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Original Research Article

Phytochemical investigation and anticancer potential of *Wattakaka volubilis* (L. Fil.) Stapf. stem: A natural Ayurvedic alternative to Murva and Jeevanti

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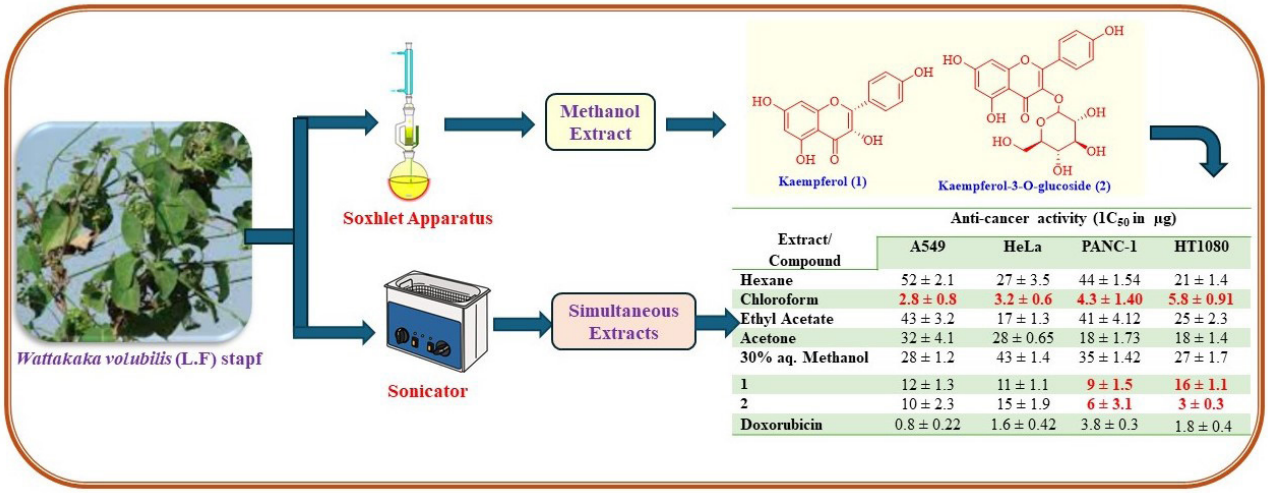
Abstract

The *Wattakaka volubilis* (L. Fil.) Stapf different plant parts have been used traditionally for different diseases like dyspepsia, diuretic, emetic, expectorant, etc. The plant is used as an alternative to the Ayurvedic herbal drugs Murva and Jeevanti. Chemical examination of the stem yielded two flavonoids: kaempferol 1 and its glycoside, kaempferol-3-O-glucoside 2. *In-vitro* cytotoxic activity of chloroform extract showed significant inhibition towards HeLa (IC_{50}: 3.2 µg) and A549 (IC_{50}: 2.8 µg) cell lines compared to standard doxorubicin (IC_{50}: 3.2 and 0.8 µg, respectively). Compound 2 showed good cytotoxicity against HT1080 (IC_{50}: 3.0µg) and PANC-1 (IC_{50}: 6.0 µg) cell lines compared to standard doxorubicin (IC_{50}: 1.8 and 3.8 µg, respectively). These findings suggest the therapeutic potential of *W. volubilis* in cancer treatment.

Keywords: Ayurveda, Cytotoxic activity, Flavonoids, Isolation, Kaempferol, *Wattakaka volubilis*



Graphical Abstract

Introduction

Wattakaka volubilis (Linn.f), a member of the Asclepiadaceae family, is a small shrub with a twining, woody vine. It is widely distributed across India, Sri Lanka, Myanmar, Indonesia, Thailand, and China (Kyaw *et al*., 2022). In India, only a single species of this plant has been identified (Moni Thomas *et al*., 1996). The flowers and leaves are consumed locally as a vegetable, and various parts of the plant have been traditionally used in South Asian countries for therapeutic purposes (Kyaw *et al*., 2022). It is also widely utilized in Ayurveda as an

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alternative source for the herbal drug Murva (Keerthana *et al*, 2021).

Traditionally, the plant's juice has been used as a sternutatory, while its roots and tender stalks serve as emetics, purgatives, and expectorants, as well as for treating lithiasis. The leaves are known to relieve rheumatic pain, cough, fever, and cold symptoms. A combination of the roots and leaves is used for treating skin diseases, diabetes (Hajirac *et al*., 2019), jaundice, poison bites, blood disorders, and respiratory ailments. Notably, this plant is particularly useful for scorpion and snake bites.

