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ANTICANCER ACTIVITY AND EVALUATION OF SYNERGISTIC ACTIVITY OF *CAPPARIS DIVARICATA* LAM. AND *NYCTANTHES ARBORTRISTIS* LINN.

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ABSTRACT
Nyctanthes arbortristis Linn is reported to have anticancer activity. The effort was made to evaluate anticancer activity of leaves of plant *Capparis divaricata* Lam & further to test the synergism effect Between ethanolic and aqueous extracts of both the plants. By using MTT assay method Percent inhibition shown by combination of both plant extracts was found to be 69.56, 70.65, 72.82 with the interval of 5, 50, 100µg/ml. Both the individual plant extracts *Capparis divaricata* Lam. and *Nyctanthes arbortristis* Linn showed percent anticancer activity. Standard drug 5 FU showed percent inhibition in 84.78%.

KEY WORDS: *Capparis divaricata Lam, Nyctanthes arbortristis Linn*, synergism, Anticancer activity.

INTRODUCTION
Capparis divaricata Lam. commonly known as caper bush, belonging to the genus *Capparis* of family Capparidaceae, found throughout the India especially in the Deccan Peninsula from Maharashtra southwards to Tamil Nadu.

The fruits, roots, and seeds of *Capparis* have been used traditionally as antirheumatic, tonic, expectorant, antispasmodic and analgesic agents in Turkey and other countries. *Capparis Divaricata* Lam also shows analgesic, Locomotor and Diuretic and anticancer activity of ethanolic extract. Phytochemical screening of Ethanolic extract of leaves of *Capparis divaricata* Lam shows presence of glycosides, saponins, alkaloids, flavonoids, tannins and phenolic compounds.[1,2]

Nyctanthes arbortristis Linn. (Oleaceae) is popularly known as 'Night Jasmine' (English) or 'Harsinghar' (Hindi) due to the fact that its flowers emit a very strong and pleasant fragrance during the whole night.[3,4] The flowers start falling after midnight and by the day break, the plant appears dull. The generic name 'Nyctanthes' has been coined from two Greek words 'Nykhta' (Night) and 'anthos' (flower). The specific name 'arbortristis' meaning 'the sad tree' is supposedly derived from dull looks of the tree during daytime. *Nyctanthes arbortristis Linn* is also called the "tree of sorrow", because the flowers lose their brightness during daytime; the scientific name arbortristis also means "sad tree".[5,6]

Different parts of *Nyctanthes arbortristis* Linn.are known to posses various ailments by tribal people of Indian subcontinent with its use in Ayurveda, Sidha and Unani systems of medicines. The flowers are used as stomachic, carminative, astringent to bowel, The powdered Stem bark is given in rheumatic joint pain, malaria and also used as expectorant.The leaves are used in Ayurvedic medicine for for the treatment of various diseases such as chronic fever, rheumatism, laxative, diuretic, and in cough reduction.[7] The Seeds are used for anthelmintics, expectorant. *Nyctanthes arbortristis* Linn Lam shows presence of Glycosides, Alkaloids, Carbohydrates, Tannins.[8,9]

MATERIALS AND METHODS
Chemicals and reagents: Reagent Grade chemicals were used for this assay., 10% MEM possessing L-glutamine (4 mmol/L), 10% fetal bovine serum (FBS), streptomycin (100 µg/mL) and penicillin (100 units/mL). It is incubated in a humidified atmosphere containing 5% CO2 with 95 % air at 37°C., MCF-7 (Breast Cancer Cell Lines free of pathogens) were purchased from NCCS pune.

Plant material: The fresh leaves of *Capparis divaricata* Lam. (Capparidaceae) were collected at the flowering stage in August from Sangli District, Maharashtra State, India. And the leaves of the *Nyctanthes arbortristis* Linn. were collected from the local areas of (Bohali) Pandharpur. Both the plants were authenticated taxonomically from the Botanical survey of India Pune.

