Pharmacognostic and Phytochemical Investigation, Molecular Docking Studies of Phytoconstituents and Anticancer Potential of Capparis Decidua (Forsk) Edgew

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Abstract

Capparis decidua (Forsk.) Edgew. belonging to family Capparidaceae is well known for curing a variety of ailments such as toothache, cough, asthma, intermittent fever and rheumatism. The present study was undertaken to investigate the pharmacognostical and phytochemical parameters Capparis decidua. The Molecular docking analysis of the target proteins with the phytochemical ligands was performed using VLifeMDS software. The docking scores and analysis of the interactions of the phytoconstituents with target proteins suggests that phytoconstituents like palmitic acid have the ability to bind to multiple targets. Brine shrimp model that may prove quite helpful as a preliminary screening to determine toxic properties. In Brine shrimp lethality bioassay, extracts produced dose dependent cytotoxicity effect to brine shrimp nauplii. Antiproliferative and cytotoxic effects of the Capparis decidua methanolic extract was determined using MTT assay and shows significant anticancer activity.

Keywords: *Capparis decidua, Pharmacognostical evaluation, Molecular docking, Brine shrimp lethality assay, Anticancer activity.*

1. INTRODUCTION

Capparis decidua (Forsk.) Edgew, commonly known as Kair, is very important medicinal plant and it belongs to the family Capparidaceae. *Capparis decidua* is very important and belongs to the Capparidaceae family, commonly referred to as Kair[1]. Kair is a glabrous densely ramified spiny shrub or small tree, found in a bare and dry habitat of sind, arabia, west and central india[2-4]. Microwave extraction method has proven to be more powerful and effective than its soxhlet

extraction method. Microwave uses electromagnetic radiation that passes through material and causes oscillation of molecule which produces heat. The phenomena of the producing heat by electromagnetic irradiation are either by conduction or collision. All the wave energy changes its polarity from +ve to -ve with each cycle of wave this cause rapid orientation and reorientation of molecule which cause heating by collision. Microwave heating produces heat in entire material in the same rate and the same time at a high speed and at a high rate of reaction. The soxhlet extraction, which is a common technique, is a continuous solvent extraction method[5-9]. In contrast with the soxhlet extraction, studies have shown that microwaves use a lower volume of solvent and sample and perform extraction at a much faster rate. Computational studies are the crucial steps in the drug designing. Docking study is the computational routine to determine probable binding manners of a ligand to the dynamic site of a receptor. It makes an image of the dynamic site with interaction points known as grid. Then it fits the ligand in the binding site either by grid search or energy search. Due to failure of ADME so it necessary to perform docking studies before pharmacological activity. An outbreak of coronavirus disease (COVID-19) caused by the novel severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) raises an unparalleled challenge in the discovery of appropriate drugs for prevention and treatment[10-23]. Given the rapid pace of scientific research and clinical data produced by the large number of people quickly infected with SARS-CoV-2, clinicians need reliable proof of successful medical care for this infection as in intial stage with help of molecular docking software it is easy to do research. The chemical modification of drug delivery system for protein and peptide drugs is important in improving both enzymatic stability and membrane permeations can help to have good biological activity from any heterocyclic compound modification. Someday soon, you might be making your own medicines at home. That’s because researchers have tailored a 3D printer to synthesize pharmaceuticals and other chemicals from simple, widely available starting compounds fed into a series. Brine Shrimp lethality bioassay is a rapid and comprehensive bioassay for the bioactive compounds of synthetic origin. The method is attractive because it is very simple, inexpensive, and low toxin amounts are sufficient to perform the test in microwell scale. Evaluation of toxic properties of a substance is crucial when considering for public health protection because exposure to chemicals can be hazardous and results to adverse effects on human being[24-35]. Breast cancer is the world's leading cause of cancer death and the most common cancer among women. Natural products have been used since ancient times for the

treatment of many diseases. Before the 20th century, 80% of all medicines used to treat human and animal illness were obtained from the leaves, barks and roots of medicinal plants. During that period, crude botanicals were percolated in readily available fluids like alcohol and the doctor will prescribe tablespoons of the fluid extract to be taken for a period. It is noteworthy to mention that about 70% of the drugs used today are models of natural products. Biodiversity and traditional medical knowledge had provided useful lead compounds for cancer chemotherapy. The present study investigates the molecular docking study, in-vitro cytotoxicity of the extract of the *Capparis decidua* and the active fractions against artemia salina nauplii and MCF-7 cell line[36].