The entire plant has been reported to exhibit anti-inflammatory (Hossain *et al*., 2010), anthelmintic, antipyretic, aphrodisiac, astringent, and anti-asthmatic properties, as well as applications in treating leukoderma (Sharma *et al*., 2014). It is regarded as the botanical equivalent of the classical Ayurvedic herb Jeevanti, which is considered one of the most potent vegetable-based medicines in various Ayurvedic texts (Sharma *et al*., 2014). Phytochemical studies indicate that *W. volubilis* is a rich source of bioactive molecules such as steroids, acids, alkaloids, sugars, and flavonoids. The flavonoids epicatechin, catechin, rutin, quercetin, and kaempferol are present in the leaves (Satapathy *et al*., 2013). The roots contain β-sitosterol, drevogenin A, 9,12-octadecadienoic acid, quinic acid, 1,2-benzenedicarboxylic acid diisooctyl ester, 5,7-dihydroxy-6,8-dimethoxyflavone, N-[4-bromo-butyl]-2-piperidinone, and digitoxose (Joshi *et al*., 2013). Additionally, an unusual novel triterpenoid ether, multiflor-7-en-12,13-ether and multiflor-7-en-12α-ol, has also been reported from this plant (Niranjan Reddy *et al*., 2002).

Although various parts of *W. volubilis* have been traditionally employed for different therapeutic purposes, the stem remains relatively underexplored compared to the roots and leaves. Despite its recognized role in traditional Ayurvedic medicine as a substitute for Murva and Jeevanti, there is a notable gap in the literature regarding the bioactive constituents and therapeutic potential of the stem. No prior studies have comprehensively evaluated the anticancer potential of isolated flavonoids from the stem. This investigation aims to fill that gap by conducting a targeted phytochemical analysis of the stem and assessing the *in-vitro* cytotoxic activity of its extracts and isolated compounds, thus providing a scientific basis for its traditional usage and identifying potential candidates for anticancer drug development.

Materials and Methods

General procedure

NMR spectra were recorded on a Bruker Avance 300 MHz spectrometer using $CDCl_3$ and methanol-d_5 as solvents, with TMS as the internal standard. Electron impact (EI) and chemical ionization mass spectra were obtained using a VG Micromass 7070H instrument. Fraction analysis was performed on Merck silica gel precoated TLC plates, with spot visualization under UV light (254 nm) and by heating with anisaldehyde-sulfuric acid spray reagent at 110°C. Silica gel (100–200 mesh) for column chromatography was sourced from Merck.

Plant collection

The stem part of the plant was collected from CSIR-CIMAP, Research Centre, Boduppal, Hyderabad, India. Taxonomic identification and authentication were carried out by Dr. Venkat Ramana, Assistant Professor, Department of Botany, Nizam College, Osmania University, Hyderabad, India. A voucher specimen (CIMAP-WV/22) has been deposited at CSIR-CIMAP, Research Centre, Boduppal, Hyderabad.

Extraction

For isolation

W. volubilis stem coarsely powder (725 grams) material was defatted with hexane at room temperature overnight, then filtered. This process was repeated two more times. The obtained filtrates were combined and concentrated under reduced pressure using a rotary evaporator to get the syrupy consistency (2.78 g). The defatted stem material was loaded in a 2.0 L soxhlet chamber, then placed into a 5 L round-bottom flask containing methanol (nearly 2000–2500 mL). The solvent was heated to reflux using a heating mantle. After completion of the extraction, the extract was filtered and concentrated under reduced pressure and resulting in 56 g of methanol extract. The methanol extract was subsequently used for the isolation of phytomolecules.

For in-vitro cytotoxic activity studies

Twenty grams of stem powder was taken into a conical flask and added 70 mL of Laboratory (LR) grade n-hexane and this was extracted by sonication at 45°C for 30 minutes. Extraction was filtered through filter paper into a round-bottom flask and the residue was returned to the conical flask. The above extraction procedure was repeated two more times. All the filtrates were mixed and concentrated under reduced pressure by using a rotary evaporator. Then the defatted material was dried and loaded in the same conical flask and extracted simultaneously with different LR grade solvents, i.e, chloroform, ethyl acetate, acetone, methanol and 20% aqueous methanol using sonicator by above mention extraction procedure. All the extract solutions were pooled in different round-bottom flasks and concentrated under reduced pressure. These extracts were used for *in-vitro* cytotoxic activity studies.

