

# **Network Pharmacology and Experimental Validation to Explore the Potential Mechanism of *Nigella sativa* for the Treatment of Breast Cancer**

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***Supplementary file***

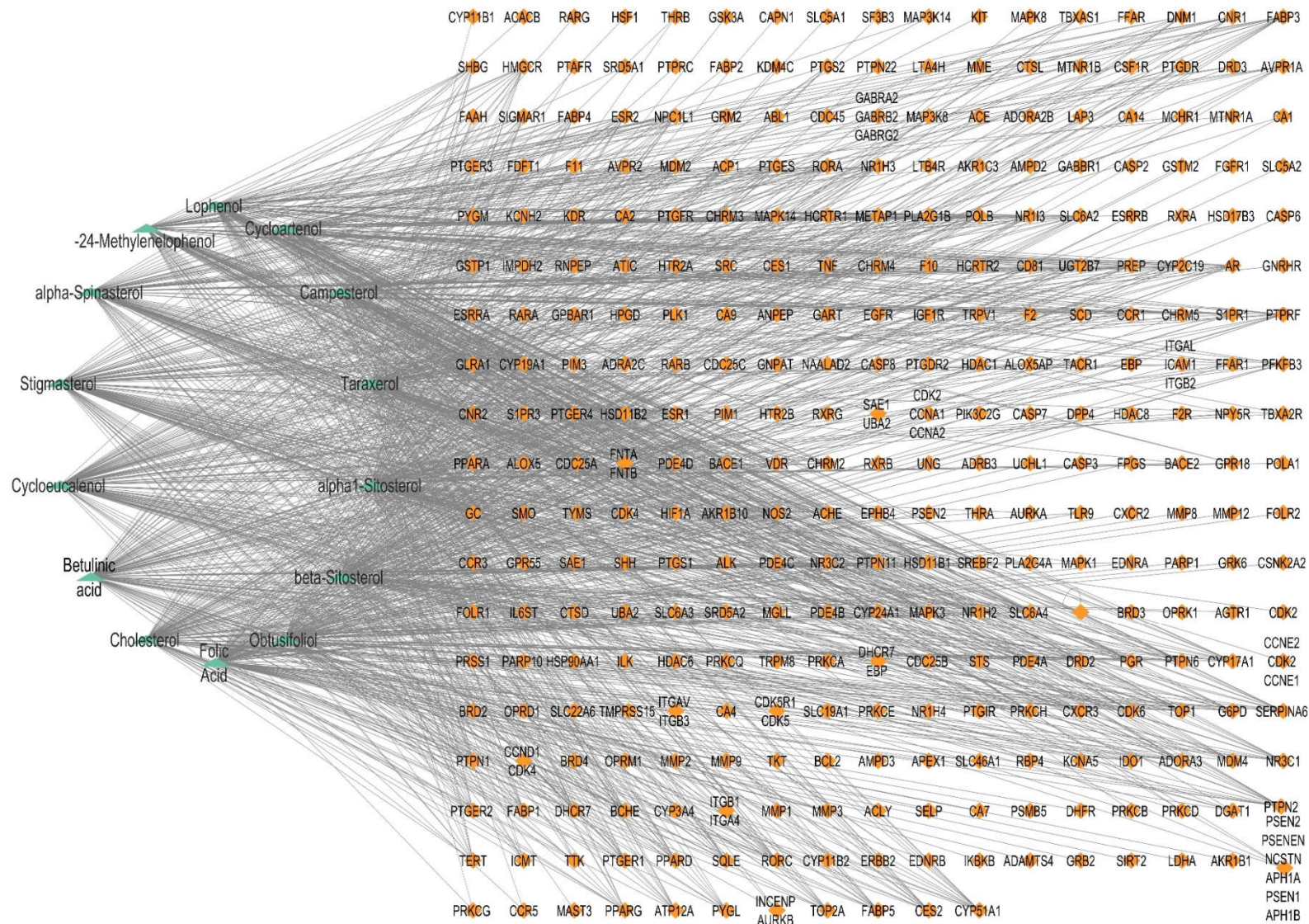


Figure S1. Compound-target network of active phytochemicals and their targets.

