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Original article

Identification of phytochemical components from *Aerva lanata* (Linn.) medicinal plants and its *in-vitro* inhibitory activity against drug resistant microbial pathogens and antioxidant properties

Mysoon Al-Ansari [a], L.A. Al-Humaid [a], P. Vijayaraghavan [b], B. Ravindran [c], S.W. Chang [c], P. Agastian [d,*], M.A. Rathi [e], B. Balamuralikrishnan [f,*]

[a] Department of Botany and Microbiology, Female Campus, College of Science King Saud University, Riyadh, Saudi Arabia
[b] Bioprocess Engineering Division, Smykon Biotech Pvt LtD, Nagercoil, Kanyakumari, Tamil Nadu 629201, India
[c] Department of Environmental Energy and Engineering, Kyonggi University, Youngtong-Gu, Suwon 16227, Republic of Korea
[d] Department of Biochemistry, Sri Narayana Guru College, KG Chavadi, Coimbatore, India
[e] Department of Plant Biology & Biotechnology, Loyola College, Chennai, Tamil Nadu 600 034, India
[f] Department of Food Science and Biotechnology, Sejong University, Seoul, Republic of Korea

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ABSTRACT

This study aimed to determine the phytochemical components, microbial inhibitory effectiveness and antioxidant properties of Aerva lanata plant extracts. The whole plant showed various medicinal applications in folklore and traditional medicine in various parts of the world. The organic extracts such as ethanol, ethyl acetate, chloroform, acetone, water and methanol were subjected for various phytochemical analysis and confirmed for the existence of flavonoids, glycosides, terpenoids and alkaloid containing components. Alternatively, the extracts were performed for the antibacterial activities against the microbial pathogens and antioxidant properties. Results indicated that, the solvent extracts showed prominent activity against the tested strains. The MIC concentrations of plant were detected from 5 mg/ml to 40 mg/ml. The plant extract was highly effective against *E. coli* and E. aerogenes and the MIC was 5 mg/ml. In addition, the extracts noted promising antioxidant activities. The antioxidant activities were dose dependent manner. In conclusion, *A. lanata* extracts showed that significant major phytochemicals and effective antioxidant and anti-microbial properties.

1. Introduction

Medicinal plants were applied for the treatment various diseases throughout the world. Medicinal plants contain various ranges of chemical molecules with pharmacological applications. In recent years, botanists, ethnophamacologist and natural-product chemist are analyzing the available medicinal plants for extracting various phytochemicals in the light of emerging various drug-resistance fungi and bacteria (Selvakumar and Rajasekar, 2017). More than 1000 bioactive principles have been identified from various medicinal plants and are referred to as phytochemicals (Dubey et al., 2004). The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds. These phytochemicals are responsible for various bioactive potentials. Antimicrobial activities of medicinal plants formed on the basis for their application in developing various drugs, alternative to various synthetic drugs (Skinner, 1995; Lis-Balchin and Deans, 1997). Infectious diseases are the important cause of death among populations. About 70% of hospital deaths are mainly due to various infectious diseases caused by bacteria, fungi or viruses (Gnanamani et al., 2003; Al-Dhabi et al., 2018a). Antibiotic resistance is a significant problematic and also some commercially available antibiotics were seriously associated with hypersensitivity and allergic reactions. Hence, scientists in a way to search various naturally available antimicrobial agents in the wake of disease resistance (Arokiyaraj et al., 2015; Valsalam et al., 2019). Among the traditional medicinal plants, herbs are

* Corresponding authors.
E-mail addresses: agastian@loyolacollege.edu (P. Agastian), bala.m.k@sejong.ac.kr (B. Balamuralikrishnan).

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frequently exploited to treat various diseases and have been documented (Dubey et al., 2004). Plant and microbial derives, or plant extracts provides an important source of novel medicinal substances (Al-Dhabi et al., 2019; Arasu et al., 2019). Antibacterial properties of various Indian medicinal plants were highlighted based on traditional information and attempts were made to study the effect of these medicinal plants against pathogenic fungi and bacteria (de Carvalho and Ferreira, 2001). In this study an attempt was made to analyze preliminary phytochemical content, antimicrobial and antioxidant potential of medicinal plant, Aerva lanata (Linn.).

2. Materials and methods

2.1. Plant material and extraction

Healthy samples of Aerva lanata (Linn.) whole plant was collected from Western Ghats, Kanyakumari, Tamil Nadu, India. It was authenticated by Botany and Biotechnology Department, Loyola College, Chennai. The whole plant of *Aerva lanata* was washed in tap water afterward rinsed in distilled water and then air-dried, ground into very fine powder at 60 °C for 24 h. Soxhlet extractor was used to extract the phytochemicals from the medicinal plant. About 50 g dried powder was used and five solvents with polar solvents were used to extract phytochemicals. Finally, the extracts were evaporated and transferred into a vial for future use.

