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Original article

In vitro anti-inflammatory and antimicrobial potential of leaf extract from *Artemisia nilagirica* (Clarke) Pamp

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ABSTRACT

In the present investigation, the bioactive compounds from the leaf extract of *Artemisia nilagirica* showed potent anti-inflammatory and antimicrobial activity. The leaf extract showed a maximum protection of human red blood cells (HRBC) with 74.63% at 20 μg/mL concentration, and the minimum hemolysis was 25.37% in a hypotonic solution with diclofenac as the control. The *in vitro* antimicrobial activity of plant extract against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Proteus vulgaris*, *Yersinia enterocolitica*, *Bacillus subtilis*, and *Candida albicans* was evaluated at various concentrations (50, 100, 150, and 200 μg). The maximum zone of inhibition was observed against *P. aeruginosa* followed by *B. subtilis*, *S. typhi*, *S. aureus* and *E. coli*. The leaf extract also showed potent activity against *C. albicans*.

1. Introduction

Plants have been widely recognized as a important source of novel therapeutic compounds since ancient times for the treatment of various diseases and were reported in traditional medicine system such as the Siddha and Ayurveda (Khanna and Chandra, 1972). Bioactive compounds are known for their antitoxin resistance and as a reliable source of antimicrobial treatments, and are widely used as anti-infective supplements and adjuncts in combination with other compounds (Blaszyle and Holley, 1998). However, the properties of natural-based mixtures need to be carefully investigated to determine their pharmacological effects on biological systems (Ushimaru et al., 2007; Viegas and Bolzani, 2006). Approximately 80% of the medicines of the world depend on plant-based bioactive components for curing various diseases (Owolabi et al., 2007). *Salvia miltiorrhiza* has been reported as an effective medication with potential pharmacological and restorative effects (Shi et al., 2005; Wang et al., 2007; Li et al., 2009). Inflammation is a normal protective reaction to tissue damage caused by physical injury and harmful chemicals. The most commonly used drugs for the management of inflammatory conditions is the non-steroidal anti-inflammatory drugs (NSAIDs), which have various adverse effects, especially gastric irritation, leading to the formation of gastric ulcers. The rich wealth of the plant kingdom represents a novel compounds with significant anti-inflammatory activities (Chandra et al., 2012). However, the most of these plant resources have not yet undergone chemical, pharmacological, and toxicological studies to investigate their bioactive compounds (Al Farug et al., 2014).

The phytochemicals such as, alkaloids, saponins, steroids, tannins, flavonoids, amino acids, and trigonillin were responsible for biological activity. The leaves and seed have been widely used to prepare concentrates and powders for restorative uses (Swati et al., 2014). Since human red blood cell (HRBC) membranes are similar to the liposomal membrane component, the prevention of hypotoxicity-induced HRBC membrane lyses has been used as a measure for estimating the anti-inflammatory property of extracts of *Gendarussa vulgaris Nees* (Saleem et al., 2011). Many important secondary metabolites and essential oils were reported from *Artemisia* sp. Essential oils of *Artemisia* spp. have been frequently used for a the treatment of various diseases for many years. *Artemisia nilagirica* (Clarke) pamp (Indian worm wood) is widely found in the hilly areas of India (Banerji et al., 1990). Many interesting studies using *Artemisia* spp. showed potent antioxidant and antimicrobial activities (Kordali et al., 2005; Juteau et al., 2002).

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The therapeutic properties of medicinal plants come from its phytochemical components. The general phytochemicals that significantly causes effective results on human health care are alkaloids, flavonoids, tannins and glycosides. The phytochemical screening of *A. nilagirica* revealed the presence of various phytochemicals such as tannins, alkaloids, flavonoids, terpenoids and glycosides (Arokiyaraj et al., 2012). In another study, the presence of phytochemicals such as, alkaloids, phenol, tannins, flavonoids, amino acids, quinines and terpenoids were reported from this plant. *A. nilagirica* has been reported to have efficiency against various neurological disorders, antimicrobial, dermal infection, antifungal, larvicidal, antiinflammatory activities (Ahameethunisa and Hopper, 2010). Also, various secondary metabolites such as, terpenids, flavonoids, polysaccharides and saponins were characterized using Gas Chromatography – Mass Spectrophotometer (GC–MS), High performance Liquid Chromatography (HPLC) and Nuclear magnetic resonance spectroscopy (NMR) (Xie et al., 2008; Avula et al., 2009). The antimicrobial compounds from *Artemisia* sp. were used as alternate medicine in food industry (Ng, 2004).