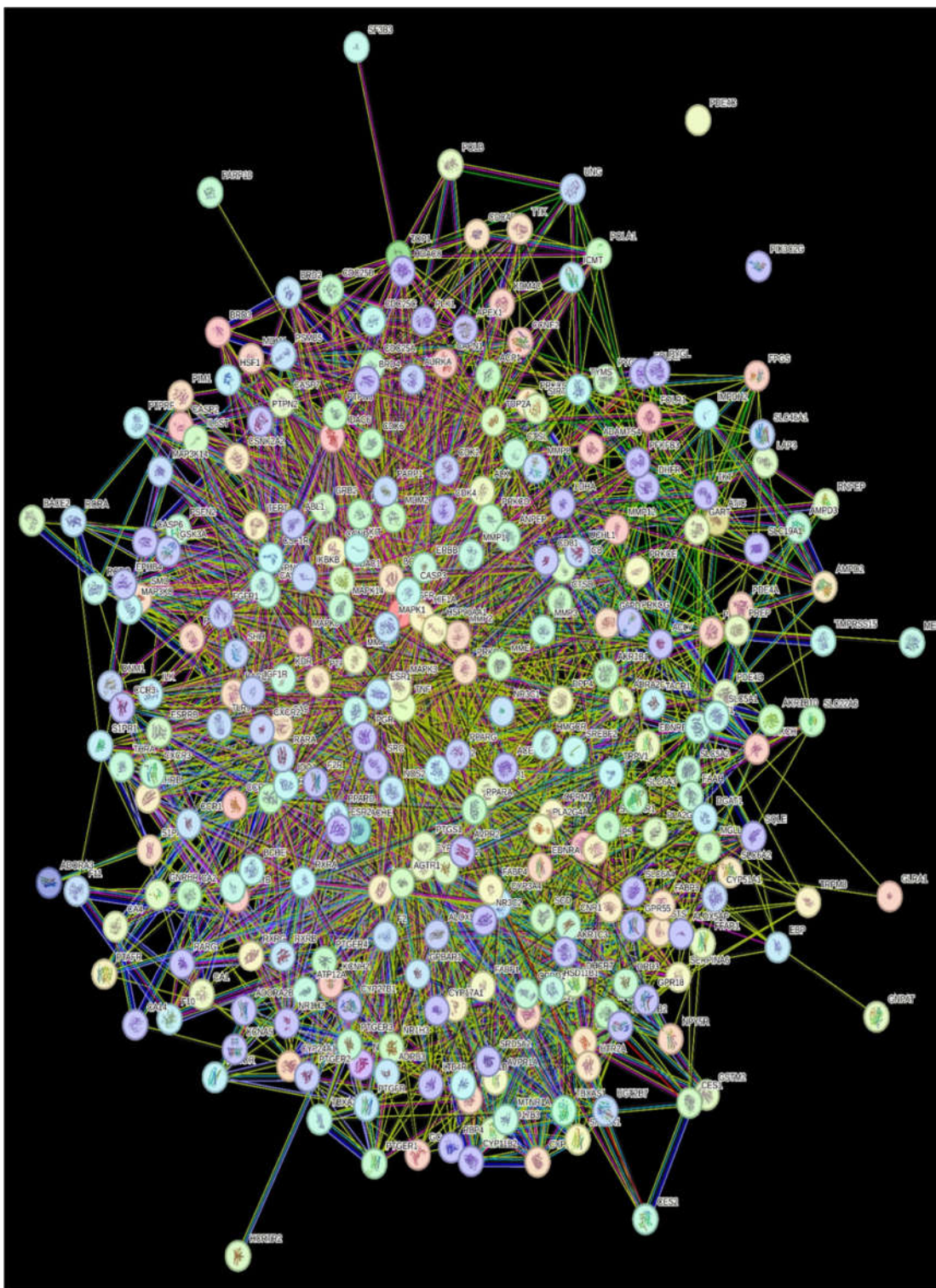


Figure S2. PPIs showing the interactions of 283 target genes, colored nodes represent query protein; white nodes represent second shell of interactors; empty nodes represent protein with unknown 3D structure; filled nodes represent protein with known or predicted 3D structure.



