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Pharmaceutical efficacy of sapanin extracted from artemisia pallens walls with reference to MCF-7 cell line

G. Renuga

School of Biological Sciences, Madurai Kamaraj University, Madurai, Tamil Nadu, India

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ABSTRACT

The use of plants in treatment of various infections, disease against pathogens in increasing interest of solvent extracts from Artemisia pallens used in folk medicine. Phytochemical screening of the leaf extract of Artemisia pallens showed the presence of alkaloid, phenols, glycosides, saponin, steroids and triterpenes. The effect of saponin were analyzed and it was observed posses highest pharmaceutical potential as antimicrobial and anticancer activity. Artemisia pallens extracts may be used to treat disease caused by these organisms such as pathogenic (Bacillus lentus, Bacillus subtilis, Escherichia coli, klebsicella pneumonia, Salmonella paratyphi and fungal strains like Aspergillus niger, Aspergillus parasiticus and Monascus purpureus has been commonly tested against extract of Artemisia pallens. The results showed that the selected organisms were susceptible to the plant extracts at concentrations of 100 µg with diameter zone of inhibition when compared with standard antibiotic disc. The extract significantly inhibited breast cancer cell MCF-7 in vitro condition the plant extract has been shown to exhibit inhibition of cell growth well known for its anticancer properties. Studies with crude extract of Artemisia pallens saponin might revealed interesting results but should be considered rather preliminary. However, it is also possible that component of the crude extract extracts interact synergistically and thus induce effects observed for pure saponins. Artemisia pallens have been a valuable source of natural products for maintaining human health. The use of plant compounds for pharmaceutical purposes has gradually increased and its properties should be investigated to safety and efficiency.

Keywords: Antimicrobial, *Artemisia pallens*, breast cancer cell line, Drug, Zone of Inhibition

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INTRODUCTION

The medicinal plant based drugs have the added advantage of being simple, effective & offering a broad spectrum of activity with an emphasis on the preventive actions of drugs. Because of these factors, the demand for plant-based medicines is increasing worldwide. Artemisia species are invariably found as small fragrant shrubs or herbs and most of them yield essential oils. Some of these oils are used as medicine such as vermifuge, stimulant and in perfumery, etc. The leaves of some species are used as culinary herbs. The plants themselves are popular among gardeners as cultivated ornamentals. *Artemisia pallens* Walls. ex DC, commonly known as Davana, is an aromatic herb found abundantly in humid habitats in the plains all over India. Artemisia is the largest genus comprising of 400 species widely distributed in South Africa and South America, and 34 species are found in India [1]. It is commercially cultivated for its fragrant leaves and flowers. It grows from seeds and cuttings and reaches maturity in four months. *Artemisia*'s leaves and flowers are highly valued in the making of floral decorations and oils. The oil possesses antispasmodic, antibacterial, antifungal and stimulant properties [2].

Artemisia abrotanum was traditionally considered as an antiseptic, astringent, emmenagogue, expectorant, febrifuge, stomachic, stimulant, and tonic, antiinflammatory, vermifuge, spasmolytic and used for treating upper respiratory tract disease. Infusions make a bitter tonic which strengthens and supports digestive functions by increasing secretions in the stomach and intestines. It has also been used against cancer, cough, fever and tumors [3]. The plant

has been screened for various pharmacological activities such as antiinflammatory, expectorant and spasmolytic [4]. To date, new generations of taxanes, anthracyclines, Vinca alkaloids, camptothecins, as well as the novel class of epothilones have been developed.

