

Evaluation Of Anticancer, Anti-Inflammatory And Antioxidant Activity Of The Aerial Parts Of *Mollugo Verticillata* Using MCF-7 Cell Line

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DOI: 10.47750/pnr.2022.13.S02.05

Abstract

Mollugo verticillata aerial parts have been used as a folk remedy to treat fever, wounds, sores, and swelling, and to alleviate premenstrual symptoms (PMS). As a result, the purpose of this study is to investigate the antioxidant, anti-inflammatory, and anticancer activity of *Mollugo verticillata* aerial parts. To obtain the extracts, the aerial parts were subjected to successive extractions with water and ethanol. The antioxidant activity was measured using the H₂O₂ scavenging assay, the reducing power assay, and the ABTS free radical scavenging assay. The anti-inflammatory activity was determined by inhibiting the protein denaturation assay. MTT assay was used to assess the plant's *in vitro* cell cytotoxicity activity, and the AO/PI dual staining method was used to assess the plant's apoptotic effect. The phytochemical composition of aqueous and ethanolic extracts of the plant showed the presence of flavonoids, saponins, glycosides, phytosterols, coumarin, tannins, and phenols. Our results revealed that *Mollugo verticillata* extracts have strong antioxidant activity against free radicals. The anti-inflammatory activity of ethanolic extract was found to be 95.098% at a concentration of 150mg/mL with an IC₅₀ value of 54.68g/mL, while the aqueous extract's was found to be 83.333% with an IC₅₀ value of 83.36g/mL. The MTT assay and AO/PI dual staining results showed that the plant extract induced apoptosis and had promising cytotoxic effects in MCF-7 cancer cells. The current study's findings have provided some information on phytochemical and pharmacological properties.

Keywords: *Mollugo verticillata*, antioxidant, anticancer, MCF cell line

INTRODUCTION:

Plants can act as the prime source of many medicines. They play an important role in both animal and human health. A variety of plants and herbs are well-known for their medicinal properties. Each plant contains several important components that can be used in the healthcare industry to produce various types of medications. Antioxidants are reducers found in common bases such as berries, vegetables, and meats¹. Antioxidants are the most common natural reducers found in everyday foods: vit C, vit E, vit A, and various polyphenols such as anthocyanins, lycopene, flavonoids, and coenzyme Q10, also known as Ubiquitin, a type of protein. Plants and herbs are among the many sources of antioxidants. Numerous studies have been conducted to establish the antioxidant and anti-cancer potential of many herbs and their extracts²⁻⁵. The majority of phytochemical-rich herbs, weeds, and plants on the market are used to treat a variety of diseases. *Mollugo verticillata* (also known as 'green carpet weed') is one of the herbs discovered. It is commonly used to treat fever, aches, and other ailments, and it has promising pharmacological activity due to the presence of phytoconstituents such as terpenoids, saponins, and flavonoids. Finally, the plant extract has tremendous medicinal value.⁶ With this in mind, we aimed to screen the phytochemical constituents from the aqueous and ethanolic extracts of aerial parts of *M. verticillata*: To assess the antioxidant activity of the extracts using H₂O₂ scavenging assay, reducing power assay, and ABTS radical scavenging assay: To evaluate the anti-inflammatory activity by the inhibition of protein denaturation method: To evaluate the *in vitro* anticancer activity of the extracts using MTT assay and AO/PI dual staining method.

METHODS:

The dried whole plant of *M. verticillata* was purchased from a local herbal shop in Kumbakonam and it was authenticated by a Botanist, from the Department of Botany, Government Arts College (Autonomous), Kumbakonam. Then the root portion of the plants was removed and coarsely powdered. The aqueous extract was made by soaking coarsely powdered plant material in distilled water; the ethanol extract was made in the same way, but 99% ethanol