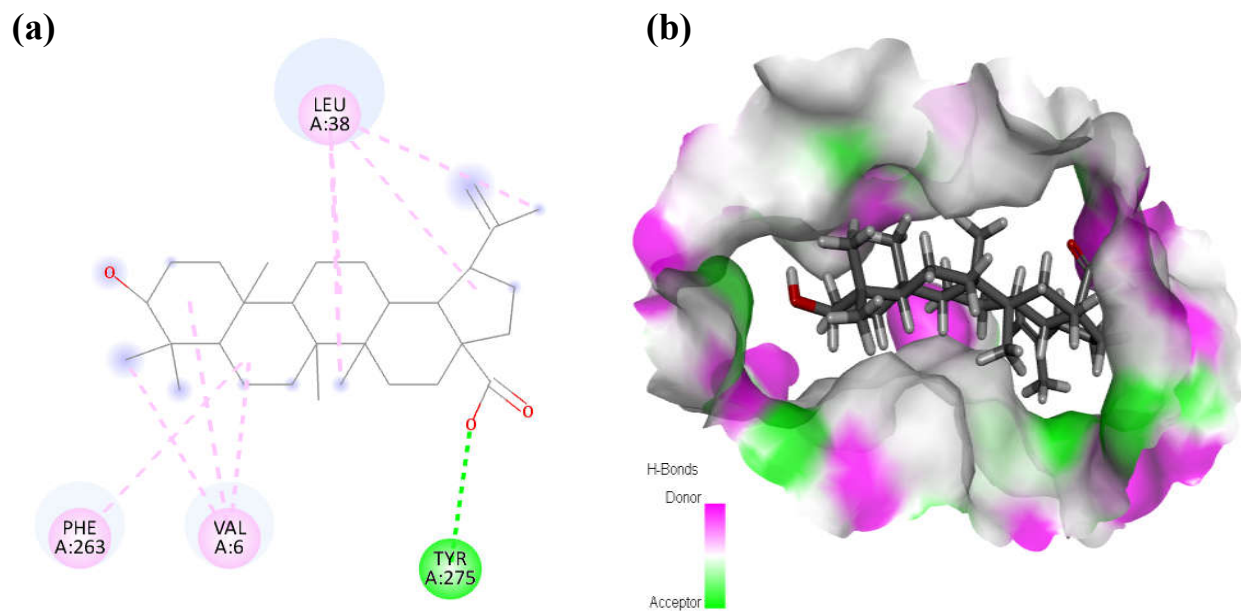


Figure S4. Binding interaction (a) and pattern (b) of betulinic acid with the EGFR as a receptor.

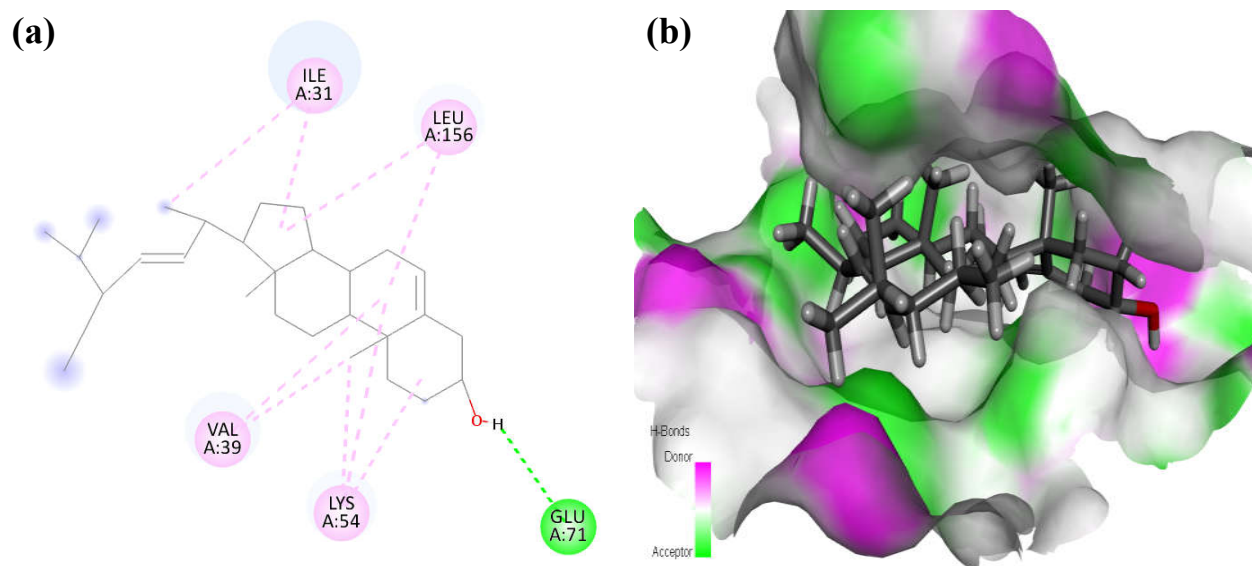


Figure S5. Binding interaction (a) and pattern (b) of stigmasterol with the MAPK1 as a receptor.

