



Research Article



Hydrosols of *Tapinanthus bangwensis* (Engl. L.K. Krause) Grown on *Azadirachta indica* and *Vitex doniana*: Chemical Composition, Cytotoxicity and Anticancer Potentials

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Article info:

Submitted: 03/07/2026

Revised: 03/09/2026

Accepted: 04/09/2026

Published: 28/09/2026

Manuscript ID: 00028

Subject: Advanced Chemistry

Abstract:

Hydrosols (HDs) are obtained from remnant water which is usually truncated after extraction of essential oils from plants by hydrodistillation. *Tapinanthus bangwensis* is used for the treatment of cancer, diabetes and hypertension in Nigeria. This research aimed at comparing the chemical composition, cytotoxicity and anticancer potentials of *T. bangwensis* grown on two different hosts. The hydrosols were obtained by partitioning the remnant water of hydrodistillation of dried leaves of the *T. bangwensis* into chloroform. Chemical composition of the HDs was determined by Gas-Chromatography (GC-MS). Toxicity potentials of the HDs were determined using brine shrimp lethality assay. Anticancer activities of the HDs were determined against MDA-MB-231 breast cancer cell line using MTT assays. The results of toxicity potentials and anticancer activities were analysed using descriptive statistics alongside appropriate inferential statistical analyses. The hydrosols of *T. bangwensis* grown on *Azadirachta indica* and *Vitex doniana* are colourless with yields of 0.22% and 0.16% (v/v) respectively. A total of seventeen (17) compounds were identified in the hydrosol of *T. bangwensis* grown on *Azadirachta indica* representing while a total of fourteen (14) compounds were identified in the hydrosol of *T. bangwensis* grown on *Vitex doniana* with nine (9) compounds common to the two hydrosols. The hydrosols are dominated by non-terpene hydrocarbons. The hydrosols of *T. bangwensis* grown on *Azadirachta indica* is dominated by Nonacos-1-ene (23.0%), E-15-Heptadecenal (13.77%), (Z)-7-

Keywords:

Hydrosol, *Tapinanthus*, Toxicity, Anticancer, Gas-Chromatography, Nonacos-1-ene

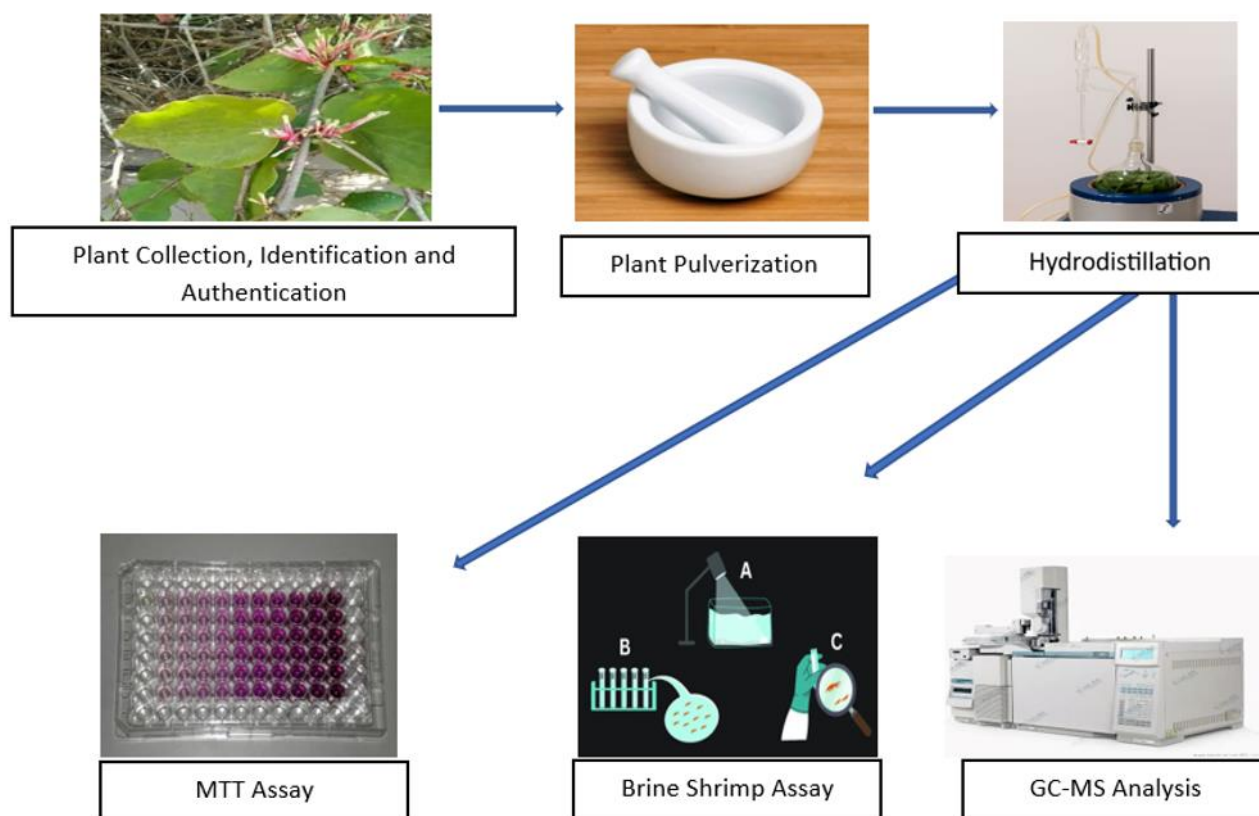
Hexadecene (12.87%), 2,4-Di-tert-butylphenol (10.63), (Z)-7-Tetradecene (9.14%) while Nonacos-1-ene (19.72%), (Z)-7-Hexadecene (15.25%), (Z)-7-Tetradecene (14.63%), 1-Heneicosanol (13.86%) and Cetylalcohol (10.04%) dominated the hydrosol of *Tapinanthus bangwensis* grown on *Vitex doniana*. The hydrosols were not toxic - *T. bangwensis* grown on *Azadirachta indica* (LC₅₀=2995.024 ppm) and *T. bangwensis* grown on *Vitex doniana* (LC₅₀=6672.169 ppm) - and showed promising anticancer activities- *T. bangwensis* grown on *Azadirachta indica* (IC₅₀=230.2357 μ L/ml) and *T. bangwensis* grown on *Vitex doniana* (IC₅₀=46.4753 μ L/ml) -against MDA-MB-231 cancer cell line. The hydrosols are reservoirs of biologically active compounds that could be explored in chemo-aromatherapy for cancer treatment.

