WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 10, 43-48. Research Article ISSN 2277– 7105

POTENTIAL IN VITRO ANTIPSEUDOMONAL ACTIVITY OF *ALOE YEMENICA*

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Article Received on
29 July 2016,

Revised on 18 August 2016,
Accepted on 07 Sept 2016

DOI: 10.20959/wjpr201610-7088

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ABSTRACT

Aloe yemenica, which is widely distributed in Yemen, is a species belonging to the plant genus Aloe (family :Aloaceae). To the best of our knowledge, there is lack of Information on the antibacterial effect of this species and hence this study was undertaken. The main objective of this study was to assess the potential antibacterial activity of aloe yemenica against pseudomonas aeruginosa which is one of the most virulent bacteria that causes serious diseases in human. Three test samples of the plant were prepared including the fresh gel of the leaf (inner leaf), powder of the dried gel and liquid extracts. The latter were prepared by either hot or cold extraction methods using either distilled water, ethanol 96 % or chloroform as extracting solvents. Results of this study showed that only the aqueous extract prepared by hot extraction has a considerable in vitro antipseudomonal activity.

KEYWORDS : *Aloe*, Yemenica, antipseudomonal, aqueous extract, hot extraction.

INTRODUCTION

Pseudomonas aeruginosa is a common gram-negative, rod-shaped bacterium that can cause serious diseases in humans. It is opportunistic and typically infects the airway, urinary tract blood, burns and wounds[1].

The plant genus *Aloe* (family : Aloaceae) has a history of economic and medicinal use that spans thousands of years and is the source of some of the oldest known herbal medicines. The name "*Aloe*" comes from the Greek (aloî), supposedly derived, in its turn, from the Hebrew allal or the similar Arabic word alloeh, both meaning bitter — a tribute to the taste of the leaf exudate .[2] The genus *Aloe* comprising of more than 360 species and sub-species are reported

from subtropical as well as temperate parts of the world.[3] *Aloe yemenica* (Fig.1 :a) , is a species belonging to that genus and is widely distributed in Yemen. The name of the species was given by the British botanist J.R.I.Wood in 1983.[4] Many of the health benefits associated with Aloe leaf preparations have been attributed to the polysaccharides contained in the inner leaf. The inner leaf, also known as gel, is obtained as gelatinous mass by cutting Aloe leaf and discarding the rind of the leaves.[2]

Preparations derived from *Aloe* vera, a common species of *Aloe*, and described as "freeze-dried juice heated for 15 minutes at 80 ^{o}C " or "liquid *Aloe* vera extract" have demonstrated in vitro bactericidal activity against a number of pathogenic organisms, including Candida albicans and Streptococcus spp and others.[5,6,7,8] Moreover, the gel was also found to have a good antipseudomonal activity against Multidrug resistant- pseudomonas aeruginosa.[9] *Aloe vera* produces at least 6 antiseptic agents such as lupeol, salicylic acid, urea nitrogen, cinnamonic acid, phenols and sulphur. All of these substances are recognized as antiseptics because they kill or control mold, bacteria, fungus and viruses, explaining why plant has the ability to eliminate many internal and external infections.[9, 10]

Most studies in the literature have focused on antimicrobial activity of *Aloe vera* or other common species. In the contrary, to the best of our knowledge, there is lack of information on such activity for *Aloe yemenica*, hence, this study was undertaken.

MATERIALS AND METHODS

Materials

Mature leaves of *Aloe* yemenica was collected from AL-Hasba area Sana`a, the capital of Yemen, within the period from September 1$^{st}$ 2015 to the 10$^{th}$ of that month. Samples containing *pseudomonas aeruginosa* was isolated from the skin of burn patients in Al-Kuwait hospital, Sana`a- Yemen. Subculturing were carried out on Nutrient agar plates and incubated at 37±0.5°C for 24 hours.

