

## RESEARCH ARTICLE

## Phytochemical analysis and anticancer activity of *Nelumbo nucifera* extracts

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### Abstract

*Nelumbo nucifera* leaf and flower solvent extracts were evaluated for phytochemical analysis, anticancer activity. Phytochemical analysis revealed major active phytoconstituents such as alkaloids, flavonoids, phenols, tannins, steroids and glycosides. Anticancer activity of methanol and acetone leaf extracts showed less activity against human breast cancer cell (MCF7). The lotus leaf showed outstanding water repellency (super-hydrophobicity) particularly on its upper side, which is more robust and less sensitive to mechanical damage than the underside. Leaf treated with 0.9% NaCl showed complete rupture of the cells and that treated with glucose showed no complete rupture of the cells.

**Keywords:** *Nelumbo nucifera*, phytochemical analysis, anticancer activity, methanol, acetone, super-hydrophobicity.

### Introduction

*Nelumbo nucifera* Gaertn. is a monogeneric plant belongs to family Nelumbonaceae, commonly known as rose of India, sacred water lily or East Indian lotus. Various pharmacologically active substances were separated from different parts of lotus mainly including alkaloids, flavonoids, triterpenoids, polyphenols, steroids and glycosides. The whole plant serves as astringent, emollient, diuretic and sudorific and possesses antifungal, antipyretic and cardiotoxic (Yu and Hu, 1997; Mukherjee *et al.*, 1997a; Mukherjee *et al.*, 1997b). The leaves and flowers of lotus plant were used to treat many bleeding disorders and consumption of flowers is recommended to promote conception (Chopra *et al.*, 1956). Flowers are useful to treat diarrhea, cholera, fever, hepatopathy and hyperdipsia (Chopra *et al.*, 1956). In folk medicines, seeds are used in the treatment of tissue inflammation, cancer, skin diseases, leprosy, poison antidote and generally prescribed to children as diuretic and refrigerant (Chopra *et al.*, 1956). The fruits and seeds of lotus are astringent and used to treat hyperdipsia, dermatopathy, halitosis, menorrhagia, leprosy and fever (Nadkarni, 1982). Seed powder mixed with honey is useful in treating cough, while roots with ghee (melted fresh butter), milk and gold promote strength, virility and intellect. Lotus seeds have been reported to possess rich antimicrobial properties (Mukherjee *et al.*, 1995; Mukherjee, 2002). Embryo of lotus seeds are used in traditional Chinese drug called 'Lian Zi Xin', which primarily helps to overcome nervous disorders, insomnia, high fevers (with restlessness) and cardiovascular diseases (e.g. hypertension, arrhythmia) (Chen *et al.*, 2007). This study was aimed to investigate the phytochemical constituents and anticancer activity of *N. nucifera* extracts. This study also investigated the super-hydrophobicity of the lotus leaf.

### Materials and methods

#### Chemicals

Analytical grade chemicals supplied by Hi-Media, Loba, Merck and Sigma were used. Methanol, acetone, hexane, phosphate buffer saline, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), Dulbecco's Modified Eagle's Medium and Acidic isopropanol were of analytical grade purchased from Merck. For Thin Layer Chromatography (TLC) aluminium sheet 20x20 cm and Silica gel 60 F<sub>254</sub> were purchased from Merck.

#### Plant material

The leaves and flowers of *N. nucifera* were collected from the different localities of Tirunelveli and Nagercoil, Tamil Nadu between Dec to Jan 2012. The plant parts were collected and then shade dried. The dried plant parts were powdered using mechanical pulveriser and subjected for extraction.

#### Solvent extracts

Air dried and powdered lotus leaf and flowers were cold macerated with different solvents (Methanol, acetone and hexane) for 3 d with occasional stirring. The extract was then filtered through Whatman filter paper (No. 1) and the solvent was removed at low temperature (40°C-50°C) under reduced pressure in a rotary evaporator.

#### Phytochemical analysis

The different phytochemical analysis was performed for establishing the profile of given extract for its chemical composition. Alkaloids, flavanoids, tannins, saponins and carbohydrates were determined according to the manual of Evans (1989; 1999). Glycosides present in the extracts were estimated according to Ramakrishnan *et al.* (1994).