The saponins are secondary metabolites that occur in wide range of plant species, stored in plant cells as inactive precursors but are readily converted into biological active antibiotics by enzymes in response to pathogen attack. Saponins are naturally occurring surface active glycosides [5] also which are produced by lower marine animals and some bacteria. Saponins consist of a sugar moiety usually containing glucose, galactose, glucuronic acid, xylose, rhamnose, glycosidically linked to a hydrophobic aglycone which may be triterpenoid or steroid in nature [6] reported that the experiments demonstrating the physiological, immunological and pharmacological properties of saponins have provoked considerable clinical interest in these substances. Recently, saponinsa are promoted commercially as dietary supplements and also used as expectorants, laxatives, diuretics, facilitate the absorption of some food and medicinal substances. *Artemisia pallens* commonly known as "Davana" has been traditionally used in Indian folk medicine for the treatment of diabetes mellitus, wound healing and immuno-modulating, antihelmintic, antipyretic, wound healing and also as stimulant [7].

Artemisia pallens has been widely used in Indian folk medicine for the treatment of diabetes mellitus. The oil possesses antispasmodic, antibacterial, Antifungal and stimulant properties [8].The plant has been screened for the following pharmacological activities, antimicrobial, antidiabetic and wound healing activity [9] have reported the methanol extracts of aerial extracts of aerial parts of Artemisia species shows antimicrobial activity against gram positive bacteria. The plant under investigation in the current study, *Artemisia pallens* wall (compositae) is an aromatic perennial shrub, traditionally used in the treatment of diabetes, inflammation and urinary problem. Hence in the current study an attempt was made to study antimicrobial activity and tumor inhibitory potential of *Artemisia pallens* extracts comparing its action with that of Ciprofloxacin, Clotrimazole and Adriamycin.

MATERIALS AND METHODS

Plant material

Atremisia pallens walls, ex Dc were collected from in and around Ootacamund, a famous hill station in southern India, belonging to the district Nilgiris of Tamil Nadu state. The plant was identified and authenticated by Medicinal Plants Survey and Collection Unit, Ootacamund, Tamil Nadu, India.

Extraction procedure:

The leaves of the plants were air-dried, grinded into powder from using mortar and pestle and. 100g each of the plant powder was suspended in 750ml, 75% ethanol for 72 hours in a conical flask. The content of plant powder was agitated daily after which was filtered using Whatman's 1filter paper. The filtrate was evaporated to dryness using steam evaporator and the extract was stored at 4^{o}C until required [10].

Apparatus and chromatographic conditions

Chromatographic separation was performed on a Shimadzu® liquid chromatographic system equipped with a LC-10AT-vp solvent delivery system (pump), SPD M-10AVP photo diode array detector and Rheodyne 7725i injector with 20 µl loop volume. Class-VP 6.01 data station was applied for data collecting and processing (Shimadzu, Japan). A Princeton SPHER C18 (150 x 4.6 mm i.d. 5 µ) was used for the separation. Mobile phase of a mixture of Acetonitrile: Water (60:40 v/v) was delivered at a flow rate of 0.5 ml/min with detection at 283 nm. The mobile phase was filtered through a 0.2 µ membrane filter and degassed. The injection volume was 10 µl. Analysis was performed at ambient temperature.

Analysis of pharmaceutical potentials:

Phytochemical screening of solvent extracted samples were carried out according to the standard methods as described by [11 and12] for alkaloids, tannins, flavonoids, steroids, glycoside, phenol saponins, terpenoids, quinons and mucilage.

Alkaloids: $1cm^3$ of 1% HCL was added to $3cm^3$ of the extract in a test tube. The mixture was then heated for 20 minutes, cooled and filtered. About 2 drops of Mayer's reagent was added to $1cm^3$ of the extract. A creamy precipitate was an indication of the presence of alkaloids.

Tannins: $1cm^3$ of freshly prepared 10% KOH was added to $1cm^3$ of the extract. Absence of dirty white precipitate showed the absence of tannins.

Phenolics: Two drops of 5% $FeCl_3$ of the extract in a test tube. Presence of greenish precipitate indicated the presence of phenolics.

Glycosides: 10cm^3 of 50% H_2SO_4 was added to 1cm^3 of the extract and the mixture heated in boiling water for about 15 minutes. 10 cm^3 of Fehling's solution was then added and the mixture boiled. A brick-red precipitate was confirmatory for the presence of glycosides.