Methods

Preparation of *Aloe yemenica* samples

Three types of *Aloe yemenica* samples were prepared; the fresh gel, powder of dried gel and liquid extracts. The fresh gel was obtained by careful cutting the leaves of the plant using a sterile scalp with discarding of the rind. The process was followed by washing with distilled water . The powder of dried gel was prepared by drying off a part of the fresh gel on sunlight

for three-day to yield yellowish crystalline solid pieces (Fig. 1: b) , which were then cut , milled and sieved to fine powder (250 µm). The Liquid extracts were of two subtypes ; those prepared by cold and those prepared by hot extraction. In cold extraction, the extracts were obtained by macerating , of 10 g of the powder of dried gel in either distilled water, ethanol 96 % and chloroform. The maceration was conducted in 250-ml conical flasks placed on an incubator shaker for 48 hours at 25^{o}C.[11] The flasks were covered by a light-resistant alumina foil with a cotton plugging the flasks mouths in order to prevent solvent evaporation. On the other hand, the extracts obtained by the hot process were prepared by Soxhlet extraction apparatus for 12 hours, using the same solvents. In both types , the process of extraction was followed by filtration to obtain clear liquid extracts.

Yield concentrations of *Aloe* sample

The yield concentrations of *Aloe* constituents in powder of dried gel was determined by as weight of the powder (g) obtained from complete evaporation of 100 g of the fresh gel. Similarly, the yield concentrations of *Aloe* in each type of liquid extracts were determined, but as grams/100 ml by weighing the residue left after complete evaporation of the solvent.

Culture media and standard control for testing activity against *Pseudomonas aeruginosa*

Mueller-Hinton agar was used as culture media for the bacteria. Gentamicin was used as standard for the purpose of comparison. Inhibition zone produced by the tested samples of ≥ 18 mm indicated susceptibility of the bacteria. 10 µg gentamicin standard disk was used for disk diffusion tests.[12] while 100 mg of a commercial brand of gentamicin 2% cream were used in the wells method.

Assessment of antipseudomonal activity

The antimicrobial activity of *Aloe* yemenica test samples against pseudomonas aeruginosa was investigated using disk diffusion method for the dried gel and liquid extracts samples while the wells method was used for testing the fresh gel. The disks were prepared from autoclaved Whatmann filter papers No .1 with diameter of 6 mm. The wells (8 mm diameter and 4 mm depth) were bored in each plate using a sterile plastic borer. Samples of 0.5 g, 100 µl, and 100 mg of the fresh gel, dried gel and liquid extracts, respectively, were tested.

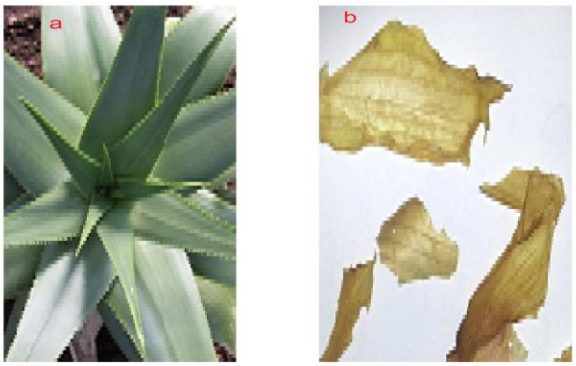

Fig. 1 Aloe yemenica: (a: plant leaves), (b: dried pieces left after complete sun drying of the fresh gel).