2. MATERIALS AND METHODS

Collection of plant material

Stem of *Capparis decidua* were collected from the residential areas of Vita, Sangli, Maharashtra, India and authenticated from the Department of Botany, Y.C. College of Science, Karad. A voucher specimen (No. RMB 001) was submitted in the department.

Preparation of plant extract

Shade drying was done for almost a month as to avoid chemical degradation due to sunlight. Grinding of the dried material was done, with the aid of a grinder and converted into coarse powder. Extraction of *Capparis decidua* was done by microwave extraction further filtered and excess solvent present was evaporated and dried extract were collected and subjected for further studies.

Chemicals and Instruments

Motic microscope, glass slides, cover slips, watch glass and other common glass ware were the basic apparatus and instruments used for the study. Solvents used for extraction includes viz. petroleum ether, methanol and ethanol, water and reagents viz. phloroglucinol, glycerine, KOH, saffarin etc.

A. Pharmacognostic and Phytochemical Evaluation

Pharmacognostical Studies

Transverse section is obtained by cutting along the radial plane of a cylindrical portion of the stem/root and perpendicular to the long axis. The stem/ root sample having a diameter of 10 to 70 cm in height and young leafs were selected. The sample is boiled in water over bunsen flame for few minutes. Microscopic sections were cut by free hand sectioning. Stained with dyes and observed under microscope.

Phytochemical Screening

Powder material of Capparis decidua was taken and the powder was mounted on clean glass slide stained the cleared powder with staining reagent like phloroglucinol and conc. HCl, Dil. Iodine solution was used as staining reagent. Keep aside slide for few seconds for staining. The glass slide was viewed under 10X of Motic digital photomicroscope to determine type of cell, nature of cell wall present and cell content. All characters were compared with standard drug characters.

Determination of Moisture or Determination of Loss on drying (LOD)

Loss on drying is the loss of mass expressed as percent w/w. The test for loss on drying determines both water and volatile matter in the crude drug. Determination of Moisture content or determination of Loss on Drying (LOD) are done by using one of the method such as gravimetric, volumetric and instrumental shown the below. An accurately weighed quantity (1.5 g) of powdered drug was taken in a porcelain dish. The sample was kept in the oven at a temperature 105^0C for 2 hrs. The dish was cooled at room temperature; the procedure was repeated till constant weight is observed. Loss on drying was calculated using the following formula.

$$\% \text{ Loss on drying} = \frac{\text{Loss in weight of sample}}{\text{Weight of sample}} \times 100$$

Determination of Total ash value

2 g of powder was weighed and taken in dish previously ignited and weighed. Plant parts were burned using muffler Furness. Heat more strongly until all carbon is burnt off. Cool in

desiccators. Weight the ash and calculate the percentage of total ash with reference to the air dried sample of the crude drug.

$$\% \text{ Total ash value} = \frac{\text{Weight of total ash}}{\text{Weight of crude drug taken}} \times 100$$

Determination of Acid insoluble ash value

Proceed as per the steps mentioned in the procedure for determination of total ash value of a crude extract. Further, using 25 ml of dilute hydrochloric acid, wash the ash from the dish used for total ash into a 100 ml beaker. The above solution was boiled for 5 minutes. The solution was filtered through filter paper and residue was washed twice with water. The porcelain was ignited, cooled and weighed. The filter with residue was heated gently until the vapour ceased and then it is heated strongly until whole carbon was removed. Cool in desicator. Weigh the residue and calculate acid- insoluble ash of the crude drug with reference to the air dried sample of the crude drug.

$$\% \text{ Acid insoluble ash value} = \frac{\text{Weight of acid – insoluble ash}}{\text{Weight of crude drug}} \times 100$$

B. Molecular Docking Study

Molecular docking study was performed using VLifeMDS 4.1 software on phytoconstituents structures of *Capparis decidua*. VLifeMDS 4.1 software provided both rigid (no torsional flexibility for a protein as well as a ligand) and flexible (torsional flexibility to a ligand with a rigid protein) docking of the molecules. The target or receptor was either experimentally known or theoretically generated through knowledge-based protein modeling. The molecular docking tool has been developed to get a preferred geometry of interaction of ligand–receptor complexes having minimum interaction energy supported different scoring functions viz. only electrostatics, the sum of steric and electrostatic (parameters from the force field), and the dock score. This utility allowed us to screen a set of compounds for lead optimization. VLifeMDS uses to minimize the interaction energy between the ligand and receptor protein.