2.2. Phytochemical analysis

The extracts such as, ethanol, ethyl acetate, chloroform, acetone, methanol and water were subjected for phytochemical analysis by following the standard methodology of Kumar et al. (2013).

2.3. In-vitro antimicrobial activity

Kirby-Bauer method was followed for the determination of antimicrobial activity of medicinal plant (Arasu et al., 2013). Mueller Hinton Agar was used, and the plates were pre-seeded with bacterial pathogens and incubated at 37 °C for standard antimicrobial activity analysis. All experiments were performed in duplicates and average value was tabulated (Rios et al., 1988). The Minimum Inhibitory Concentration (MIC) was evaluated as described previously.

2.4. In-vitro antioxidant activity

In-vitro antioxidant potential of the extracts were determined by DPPH method (Simona et al., 2014. DPPH assay DPPH assay was performed by preparing fresh DPPH solution, prepared using ice cold 100% methanol. Briefly, different concentrations of the extracts (20, 40, 60, 80 and 100 μg) was mixed with 1 ml of the DPPH reagent and kept under dark and shaking condition. After the incubation, time, the reaction mixture was subjected to calorimetric test. Separately, standard vitamin C was used as the positive control. The antioxidant potential of the extract was determined by following the formula

$$\text{Percentage of the activity} = \{(\text{absorbance at blank}) - (\text{absorbance at test}) / (\text{absorbance at blank})\} \times 100$$

3. Results and discussion

Antibacterial and antifungal activities of medicinal plants are being increasing in recent years (Raja et al., 2010; Arasu et al., 2017). Presently, researchers are concentrating on the active compound from the natural sources to develop newer drugs. Though, the pilot pharmacological study is an important process to achieve earlier or after the seclusion of the bioactive molecule. Earlier, a variety of *A. lanata* extracts of different parts have been stated to possess different medicinal properties viz, antimicrobial activity, antidiabetic activity, antioxidant activity (Kumar et al., 2013). It has been reported that that the active phytochemical components are predictable to be more focused in the dried material than in the fresh plant material (Deepak et al., 2019; Zhao et al., 2015). In this study, the extracts of A. lanata revealed activity towards all the tested microbial strains. The extracts such as, ethanol, ethyl acet-

Table 1
Qualitative determination of the phytochemicals present in the *A. lanata* extracts.

Phytochemicals	Different solvents					
	Ethanol	Methanol	Chloroform	Hexane	Ethyl acetate	Water
Flavanoids	–	+	+	+	–	–
Alkaloids	+	–	–	–	–	–
Phenolic compounds	–	–	–	–	+	+
Tannins	+	–	–	–	–	–
Steroids	–	–	–	–	–	–
Saponins	–	+	+	+	+	+
Terpenoids	–	–	–	–	–	–
Glucosides	–	–	–	+	+	–

+: This phytochemical is present in the extracts; –: This phytochemical is absent in the extracts.

Table 2
Antibacterial activity of *A. lanata* extracts against various pathogenic bacteria.

Test organisms	Antimicrobial activity (mm)					
	Ethanol	Methanol	Chloroform	Hexane	Ethyl acetate	Water
P. aeroginosa	9	24	8	7	10	9
S. aureus	11	18	13	11	13	11
E. coli	7	9	8	12	10	11
B. subtilis	10	21	9	11	14	10
E. aerogens	10	14	11	9	10	11

Antimicrobial activity was assessed by determining the zone of inhibition.