RESULTS AND DISCUSSION

Yield concentrations of *Aloe* yemenica sample

The yield concentration, as average ± standard deviation, of *Aloe yemenica* powder from the fresh gel , was 10.1 ± 0.27 (g/100g) , (C.I. 95 % : 9.8-10.4 %). Regarding liquid extracts, the yield concentration, as g/100ml, of *Aloe* in extracts obtained by the hot process , were 18.1 ± 0.21 (C.I. 95 % : 17.8 -18.3), 12.6 ± 0.56 (C.I. 95 % : 12 -13.2), 9.9 ± 0.57 (C.I. 95 % : 9.2 -10.5), in aqueous, alcoholic and chloroform extracts, respectively, while in those obtained by cold extraction, those values were 6.3 ± 1.17 (C.I. 95 % : 5 – 7.7), 10.4 ± 0.45 (C.I. 95 % : 9.9 -10.9) and 8.9 ± 0.61 (C.I. 95 % : 7.9 -9.3), respectively. These findings showed that, quantitively, water , as extracting solvent, was more efficient in extraction of *Aloe* constituents compared to other used extracting solvents. Moreover, the results demonstrated that hot extraction, quantitively, was better than cold extraction in extraction of *Aloe* yemenica constituents regardless the type of solvent used.

In-vitro Antipseudomonal activity of *Aloe* yemenica

Among all tested *Aloe* yemenica samples, no one showed a proper activity against the bacteria except the hot aqueous extract. The finding revealed that, qualitatively, water and hot extraction were superior in extraction efficiency of the antipseudomonal constituents of Aloe to other solvents used and to the cold extraction process, respectively. The result also indicated that the constituents of *Aloe* yemenica having a considerable activity against the bacteria were soluble only in hot water. The results of this study was in contrast to those reported in the literature such as Saba Irshad et al.[11] which demonstrated that the methanolic and ethanolic extracts in *Aloe vera* had superior activity against bacteria when compared to that of aqueous extract. This conflict may be attributed to the use of cold extraction process in

those studies and to the probable variations in active constituents between *Aloe vera* and *Aloe yemenica*. The inhibition zone produced by *Aloe* yemenica extract obtained by hot aqueous extraction with a yield concentration of 18.1 g/100 ml, as shown in Fig.2, was 22 ± 2.26 mm (C.I. 95 % : 19.7 -24.3). The extract was then subjected to serial dilution to prepare extracts with lower concentrations of 9 , 4.5 2.25 and 1.125 g/100ml. Thereafter, the antipseudomonal activity of those diluted extracts were tested. The yield concentration of *Aloe* constituents in those extract that showed the minimum activity against the bacteria was 2.25 g/100 ml with inhibition zone, as shown in Fig.2, of 18.1 ± 0.2 mm (C.I. 95 % : 17.9 -18.3).

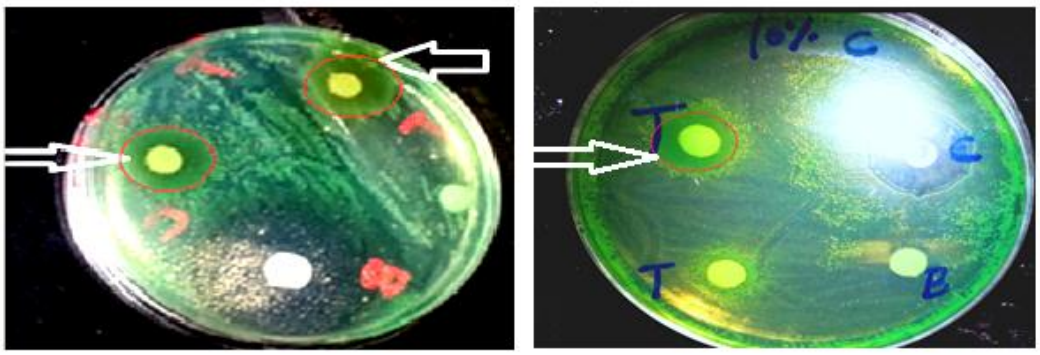

Fig. 2 Results of In vitro testing of antipseudomonal activity of aqueous extract of *Aloe* yemenica prepared by hot extraction (left : 18.1 g/100ml; Right 2.25 g/100ml); (Red circles).

ACKNOWLEDGMENT

The authors are thankful to photochemistry and microbiology laboratories at medical science college- Al-Razi university for their assistance to accomplish this study.

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