was used instead of distilled water. Both extracts were kept at room temperature for 72 hours before being filtered through Whatman No.1 filter paper. To remove the excess solvent from the extract, the filtrates were evaporated in a water bath. The dried extracts were then weighed and refrigerated for further analysis. Phytochemicals are plant components with unique bioactivities for animal biochemistry and metabolism that are being extensively researched for their potential to have positive health benefits. According to Harborne ⁷(1990), the plant chemicals are categorized into three major classes: (1) terpenoids, (2) phenolic metabolites, (3) alkaloids and other nitrogen-containing plant constituents. For the initial qualitative examination, a number of chemical tests^{8,9} were carried out as described by Tiwari *et al.*, (2011) and Rufai *et al.*, (2016) to determine the presence of various phytochemicals in the prepared aqueous and ethanolic extracts of *M. verticillata*. The results were evaluated as a change in colour or precipitation by inspecting them visually.

Hydrogen peroxide assay: A solution of freshly prepared 4 mM hydrogen peroxide in 0.1 M phosphate buffer (pH 7.4) was used in this assay. 850µL of aqueous and ethanolic extracts of *M. verticillata* of varying concentrations (25-150µg/mL) was added to 150µL of H₂O₂ solution in phosphate buffer. The tubes were then kept aside for 10 minutes and the absorbance is read at 230nm. Ascorbic acid was used as positive control and the experiment was carried out in triplicates.

Reducing power assay: (Valarmathi *et al.*, 2015)

Varying concentrations (25-150µg/mL) of 1mL of both aqueous and ethanol extracts of *M. verticillata* and different concentrations (25-150µg/mL) of 1mL of standard (ascorbic acid) were taken in a series of test tubes. Then to all the tubes, 2.5mL of phosphate buffer (0.2M, pH 6.6) and 2.5mL of 1% potassium ferricyanide were added. The tubes were then incubated at 50 °C for 20 minutes, followed by the addition of 2.5mL of 10% TCA. Then, centrifugation of the reaction mixture was carried out at 3000rpm for 10 minutes.

The resulting supernatant (2.5mL) was taken in a dry tube mixed with 2.5mL of distilled water and 0.5mL of freshly prepared 1% ferric chloride solution was added. Distilled water instead of extracts with the above mixture was used as a negative control. The experiment was carried out in triplicate and the absorbance was then measured calorimetrically at 700 nm⁷.

Inhibition of protein denaturation assay: (Jayasuriya *et al.*, 2017)

1mL of *M. verticillata* extract in concentrations ranging from 25 to 150µg/mL, 0.45mL of bovine serum albumin (5% aqueous solution), and 1.4mL of phosphate-buffered saline make up the reaction mixture. Instead of the extract from the aforementioned mixture, distilled water was employed as a negative control⁸.

The mixture was then heated at 70 °C for 10 minutes after 15 minutes of incubation at 37°C. At 660 nm, the absorbance was measured after cooling under running water. As a positive control, diclofenac sodium was used, and the experiment was carried out three times.

The human breast cancer cell line MCF-7 was procured from the National Centre for Cell Sciences (NCCS) in Pune, India. The cells were grown in Dulbecco's modified Eagle medium (DMEM) with 10% FBS and antibiotics (penicillin 100 U/mL and streptomycin 100g/mL). They were kept at 37°C with a relative humidity of 95% and 5% CO₂. MCF-7 cells were plated in a 96-well microplate at a cell density of 1x10⁶ cells/mL and allowed to grow at 37°C for 24 hours. After incubation, the medium was replaced, and the MCF-7 cells were treated with ethanolic extracts of *M. verticillata* at concentrations of 25, 50, 75, 100, and 150g/mL. The samples were examined after a 24-hour incubation period, and photographs of the morphological differences between treated and untreated cells were taken using an inverted microscope (Magnus INVI; 20 magnification). The cells were washed with phosphate-buffered saline (PBS, pH 7.4) before being loaded into each well with 20L of MTT solution (5 mg/mL) and left in the dark at 37 °C for an additional 4 hours. Following the addition of 100µL of DMSO to dissolve the formazan crystals, an ELISA plate reader was used to read the absorbance spectroscopically in the 570 nm range. The viability calculations were done according to established techniques.

