid insoluble ash, Water soluble ash and Loss on drying of fruits of *Jasminum malabaricum wight* were found to be 2.7%, 0.7%, 0.8% and 11.1% respectively. (Table. 1)

Preliminary Phytochemical tests

The methanol extract showed the presence of Steroid, Alkaloid, Flavanoid, Saponins, Terpenoids, Reducing sugars, Phenolic compounds, Xanthoprotiens, Carboxylic acids and Glycosides. The aqueous extract showed positive results for test for Alkaloid, Saponins, Tannins, Terpenoids, Reducing Sugar, Phenolic compounds, Xanthoprotiens, Carboxylic acids and Glycosides.

Antioxidant Activity

In-vitro antioxidant activity of aqueous extract of *Jasminum Malabricum Wight* by Hydrogen peroxide scavenging assay was presented in table 3.

The Hydrogen peroxide scavenging assay exhibited effective inhibition activity of aqueous extract of the plant *Jasminum malabaricum wight* and ascorbic acid. The antioxidant activity of aqueous extract of *J. malabaricum wight* was found to increase in a dose dependent manner. The extract showed maximum inhibition of 66.6% at 2000µg/ml. The antioxidant activity of extracts may be due to the presence of phytochemicals. Flavonoids, Tannins and Phenolic compounds are a major group of compounds that act as primary antioxidants of free radical scavengers. Similarly, Terpenoids and vitamins, act as regulators of metabolism and play a protective role as antioxidants [10].

Anticoagulant Activity

In-vitro anticoagulant activity of water extract of *J.malabricum wight* by Prothrombin Time method was presented in table.4. The aqueous extracts of plant *Jasminum malabaricum wight* was tested for blood coagulation effects in normal human blood plasma and was found to prolong the Prothrombin Time (PT) of normal human blood plasma. The results were presented in Table 3, it can be seen that the extract showed good anticoagulant activity, the activity of 500µg/ml concentration solution of plant extract showed little higher prolongation than control but non concentrations showed higher activity than standard heparin. This may be due to the presence of sterols, flavanoids, terpenoids, and cardiac glycosides[11].Thus, it can be said that the plant *J. malabaricum wight* exhibits satisfactory anticoagulation activity.

Anticancer activity

In-vitro anti-cancer activity of methanol extract of *J.malabricum wight* against human breast cancer cell line (MDA MB 231) using MTT assay method was presented in Table.5.

S.no	Parameters	Values
1	Total ash value	2.7%
2	Acid insoluble ash	0.7%
3	Water soluble ash	0.8%
4	Loss on drying	11.1%
5	Extractive values:	
	1. Per Ether	1.4%
	2. Chloroform	2.6%
	3. Methanol	7.4%
	4. Water	5.6%

Table 1. Physico-Chemical Characters of the plant *Jasminum malabaricum wight.*

S .No	Phytochemical Constituents	Presence(+)/Absence(-)	
		Methanol Extract	Water Extract
1	Steroids	+	-
2	Alkaloids	+	+
3	Flavanoids	+	-
4	Saponins	+	+
5	Tannins	-	+
6	Terpenoids	+	+
7	Triterpenoids	-	-
8	Anthroquinones	-	-
9	Reducing sugars	+	+
10	Phenolic compounds	+	+
11	Xenthoproteins	+	+
12	Carbohydrates	-	-
13	Carboxylic acids	+	+
14	Proteins	-	-
15	Cardinoloids	+	+

Table 2. Preliminary Phytochemical studies of the Methanol and Aqueous extract of the plant *Jasminum malabaricum wight.*

Concentration (µg/ml)	% Free radical scavenging activity
500	7.0%
100	22.2%
1500	44.4%
2000	66.6%
Standard (Ascorbic acid 200µg/ml)	86%

Table.3. *In vitro* Antioxidant studies of aqueous extract of Jasminum Malabaricum Wight

Concentration (µg/ml)	Coagulation time (PT time) in mins
200	1.37
300	1.43
400	2.06
500	2.11
Control (Saline)	2.10
Standard (Heparine)	2.26

Table.4. *In vitro* Anticoagulant activity of aqueous extract of J.malabaricum wight

S.No.	Sample	Concentration	Absorbance	Results as observed (% lysis)	IC_{50} (µg)
1	JMW	10	1.013	0	50µg
2		20	1.004	0.88	
3		25	0.974	3.84	
4		30	0.693	31.58	
5		50	0.502	50.44	
6	Control	0	1.013		

Table.5.*In-vitro* Anticancer properties of the methanol extract of *J.malabaricum wight* against MDA MB 231 cell lines of human breast cancer.

The present study shows a significant *In-vitro* anticancer activity of methanol extract of the plant *Jasminum Malabricum Wight* on human breast cancer cell line (MDA MB 231) at increasing concentrations. IC_{50} was found to be 50µg/ml. Phyto-constituents such as terpene, flavonoids and tannins are biologically active against different human cancer cell lines[12]. The presence of these phytoconstituents may be responsible for the anticancer activity of *Jasminum Malabaricum Wight.*

CONCLUSION

The investigation suggests that the aqueous extract of plant *Jasmunum malabaricum wight* possess significant antioxidant and anticoagulant activity. This study also concludes that the methanol extracts of *Jasminum malabaricum wight* possess significant *Invitro* anticancer activity with increasing concentrations. It is anticipated that this plant would be useful pharmaceutical material to treat breast cancer. Future research should focus on the molecular mechanism of *Jasminum malabaricum wight*. There is a need for further investigation of this plant in order to identify and isolate its active principle(s) to treat Cancer.

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