The bacterial species such as, *Salmonella*, *Pseudomonas* and *Staphylococcus* cause various diseases. *Pseudomonas aeruginosa* causes respiratory tract infections or sepsis in patients with cystic fibrosis or suppression of the immune system (Esen et al., 2001). *Salmonella enterica* serovar Typhi causes typhoid fever (Dougan and Baker, 2014). The genus *Proteus* includes facultative anaerobic, Gram-negative, proteolytic, and heterotrophic rods being human opportunistic pathogens (Drzewiecka, 2016). In recent years, the rates of antibiotic resistance in *Pseudomonas aeruginosa* are increasing throughout the world. The multidrug-resistant phenotype in *P. aeruginosa* could generally be mediated by various mechanisms including enzyme production, multidrug efflux systems, loss and target mutations and outer membrane protein (Hirsch and Tam, 2010). Multiple drug resistance is a major health problem in the treatment of staphylococcal infections, mainly infections of methicillin-resistant *Staphylococcus aureus* which occurs mainly due to the extensive use of antimicrobial substances, coupled with the transmission of pathogenic organism by person-to-person contacts (Okeke and Lamikanra, 2003). Hence, effective control of applications of antibiotics and prevention of the transmission of these pathogenic strains are essential to eradicate this highly infectious organism. Among medicinal plants of the world, the biological activity of the genus *Artemisiais* comparatively less explored against various pathogenic bacteria and anti-inflammatory activity. Hence, in this study, the anti-inflammatory property and antimicrobial properties was carried out.

2. Materials and methods

2.1. Plant material

The leaves of *A. nilagirica* (Clarke) Pamp were collected at Theni District, Tamil Nadu, India. The plant material was identified and authenticated by the Department of Plant Anatomy Research Centre, Chennai, and a voucher specimen (No.PARC/2014/2208) was deposited in the Herbarium of the Department of Plant Anatomy Research Centre, Chennai, Tamil Nadu, India.

2.2. Preparation of extracts

The leaves were air-dried, powdered, and extracted with methanol. Then, the solvent was distilled off, and the extract was concentrated on a water bath to obtain a dry residue that was stored in desiccators.

2.3. In vitro anti-inflammatory activity

The HRBC membrane stabilization was used as a method to evaluate the anti-inflammatory activity (Gandhisan et al., 1991). The collected blood was mixed with an equal volume of sterilized Alsever medium (2%, (w/v) dextrose, 0.8% sodium citrate, 0.5% citric acid, and 0.42% sodium chloride in water). The blood was further centrifuged at 3000 rpm for 10 min, and the packed cells were washed with isosaline (0.85%, pH 7.2) and finally 10% (v/v) suspension was made with isosaline. The assay mixture contained the secondary metabolite from the plat extract, 1 mL phosphate buffer (0.15 M, pH 7.4), 2 mL hyposaline (0.36%), and 0.5 mL HRBC suspension. Diclofenac was used as a reference drug. Instead of the hyposaline, 2 mL distilled water was used as the control. The assay mixtures were incubated at 37 °C for 30 min and centrifuged at 3000 rpm for 10 min. The hemoglobin content in the supernatant was estimated using a UV–Visible spectrophotometer at 560 nm (Anosike et al., 2012). The percentage hemolysis was calculated using the following equation:

$$\text{Hemolysis}(\%) = (\text{Optical density of test sample}/\text{Optical density of control}) \times 100$$

$$\text{Protection}(\%)100 - [(\text{Optical density of test sample}/\text{Optical density of control})] \times 100$$