Selection of receptor

The crystal structure potent inhibitors of NUDT5 silence hormone signaling in breast (PDB Code-5NQR) was downloaded from protein data bank and water molecules in the crystal structure were deleted. The optimized receptor was then saved as mol file and used for docking simulation.

Ligand preparation

The 2D structures of the compounds were built and then converted into the 3D. The 3D structures were then energetically minimized up to the rms gradient of 0.01 using MMFF.

Identification of cavities

By using cavity determination option of software, cavities of enzyme were determined. The cavities in the receptor were mapped to assign an appropriate active site. The basic feature used to map the cavities were the surface mapping of the receptor and identifying the geometric voids as well as scaling the void for its hydrophobic characteristics. Hence, all the cavities that are present in receptor are identified and ranked based on their size and hydrophobic surface area considering the dimensions and hydrophobic surface area, cavity with found to be the best void as an active site.

Scoring function

Distinction of good or bad docked conformation is based on scoring or fitness function. MDS uses fitness functions on only electrostatic and both steric and electrostatic interactions between receptor ligand as well as dock score scoring function. The dock score compute binding affinity of a given protein-ligand complex with known 3D structure[37].

C. Brine Shrimp Lethality Assay

For the bioactive compound of either natural or synthetic origin, this is a rapid and comprehensive test. It is also an inexpensive and simple test as no aseptic techniques are required. It easily utilizes a large number of organisms for statistical validation and requires no special equipment and relatively small amount of sample (2-20 mg or less) is necessary.

Preparation of reagents

Serial dilution of extract: Clean test tubes were taken and labelled. Plant extract of 10 mg was weighed by an analytical balance. Then stock solution was prepared by dissolving 10 mg of plant extract (soluble in water) in 1 ml of water. Concentrations of 100 µg/ml, 500µg/ml and 1000µg/ml were prepared by serial dilution from the stock solution. Five test tubes were labelled as 1-3. Then 1 ml of prepared solution was taken into the respect test tubes containing 10 nauplii and 1 ml of seawater. The number of dead nauplii was counted after 24 hours.

Hatching brine shrimp

Measured 3 liters of water using measuring cylinder and pour into the rectangular jar. Weighed about 27 g of table salt by an balance and add it into the jar containing water. Mix the water with a spatula. Place the tip of an airline from a air pump into the bottom of the jar maintaining proper aeration. Add about 15 g of brine shrimp eggs at the top water level of the jar and mix with the water. Switch on a light (60-100 Watt bulb) placed a few inches away from the jar. After 20-24 hours, the nauplii will hatch. Observe the eggs and nauplii. Collect the nauplii after the next 24 hours. Hatched nauplii must be separated from the empty egg. It can be done by turn off the air and switch off the lamp. The empty egg will float while the brine shrimp will concentrate in the water column. Transfer 10 nauplii to a test tube using a Pasteur pipette. Expose the nauplii to different concentrations of the plant extract. Count the number of survivors and calculate the percentage of death after 24 hours.

Calculations

The percent of lethality of the nauplii for each concentration was calculated. For each tube, count the number of dead and number of live nauplii, and determine the % death and LC_{50} values were calculated[38].

$$\% \text{ death} = \frac{\text{Number of dead nauplii}}{\text{Number of dead nauplii} + \text{Number of live nauplii}} \times 100$$

D. Anticancer Evaluation

MTT assay and Anti proliferative activity

The in-vitro anti-proliferative activity was carried out on human carcinoma cell lines namely MCF-7. All the cell lines were grown in DMEM-HG supplemented with 10% heat inactivated FBS, 2% Penicillin-Streptomycin and 2.5 µg/mL Amphotericin-B solutions. Cell lines were incubated at 37°C in a humidified atmosphere of 95% air, 5% CO_2. Following 24-48 hrs. of incubation period, the adherent cells were detached using Trypsin-EDTA solution. Cell count was determined using the Luna automated cell counter based on trypan blue dye exclusion method. Cytotoxicity of *Capparis decidua* extract have been determined using MTT 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay.

Cell Viability Assay (MTT Assay)

200µL cell suspension was seeded in 96-well microplates at a density of 25,000 cells/well and incubated for 24hrs, all cells were seeded in duplicates with *Capparis decidua* extract having range of concentrations from 50µM-500µM, incubated in a CO_2 incubator at 37°C. Treated cells were thereafter incubated with 10% MTT (5mg/ml) for 3 hrs. The culture medium was then aspirated and 200µL Dimethyl sulfoxide (DMSO; Sigma-Aldrich, India) was added. 5-Fluorouracil (5-FU) was used as standard. Cell viability was determined by measuring the absorbance on a microplate reader at 570nm. Cell viability was calculated as a percentage of viable cells at different test concentrations relative to the control (5-FU) cells [% cell viability = (A570 of treated cells /A570 of control cells) ×100%][39-40].