Collection No.RVP01(2012) & PFA 07. The leaves of the plant *Capparis divaricata* Lam were then dried in shade at room temperature for about 30 to 45 days, after which these parts were chopped and ground. The leaves of the plant *Nyctanthes arbortristis* Linn. were sorted carefully and washed thoroughly to remove dirt and debris. The materials were dried in the open air/shade drying.

Preparation of the extract[10,11]

A. *Capparis divaricata* leaves Extract: For the preparation of extract about 100 g of air dried, powdered leaves were charged in to Soxhlet's apparatus and successively extracted with 95% ethanol at room temperature for 7 days. The extract was evaporated to dryness in rotary evaporator. The yield of ethanolic extract was obtained as 2.5 % w/w. Moreover, the extract was subjected to preliminary phytochemical screening for the detection of various plant constituents.

***B. Nyctanthes arbortristis* Linn. Extract:** Shade dried leaves of *Nyctanthes arbortristis* Linn were used for extraction The extraction of leaves of plant material were done by Decoction method by using water as a solvent.Decoction is done by boiling the herb in water.50 gm of deffated leaves were taken in to a 1000 ml beaker and decoction extraction by using water solvents. It was heated on a boiling water bath for 30 minutes. and filtered. The excess of solvent was removed. The extract was subjected to preliminary phytochemical screening for the detection of various plant constituents.

Procedure: The **MCF-7** cell line was maintained in DMEM medium supplemented with 10 % fetal bovine serum. The cells were plated at a density of 1×10^4 cells per well in a 96-well plate, and cultured for 24 h at 37 °C. The cells were subsequently exposed to 5µg/ml, 50µg/ml , 100µg/ml concentration of *Capparis divaricata Lam Nyctanthes arbortristi*sis Linn Combination of both plant . After drug incubation add 50 µL TCA (50%) and kept for 1 hour in 4^0C then plate washed with TDW (triple distilled water) and air dry the plate. Then add 100 µLSRB dye in each well and kept for 30min at room temperature. Again wash three times with 1% acetic acid and air dry the plate the add 200 µL tris buffer, and absorbance was read at 490 nm. The results were compared with the standard drug inhibitors 5 flurouracil. (20µg/ml).[12]

Lastly percent cytotoxicity of the compounds was calculated by using following formula.

Percent Cytotoxicity = Reading of control - Reading of treated cells / Reading of control X 100.

RESULTS AND DISCUSSION

Table no. 1 Synergistic anticancer activity of *Capparis divaricata Lam and Nyctanthes arbortristi*sis Linn on MCF-7 cell line (Human breast cancer)

Extracts	Reading	% inhibition
Negative control	0.092	
Positive control (20µg/ml**)**	0.014	84.78
Capparis divaricata Lam (5µg/ml)	0.064	30.43
Capparis divaricata Lam (50µg/ml)	0.060	34.78
Capparis divaricata Lam (100µg/ml)	0.048	47.82
*Nyctanthes arbortristi*sis Linn (5µg/ml)	0.055	40.21
*Nyctanthes arbortristi*sis Linn (50µg/ml)	0.047	48.91
*Nyctanthes arbortristi*sis Linn (100µg/ml)	0.036	60.86
Combination (5µg/ml)	0.028	69.56
Combination (50 µg/ml)	0.027	70.65
Combination (100µg/ml)	0.025	72.82

The results anticancer activity [Table 1] showed that the combination extracts of *Capparis divaricata Lam* (5,50 & 100 µg/ml), (*Nyctanthes arbortristis* Linn 5,50 &100 µg/ml), combination (5,50 & 100 µg/ml) significantly increased anticancer activity in comparison with 5-flurouracil (20µg/ml).

DISCUSSION

All the observations and results of the proposed work conclude that extract of *Nyctanthes arbortristis* Linn has anticancer activity against breast cancer while in combination of extracts of *Capparis divaricata* Lam. and *Nyctanthes arbortristis* Linn Linn reveal synergism in anticancer activity action much better as compared to standard drug. This effect may be attributed to the presence of flavones and tannins present in both the extracts.

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