How to Cite this article: A.A. Azeez, A. Bhatnagar, S.H. Makwana, C.C. Mandal, V. Kumar, A.T. Abimbola, M.O. Abdullahi, S.A. Aboaba. Hydrosols of *Tapinanthus bangwensis* (Engl. L.K. Krause) Grown on *Azadirachta indica* and *Vitex doniana*: Chemical Composition, Cytotoxicity and Anticancer Potentials. *J. PhytoPharm. Adv. Chem.* **2026**, 01 [03], 178-189. DOI: <https://doi.org/10.66220/wjwdkf98>

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Graphical Abstract



1. Introduction

Plant materials have been used in the preparation of medicinal products since prehistoric times. Various parts of plants, including leaves, flowers, fruits, rhizomes, roots, stems, and bark, have been used for medicinal purposes in different forms [1,2]. In recent years, interest in medicinal plants as a complementary and alternative source of healthcare has increased, partly due to the rising cost of prescription drugs used to maintain health and well-being [3]. Herbal preparations and homemade remedies derived from medicinal plants are generally more affordable than synthetic drugs and are often more accessible and acceptable in rural and underserved communities [4].

The genus *Tapinanthus*, commonly known as African mistletoe, belongs to the family Loranthaceae. In Nigeria, it is locally known as *Afomo* among the Yoruba people. *Tapinanthus bangwensis* is a woody, parasitic shrub with pendulous stems that can reach 1–2 m in length and is widely distributed in forested areas of Africa. In Nigeria, the plant is traditionally used in the treatment of diabetes, hypertension, and certain forms of cancer [5,6,7].

Hydrosols are aqueous products obtained as co-products during the steam distillation of plants for the extraction of essential oils. Unlike essential oils, however, hydrosols have received relatively limited research attention, despite their reported applications in the phytotherapeutic, cosmetic, and food industries, particularly as natural alternatives to synthetic preservatives [8,9]. They contain complex mixtures of trace amounts of essential oils and water-soluble phytochemicals, some of which possess significant biological activities [10]. Hydrosols were initially regarded as by-products or waste materials of essential oil production. However, recent research comparing their chemical composition and biological properties with those of essential oils has increased interest in their potential applications. Consequently, hydrosols are increasingly recognized as valuable products that support biorefinery and green extraction approaches rather than being considered waste [11].

Hydrosols represent a promising yet underutilized by-product of essential oil (EO) extraction. These aqueous solutions contain complex mixtures of trace amounts of essential oils and water-soluble compounds and have been reported to exhibit significant biological activities [12].

Evaluating the hydrosols of *T. bangwensis* is significant because it may reveal bioactive constituents that are not adequately represented in the corresponding essential oils, while also supporting green extraction and biorefinery principles by valorizing an often-overlooked co-product. The study further provides insight into the influence of host plants, *Azadirachta indica* and *Vitex doniana*, on the chemical composition and biological activities of *T. bangwensis* hydrosols. Given the limited information on the chemical characterization and cytotoxic and anticancer activities of these hydrosols, particularly against the MDA-MB-231 breast cancer cell line, this investigation contributes novel information on their potential as sustainable sources of biologically active compounds for future therapeutic applications.

2. Material and Methods

2.1 Plants Collection, Identification and Authentication

The leaves and floral parts of *Tapinanthus bangwensis* (Engl. & L.K. Krause) growing on *Azadirachta indica* (TBD) and *Vitex doniana* (TBK) were collected from Oba Adeyemi High School, Oyo, Nigeria. The plant materials were identified, authenticated and deposited at the Herbarium, University of Ibadan, Nigeria, and assigned the voucher specimen number UIH-23177 by Mr. D. P. O. Esimekhuai, the plant taxonomist of the herbarium.

2.2 Extraction of Hydrosols

Hydrosols of the *Tapinanthus* species were obtained via liquid-liquid extraction method for partitioning of chemical constituents of the remnant aqueous distillates recovered after essential oil collection from the Clevenger

through hydrodistillation. At room temperature, 20 mL of the aqueous distillates and 4 mL chloroform were mixed for 5 minutes and left to subside. The chloroform layers were collected, dried over anhydrous sodium sulphate and kept in refrigerator for further analyses [12].

2.3 Analysis of the Hydrosols

The hydrosols were analysed by Shimadzu (GC-2030) series. The GC-MS was equipped with Headspace (HS-20) and QQQ Mass Spectrometer GC-TQ8040NX. The Capillary column type was SH-Rxi-5 SILMS (0.25 x 30 x 0.25). The carrier gas was Helium at constant flow rate of 1.00 ml/min. The column initial temperature was programmed at 60 °C and held for 3 minutes, then increased to 120 °C at the rate of 10° C held for 3 minutes then increased to 180 °C at the rate of 15 °C held for 3 minutes through the split ratio (50:50) mode. The management of the GC-MS system, parameter settings for GC and MS, and data receipt and processing were performed using Shimadzu Realtime Analysis.

The compounds were identified using NIST library by matching their retention times with those of standards, comparing their retention indices with the series of n-hydrocarbon and their mass spectra with published spectra and reference compounds. The relative percentages of each compound found in the sample were determined by integrating the Gas Chromatogram peak areas.

2.4 Cytotoxicity Bioassay

2.4.1 Brine Shrimp Lethality Test

The cytotoxicity of the essential oils was examined using the Brine shrimp lethality assay proposed by Michael *et al*, (1956) which was later developed and improved upon by Sleet and Brendel (1988) [13,14].

The brine shrimp eggs were hatched in a bowl with two compartments, one illuminated and the other un-illuminated. The eggs were introduced into the un-illuminated compartment and were kept for 48 hours. After hatching the shrimps swarm into the illuminated compartment.