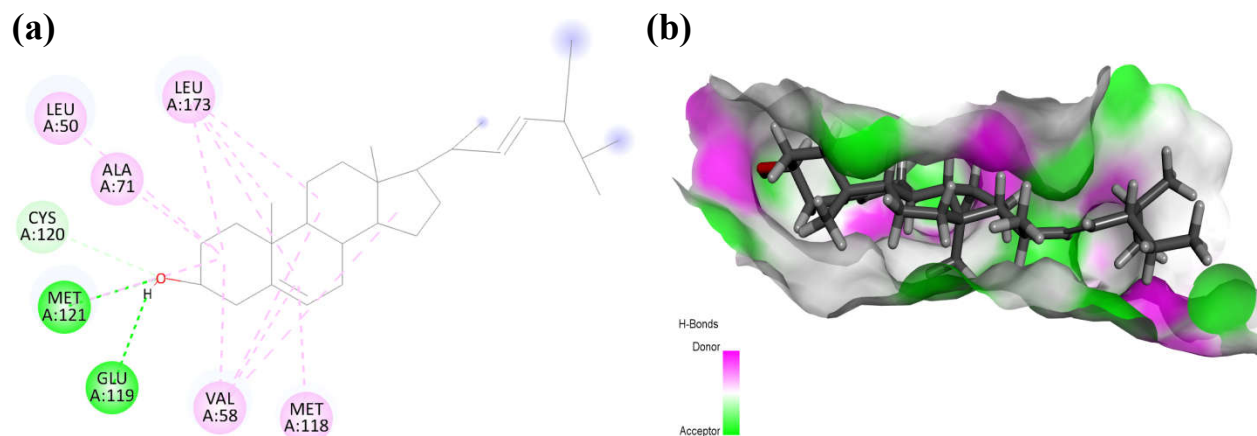


Figure S6. Interaction (a) and binding pattern (b) of stigmasterol with the MAPK3 as a receptor.

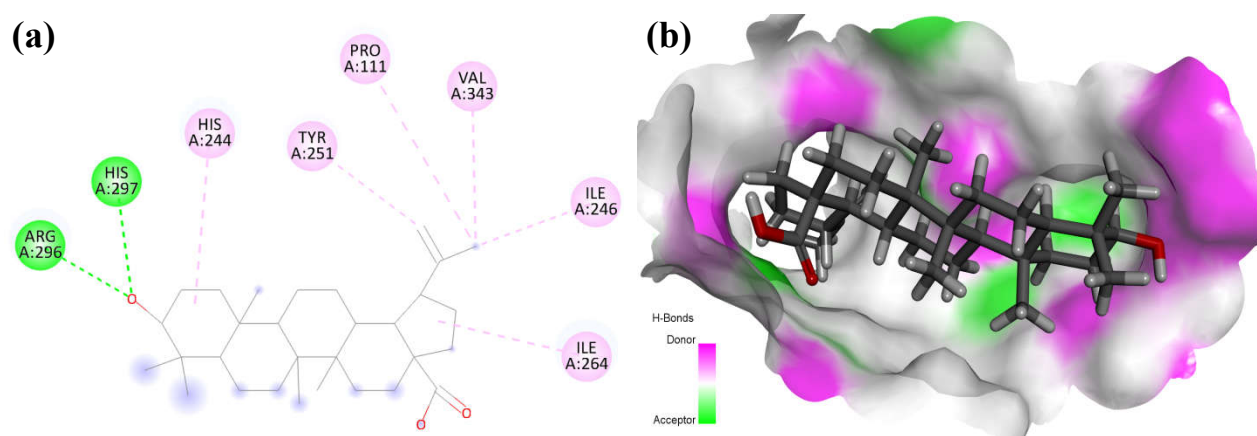


Figure S7. Interaction (a) and binding pattern (b) of betulinic acid with the PTGS2 as a receptor.