Isolation of phytomolecules

The stem methanol extract (50 g) was purified using

column chromatography with silica gel (100–200 mesh) as the stationary phase and hexane and mixtures of ethyl acetate in hexane as the mobile phase. The column was initially eluted with hexane, followed by a gradual increase in ethyl acetate concentration in hexane, and finally with 10% methanol in ethyl acetate. The eluents were collected in fractions of 250 mL each and concentrated. The identical 50% ethyl acetate in hexane fractions (88–108) are mixed, concentrated and washed repeatedly with hexane and a minimum amount of cold chloroform, which yielded pale yellow compound 1 (80 mg) in pure form. Similarly, the identical 10% methanol in ethyl acetate fractions (119–130) are mixed, concentrated and washed repeatedly with cold chloroform, later washed with a minimum amount of methanol, yielding yellow colored compound 2 (40 mg) in pure form.

In-vitro cytotoxic activity

Cell culture, maintenance, and anti-proliferative evaluation

All cell lines used in this study were obtained from the American Type Culture Collection (ATCC, USA). The cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) in a humidified atmosphere of 5% CO_2 at 37°C. When sub-confluent, cells were trypsinized from T25 flasks or 60 mm dishes and seeded into 96-well plates for further experiments.

The test compounds were assessed for *in-vitro* anti-proliferative activity against four different human cancer cell lines using a 48-hour continuous drug exposure protocol. Cell viability and proliferation were evaluated using the sulforhodamine B (SRB) assay. Cells were seeded into 96-well microtiter plates at densities appropriate for their doubling times in 200 µL aliquots of their respective media containing 10% FBS. The plates were incubated at 37°C with 5% CO_2, 95% air, and 100% relative humidity for 24 hours before the addition of test compounds.

Test compounds (2 µL) were added to each well containing cells in 198 µL of media, ensuring the required final drug concentrations. Five concentrations (0.01, 0.1, 1, 10, and 100 µM or µg/mL) were tested, and all experiments were performed in triplicate. After 48 hours of incubation, the assay was terminated by adding 10% trichloroacetic acid (TCA) and incubating for 60 minutes at 4°C. The plates were then air-dried and washed three times with 1× PBS. Cells were stained with 0.57% sulforhodamine B dye for 1 hour at 37°C, followed by three washes with 1% acetic acid. After air-drying, the bound dye was solubilized using 50 µL of 10 mM Tris buffer (pH 8.0), and absorbance was measured at 510 nm using an EnSpire multimode reader.

A separate plate was terminated at 24 hours to serve as the time-zero (t_0) control. After 48 hours, treated plates included a DMSO-treated control (tc) and five-dose-treated groups. The IC_{50} values were determined using linear interpolation from plots of percent growth *versus* the logarithm of compound concentrations (µM). Results are presented as mean ± standard deviation (SD) from three independent experiments.

Results and Discussions

Chemistry

Compound 1 was obtained as a yellow amorphous powder. Based on mass spectral data (m/z 287.4 $[M+1]^+$), its molecular formula was determined to be $C_{15}H_{10}O_6$. The ^{1}H-NMR spectrum of compound 1 exhibited meta-coupled aromatic protons at δH 6.28 (1H, d, J = 2.0 Hz) and 6.52 (1H, d, J = 2.0 Hz), corresponding to H-6 and H-8, respectively. Additionally, two doublet signals were observed at δH 8.04 (2H, d, J = 2.8, 11.5 Hz, H-2′ and H-6′) and 6.95 (2H, d, J = 2.8, 9.7 Hz, H-3′ and H-5′), indicating the presence of four aromatic protons in ring B, characteristic of a 1′,4′-disubstituted flavone system. The ^{13}C-NMR spectrum displayed fifteen carbon signals, including a distinct carbonyl signal at δC 176.6. The degree of unsaturation accounted for eight out of eleven double bond equivalents, with the remaining three degrees of unsaturation consistent with a flavonol structure. A comparison of the obtained NMR data with previously reported literature confirmed that the

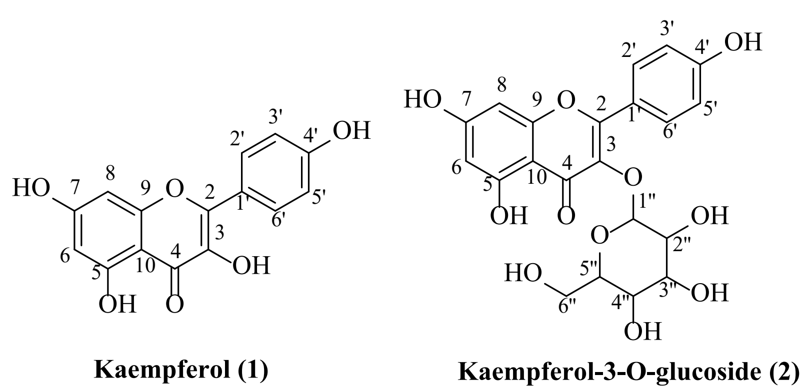

Figure 1: Structures of isolated flavonoids from stem.