Acridine orange/propidium iodide (AO/PI) dual staining: (Kowsalya *et al.*, 2020)

The IC₅₀ concentration of ethanolic extract of *M. verticillata* treated cells were stained with a combination of acridine orange and propidium iodide (AO/PI) to look for morphological signs of apoptosis. MCF-7 cells were placed in a 6-well plate (1 105 cells per well) and treated for 24 hours with the IC₅₀ concentration of *M. verticillata* ethanolic extract⁹. The untreated MCF-7 cells served as the control. After being exposed to IC₅₀, the cells were rinsed with PBS before being stained for 5 minutes with 20L of AO/PI staining solution (100g/mL AO and 100g/mL PI). The stained cells were then examined under a fluorescence microscope (Invitrogen EVOS FL Cell Imaging; 40x)

RESULTS:

Table 1 summarises the preliminary phytochemical analysis of aqueous and ethanolic extracts of *M. verticillata* aerial portions. The presence of flavonoids, phenols, phytosterols, glycosides, coumarin, carbohydrates, and proteins was found in both the aqueous and ethanolic extracts of *M. verticillata*. In contrast to aqueous extract, ethanolic extract contained saponins, whereas tannins were only found in aqueous extract. Both extracts contained no anthraquinones or alkaloids.

Table 1: Phytochemical analysis of *Mollugo verticillata*.

S.No	Phytoconstituents	Aqueous extract	Ethanol extract
1	Flavonoids	+	+
2	Alkaloids	-	-
3	Phenols	+	+
4	Steroids	+	+
5	Terpenoids	+	+
6	Glycosides	+	+
7	Coumarin	+	+
8	Anthraquinones	-	-
9	Saponins	-	+
10	Tannins	+	-
11	Carbohydrates	+	+
12	Protein	+	+

The free radical scavenging activity of *M. verticillata* was assessed by the hydrogen peroxide scavenging method. From the results, a concentration-dependent activity was observed, the H₂O₂ scavenging effect of the aqueous and ethanolic extracts was found to be 89% and 93%, respectively, at a concentration of 150µg/mL.

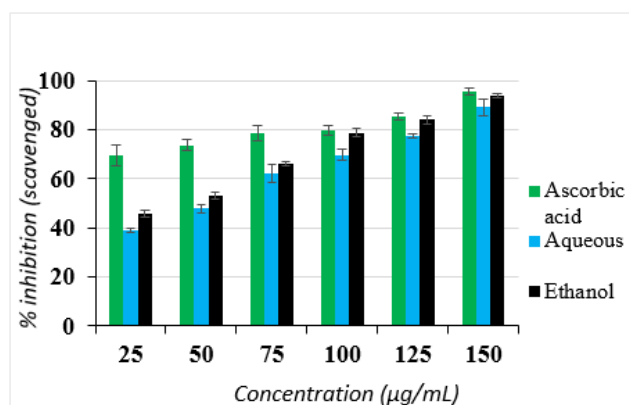


Fig. 1. Effect of *M. verticillata* extract on H₂O₂ free radical scavenging.

M. verticillata's reductive abilities were compared to those of ascorbic acid. At a concentration of 150g/mL, the reducing power of the aqueous and ethanolic plant extracts was 88% and 90%, respectively, whereas the reducing power of standard ascorbic acid was 93%. The percentage of reduction of the plant extracts at different concentrations is shown in Fig. 2, and the IC₅₀ of aqueous and ethanolic extracts was found to be 50.97g/mL and 40.17g/mL, respectively. A significant difference between the control was observed with the lower concentrations while higher concentrations were slightly similar to control groups.

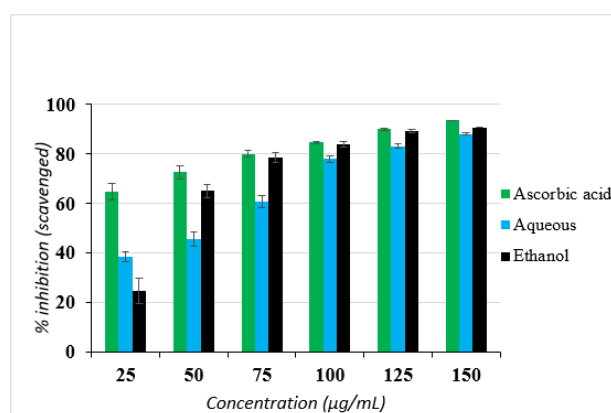


Fig 2. Effect of *M. verticillata* extract on reducing activity.