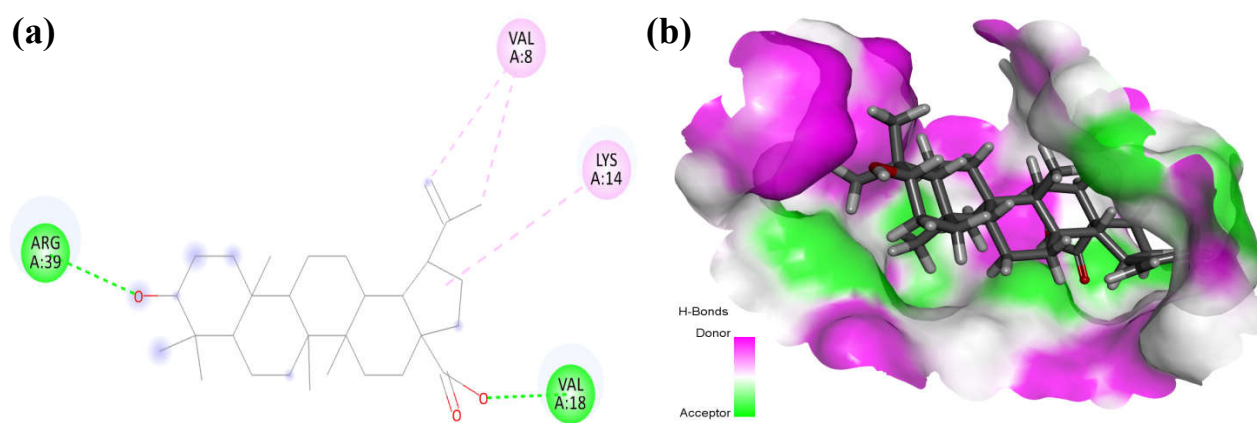


Figure S8. Interaction (a) and binding pattern (b) of betulinic acid with the ESR1 as a receptor.