Flavonoid: 1 cm^3 of 10% NaOH was added to 3cm^3 of the extract. There was yellow colouration which is indicative the presences of flavonoids.

Steriods: Salkowski test: 5 drops of concentrated H_2SO_4 was added to 1 cm^3 of the extract in a test tube. Red colouration was observed which is indicative for the presence of steroids.

Saponins: (i) Frothing test: 2cm^3 of the extract was vigorously shaken in the test tube for 2 minutes. Frothing was observed. (ii) Emulsion test: 5 drops of olive oil was added to 3cm^3 of the extract in the test tube and vigorously shaken. Presence of stable emulsion formed indicates the presence of saponins.

Antimicrobial studies were carried out against six bacterial strains such as (*Bacillus lentus, Bacillus subtilis, Escherichia coli, klebsicella pneumonia, Salmonella paratyphi* and fungal strains like *Aspergillus niger, Aspergillus parasiticus and Monascus purpureus.* The paper disc diffusion method was employed. Samples of each extracts (100µg/disc) were dissolved in respective solvents (1 ml). Sterile 5 mm diameter filter paper discs were impregnated with 40 µL of these solvent extracts (8 mg /disc). The bacterial strains were inoculated on nutrient broth and incubated for 24 hours at 37 ± 0.1 °C. The test organisms (0.1 ml) were inoculated with a sterile spreader on the surface of solid medium in plates. The agar plates inoculated with test organism were incubated for one hour before placing the extract impregnated paper discs on the plates. Following this, the sterile discs impregnated with different extracts were placed on agar plates. The bacterial plates were incubated at 37 ± 0.1 °C for 24 hours, after incubation all the plates were observed for zones of growth of inhibition and the diameters of these zones were measured in millimeters. All tests were performed under sterile conditions. Ciprofloxacin discs (10 µg/disc) and Clotrimazole discs (10µg/disc) were used as positive controls. Disc of standard drugs were used against the test organisms to compare the activity of the plant extracts with the commercially prepared antibiotics.

The anti proliferative effect of the plant extract on human breast cancer cell line – MCF 7 was assessed by inhibition of cell growth. All the following procedures were done in a sterile area using a Laminar flow cabinet biosafety class II level (Baker, SG403INT, Sanford, ME, U 10 days, then seeded at concentration of $10x10^3$ cells/well in fresh complete growth medium in 96 well microtiter plastic plates at 37^oC for 24 h under 5% CO_2 using a water jacketed carbon dioxide incubator (Sheldon, TC 2323, Cornelius, OR, USA). Media were aspirated, fresh medium (without serum) was added and cells were incubated either alone (negative control).A positive control adrinamycin (Doxorubicin) [Mw= 579.99] composed of 100 µg/ml was used as a known cytotoxic natural agent that gives 100% lethality under the same conditions [13].

RESULTS AND DISCUSSION

Atremisia pallens walls, ex Dc commonly known as Davana, is an aromatic herb found abundantly in humid habitats in the plains all over India. It is cultivated for its fragrant from leaves and flowers and oil contents were maximum level in the flower head and less in the leaf and stem. Although secondary products can have a variety of functions in plants, may have some bearing on potential medicinal effects for humans. For example, secondary products involved in plant defense through cytotoxicity toward microbial pathogens could prove useful as antimicrobial medicines in humans.

Solvent extract of plants has screened to identify compound of therapeutic potential source for the drugs against human illnesses. Extract of *Artemisia pallens* were contributed different compounds (Table1) particularly saponins contain a steroid, steroid alkaloid or triterpene core structure so called aglycone.