### Thin Layer Chromatography (TLC)

Silica gel 60 F<sub>254</sub> was used for TLC. The crude methanol, acetone and hexane extracts of lotus leaf and flowers were spotted at 1 cm from the edge of the sheet. The chromatogram was developed in a mixture of suitable solvent system and dried at room temperature. The spots were visualized with UV light at 365 nm (long wavelength) and 254 nm (short wavelength). Alternatively, the developed TLC plates were visualized with methanol and sulphuric acid in the ratio 4:1. The relationship between the distance travelled by the solvent front and the substance is usually expressed as the R<sub>f</sub> value:

$$R_f = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

### Cytotoxicity determination by MTT assay

Methanol and acetone extract of lotus leaf were evaluated for anticancer activity. Cytotoxicity/anticancer assay, MCF-7 breast cancer cell line was used. Cells were maintained in DMEM (Dulbeccos modified eagles medium) supplemented with FBS (foetal bovine serum) and penicillin/streptomycin-L-glutamine and cultured in a humidified atmosphere of 5% CO<sub>2</sub> and 95% air at 37°C in Thermo Hera Cell 150 incubator. Cells were seeded in 96 well plates at the density of 5000 cells/well in 100 µL of RPMI 1640 medium. Then various concentrations of the crude extract were added to the cells in 100 µL medium. Cells were incubated for 24 h with test extract concentrations. Each concentration was tested in triplicate.

### Cell viability test

MTT assay was used to determine cell viability. The MTT assay measures changes in colour for measuring the activity of enzyme that reduce MTT to formazan, giving a purple colour. Yellow MTT (a tetrazole) reduce to purple formazan in living cells (Mosmann, 1983). After 24 h incubation, 10 µL of MTT was added to each well and incubated for additional 4 h. Then 100 µL of DMSO solution was added to each well to solubilize the formazan crystals. The plates were read for optical density at 570 nm using a plate reader. By using optical density, the percentage inhibition of MCF-7 cells was calculated. The percentage viability was calculated as follows:

$$\text{Cell viability} = \text{OD of samples} / \text{OD of control} \times 100.$$

### Scanning Electron Microscopy (SEM)

Lotus leaf was cut into pieces of equal size (2 cm in dia.) and treated with 0.9% NaCl, 0.45% NaCl and 0.1% glucose solution for 3 d at room temperature in light conditions. After 3 d, the samples were freeze-dried using lyophilizer and analyzed for scanning electron microscopy.

## Results and discussion

When compared with different extracts, white flower extract was found to contain major active phytoconstituents such as alkaloids, flavonoids, phenols, tannins, steroids, glycosides and saponins. In white flowers, relatively higher amounts of phytochemicals were present. In leaf extract, the phytochemicals are present in relatively lower amounts when compared to the flower extracts (Table 1).

Table 1. Phytochemical analysis of *N. nucifera*.

| Chemical test | Leaf | White flower | Pink flower |
|---------------|------|--------------|-------------|
| Alkaloids     | +    | +            | +           |
| Flavonoids    | +    | +            | +           |
| Phenols       | +    | +            | +           |
| Tannins       | -    | +            | +           |
| Saponins      | +    | -            | -           |
| Glycosides    | -    | +            | +           |

Thin Layer Chromatography profile for methanol, acetone and hexane extracts of leaf and flowers of *N. nucifera* were thoroughly analyzed using four solvent systems. Solvent systems used were

1. Chloroform: Methanol = 9:1
2. Chloroform: Ethyl acetate = 1:1,
3. Hexane: Ethyl acetate = 9:1
4. Hexane: Dichloromethane = 5:5.

The spots were visualized with UV light at 365 nm and 254 nm. From the TLC profile it was found that methanol and acetone extract showed discrete bands in Chloroform: Methanol (9:1) solvent system (Fig.1 and Table 2). Hexane extracts showed fluorescent bands in both the solvent systems. It was found that the bands eluted better in Hexane: Ethyl acetate solvent system. The bands in the methanol extract and ethyl acetone extracts might be due to polar compounds when compared to the other extracts and solvent systems. However further experiments are need to be done to confirm the identity of the single compounds.