The hydrosols were diluted into a concentration of 1000, 100 and 10 ppm. The 1000 ppm stock solution was prepared by adding 0.2 mL of the hydrosol into a solvent comprising 1.6 mL of sea water and 0.2 mL of DMSO, the 100 ppm stock solution was prepared by introducing 0.2 mL of 1000 ppm into 1.8 mL of sea water and the 10 mL stock was prepared by putting 0.2 mL of 100 ppm in 1.8 mL of sea water.

Ten shrimps were introduced into 0.5 mL of each of the triplicates of the stock solutions and the solutions containing the shrimps were made up to 5 mL by sea water. The record of number of shrimps that survived after 24 hours was taken and used to determine LC₅₀ of the essential oils.

2.5 Anticancer Study

2.5.1 MTT Assay

To assess anti-cancer activity of the plant extracts, MTT assay was performed. The impact of these extracts on MDA-MB-231 cell viability was done by seeding 10×10⁴ MDA-MB-231 cells in a 96-well plate in 10% DMEM. The treatment of various plant extract was administered to the cells and incubated for 24 hours in a 5% CO₂ incubator, after which 10 µL MTT solution (5 mg/mL) was added and the cells were incubated at 37°C for 2 hours. To dissolve the formazan crystals generated during MTT reduction, DMSO is added which leads to purple colouration being developed. Microplate reader is used to observe the absorbance of the sample at 530nm and calculations were done [15].

3. Results and Discussion

The hydrodistillation of leaves of *Tapinanthus bangwensis* grown on *Azadirachta indica* and *Tapinanthus bangwensis* grown on *Vitex doniana* gave colourless hydrosols with yield of 0.22% and 0.16% (v/v) respectively. The identified constituents from the hydrosols and their percentage composition are summarised in Table 1.

Table 1. Chemical Composition of Hydrosols of the *Tapinanthus bangwensis*

S/N	Retention Time (Min)	Compound	Chemical group	TBD2	TBK2
1	6.015	Benzaldehyde	Aldehyde	2.54	--
2	7.400	Decane	Hydrocarbon	1.16	1.38
3	8.541	α -Cresol	Phenolics	2.20	1.94
4	9.554	Perchloroethane	Halogenated compound	--	1.82
5	13.980	1-Dodecene	Hydrocarbon	4.42	--
6	13.980	1-methyl-2-octylcyclopropane	Hydrocarbon	--	5.19
7	14.268	Dodecane	Hydrocarbon	1.56	1.76
8	15.674	5-methyl-2-(1-methylethyl)-2-cyclohexen-1-one	Ketone	2.13	--
9	20.473	Cetylalcohol	Alcohol	--	10.04
10	20.476	(Z)-7-Tetradecene	Hydrocarbon	9.14	14.63
11	23.206	Allo-aromadendrene epoxide	Terpenoid	1.33	--
12	24.151	2,4-Di-tert-butylphenol	Phenolics	10.63	9.24
13	26.361	(Z)-7-Hexadecene	Hydrocarbon	12.87	15.25
14	26.569	Cetane	Hydrocarbon	1.00	--
15	31.670	E-15-Heptadecenal	Aldehyde	13.77	--
16	31.848	2,6,10-trimethyltetradecane	Hydrocarbon	1.03	--
17	36.496	1-Heneicosanol	Alcohol	--	13.86
18	36.499	Heptacos-1-ene	Hydrocarbon	8.61	--
19	40.916	Nonacos-1-ene	Hydrocarbon	23.00	19.72
20	48.837	2-(Octadecyloxy)-ethanol	Ether	--	0.07
21	52.241	Octatriaconylpentafluoropropionate	Ester	2.63	3.57
22	56.182	17-Pentatriacontene	Hydrocarbon	1.50	1.53
Total				100.0	100.0

TBD2= Hydrosol of *Tapinanthus bangwensis* grown on *Azadirachta indica*

TBK2= Hydrosol of *Tapinanthus bangwensis* grown on *Vitex doniana*

The GC-MS analysis revealed a diverse range of chemical constituents belonging to nine major chemical classes: *hydrocarbons, alcohols, aldehydes, ketones, phenolics, ethers, esters, epoxides/terpenoids, and halogenated compounds*. The distribution of these classes indicates that the samples contain predominantly hydrocarbon constituents, together with oxygenated compounds that may contribute substantially to their characteristic chemical and biological properties.

A total of seventeen (17) compounds were identified in the hydrosol of *Tapinanthus bangwensis* growing on *Azadirachta indica*, while fourteen (14) compounds were identified in the hydrosol of *Tapinanthus bangwensis* growing on *Vitex doniana*. The identified compounds accounted for 100.0% of the total composition of each hydrosol. Nine (9) compounds were common to both hydrosols, accounting for 64.69% of the total composition of

the hydrosol from *T. bangwensis* growing on *Azadirachta indica* and 69.02% of that from *T. bangwensis* growing on *Vitex doniana*, as shown in Table 2.

Although parasitic plants depend on their host plants for water, minerals, and other nutrients, they retain the ability to synthesize certain primary and secondary metabolites independently [17]. Consequently, the phytochemical profile of a parasitic plant may reflect a combination of compounds synthesized intrinsically by the parasite and those whose production or accumulation is influenced by the host plant. Differences in host plants may therefore alter the availability of precursors, nutrients, and signaling molecules, potentially affecting the biosynthesis and accumulation of bioactive secondary metabolites in the parasite. This host-parasite interaction can ultimately influence the chemical composition and, consequently, the biological activities of parasitic plants such as *Tapinanthus bangwensis*.

The occurrence of several common compounds in the two hydrosols may be attributed to the similar geographical location and environmental conditions under which the host plants and *Tapinanthus bangwensis* were grown.