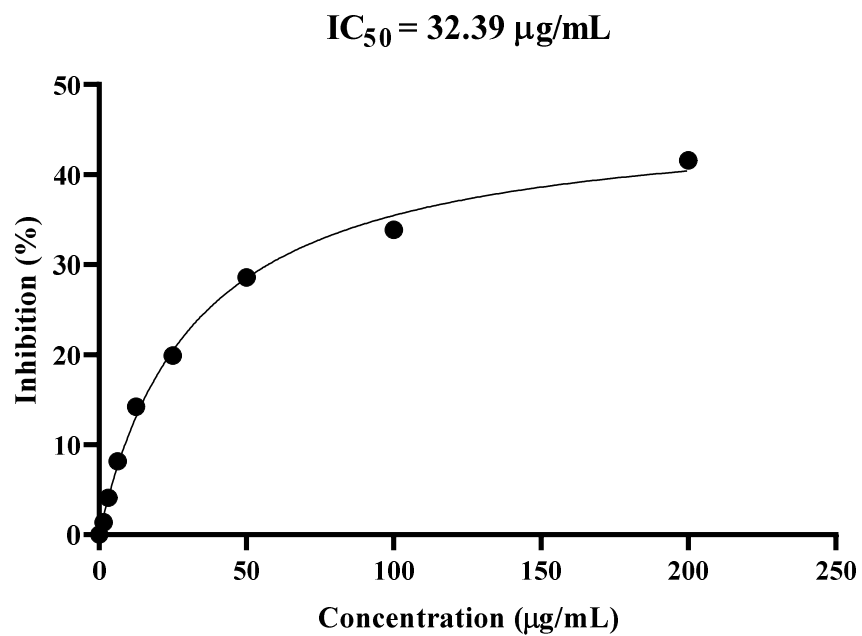


Figure S9. Folic acid  $IC_{50}$  curve

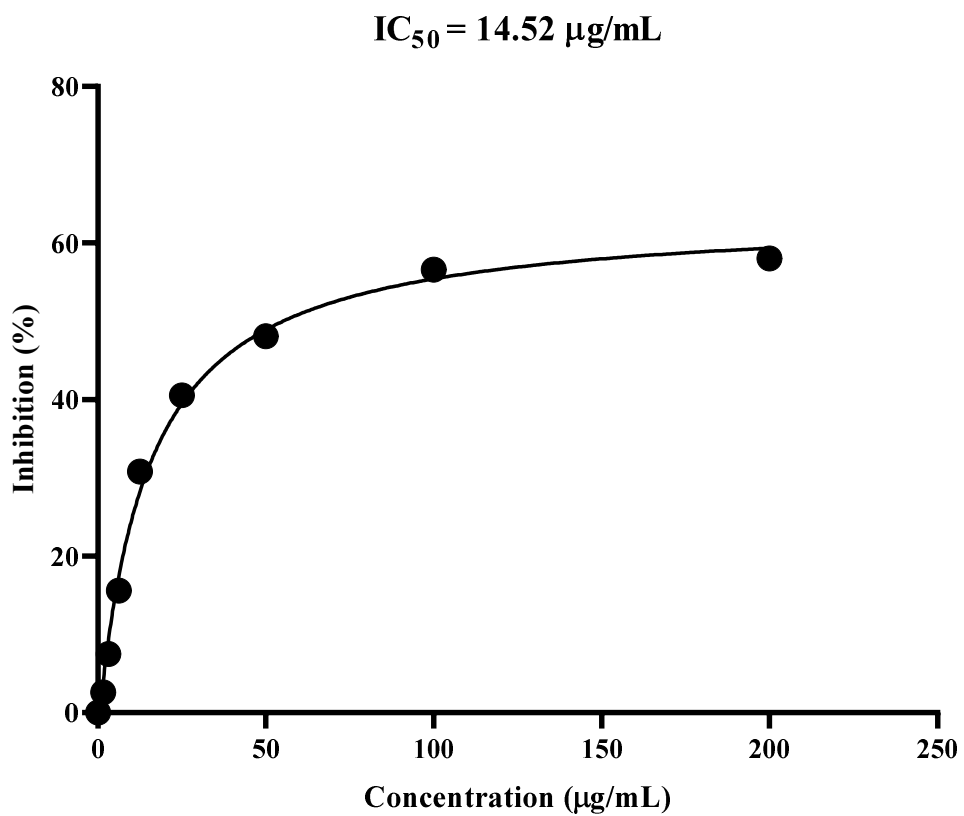


Figure S10. Betulinic acid  $IC_{50}$  curve.

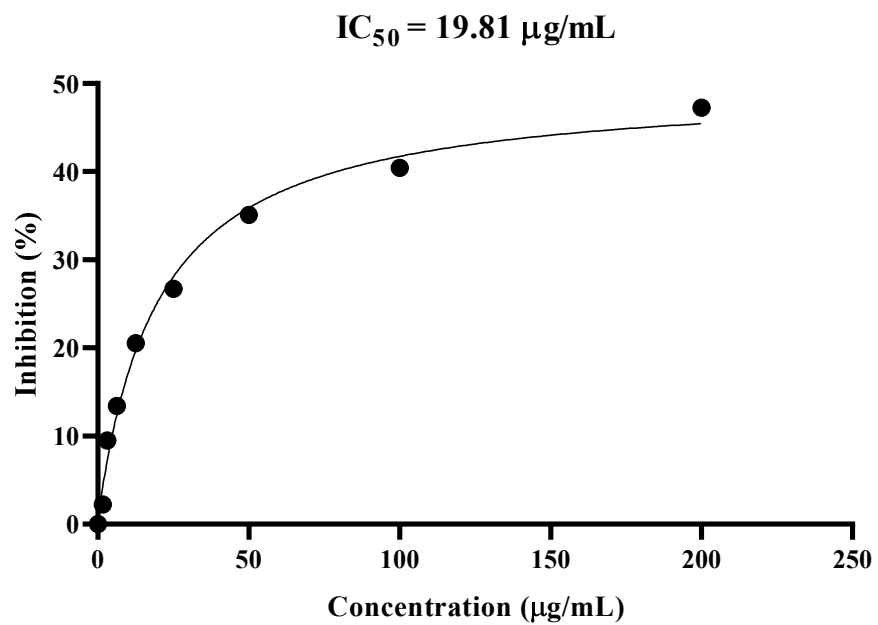
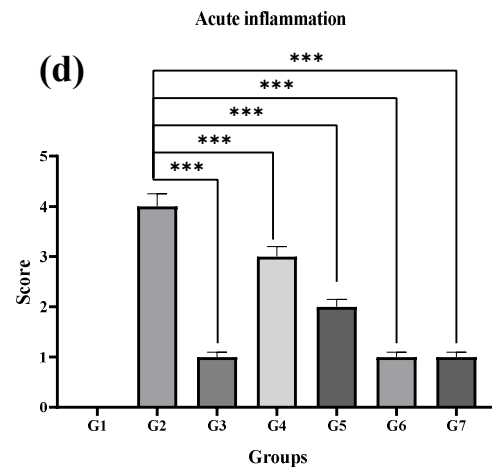
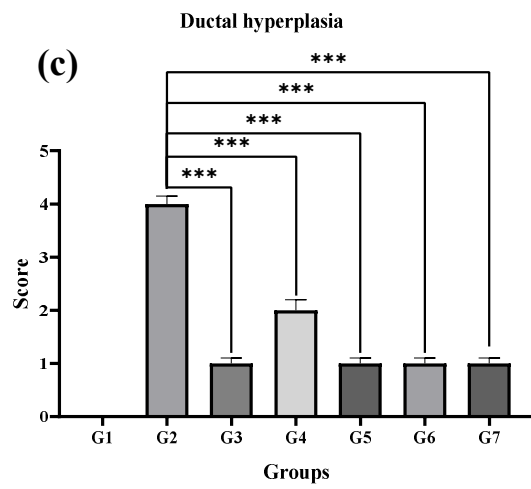
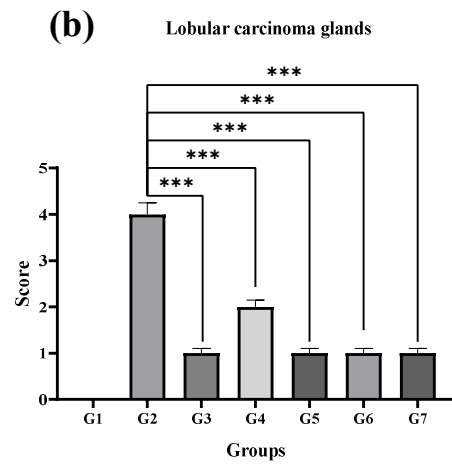
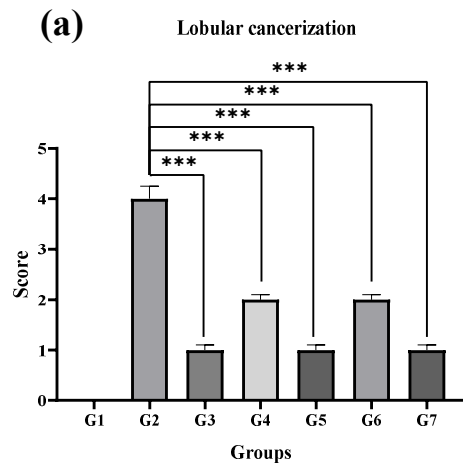