2.4. In vitro antimicrobial activity

Seven bacterial and a fungal culture (*E. coli, S. aureus*, *P. aeruginosa*, *S. typhi*, *P. vulgaris*, *Y. enterocolitica*, *B. subtilis,* and *C. albicans*) were used in this study. The bacterial strains were grown in nutrient broth (Himedia, Mumbai, India) at 37 °C (4×10^6 cells/ml), and they were subcultured on nutrient agar slants for future use. The fungal strains were cultured in potato dextrose broth (Himedia, Mumbai, India) at 27 ± 2 °C (3×10^5 spore/ml), and they were further subcultured on potato dextrose agar slants. Different concentrations of the samples (50, 100, 150, and 200 μg/well) were aseptically loaded into the wells. Muller Hinton agar (Himedia, Mumbai, India) (MHA) plates were inoculated with the test organisms. The plates were evenly spread out, and wells were cut in the plates using a cork borer (6 mm diameter). Each well was loaded with 0.020 mL of sample and ketoconazole was used as a positive control. The plates were incubated for 24 h at 37 ± 2 °C, and the zone of inhibition was measured after 48 h for bacteria and fungus, respectively.

3. Results and discussion

Aromatic and medicinal plants are very important sources of secondary metabolites, which have a range of applications in control of human and plant diseases, pharmaceutical industry and cosmetics (Pandey and Tripathi, 2011). There are many species of *Artemisia* that have been widely studied for their antioxidant, antimicrobial, cytotoxic, repellent, insecticidal and anticonvulsant agents (Tan et al., 1998). Leaves of *Artemisia nilagirica* showed the presence of phytochemicals such as, tannins, flavonoid, alkaloids, saponins, coumarins, steroids and phenols (Parameswari and Devika, 2014; Farahani et al., 2017). The phytochemical investigations have revealed more than 839 compounds from the parts such as, stem, root and leaves of fourteen *Artemisia* species viz. *A. abrotanum L., A. absinthium L., A. arborescens, A. chamaemelifolia, A. capillaris Thunb., A. indica Willd., A. vulgaris, A. afra, A. annua L., A. caruifolia, A. cina, A. dracunculus L., A. herba-alba, A. japonica*

Thunb. These species contain the phytochemicals such as, coumarins, terpenoids, flavonoids, caffeoylquinic acids, coumarins, acetylenes and sterols (Martinez-Diaz et al., 2015; Singh et al., 1989; Suresh et al., 2011).

3.1. In vitro anti-inflammatory activity

The extract from the leaves of *A. nilagirica* (Clarke) Pamp was studied for *invitro* anti-inflammatory activity. The leaf extract showed maximum protection and minimum hemolysis of the HRBC (74.63% and 25.37%, respectively) at a concentration of 200 μg/mL in hypotonic solution. Furthermore, at 50 μg/mL, the extract showed maximum hemolysis and minimum protection of 52.89 and 47.11%, respectively. The results were compared with the standard diclofenac, which showed a <91.18% protection (Fig. 1) and 16.68% hemolysis (Fig. 2). The extract exhibited membrane stabilization by inhibiting hypotonicity-induced lyses of the erythrocyte membrane (Chou, 1997), and its stabilization implies that the extract may stabilize lysosomal membranes. Stabilization of liposomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage by extracellular release (Murugasan et al., 1981). The extract may inhibit these processes, which may stimulate or enhance the efflux of these intracellular components (Iwueke et al., 2006).