Table 2. Common compounds in the *Tapinanthus* species

S/N	Retention Time(Min)	Compound	TBD2	TBK2
1	7.400	Decane	1.16	1.38
2	8.541	α -Cresol	2.20	1.94
3	14.268	Dodecane	1.56	1.76
4	20.476	(Z)-7-Tetradecene	9.14	14.63
5	24.151	2,4-Di-tert-butylphenol	10.63	9.24
6	26.361	(Z)-7-Hexadecene	12.87	15.25
7	40.916	Nonacos-1-ene	23.00	19.72
8	52.241	Octatriaonylpentafluoropropionate	2.63	3.57
9	56.182	17-Pentatriacontene	1.50	1.53
		Total	64.69	69.02

The hydrosols are dominated by non-terpene hydrocarbons. Nonacos-1-ene (23.0%), E-15-Heptadecenal (13.77%), (Z)-7-Hexadecene (12.87%), 2,4-Di-tert-butylphenol (10.63), (Z)-7-Tetradecene (9.14%) are the dominant compounds in the hydrosol of *Tapinanthus bangwensis* grown on *Azadirachta indica* while Nonacos-1-ene (19.72%), (Z)-7-Hexadecene (15.25%), (Z)-7-Tetradecene (14.63%), 1-Heneicosanol (13.86%) and Cetylalcohol (10.04%) dominated the hydrosol of *Tapinanthus bangwensis* grown on *Vitex doniana*. Essential oils of *Tapinanthus bangwensis* have been reported to be dominated by non-terpene hydrocarbons [16] with some of these compounds such as heneicosane, pentadecane and hexacosane being analogues of the compounds 1-Heneicosanol, decane and dodecane found in the hydrosols the *Tapinanthus bangwensis* in our current study.

2,4-Di-tert-butylphenol demonstrated significant cytotoxicity against HeLa cells, with an IC₅₀ value of 10 μ g/mL and showed a strong ability to induce apoptotic gene expression in cancer cell lines, comparable to the effects of the standard drug Cisplatin [17,18]. 2,4-Di-tert-butylphenol, extracted from the thermophilic *Bacillus licheniformis* found in an Algerian hot spring, demonstrated activity against two multidrug-resistant bacteria, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, both in pure and mixed cultures. Dodecane has been reported to possess antimicrobial activities [19].

A skin cleanser formulated with cetyl alcohol- one of dominant compounds of hydrosol of *Tapinanthus bangwensis* grown on *Vitex doniana*, stearyl alcohol, colloidal oatmeal, castoryl maleate, and vitamin E effectively removed excess oil, dirt, dead skin cells, microorganisms, sweat, and dust, while offering a soothing and gentle

cleansing experience [20]. The structures of compounds that are common to the two hydrosols are shown in figure 1 below:

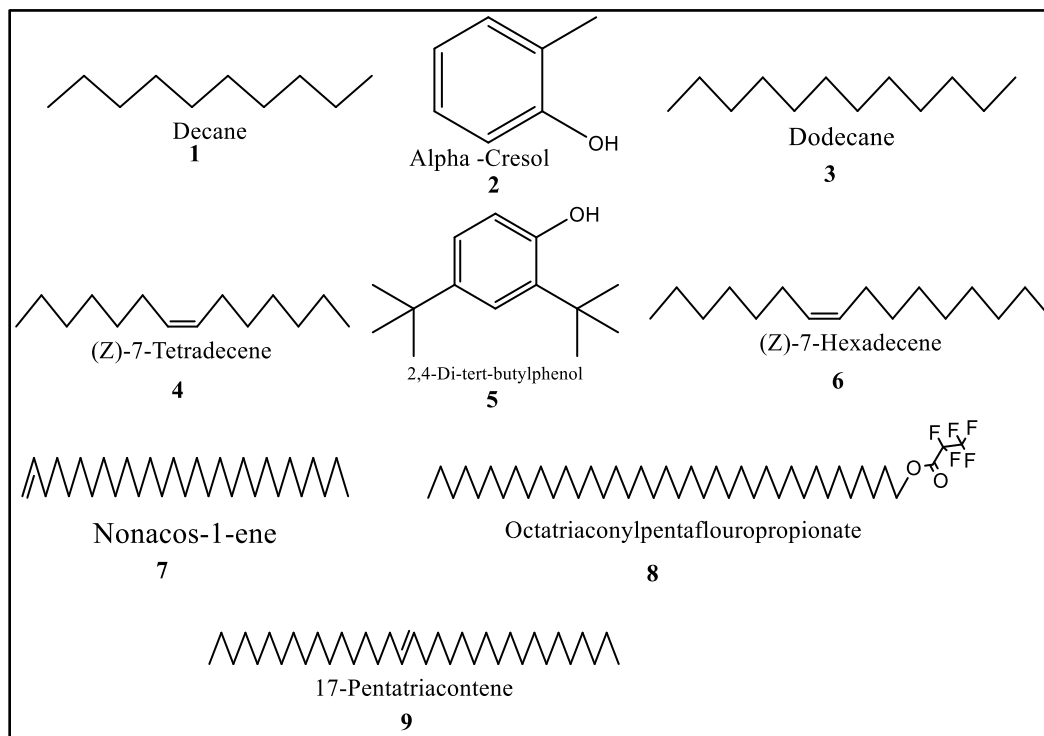


Figure 1. Structures of the Common Compounds in the Hydrosols

3.1 Cytotoxicity Activities of Hydrosols of the Selected *Tapinanthus* Species

Table 3 and Figure 2 present the results of the cytotoxicity assessment of the hydrosols obtained from the two *Tapinanthus* samples. The results indicate that both hydrosols exhibited concentration-dependent cytotoxicity against *Artemia salina*. As shown in Table 3, the percentage mortality increased progressively with increasing hydrosol concentration. For TBD2, mortality increased from $10.00 \pm 0.10\%$ at 10 ppm to $23.33 \pm 0.0081\%$ at 100 ppm and further to $40.00 \pm 0.50\%$ at 1,000 ppm. Similarly, TBK2 produced $29.99 \pm 0.74\%$, $33.33 \pm 0.33\%$, and $50.00 \pm 1.00\%$ mortality at 10, 100, and 1,000 ppm, respectively. Statistical analysis was performed at a 95% confidence level, with differences considered statistically significant at $p < 0.05$. The observed variation in mortality across the tested concentrations indicates a concentration-dependent response; however, statistical significance should be interpreted based on the results of the one-way ANOVA and Duncan's multiple range test. Where significant differences were observed ($p < 0.05$), these indicate that the corresponding treatment concentrations produced significantly different cytotoxic responses.