3. RESULTS AND DISCUSSION

A. Pharmacognostic and Phytochemical Evaluation

Macroscopical Characters of stem

For organoleptic testing, the plant was morphologically examined such as colour, odour, taste, fracture, form and structure. The morphological examination of the stem of *Capparis decidua* is shown below in the Table 1

Fig 1. *Capparis decidua*

Table 1. Morphological examination of stem of *Capparis decidua*

Characters	Observation
Shape	Each branch smooth, terete and spinous
Colour	Green
Taste	Characteristic
Odour	Odorless
Fracture	Rough and corky covered with paired thorns

Microscopical characters of stem

The transversal section and the longitudinal section were produced and installed in glass slide as part of quantitative microscopy in an effort to identify internal structures such as vascular bundles, the cortex and other elements using iodine and safranine solution by using fresh stem bark of plant. The stalk was cut into small pieces and allowed to dry in the shade. Dry materials have been processed in a coarse powder mixer. For the identification of powder microscopic characters, material from the dried stem was used. Phloroglucinol, saffarin, glycerin and iodine

were treated separately, with the exception of lignified cells, calcium oxalate crystals and starch grains.

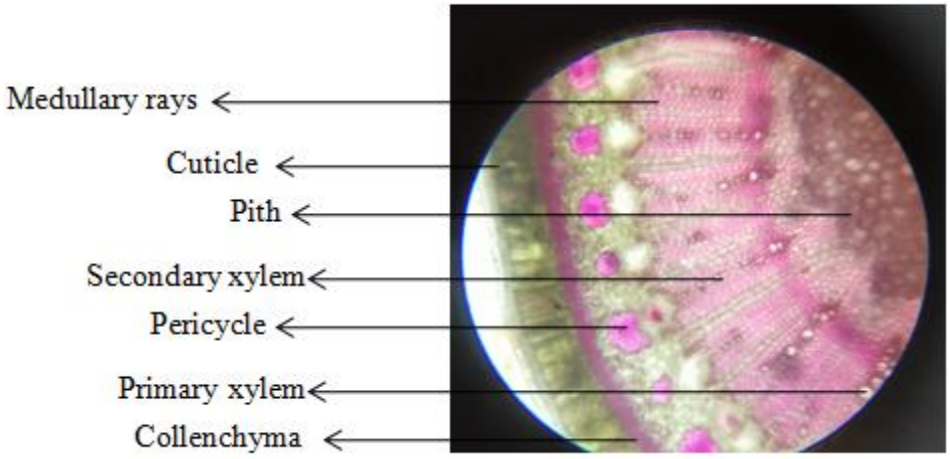

Fig 2. T. S of *Capparis deciduas* stem

Powder microscopy

Powdered microscopy showed the presence of trichomes, fibers, calcium crystals, starch grains, xylem vessels and oil globules and tracheids.

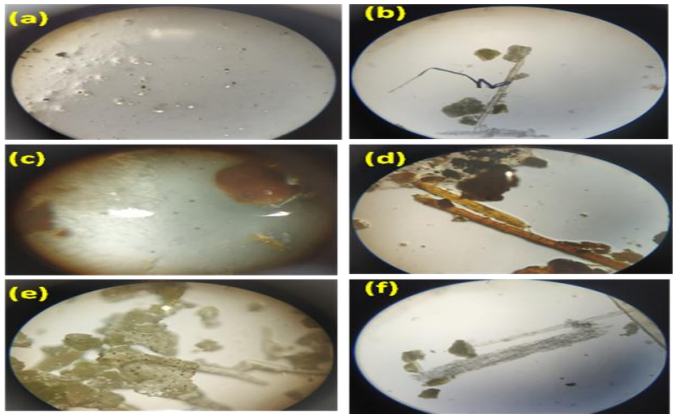

Fig 3. (a-f): Powder characteristics of *Capparis decidua* showing (a) Calcium oxalate crystals, (b) Fibre, (c) Starch grains, (d) Pericyclic fibres, (e) Tracheids, (f) Xylem vessels

Qualitative phytochemical analysis

The powder of dried stem was subjected to successively microwave extraction with organic solvent methanol respectively. After concentration and drying of extract identification of phytoconstituents was carried out using chemical test as shown in Table 2.