Figure 1: Plant view of *Atremisia pallens* walls ex DC

Table: 1 Phytochemical Screening of *Artemisia pallens* extracts

Alkaloids	+
Glycosides	+
Flavonoids	+
Steroids	-
Tannins	+
Phenols	+
Terpenoids	+
Quinons	+
Mucilage	+
Saponins	+

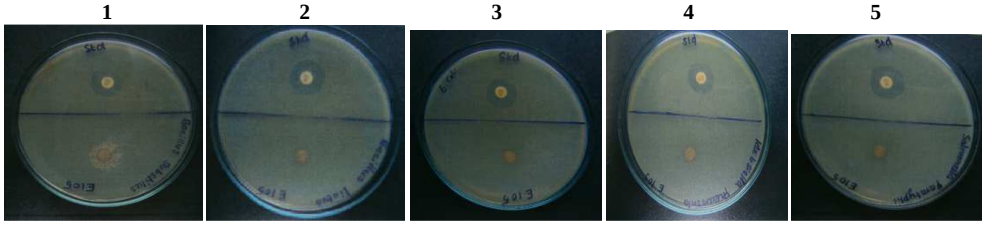
+ Positive indicates the presence of the compound; - Negative indicates the absence of the compound;

The data pertaining to the antimicrobial potential of the plant extracts and phytochemical was saponin presents in (Table1). Saponins are plant glycosides and possess great diversity in their structure. The diversity of saponin is a result of differences in the aglycone structure and the amount and composition of the sugar side chains. One of the first studies with saponin for the treatment of cancer was described by [14]. This saponin had a potent anti tumorigenic effect in a mouse skin tumor model. The number of studies on saponins as anti tumor drugs has increased drastically in the last decade therefore one has to be careful when considering a certain saponin whose efficacy was only demonstrated on the basis of its high toxicity on tumor cell lines.

Antibacterial activity:

Figure 2 results revealed tested microorganism showed moderate sensitivity of plant extract samples were against antibiotic standard disc. According to the zone of inhibition, the tested organisms like *Bacillus lentus* and *salmonella paratyphi* showed sensitivity response towards plant extract containing saponin subsequently other organism such as *Bacillus subtilis*, *Escherichia coli* and *Klebsicilla pneumonia* were considered as moderate sensitivity in response to the saponin content in the plant samples extract was compared with ciprofloxacin. The results showed the zone of inhibition of the100 µg of sample extract when compare with 10 µg of standard were susceptible to antibiotic which shows the sample sensitivity against antibiotic (ciprofloxacin disc).

Figure 2: Antibacterial activity of *Artemisia pallens* extract

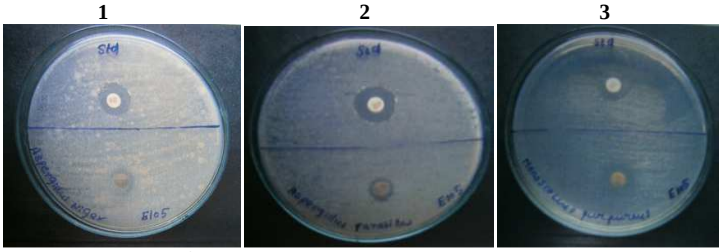


1- Indicated the presence of Bacillus lentus inhibited zone size in diameter 11mm
2- Indicated the presence of Bacillus subtilis inhibited zone size in diameter 7mm
3- Indicated the presence of Escherichia coli inhibited zone size in diameter 8mm
4- Indicated the presence of Klebsicilla pneumoniae inhibited zone size in diameter 8mm
5- Indicated the presence of salmonella paratyphi inhibited zone size in diameter 9mm

Antifungal activity:

Efficiency of anti fungal effects of *Artemisia pallens* sample were given in Figure 3 results revealed tested strains such as *Aspergillus niger* (11mm), *Aspergillus parasiticus* (10)and *Monascus purpureus* (15) showed sensitivity in response to the plant extract samples when compared to standard drug clotrimazole antibiotic disc.