However, the hydrosols were considered non-toxic, as their LC_{50} values were greater than 1,000 ppm. The hydrosol of *Tapinanthus bangwensis* growing on *Azadirachta indica* exhibited an LC_{50} value of 2,995.024 ppm, while that of *T. bangwensis* growing on *Vitex doniana* had an LC_{50} value of 6,672.169 ppm [23]. The higher LC_{50} value recorded for the hydrosol obtained from *T. bangwensis* growing on *V. doniana* indicates comparatively lower toxicity toward *Artemia salina* than the hydrosol obtained from the *A. indica* host. This difference suggests that the host plant may influence the composition and consequently the biological activity of the hydrosol.

Some compounds identified in the hydrosols, including α -cresol, 2,4-di-tert-butylphenol, (Z)-7-tetradecene, (Z)-3-hexadecene, and E-15-heptadecenal, have previously been reported to exhibit cytotoxic or toxic effects against different cell types [24,25,26]. However, the cytotoxic effects of these individual constituents may have been

attenuated by interactions with other compounds present in the hydrosols, potentially through antagonistic effects. This may explain the relatively low overall cytotoxicity observed for the hydrosols despite the presence of some potentially bioactive compounds at appreciable concentrations. The relatively high LC₅₀ values obtained for both hydrosols further support their low toxicity in the *Artemia salina* assay and suggest that the hydrosols may have a comparatively favourable toxicity profile under the conditions employed in this study.

Table 3. Cytotoxicity Assay of Hydrosols of the *Tapinanthus* Species

Conc. (ppm)	TBD2 (Mean ± SD)	TBK (Mean ± SD)
10	10.00 ± 0.1000	29.99 ± 0.7400
100	23.33 ± 0.0081	33.33 ± 0.3300
1000	40.00 ± 0.5000	50.00 ± 1.0000

TBD2= *Tapinanthus bangwensis* grown on *Azadirachta indica* (LC₅₀=2995.024 ppm)

TBK2= *Tapinanthus bangwensis* grown on *Vitex doniana* (LC₅₀=6672.169 ppm)

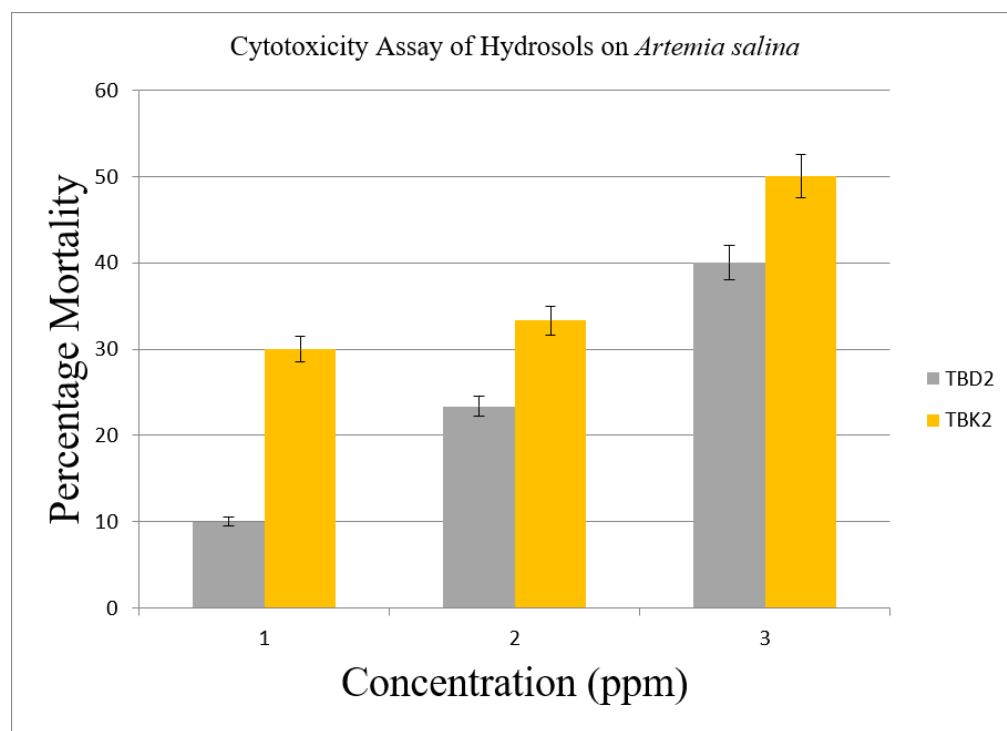


Figure 2. Cytotoxicity Assay of Hydrosols of the *Tapinanthus* Species [TBD2= *Tapinanthus bangwensis* grown on *Azadirachta indica* (LC₅₀=2995.024 ppm), TBK2= *Tapinanthus bangwensis* grown on *Vitex doniana* (LC₅₀=6672.169 ppm)]

3. 2 Anticancer Activities of Hydrosols of the Selected *Tapinanthus* Species

The hydrosols of the selected *Tapinanthus* samples exhibited promising anticancer activity against the MDA-MB-231 breast cancer cell line, an aggressive subtype of breast cancer, in a concentration-dependent manner (Table 4 and figure 3). The hydrosol of *Tapinanthus bangwensis* growing on *Azadirachta indica* exhibited an IC₅₀ value of 230.2357 µL/mL, while the hydrosol of *T. bangwensis* growing on *Vitex doniana* showed a lower IC₅₀ value of 46.4753 µL/mL, indicating greater cytotoxic potency. The difference in IC₅₀ values between the two hydrosols was statistically significant at the 95% confidence level ($p < 0.05$), indicating that the hydrosol obtained from *T. bangwensis* growing on *V. doniana* exhibited significantly greater cytotoxic potency against MDA-MB-231 cells than that obtained from the *A. indica* host. These activities may be attributed to the presence of biologically active compounds in the hydrosols.