ABTS radical scavenging activity of the extracts of *M. verticillata*. The ABTS radical scavenging potential of aqueous and ethanolic extracts of aerial parts of *M. verticillata* was determined at various concentrations varying from 25-150µg/mL. A gradual increase in the scavenging potential of the extracts was obtained with an increase in concentration. (fig 3) IC₅₀ of aqueous and ethanolic extract was found to be 35.72µg/mL and 38.23µg/mL, respectively.

An inhibition of protein denaturation is equivalent to anti-inflammatory activity

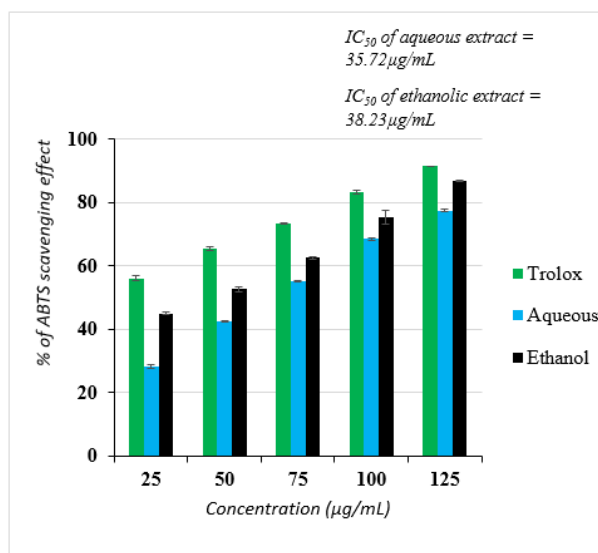


Fig 3. Effect of *M. verticillate* on ABTS scavenging activity.

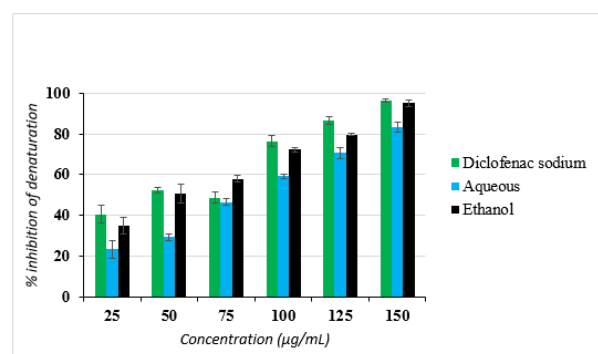


Fig 4. Effect of *M. verticillate* on anti-inflammatory activity.

MTT assay of ethanolic extract of *M. verticillata* against MCF-7 cell line. The cytotoxic activity of ethanolic extracts of aerial parts of *M. verticillata* was determined at various concentrations varying from 25 to 150µg/mL. A gradual decrease in the percentage of cell viability of the extract was obtained with an increase in concentration. IC₅₀ of the ethanolic extract was found to be 97.03µg/mL.

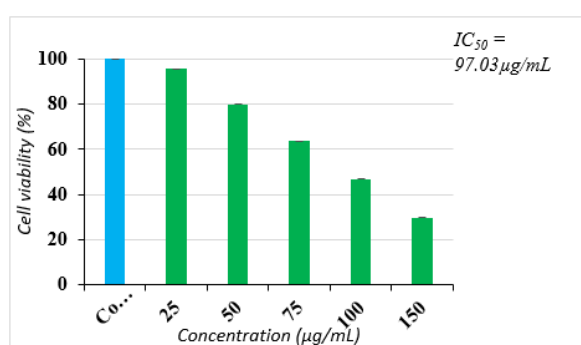


Fig 5. Cytotoxic effect of ethanolic extracts of *M. verticillate* on MCF7 cells.