Table 1: Anticancer activity studies

Extract/Compound	*Anticancer activity (IC_{50} in μg)*			
	A549	*HeLa*	*PANC-1*	*HT1080*
Hexane	52 ± 2.1	27 ± 3.5	44 ± 1.54	21 ± 1.4
Chloroform	2.8 ± 0.8	3.2 ± 0.6	4.3 ± 1.40	5.8 ± 0.91
Ethyl acetate	43 ± 3.2	17 ± 1.3	41 ± 4.12	25 ± 2.3
Acetone	32 ± 4.1	28 ± 0.65	18 ± 1.73	18 ± 1.4
30% aq. Methanol	28 ± 1.2	43 ± 1.4	35 ± 1.42	27 ± 1.7
Kaempferol (1)	12 ± 1.3	11 ± 1.1	9 ± 1.5	16 ± 1.1
Kaempferol-3-O-glucoside (2)	10 ± 2.3	15 ± 1.9	6 ± 3.1	3 ± 0.3
Doxorubicin	0.8 ± 0.22	1.6 ± 0.42	3.8 ± 0.3	1.8 ± 0.4

structure of compound 1 corresponds to kaempferol (Figure 1).

Compound 2 was also obtained as a yellow powder. Its ^{1}H and ^{13}C-NMR spectra (300 MHz, Acetone) showed a close resemblance to compound 1 (Kaempferol), except for additional signals indicating the presence of a glucose moiety. The ^{1}H-NMR spectrum displayed aromatic proton signals at δH 6.29 (1H, CH, C-6), 6.29 (1H, CH, C-8), 6.53 (1H, CH, C-5′), 6.97 (1H, CH, C-6′), and 6.99 (1H, CH, C-2′), confirming the flavonoid core structure. The presence of a glucose moiety was inferred from characteristic additional signals. The ^{13}C-NMR spectrum exhibited 19 distinct signals, with the highest chemical shift at δC 178.24 (carbonyl, C=O). Signals corresponding to C=C bonds attached to hydroxyl (-OH) groups appeared in the range 164.36–157.77 ppm, while glucose-related carbons attached to hydroxyl groups were observed in the range 77.07–61.83 ppm. A comparison of the ^{1}H and ^{13}C-NMR data with previously reported literature confirmed that compound 2 is kaempferol-3-O-glucoside (Astragalin) (Figure 1).

In-vitro cytotoxic activity

The *in-vitro* cytotoxic activity of the individual stem extracts and the isolated compounds kaempferol (1) and kaempferol-3-O-glucoside (2) were evaluated against four different cancer cell lines: A549 (human lung carcinoma cells), HeLa (human cervical carcinoma cells), PANC-1 (human pancreatic adenocarcinoma cells), and HT1080 (human fibrosarcoma cells) (Table 1). The results indicate that the chloroform extract exhibited notable anticancer activity against all tested cell lines, with particularly significant inhibition against HeLa cells (IC_{50}: 3.2 ± 0.6 μg) compared to the standard drug doxorubicin (IC_{50}: 1.6 ± 0.42 μg). Among the isolated compounds, kaempferol-3-O-glucoside 2 demonstrated significant cytotoxic activity against HT1080 cells (IC_{50}: 3 ± 0.3 μg; doxorubicin: 1.8 ± 0.4 μg) and PANC-1 cells (IC_{50}: 6 ± 3.1 μg; doxorubicin: 3.8 ± 0.3 μg). Similarly, kaempferol (1) showed notable anticancer activity against PANC-1 cells (IC_{50}: 9 ± 1.5 μg; doxorubicin: 3.8 ± 0.3 μg).

Conclusion

In this study, the methanol extract of the stem of *W. volubilis* was subjected to phytochemical investigation, leading to the isolation of two flavonoids: Kaempferol (1) and kaempferol-3-O-glucoside (2), the latter being reported for the first time from this plant. Among the tested extracts, the chloroform fraction exhibited notable *in-vitro* cytotoxic activity against A549 and HeLa cell lines. Both isolated compounds also demonstrated cytotoxic potential, particularly kaempferol-3-O-glucoside against HT1080 and PANC-1 cells. These findings support the presence of bioactive constituents in the stem of *W. volubilis*, providing preliminary evidence of anticancer activity *in-vitro*. However, the therapeutic relevance of these results remains to be established. Further investigations, including *in-vivo* efficacy studies and mechanistic evaluations, are necessary before any conclusions can be drawn about potential clinical applications.

Conflict of Interest

The authors declare no conflict of interest.

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