2,4-Di-tert-butylphenol, a compound common to both hydrosols, has previously been reported to induce apoptosis in MCF-7 breast cancer cells, with an IC_{50} value of 70 μ M [27]. In addition, the essential oil of *Cleome amblyocarpa* has been reported to exhibit significant anti-inflammatory activity through the inhibition of lipoxygenase (LOX) and cyclooxygenases (COX-1 and COX-2), as well as through membrane stabilization. This essential oil contained alloaromadendrene epoxide, which was also identified in the hydrosol of *T. bangwensis* growing on *Azadirachta indica* [28].

The observed anticancer activity of the hydrosols may therefore be associated, at least in part, with the presence of these and other bioactive constituents. However, further studies are required to establish the specific contributions and possible synergistic or antagonistic interactions of the individual compounds.

Table 4. MTT Assay of Hydrosols of the *Tapinanthus* Species

Conc. (μ L/L)	TBD2 Mean $\pm$ SD	TBK2 Mean $\pm$ SD
0	100.00 $\pm$ 0.0000	100.00 $\pm$ 0.0000
50	65.67 $\pm$ 0.6700	63.9386 $\pm$ 0.9300
75	63.13 $\pm$ 0.0048	50.21624 $\pm$ 0.2100
100	60.95 $\pm$ 0.9000	45.5835 $\pm$ 0.4200
125	52.39 $\pm$ 0.3900	40.00 $\pm$ 0.20000
150	30.00 $\pm$ 1.0000	32.3863 $\pm$ 0.0063

TBD2= *Tapinanthus bangwensis* grown on *Azadirachta indica* (IC_{50} =230.2357 μ L/ml)

TBK2= *Tapinanthus bangwensis* grown on *Vitex doniana* (IC_{50} =46.4753 μ L/ml)

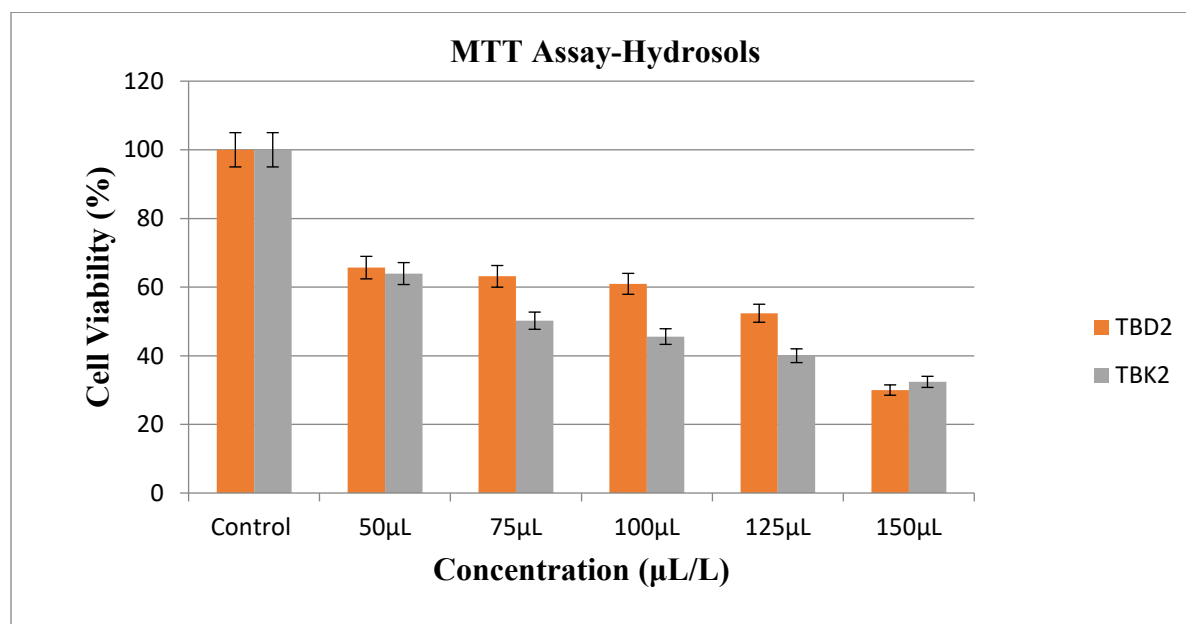


Figure 3. MTT Assay of Hydrosols of the *Tapinanthus* Species [TBD2= *Tapinanthus bangwensis* grown on *Azadirachta indica* (IC_{50} =230.2357 μ L/ml), TBK2= *Tapinanthus bangwensis* grown on *Vitex doniana* (IC_{50} =46.4753 μ L/ml)]

4. Conclusion

The current study established the chemical compositions of the hydrosols of *Tapinanthus bangwensis* grown on *Azadirachta indica* and *Tapinanthus bangwensis* grown on *Vitex doniana*, showed that the hydrosols were not toxic to *Artamia salina* but possess promising anticancer potentials against MDA-MB-231 cell line, an aggressive breast cancer cell line. The hydrosols are reservoirs of biologically active compounds that could be explored in chemo-aromatherapy.

Limitations of the Study

Despite the promising anticancer activity observed for the *Tapinanthus bangwensis* hydrosols, several limitations should be acknowledged. First, the anticancer evaluation was performed using only one cancer cell line, MDA-MB-231 breast cancer cells. Although this cell line represents an aggressive subtype of breast cancer, the findings cannot be generalized to other breast cancer subtypes or different cancer types. Second, the study was limited to an MTT-based assessment of cell viability and did not investigate the underlying molecular mechanisms responsible for the observed cytotoxic effects. Thus, it remains unclear whether the activity involves apoptosis, oxidative stress, cell-cycle arrest, or other cellular pathways. Third, the study did not include normal, non-cancerous cell lines; therefore, the selectivity of the hydrosols toward cancer cells relative to normal cells could not be established. The brine shrimp lethality assay provides a preliminary indication of general toxicity but cannot substitute for a mammalian normal-cell selectivity assessment. Finally, the anticancer activity was demonstrated only under *in vitro* conditions, with no *in vivo* animal studies to confirm efficacy, bioavailability, pharmacological activity, or systemic safety. Consequently, the reported IC₅₀ values should be interpreted as preliminary evidence of anticancer potential rather than proof of therapeutic effectiveness. Future studies should therefore employ multiple cancer cell lines, appropriate normal-cell controls, mechanistic assays, isolation and characterization of the active constituents, and *in vivo* models to validate the therapeutic potential and safety of the hydrosols. This is particularly important because the present study itself recognizes that further work is needed to establish the individual and possible synergistic or antagonistic contributions of the identified compounds.