The apoptosis-inducing ability of the ethanolic extract of aerial parts of *M. verticillata* in MCF-7 cell lines was examined. Apoptosis-induced morphological alterations in breast cancer cells were observed using AO/PI dual staining. After 24 hours of treatment, the treated MCF-7 cell lines showed signs of early apoptosis, including membrane blebbing and nuclear condensation, at concentrations as low as 97.03µg/mL

DISCUSSION:

The extract has got significant antioxidant, anti-inflammatory, radical scavenging and anti-cancer activities in our in vitro study. The investigation by Antonio and Vivit (2017) was supported by the *Mollugo verticillata* extracts' inclusion of the aforementioned medicinally significant ingredients¹⁰. These phytochemicals were also responsible for

its broad spectrum of pharmacological activity, which includes antioxidant, anti-inflammatory, antimicrobial, and anticancer activities, and made it as a useful herbal remedy in treating wounds, inflammation, and fever.

Fernando and Soysa (2015) measured the H₂O₂ scavenging activity of *Mollugo cerviana* using a simple enzymatic colorimetric experiment, and the results revealed an EC₅₀ value of 1480.3g/mL¹¹. They also stated that H₂O₂ can be produced *in vivo* by a variety of oxidising enzymes, which can inactivate enzymes involved in cellular energy production, such as glyceraldehyde-3-phosphate dehydrogenase from glycolysis and aconitase and -ketoglutarate dehydrogenase from the Krebs cycle. The ability of antioxidants to scavenge H₂O₂ is thus regarded as a critical feature.

At a concentration of 2.5 mg/mL, *Mollugo nudicaulis* was shown to have a reducing power of 45%. Reducing power is a measure of a reducing agent's capacity to interact with free radicals and interrupt the chain reaction that would otherwise result in more unstable metabolites by contributing electrons (Gopalakrishnan *et al.*, 2011).¹² Appropriately, *Mollugo verticillata* might have a large number of reductants that could interact with the free radicals to become stable and stop the free radical chain reaction.

Napagoda *et al.*, (2016) found similar results when they investigated the ABTS radical scavenging activity of a hydro-methanolic extract of *Mollugo cerviana*¹³. The extract's ABTS radical scavenging activity was quantified at 100 0.005 millimolar Trolox equivalents per milligramme, according to the findings.

The MTT assay was used to evaluate the anticancer activity of *Mollugo nudicaulis* n-hexane extract *in vitro* against A2780 cell lines at various doses ranging from 3.12 to 200g/mL. The plant extract impeded 86% of the bacteria at a maximum dosage of 200g/mL, with an IC₅₀ value of 32.46 0.92g/mL. Palanisamy *et al.*, (2021) discovered ¹⁴that *M. nudicaulis* n-hexane extract has powerful anti-cancer activity. We have demonstrated with other extracts.

Eventhough there are a lot of such studies, ours is unique in that the plant showed anti inflammatory and anti cancer effects with traditional extracts.

CONCLUSION:

The antioxidant efficacy of the ethanolic extract, which is equivalent to the standard drug at a concentration of 150mg/mL, suggests that this plant has a lot of potential for isolating and identifying important antioxidant molecules that can be formulated into antioxidant dosage forms. Even though it was weaker than the standard drug, the aqueous extract demonstrated assuring antioxidant activity. Furthermore, this plant demonstrated promising anti-inflammatory properties comparable to those of the commonly used medication, diclofenac sodium. The ethanolic extract was also tested for cell cytotoxicity *in vitro* using the MTT assay, and the results show that the plant is highly stable and biocompatible for anticancer activity against MCF-7 cells. The ethanolic extract of *M. verticillata* promoted apoptosis and necrosis in breast cancer cells by inducing DNA damage. The AO/PI dual staining fluorescence assay confirmed the plant extract's apoptotic effects.

Conflict of interest – NIL

Financial aid - No external funding

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