3.2. Invitro antimicrobial activity

The plant extract showed activity against, *E. coli, S. aureus, P. aeruginosa, S. typhi, P. vulgaris, Y. enterocolitica, B. subtilis,* and *C. albicans*. In our study, *P. aeruginosa* exhibited the largest inhibition zones of 16, 17, and 19 mm at 100, 150, and 200 μg concentrations, respectively. *B. subtilis* and *S. typhi* exhibited the second highest inhibition zones of 14, 16, and 18 mm, followed by *S. aureus* with 13, 15, and 18 mm at three different concentrations (100, 150, and 200 μg, respectively). *E. coli* showed the highest zone of inhibition (14, 15, and 17 mm) at 100,150, and 200 μg, respectively. The plant extract showed antifungal activity against *C. albicans* and exhibited the highest reduction in the zone of inhibition (6, 8, and 10 mm) at 100, 150, and 200 μg, respectively (Table 1). The microbial properties of *A. nilagirica* were studied previously by various research groups. Rao et al. (2006) reported that *A. nilagirica* has potential antibacterial effect against *Staphylococcus aureus*, *Bacillus subtilis*, and *Klebsiella pneumonia*. Chowdhury et al. (2003) found the antifungal activity of essential oils from *A. nilagirica* against various fungal isolates and reported mycelia inhibition activity. The antimicrobial potential of leaf extract of *Artemisia nilagirica* was studied and reported activity against various Gram-negative and Gram-positive bacteria and showed no inhibitory effect against *Staphylococcus aureus*, *Klebsiella pneumonia* and *Staphylococcus aureus* (Ahameethunisa and Hopper, 2010).

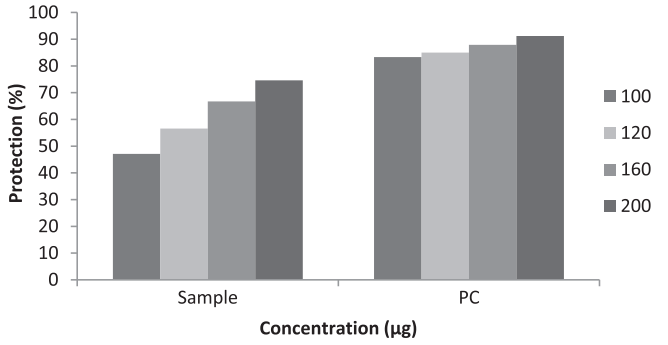

Fig. 1. Percentage protection of *Artemisia nilagirica* (Clarke) Pamp leaf extract. Diclofenac was used as the control. The hemoglobin content in the supernatant was estimated using a UV–Visible spectrophotometer.

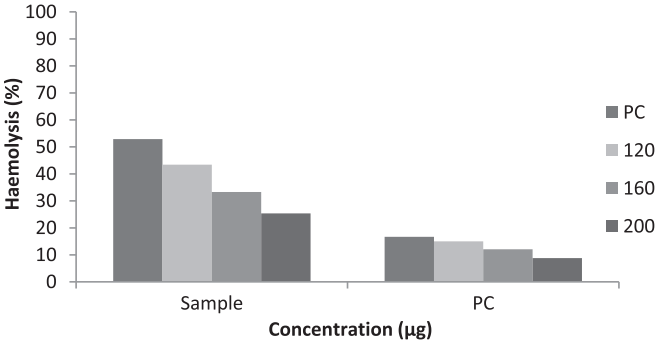

Fig. 2. Percentage haemolytic effect of *Artemisia nilagirica* (Clarke) Pamp leaf extract. Diclofenac was used as the control. The hemoglobin content in the supernatant was estimated using a UV–Visible spectrophotometer.

Table 1
Antimicrobial activity of plant extract from *Artemisia nilagirica* (Clarke) Pamp.

Bacterial pathogens	Concentration of the leaf extract (μg)				Standard
	50	100	150	200	
E. coli	12	14	15	17	19
S. aureus	11	13	15	18	20
P. aeruginosa	13	16	17	19	43
B. subtilis	12	14	16	18	40
S. typhi	12	14	16	18	33
P. vulgaris	–	–	–	–	13
Y. enterocolitica	–	–	–	–	16
C. albicans	3	6	8	10	12

4. Conclusions

Artemisia nilagirica (Clarke) Pamp is wide spread in India. This plant has potential anti- inflammatory, antifungal, antimicrobial, antiulcer, antifilarial, anticancer and antioxidant activity. The antibacterial and antifungal study confirms the therapeutic potential of *A. nilagirica*. This plant is important source of various phytochemicals with pharmaceutical potential. The leaf extract showed potent activity against various bacteria and fungus indicate that the plant could "lead" for the isolation of novel agents with good efficacy to treat various diseases and disorders.

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