Acknowledgements

The authors acknowledge the Government of India for funding this project through Sir C.V. Raman International Fellowship for African Researchers.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Contribution

Abeeb A. Azeez conceived and designed the study, performed the experiments, analyzed the data, and drafted the manuscript. A. Bhatnagar, S.H. Makwana, C.C. Mandal, and V. Kumar supervised the study, validated the findings, and critically revised the manuscript. Adeolu T. Abimbola contributed to plant collection and authentication. Mustopha O. Abdullahi assisted with the chemical composition analysis. S.A. Aboaba participated in the investigation and data analysis. All authors reviewed, approved, and agreed to the published version of the manuscript.

Funding

This research received no external funding

References

1. Namkoong J., Kern D., Knaggs H. E. (2018). [Assessment of human skin gene expression by different blends of plant extracts with implications to periorbital skin aging](#). International Journal of Molecular Sciences, 19(11), 3349. doi: [10.3390/ijms19113349](https://doi.org/10.3390/ijms19113349).
2. Sitarek P., Merecz-Sadowska A., Kowalczyk T., Wieczfinska J., Zajdel R., Sliwiński T. (2020). [Potential synergistic action of bioactive compounds from plant extracts against skin infecting microorganisms](#). International Journal of Molecular Sciences, 21(14), 5105. doi: [10.3390/ijms21145105](https://doi.org/10.3390/ijms21145105).
3. Hoareau L., DaSilva E. J. (1999). [Medicinal plants: A re-emerging health aid](#). Electronic Journal of Biotechnology, 2(2). doi: [10.2225/VOL2-ISSUE2-FULLTEXT-2](https://doi.org/10.2225/VOL2-ISSUE2-FULLTEXT-2).
4. Smith P. M. (2005). [When your hormones go haywire: Solutions for women over 40](#) (p. 109).

5. Burkil H. M. (1985). [Useful plants of West Tropical Africa](#) (Vol. 3, pp. 548–560). Royal Botanical Gardens Kew.
6. Grossarth-Maticek R., Ziegler R. (2007). [Prospective controlled cohort studies on long-term therapy of ovarian cancer patients with mistletoe \(*Viscum album* L.\) extracts Iscador](#). *Arzneimittel Forschung*, 57, 665–678. doi: [10.1055/s-0031-1296666](https://doi.org/10.1055/s-0031-1296666).
7. Osadebe P. O., Omeje E. O., Nworu S. C., Esimone C. O., Uzor P. F., David E. K., Uzoma J. U. (2010). [Anti-diabetic principles of *Loranthus micranthus* \(Linn.\) parasitic on *Persea americana*](#). *Asian Pacific Journal of Tropical Medicine*, 1, 619–623. doi: [10.1016/S1995-7645\(10\)60150-2](https://doi.org/10.1016/S1995-7645(10)60150-2).
8. Tornuk F., Cankurt H., Ozturk I., Sagdic O., Bayram O., Yetim H. (2011). [Efficacy of various plant hydrosols as natural food sanitizers in reducing *Escherichia coli* O157:H7 and *Salmonella typhimurium* on fresh cut carrots and apples](#). *International Journal of Food Microbiology*, 148, 30–35. doi: [10.1016/j.ijfoodmicro.2011.04.022](https://doi.org/10.1016/j.ijfoodmicro.2011.04.022).
9. Hamed A., Afifi M., Etemadfar, H. (2017). [Investigating chemical composition and indications of hydrosol soft drinks \(aromatic waters\) used in Persian folk medicine for women's hormonal and reproductive health conditions](#). *Journal of Evidence-Based Complementary & Alternative Medicine*, 22(4), 824–829. doi: [10.1177/2156587217717413](https://doi.org/10.1177/2156587217717413).
10. Almeida H. H. S., Fernandes I. P., Amaral J. S., Rodrigues A. E., Barreiro M.-F. (2024). [Unlocking the potential of hydrosols: Transforming essential oil byproducts into valuable resources](#). *Molecules*, 29(19), 4660. doi: [10.3390/molecules29194660](https://doi.org/10.3390/molecules29194660).
11. Shafie M. H., Kamal M. L., Abdul Razak N. A., Hasan S., Uyup N. H., Abdul Rashid N. F., Zafarina Z. (2022). [Antioxidant and antimicrobial activity of plant hydrosol and its potential application in cosmeceutical products](#). *Jundishapur Journal of Natural Pharmaceutical Products*, 17(4), e124018. doi: [10.5812/jjnpp-124018](https://doi.org/10.5812/jjnpp-124018)
12. Afassi F., Charki A., El Youssfi C., El Loukili H., Labhar A., Ahari M., Amhamdi H., Mourabit F., Ez Zoubi Y., Salhi A., Elyoussfi A. (2026). [Chemical composition and biological activities of essential oils from medicinal plants: a comprehensive review and bibliometric analysis](#). *Results in Engineering*, 32, 112439. doi: [10.1016/j.rineng.2026.112439](https://doi.org/10.1016/j.rineng.2026.112439).
13. Yann-Olivier H., Miguel A. A., Luis G. S., Catherine B., Christine R, 2018. [Evaluation of combinations of essential oils and essential oils with hydrosols on antimicrobial and antioxidant activities](#). *Journal of Pharmacy & Pharmacognosy Research*, 6 (3), 216-230. doi: [10.56499/jppres17.310_6.3.216](https://doi.org/10.56499/jppres17.310_6.3.216).
14. Michael A. S., Thompson C. G., Abramowitz M. (1951). [Artenia salina as a test organism for bioassay](#). *Science*, 123, 464. doi: [10.1126/science.123.3194.464](https://doi.org/10.1126/science.123.3194.464).
15. Sleet R. B., Brendel K. (1988). [Improved methods for harvesting and counting synchronous populations of *Artemia salina* for use in developmental toxicology](#). *Ecotoxicology and Environmental Safety*, 7, 435–446. doi: [10.1016/0147-6513\(83\)90082-9](https://doi.org/10.1016/0147-6513(83)90082-9).
16. Emran H., Sepehrara A., Arabnozari H., Sharifianjazi F., Enderami S. E., Sarker S. D., Hassannia H., Nahar L. (2024). [Alkaloid-rich extract of jujube seed regenerates the antiproliferative effect of paclitaxel on paclitaxel-resistant MDA-MB-231 breast cancer cell line in 2D and 3D cultures](#). *Journal of Agriculture and Food Research*, 18, 101438. doi: [10.1016/j.jafr.2024.101438](https://doi.org/10.1016/j.jafr.2024.101438).
17. Alwan S., Al-Khesraji T., Al-Samarrai G. (2025). [Effect of the host type and the parasite species on the phytochemicals of two dodder species, compared to healthy hosts](#). *IOP Conference Series: Earth and Environmental Science*, 1549, 012132. doi: [10.1088/1755-1315/1549/1/012132](https://doi.org/10.1088/1755-1315/1549/1/012132).
18. Atewolara-Odule O. C., Aiyelaagbe O. O., Olubomehin O. O., Ogunmoye A. O., Feyisola R. T. 2020. [Antioxidant Activity of Some Secondary Metabolites from *Tapinanthus bangwensis* \(Engl., and K.Krause\) \[Loranthaceae\] Grown in Nigeria](#). *Scientific Africa*, 8:348-353. doi: [10.1016/j.sciaf.2020.e00348](https://doi.org/10.1016/j.sciaf.2020.e00348).