Figure S11. Stigmasterol  $IC_{50}$  curve.



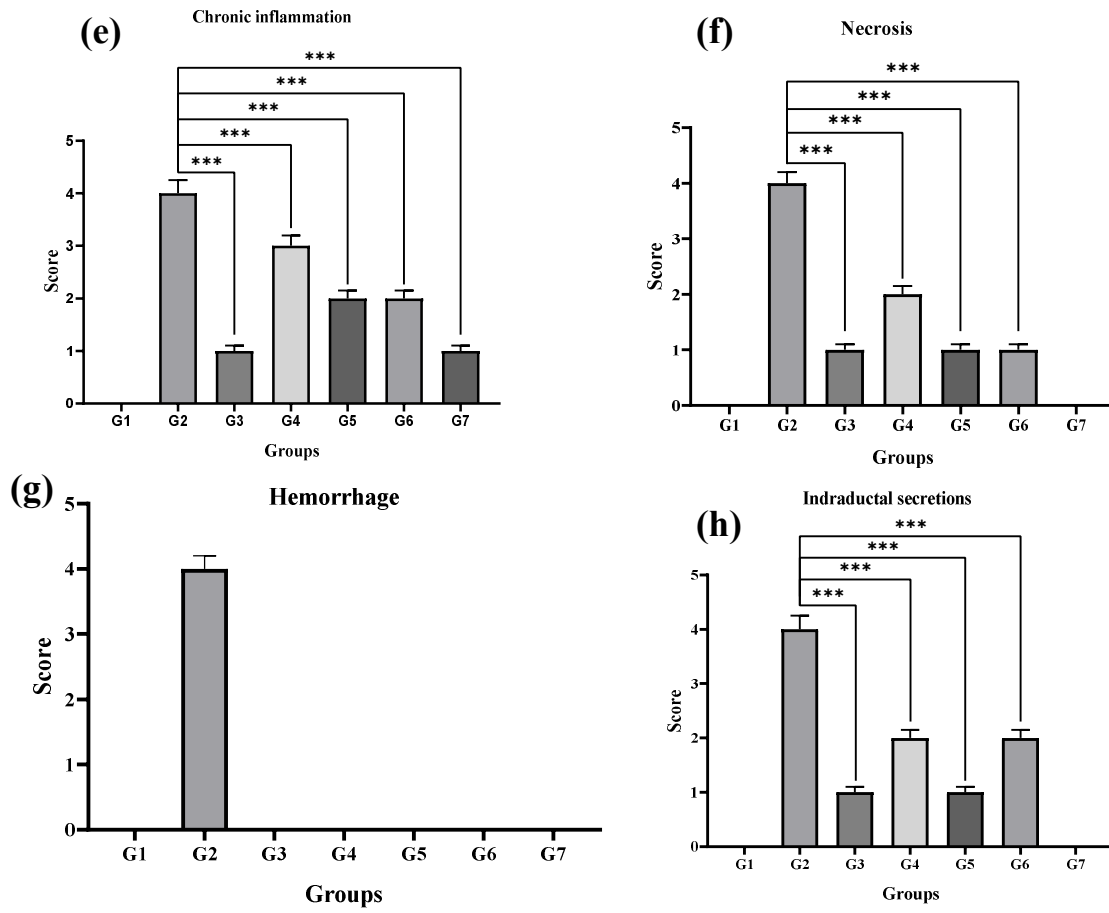


Figure S12. Histological features of different groups of breast tissues with statistical significance.