Table 2. Qualitative phytochemical analysis of Capparis deidua stem extracts

Sr. no	Test	Observation	Inference
1	Molish test	Violet ring at the junction of two liquid	Carbohydrate present
2	Benedicts test	Green colour	Reducing sugar present
3	Barfoed test	Red ppt.	Monosaccaride sugar present
4	Test for pentose sugar	Red colour	pentose sugar present
5	Test for alkaloids (dragendrof's test)	Reddish ppt.	Alkaloids present
6	Test for proteins (precipitation test)	White colloidal ppt.	Proteins present
7	Test for saponin glycosides	Foam observed	Glycosides present

Physiochemical evaluation

To determine the different ash values and extractive values of each powered drug, procedures from herbal and ayurvedic pharmacopeia were followed. Determination of ash values: Determination of ash values are meant for detecting low grade products, exhausted, sandy or earthy matter and inorganic matters. It can also be utilized to detect the chemical constituents by using water soluble ash and acid insoluble ash.

Table 3. Loss on drying of plant powder

Sr no.	Extract	Loss on drying (w/w)
1	Chloroform	10%
2	Aqueous	18%
3	Methanol	12%
4	Ethanol	16%
	Petroleum Ether	14%

Table 4. Total ash values of plant powder

Sr no.	Extract	Total ash values (w/w)
1	Chloroform	12%
2	Aqueous	4%
3	Methanol	10%
4	Ethanol	14%
5	Petroleum Ether	18%

Table 5. Acid insoluble ash values of plant powder

Sr no.	Extract	Acid insoluble ash values (w/w)
1	Chloroform	10%
2	Aqueous	9%
3	Methanol	7%
4	Ethanol	4%
5	Petroleum ether	6%

B. Molecular Docking Results

The structure of phytoconstituents of capparis decidua were evaluated for their anticancer activity against breast cancer. The dock score of compound palmitic acid has shown in table 6 and in that phytoconstituents showed good binding score of -52.99 shown minimum dock score than other compounds. As we compared result with other phytoconstituents to the literature this docking score indicate that the designed compounds have good binding affinity for binding to receptor potent inhibitors of NUDT5 silence hormones signaling in Breast cancer (PDB Code-5NQR). The best pose obtained by docking results is reported where main interaction between ligand and receptor can be observed. All designed compound adopt a very similar conformation at binding pocket, showing hydrogen bond interaction with amino acid of TRP355A, THR356A, LEU357A, SER446AVander Waals binding with amino acid ofTRP64A, ARG94A, PHE301A, TRP355A, LEU357A, ASP358A, ALA486B which shown by 2D representation diagram (Fig 4). Superimpose image of palmitic acid with receptor show in diagram (Fig 6). The dock score of standard drug 5-fluorouracil was found -53.65.

Table 6. Anticancer activity result of molecular docking studies by using GRIP batch docking

Sr no.	**Phytoconstituents**	**Dock scores (Kcal/mol)**
1	Capparisine	-38.40
2	Palmitic acid	-52.99
3	Isocodonocarpine	-29.42
4	Stachydrine	-11.20
5	N-hexadecanoic acid	-30.14
6	Arachidic acid	-18.82
7	Stearic acid	-25.81
8	Stachydrine	-35.64