19. Varsha K. K., Devendra L., Shilpa G., Priya S., Pandey A., Nampoothiri K. M. (2015). [2,4-Di-tert-butyl phenol as the antifungal, antioxidant bioactive purified from a newly isolated Lactococcus sp.](#) *International Journal of Food Microbiology*, 211, 44–50. doi: [10.1016/j.ijfoodmicro.2015.06.025](https://doi.org/10.1016/j.ijfoodmicro.2015.06.025).
20. Nair R. V. R., Jayasree D. V., Biju P. G., Baby, S. (2018). [Anti-inflammatory and anticancer activities of erythrodiol-3-acetate and 2,4-di-tert-butylphenol isolated from Humboldtia unijuga.](#) *Natural Product Research*, 26, 1–4. doi: [10.1080/14786419.2018.1531406](https://doi.org/10.1080/14786419.2018.1531406).
21. Padma M., Ganesan S., Jayaseelan T., Azhagumadhavan S., Sasikala P., Senthilkumar S., Mani, P. (2018). [Phytochemical screening and GC-MS analysis of bioactive compounds present in ethanolic leaves extract of Silybum marianum \(L\).](#) *Journal of Drug Delivery and Therapeutics*, 8(5), 168–175. doi: [10.22270/jddt.v9i1.2174](https://doi.org/10.22270/jddt.v9i1.2174).
22. Gupta S., Shah H. B., Bhardwaj P., Holani A., Singh C., Yadav S., Nair R., Korukonda K. (2023). [Cetyl alcohol, stearyl alcohol and colloidal oatmeal-based gentle skin cleanser in management of dry and sensitive skin: A cross-sectional study.](#) *International Journal of Research in Dermatology*, 9(6), 353–361. doi: [10.18203/issn.2455-4529.IntJResDermatol20233179](https://doi.org/10.18203/issn.2455-4529.IntJResDermatol20233179).
23. Silva M.L., Silva F.J.. 2023. [Bioassay With Artemia Salina L.: A Gateway to Understanding the Toxicity of Medicinal Plant Extracts.](#) doi: [10.37885/230814206](https://doi.org/10.37885/230814206).
24. Supardy N., Afifah N., Ibrahim D., Sulaiman S., Zakaria N. A. (2012). [Inhibition of Klebsiella pneumoniae ATCC 13883 cells by hexane extract of Halimeda discoidea \(Decaisne\) and the identification of its potential bioactive compounds.](#) *Journal of Microbiology and Biotechnology*, 22(6), 872-881. doi: [10.4014/jmb.1111.11053](https://doi.org/10.4014/jmb.1111.11053).
25. Chang M. C., Chang H. H., Chan C. P., Yeung S. Y., Hsien H. C., Lin B. R., Yeh C. Y., Tseng W. Y., Tseng S. K., Jeng J. H. (2014). [p-Cresol affects reactive oxygen species generation, cell cycle arrest, cytotoxicity and inflammation/atherosclerosis-related modulators production in endothelial cells and mononuclear cells.](#) *PLoS One*, 9(12), e114446. doi: [10.1371/journal.pone.0114446](https://doi.org/10.1371/journal.pone.0114446).
26. Ahmed R., Abdel-Motaal F., Abou-Ellail M., et al. (2022). [Antioxidant and cytotoxic activity of ethyl acetate extract from Thermomyces lanuginosus and Aspergillus nidulans isolated from rhizospheric region of peanut \(Arachis hypogaea\).](#) *Research Square Platform LLC*, 2022. doi: <https://doi.org/10.21203/rs.3.rs-2315764/v1>
27. Annamalai A., Aravinth P., Perumal P., Rajaram R., Dhanasundaram S., Narayanan M., Maharaja S., Manikumar A. (2023). [Isolation and characterization of 2,4-di-tert-butyl phenol from the brown seaweed, Dictyota ciliolata and assessment of its antioxidant and anticancer characteristics.](#) *Biocatalysis and Agricultural Biotechnology*, 54, 102933. doi: [10.1016/j.bcab.2023.102933](https://doi.org/10.1016/j.bcab.2023.102933).
28. Abd-ElGawad A. M., Elgamal A. M., El-Amier Y. A., Mohamed T. A., El Gendy A. E.-N. G., Elshamy A. I. (2021). [Chemical composition, allelopathic, antioxidant, and anti-inflammatory activities of sesquiterpenes rich essential oil of Cleome amblyocarpa Barratte & Murb.](#) *Plants*, 10(7), 1294. doi: [10.3390/plants10071294](https://doi.org/10.3390/plants10071294).