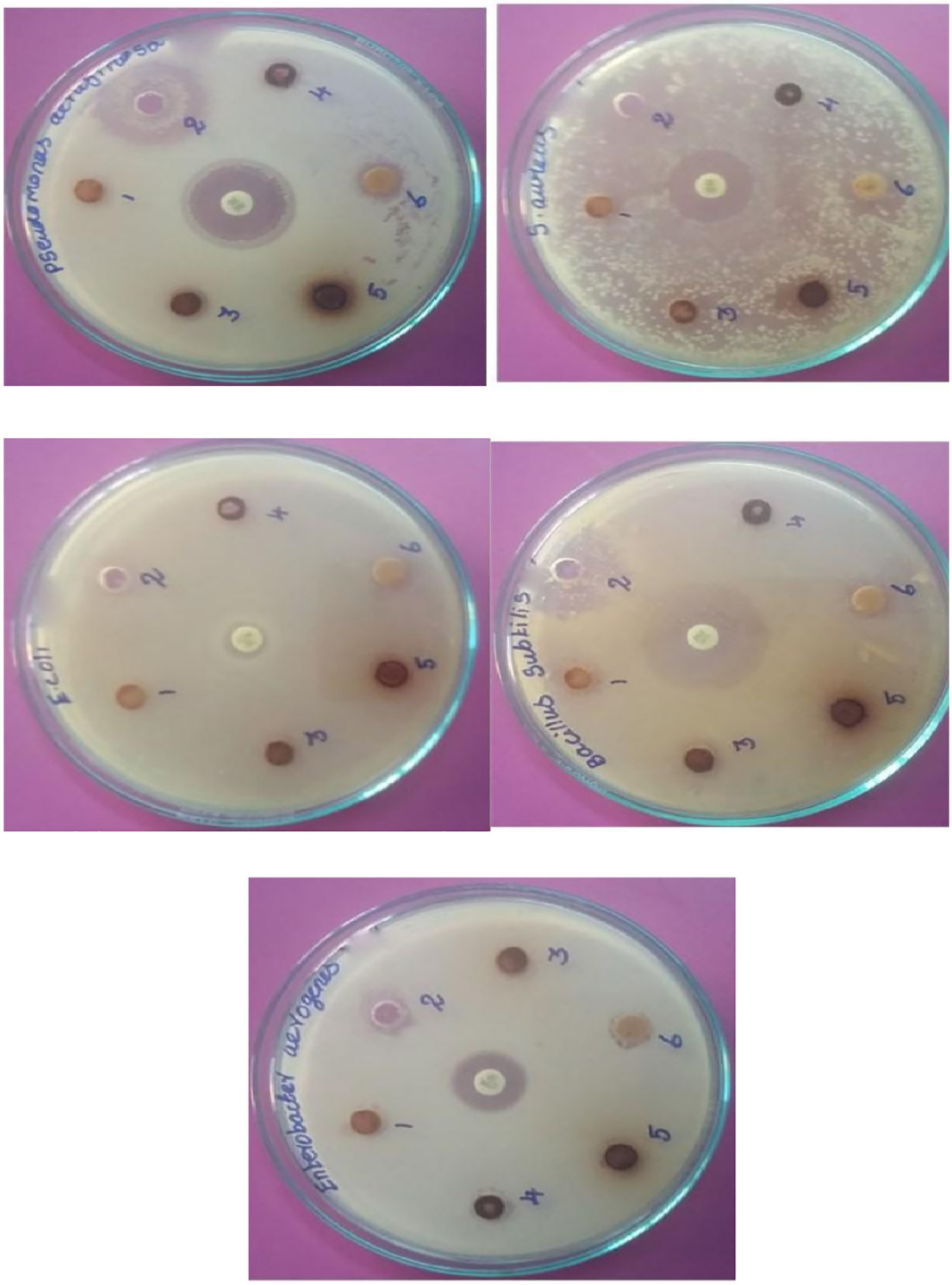

Fig. 1. Antibacterial activity of Aerva lanata (Linn.) extracted with various solvents against the selected bacterial pathogens. (1 = ethanol extract, 2 = methanol extract, 3 = chloroform extract, 4 = hexane extract and 5 = ethyl acetate extract).

ate, chroroform, acetone, water and methanol were subjected for various phytochemical analysis and confirmed the existence of flavonoids, glycosides, tannins, steroids, saponins, phenolics, terpenoids and alkaloid containing components (Table 1). Similarly, confirmed major phytochemicals in various extracts of *A. lanata* of stem and leaf (Kumar et al., 2013; Zhao et al., 2015). Phytochemicals are the non-nutritive compounds, are formed by the plants to defend from insects, bugs and environmental stress factors. These phytochemical compounds are the important for the medicinal value of *A. lanata*. Medicinal plants are rich in phenolic compounds and tannins have been shown to have antimicrobial properties against various bacterial organisms (Selvakumar and Rajasekar, 2017). A substantial number of glycosides are prodigious medicinal worth, all of which are of natural source. In the present study, confirmed that glycosides presence in hexane and ethyle acetate extracts (Table 1). Nevertheless, observed the glycosides present in the methanolic extracts of various parts of *A. lanata* (Yamunadevi et al., 2011).

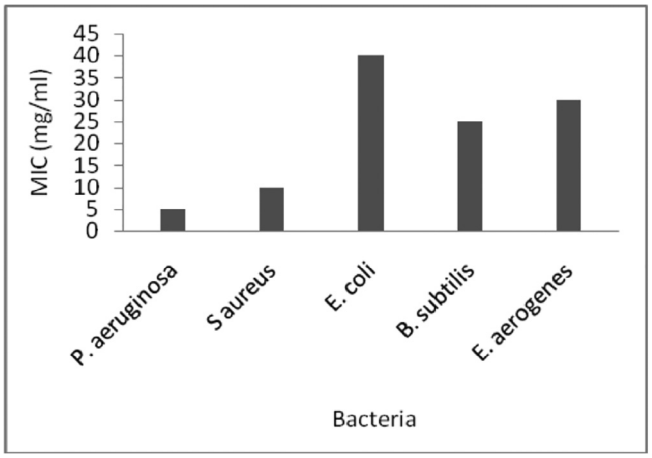

Fig. 2. Minimum Inhibitory Concentration (MIC) of *A. lanata* extracted with ethanol for antibacterial activity.