9	5-fluorouracil (std)	-53.65

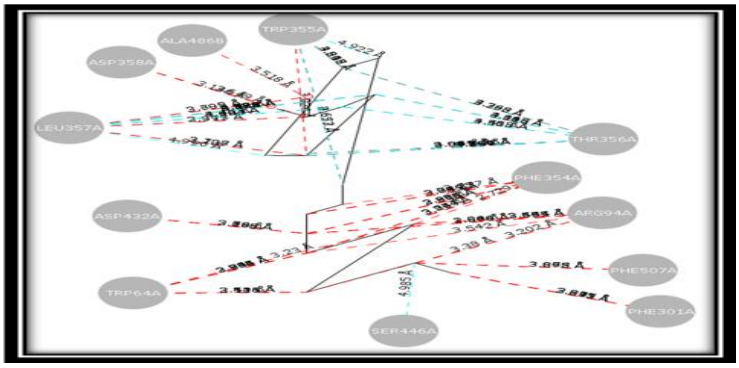

Fig 4. 2D representation of docking poses palmitic acid

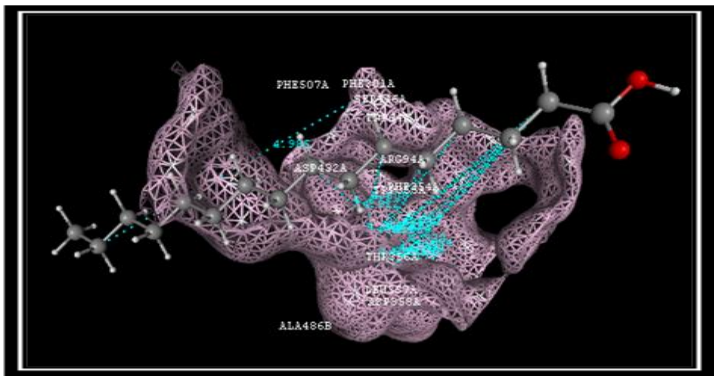

Fig 5. 3D Docking Poses of palmitic acid

Fig 6. Superimpose image representation of docking poses of palmitic acid

C. Brine Shrimp Lethality Assay

The brine shrimp lethality bioassay is found to have a good correlation with cytotoxic activity in some human solid tumors and with pesticidal activity, and led to the discovery of new class of natural pesticides and active antitumour agents. The observed cytotoxic activity for these extracts may be due one of these mechanisms. From Table 7 it is evident that methanolic extract was found to be significant activity against brine shrimp nauplii and LC_{50} was found 161.5 µg/ml.

Table 7. Brine shrimp lethality bioassay of *capparis decidua extract*

Fractions	**Concentration**	**Total number of shrimp/ tube**	**Shrimp survived**			**Total number of Shrimp survived**	**% inhibition**	**LC_{50}**
			T1	**T2**	**T3**			
Chloroform	100 µg/ ml	10	5	4	6	15	50.00	655
	500 µg/ ml		3	4	5	12	60.00	
	1000 µg/ ml		7	6	5	18	40.00	
Aqueous	100 µg/ ml	10	9	8	8	25	16.67	>1000
	500 µg/ ml		7	6	8	21	30.00	
	1000 µg/ ml		8	6	6	20	33.33	
Methanol	100 µg/ ml	10	3	5	6	14	53.34	161.5
	500 µg/ ml		7	6	7	20	33.33	
	1000 µg/ ml		8	7	7	22	26.66	
Ethanol	100 µg/ ml	10	6	6	5	17	43.44	>1000
	500 µg/ ml		6	7	6	19	36.67	
	1000 µg/ ml		3	6	3	12	60.00	
Petroleum ether	100 µg/ ml	10	3	5	6	14	53.34	348
	500 µg/ ml		6	2	7	15	50.00	

	1000 µg/ ml		7	8	8	23	23.34	

D. Anticancer activity

The extracts of *Capparis decidua* were evaluated their cytotoxicity on MCF-7 cell lines (50-500µM) in order to obtain the effective concentration at 50% of the inhibited cells. The results are expressed as 50% of the total available cells inhibited after 72hrs. of incubation. The methanolic extract showed good cytotoxicity having IC_{50} of 2.58µg/ml on MCF-7 cell lines respectively.

Table 8. IC_{50} values fractions of *capparis decidua* for anticancer activity

Sr. no	**Fractions**	**IC_{50} values of in µM (MCF-7 Cell line)**
1	Chloroform	12.52
2	Aqueous	35.18
3	Methanol	2.58
4	Ethanol	40.81
5	Petroleum ether	3.45
6	5-fluorouracil	8.2

4. CONCLUSION

Evaluation of crude drug involves the determination of identity, purity and quality. Macorscopic and microscopic evaluation is an important parameter in accessing the identity of herbal raw material. To conclude, various macroscopic, microscopic, physical and phytochemical

aspects/parameters listed here for *capparis decidua* in the present work can be used with respect to its identification, authentication and standardization. Molecular docking studies identify phytoconstituents which can bind to the target cancer. The docking scores and analysis of the interactions of the compounds suggest that most of the compounds have the ability to bind to multiple targets involved in breast cancer and it modulation. The brine shrimp lethality bioassay as preliminary assessment of toxicity *and* extracts produced dose dependent cytotoxicity effect to brine shrimp nauplii. All the extracts subjected for anticancer activity among all, fractions namely methanol fraction of *capparis deciduas* displayed significant cytotoxic activity. However, additional research to confirm this and/or to identify novel natural products, that in fact, are the cytotoxic compounds of interest is ongoing.

5. ACKNOWLEDGEMENT

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