Methanol and acetone leaf extracts were used for anticancer activity by MTT assay. About 6.25 µg/mL to 100 µg/mL of sample was used for MTT assay. Methanol leaf extract showed 27% and acetone leaf extract showed 7% in 100 µg/mL of MCF-7 breast cancer cell line (Fig. 2). Both extracts showed less anticancer activity against breast cancer. According to Weng *et al.* (2009), arnepavine (Arm, C<sub>19</sub>H<sub>23</sub>O<sub>3</sub>N), an active compound from *N. nucifera*, has been shown to exert immunosuppressive effects in *in vitro*. Arm (1-10 µM) concentration dependently attenuated TNF-α- and LPS-stimulated α-SMA protein expression and AP-1 activation by HSC-T6 cells without adverse cytotoxicity (Weng *et al.*, 2009). Arm also suppressed TNF-α-induced collagen deposition, NFκB activation and MAPK (p38, ERK1/2 and JNK) phosphorylation. Ahmed *et al.* (2009) who studied effects of different concentrations of *Caralluma tuberculata* crude extract against MCF-7 cell line indicated maximum growth inhibition of 82% at a concentration of 500 µg/mL.

Srivastava *et al.* (2012) reported alkaloids, glycosides, flavonoids, saponins and carbohydrates in phytochemical analysis of *N. nucifera* leaf and absence of tannins and steroids in lotus leaf. From the results, it was evident that both methanol and acetone extracts of lotus leaf exhibited less cytotoxic activity on breast cancer cell lines (MCF-7). Folk history says that sacred water lotus can be used to treat cancer. Modern research has isolated certain compounds from the plant that showed anticancer activity. However, there is no report showing the solvent extracts of *N. nucifera* can mitigate breast cancer. Future attempts can be done to assess its anticancer activity on other cell lines also.

To check the superhydrophobicity of lotus leaf by SEM, the leaf was cut into pieces of equal size (2 cm in dia.) which were treated with 0.9% NaCl, 0.45% NaCl and 0.1% glucose solution for 3 d at room temperature in light conditions. After 3 d, the samples were freeze-dried using lyophilizer and analyzed for SEM. SEM analysis showed the difference in nanoparticulate surface of the leaf when treated with different solutions in comparison with the fresh leaf and dried powder. The lotus leaf shows outstanding water repellency particularly on its upper side, which is more robust and less sensitive to mechanical damage than the underside. This water repellency relies on the combination of optimized features such as the surface topography, robustness and the unique properties of the nanoscopic wax crystals. It was inferred that the leaf treated with 0.9% NaCl showed complete rupture of the cells and that treated with glucose showed no complete rupture of the cells (Fig.3). According to Ensikat *et al.* (2011), lotus leaves have become an icon for superhydrophobicity and self-cleaning surfaces and have led to the concept of the 'Lotus effect'. Although many other plants have superhydrophobic surfaces with almost similar contact angles, lotus shows better stability and perfection of its water repellency. The lotus plant has successfully developed an excellent protection for this delicate epistomatic surface of its leaves.

Fig. 1. TLC profiles of methanol, acetone and hexane extracts of leaf, pink and white flowers of *N. nucifera*.