Figure 3: Antifungal activity of *Artemisia pallens* extract



1- Indicated the presence of Aspergillus niger inhibited zone size in diameter 11mm
2- Indicated the presence of Aspergillus parasiticus inhibited zone size in diameter 10mm
3- Indicated the presence of Monascus purpureus inhibited zone size in diameter 15mm

Another important effect of saponin is their membrane permeabilizing property. The pore formation is ascribed to an interaction between saponin and membrane bound cholesterol. The amount of cholesterol in the membrane has been shown to be important for this interaction [15].

Anticancer activity:

Saponins are plant glycosides with favorable antitumorigenic properties as inhibit tumor cell growth by cell cycle arrest and apotosis with IC_{50} valve of up to 10µg/ml. A reduction to the expression of the anti-apototic protein Bcl-2 together with caspase activation was observed. Paclitaxel, which has become one of the most effective drugs against breast and ovarian cancer and has been approved worldwide for the clinical treatment of cancer patients, paclitaxel exerts its anticancer activity by inhibiting mitosis. The member of newly isolated and described saponin is increasing constantly and many further saponin will be identified due to improved methods of purification and detection. Saponins possess impressive anticancer effects and might help to develop improved anticancer regimens.

Figure 4: In vitro cell line MCF-7treated with Saponin from *Artemisia pallens*

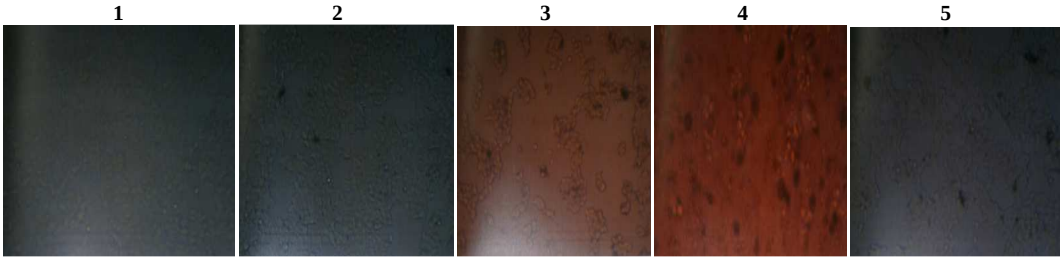


Plate 1: Control showed the presence human breast cancer cell line without any treatment
Plate 2: 25µg concentrations of plant extracts treated on MCF-7 cell line
Plate 3: 50µg concentrations of plant extracts treated on MCF-7 cell line
Plate 4: 75µg concentrations of plant extracts treated on MCF-7 cell line
Plate 5: Standard Drug treated on MCF-7 cell line

Adriamycin have a different effect on the cell cycle inhibits the cell-cycle traverse at the G2-M phase junction while docetaxel produces its maximum cell-killing effect against cells in the S phase [16]. Other potential anti-tumour effects of taxanes not directly associated with the classical anti-microtubule action have been reported. The apoptosis induced by paclitaxel and docetaxel has also been associated with enhanced phosphorylation of Bcl-2. In addition paclitaxel induces the release of tumour necrosis factor- (TNF-) and a decrease in expression of TNF receptors.

CONCLUSION

For a long period of time plants have been a valuable source of natural products for maintaining human health. The results of this study revealed that the extracts of *Artemisia pallens* have inhibitory properties on all the test organisms. The leaf extracts revealed the presence of saponin active component which could be responsible for the antibacterial activity of the extracts against the tested organisms. The leaf extracts of the medicinal plants used in this study could be a source of important chemical agents to be used to treat bacterial diseases and infections caused by *Bacillus lentus, Bacillus subtilis* and gram-ve bacteria viz., *Escherichia coli, Klebsiella pneumonia, Salmonella paratyphi.* The combined application of saponins with other antitumor drugs offers an interesting development in cancer treatment since in our other studies reports additive or even synergistic effects between saponins and other drugs have been observed. These combinations will lead to essentially improved possibilities for the treatment of cancer. Most important is the saponin mediated potentiation of tumor growth inhibition and the possibility to circumvent drug resistance,However detailed information on this basis is necessary for a directed improvement of saponin based tumor therapies in the future.

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