Table S1. The characteristics of the best selected phytochemicals

| Sr. No. | Phytochemical         | MF  | OB > 30% | DL >0.18 | MW (g/mol) | PubChem ID |
|---------|-----------------------|---|----------|----------|------------|------------|
| 1       | Cycloeucalenol        | C <sub>30</sub> H <sub>50</sub> O                             | 39.73    | 0.79     | 426.7      | 101690     |
| 2       | Cholesterol           | C <sub>27</sub> H <sub>46</sub> O                             | 37.87    | 0.68     | 386.7      | 5997       |
| 3       | Obtusifoliol          | C <sub>30</sub> H <sub>52</sub> O                             | 42.55    | 0.76     | 426.7      | 65252      |
| 4       | Lophenol              | C <sub>30</sub> H <sub>50</sub> O                             | 38.13    | 0.71     | 400.7      | 160482     |
| 5       | $\alpha$ -spinasterol | C <sub>29</sub> H <sub>48</sub> O                             | 42.98    | 0.76     | 412.7      | 5281331    |
| 6       | Cycloartenol          | C <sub>30</sub> H <sub>50</sub> O                             | 38.69    | 0.78     | 426.7      | 92110      |
| 7       | $\alpha$ 1-sitosterol | C <sub>29</sub> H <sub>50</sub> O                             | 43.28    | 0.78     | 426.7      | 9548595    |
| 8       | Taraxerol             | C <sub>30</sub> H <sub>52</sub> O                             | 38.4     | 0.77     | 426.7      | 92097      |
| 9       | Campesterol           | C <sub>28</sub> H <sub>48</sub> O                             | 37.58    | 0.71     | 400.7      | 173183     |
| 10      | 24-methylenelophenol  | C <sub>19</sub> H <sub>48</sub> O                             | 44.19    | 0.75     | 412.7      | 5283640    |
| 11      | $\beta$ -sitosterol   | C <sub>29</sub> H <sub>50</sub> O                             | 36.91    | 0.75     | 414.7      | 222284     |
| 12      | Stigmasterol          | C <sub>29</sub> H <sub>48</sub> O                             | 43.83    | 0.76     | 412.7      | 5280794    |
| 13      | Folic acid            | C <sub>19</sub> H <sub>19</sub> N <sub>7</sub> O <sub>6</sub> | 68.96    | 0.71     | 441.4      | 135398658  |
| 14      | Betulinic acid        | C <sub>30</sub> H <sub>48</sub> O <sub>3</sub>                | 55.38    | 0.78     | 456.7      | 64971      |

MF: Molecular formula; OB: Oral Bioavailability; DL: Drug-likeness; MW: Molecular weight

Table S2. Indication of each color interaction in the PPI network of STRING.









| Interaction type       | Color representation  | Indication                |
|------------------------|---|---------------------------|
| Known interactions     |  | From curated database     |
|                        |  | Experimentally determined |
| Predicted interactions |  | Gene neighbourhood        |
|                        |  | Gene fusion               |
|                        |  | Gene co-occurrence        |
| Others                 |  | Textmining                |
|                        |  | C0-expression             |
|                        |  | Protein homology          |

Table S3. Drug-like parameters or ADMET profiling of the best selected phytochemicals

| Parameters          | Phytochemicals |                |              |
|---------------------|----------------|----------------|--------------|
|                     | Folic acid     | Betulinic acid | Stigmasterol |
| Absorption          |                |                |              |
| BBB                 | +              | –              | +            |
| HIA                 | +              | +              | +            |
| Caco-2 permeability | –              | –              | +            |
| PGS                 | +              | –              | +            |
| PGI                 | –              | –              | –            |
| Metabolism          |                |                |              |
| CYP3A4 Substrate    | +              | +              | +            |
| CYP2C9 Substrate    | –              | –              | –            |
| CYP2D6 Substrate    | –              | –              | –            |
| CYP3A4 Inhibitor    | –              | –              | –            |
| CYP2C9 Inhibitor    | –              | –              | –            |
| CYP2C19 Inhibitor   | –              | –              | –            |
| CYP2D6 Inhibitor    | –              | –              | –            |
| CYP1A2 Inhibitor    | –              | –              | –            |
| Toxicity            |                |                |              |
| AMES Toxic          | –              | –              | –            |
| Carcinogenic        | –              | –              | –            |