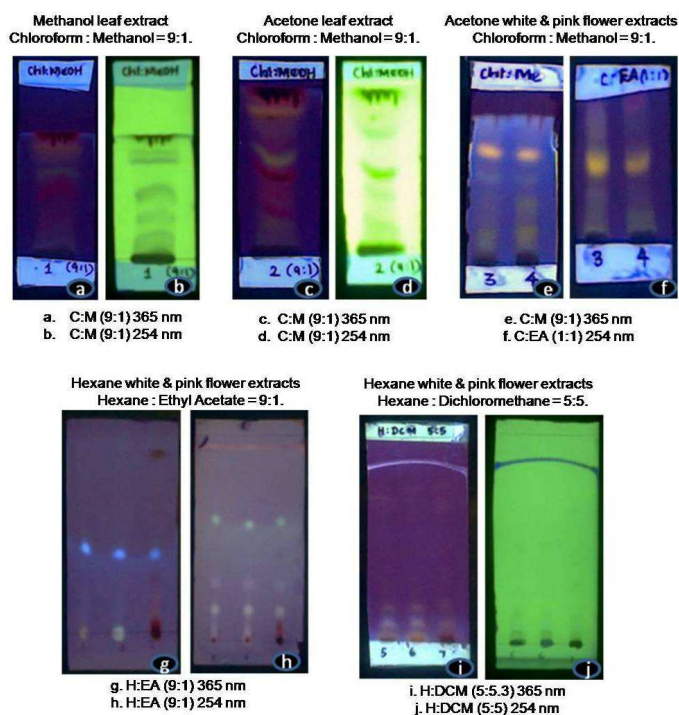


Fig. 2. Inhibition percentage of the leaf extracts on MCF-7 cells.

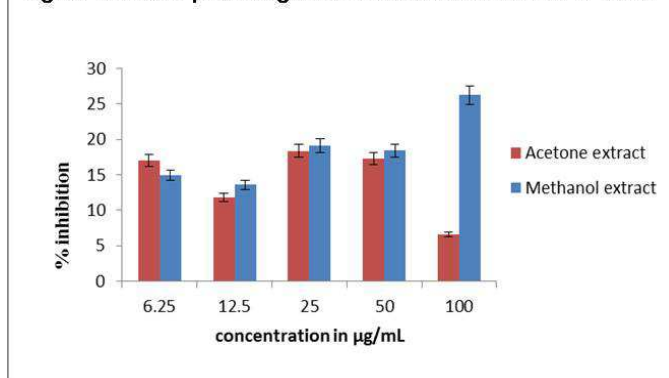
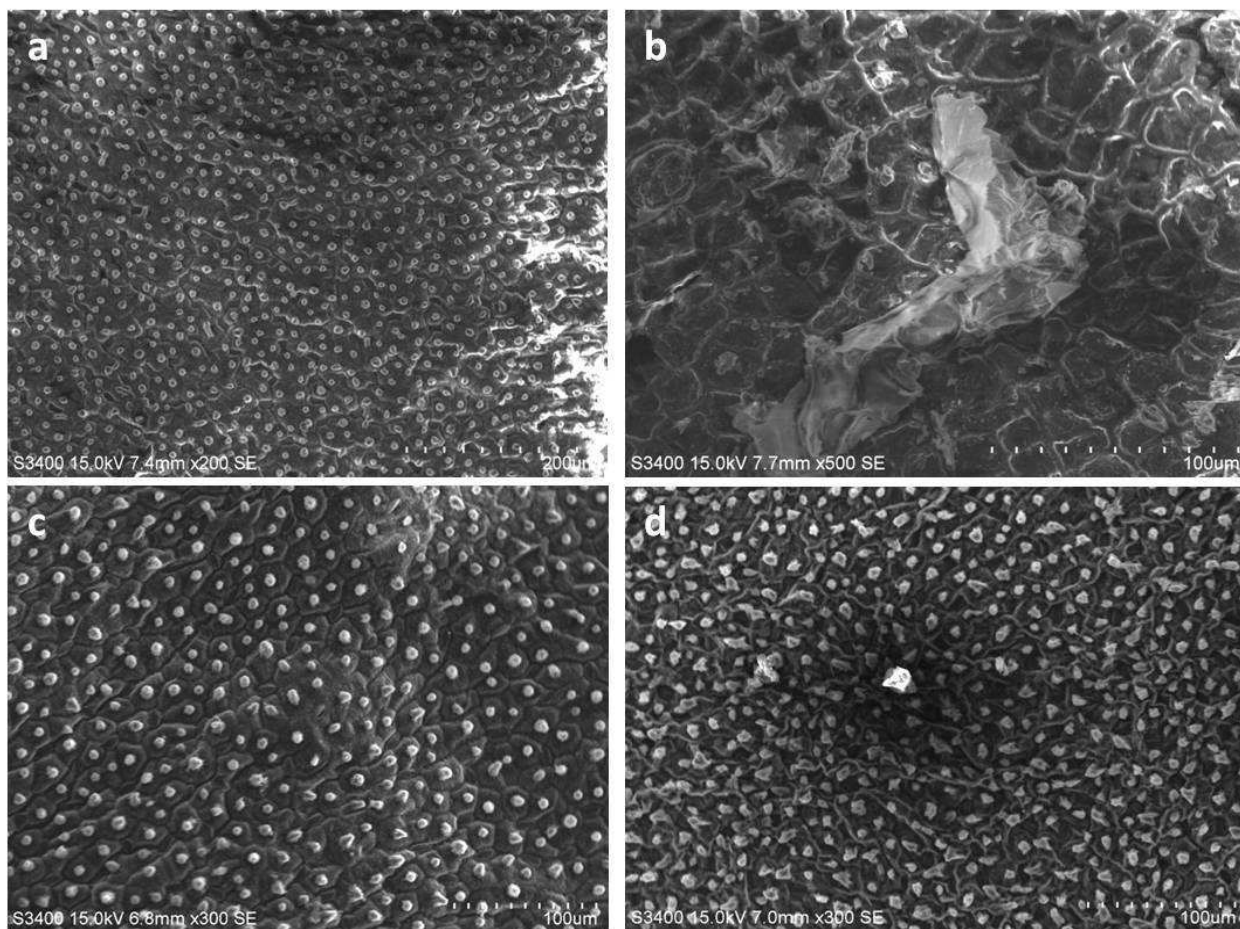