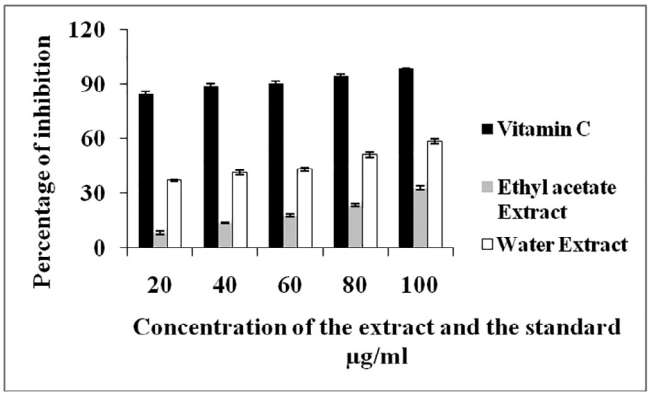

Fig. 3. In-vitro antioxidant activities of the extracts of *A. lanata.*

The obtained extracts effectively suppressed the microbial pathogens (Table 2, Fig. 1). In the present study, methanolic extract of A. lanata revealed antimicrobial activity against various bacteria, which are in agreement with Chowdhury et al. (2002) reported that *A. lanata* whole plant ethyl acetate and methanol extracts displayed antimicrobial activities. The results MIC concentrations of plant were 5–40 mg/ml level. The plant extract with methanol was highly effective against E. aerogenes and the MIC was 5 mg/ml (Fig. 2) Medicinal plants showed antibacterial activity against various bacteria and fungi (Khan et al., 2014; Rejiniemon et al., 2014). The ethyl acetate extract showed potent activity against Vibrio mimicus, V. cholerae and V. alginolyticus (Anita and Retna, 2013). A dose dependent antimicrobial activity was described previously (Yamunadevi et al., 2012). In the present study, ethyl acetate extract completely suppressed S. aureus and E. coli (Fig. 1).

According to previous studies, ethyl acetate solvent fraction showed more activity against E. coli, Bacillus sp., S. aureus and Proteus species however, n-butanol extract showed activity against Bacillus species, Proteus sp., and S. aureus (Dinnimath and Jalalpure, 2013) and methanolic extract showed antimicrobial activity against Mycobacterium phlei (Rajakaruna et al., 2002). Although, Aerva lanata showed activity against various fungi, including, Cryptococcus neoformans, Candida albicans and Aspergillus flavus (Suresh et al., 2010), green synthesis of silver nanoparticles using Aerva lanata detected effective activity for the infectious Gram-positive bacteria S. aureus (Safana Farjeen et al., 2014; Selvakumar and Rajasekar, 2017). The reason behind different results of antibacterial and antifungal activities of medicinal plants mainly depend on various factors such as, climatic condition, environmental factor under with the medicinal plant growth, the choice of extraction method and the solvent that used for extraction (Janssen et al., 1987).

The *In-vitro* antioxidant properties is an additional importance of the plant extracts towards the development of the herbal based formulations. In general, medicinal plants exhibited comparatively moderate antioxidant activities (Aruoma, 1998; Maia et al., 2006; Valko et al., 2007). In agreeing to the report of many researchers, in the present study, the plant extracts revealed dose dependent antioxidant activities (Simona et al., 2014). Especially, 100 µg/ml concentrations of the ethyl acetate and water extract revealed respectively 33% 58.5 percentage antioxidant properties (Fig. 3). This clearly indicated that the phytochemcial components responsible for the antioxidant potentials might be present in both the ethylacetate and water extracts. It is predicted that the total polyphenols content of *A. lanata* were the major responsible components for the antioxidant potentials, in addition, it was evidenced that different steps of reactive oxygen species (ROS) namely, as hydrogen peroxide, superoxide anion and hydroxyl radicals generated from the system were controlled by the various antioxidants, thereby preventing the cell damages (Yamunadevi et al., 2011). Therefore, the antioxidant potential of the *A. lanata* were need to be considered for the novel herbal formulations.

4. Conclusion

The discovery of a phytomedicine will be great interest in various treatments against infectious diseases. The results of present finding strongly indicate the importance of traditional medicine and this plant extract could serve as very useful source for novel antibacterial substance antioxidant components. Furthermore, isolation of active metabolites and structural characterization from this plant is required to characterize the potent molecule in pharmaceutical point of view in future studies.

Conflict of interest

The authors declare no competing interests.

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