Table 2. Retention factors for samples in various solvent systems in TLC.

| Extracts and solvent systems | Retention factor ( $R_f$ values)   |                              |
|------------------------------|------------------------------------|------------------------------|
|                              | 365 nm (UV long)                   | 254 nm (UV short)            |
| 1. C:M (9:1)                 | 0.25, 0.31, 0.42, 0.69, 0.77, 0.89 | 0.23, 0.44, 0.69, 0.79, 0.89 |
| 2. C:M (9:1)                 | 0.24, 0.6, 0.89, 0.31, 0.49        | 0.13, 0.33, 0.49, 0.71       |
| 3. C:M (9:1)                 | 0.23, 0.63, 0.77                   | -                            |
| C:EA (1:1)                   | 0.16, 0.3, 0.43, 0.83              | 0.33, 0.76                   |
| 4. C:M (9:1)                 | 0.23, 0.46, 0.63, 0.8              | -                            |
| C:EA (1:1)                   | 0.16, 0.26, 0.5, 0.83              | 0.36, 0.8                    |
| 5. H:EA (9:1)                | 0.16, 0.29, 0.62                   | 0.15, 0.28                   |
| H:DCM (5:5)                  | 0.07, 0.18, 0.86                   | 0.07, 0.18                   |
| 6. H:EA (9:1)                | 0.15, 0.28, 0.6                    | 0.15, 0.275                  |
| H:DCM (5:5)                  | 0.08, 0.18                         | 0.08, 0.18                   |
| 7. H:EA (9:1)                | 0.16, 0.29, 0.62                   | 0.16, 0.29                   |
| H:DCM (5:5)                  | 0.08, 0.2                          | 0.08, 0.2                    |

1. Methanol leaf extract, 2. Acetone leaf extract, 3. Acetone white flower extract, 4. Acetone pink flower extract, 5. Hexane pink flower extract, 6. Hexane white flower extract, 7. Hexane leaf extract.

**Fig. 3. SEM images of *nelumbo nucifera* leaf.**

**a. SEM image of fresh leaf at 200 µm, b. SEM image of dried leaf powder  
c. SEM image of leaf surface treated with 0.1% glucose solution  
d. SEM image of leaf surface treated with 0.9% NaCl solution**

## Conclusion

The phytochemical analysis of extracts of the lotus plants contained many bioactive chemical constituents including alkaloids, glycosides, terpenoids, steroids, flavonoids and tannins. TLC profile of plant extracts in different solvent system confirmed the presence of diverse group of phytochemicals. Both methanol and acetone leaf extracts showed less anticancer activity against breast cancer. Lotus leaves with unrivaled superhydrophobicity and self-cleaning properties as an effective protection of the delicate epistomatic surface of *N. nucifera*. To conclude, further investigations are necessary to find out the active isolate the active compounds responsible anticancer activity.

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