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Preliminary Investigation on the *in vitro* Antibacterial Activity of Ethanolic Extract & Essential Oil of *Aloe vera* and *Opuntia dillenii* on Human Bacterial Pathogens

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ABSTRACT

Opuntia dillenii and *Aloe vera* are used as medicinal plants. *Opuntia dillenii* belongs to Cactaceae and used for treatment of cough and bronchial troubles. *Aloe vera* belongs to Aloaceae and it is well established for its different medicinal uses ranging from treatment of skin burns to anti metastatic and antimicrobial activity. The aim of the present study was to evaluate the antimicrobial activity of ethanol extracts and essential oils of *Aloe vera* and *Opuntia dillenii* against selected human pathogens *viz., Staphylococcus aureus*, *Streptococcus pneumoniae*, *Bacillus* spp., *Klebsiella pneumoniae*, *Salmonella typhi* and *Shigella dysenteriae* by disk diffusion method and Triphenyl Tetrazolium Chloride (TTC) staining. The ethanol extract of *Opuntia dillenni* and *Aloe vera* showed good antibacterial activity against all the pathogens tested. Lower Minimum Inhibitory Concentration (MIC) values were obtained for ethanol extracts of both the medicinal plants against the bacterial pathogens except *Shigella dysenteriae* which was not inhibited at lower concentrations. The essential oils of *Opuntia dillenni* and *Aloe vera* also exhibited excellent antimicrobial activity against bacterial pathogens where *Shigella dysenteriae* was inhibited only at higher concentrations.

KEYWORDS: *Opuntia dillenni, Aloe vera*, Ethanol extract, Essential oils, Antibacterial activity, TTC staining

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1. INTRODUCTION

Man has used plants since time immemorial to treat common infectious diseases and some of these traditional medicines are still included as part of the habitual treatment of various maladies. Herbal medicines form an integral part of healing practiced by the traditional healers *(Souza et al., 2003).* Plants contain a spectrum of secondary metabolites such as phenols, alkaloids, flavonoids, quinines, tannins and their glycosides and their essential oils. Importance of these substances as antimicrobial agents against pathogens has been emphasized by several workers. These are generally obtained from plant materials by steam distillation or by extraction with organic aqueous solvents and they are relatively low in molecular weight generally less than 2000. Essential oil extracted from a plant can be defined as any concentrated, hydrophobic and viscous liquid that may contain volatile aromatic compounds. They are also known as volatile or ethereal oils, or simply as the "oil of" the plant material from which they were extracted, such as oil of clove (*Vaijayanthimala et al., 2001).* *Opuntia dillenii* (Ker-Gawl) Haw is a cactus belonging to the family Opuntiae which usually grows in semi-desert regions in the tropics and subtropics, including the Canary Islands *(Reza et al., 2019 and Zeb et al., 2015)*. Canarian folk medicine has shown much evidence that the crude extract prepared from fruits of *Opuntia dillenii* is useful in the treatment of gastrointestinal and liver disturbances (diabetes, hepatitis, intestinal spasm, etc.) *(Kedanath et al., 2013 and Saket et al., 2017)*. *Aloe barbadensis* Mill. is an important medicinal plant belonging to the family Liliaceae. *Aloe vera* treated diabetic rats showed a marked increase in body weight, liver glycogen and decrease in blood glucose, urine sugar levels and serum lipids when compared to other groups. In wound healing experiment, the progress in the healing of the wound treated with phenolic anthraquinones of *Aloe vera* sap was faster than the untreated control *(Rajendran et al., 2007).*

Antibiotic resistance in bacteria has become a global threat and cause of concern for public health. To counterattack this danger, the search for new therapeutic agents with novel modes of action from natural resources is in full swing. Thus, these two medicinal plants were used for the present study against bacterial pathogens with the aim that the screening and further investigation into the components of its extract for their antimicrobial activity may reveal many interesting therapeutic properties.

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2. MATERIALS AND METHODS

2.1. Sample Collection

Healthy plant samples of *Aloe vera* and *Opuntia dillenii* were collected in zip lock covers from different locations of the village Koovanoor, near Natchiyar Kovil, Kumbakonam, Thanjavur, Tamil Nadu, India. The plants were identified by Dr. V. Mohan, Scientist G, Institute of Forest Genetics and Tree Breeding, Coimbatore, Tamil Nadu. The collected plant materials were immediately transported to the Laboratory and maintained at 4°C. The plants samples were processed within 12 hours to maintain the freshness.

2.2. Preparation of *Aloe vera* essential oil

Fresh healthy leaves of plants were chosen. The leaves were washed thoroughly 2-3 times with running tap water and finally with sterile distilled water. Then these were air-dried under shade. 300g of air dried leaves were cut into small pieces and were macerated in mortar and pestle. They were placed in 2L flask together with 1L distilled water for steam distillation. After steam distillation, the 100% pure essential oil was collected, dispensed into dark bottles and stored at 4°C until further use *(Nanasombat and Lohasupthawee, 2005).*

2.3. Preparation of crude ethanol extracts

20g of dried leaves were soaked in 100ml of 95% ethanol and shaken at 150rpm for 4days at ambient temperature. The mixture was then filtered. The filtrates were then evaporated and dried. Stock solutions of crude ethanol extracts were prepared by diluting it with 10% Dimethyl sulfoxide (DMSO) solution to obtain a final concentration of 400mg/ml *(Nanasombat and Lohasupthawee, 2005).*

2.4. Collection and maintenance of bacterial stock cultures

Six bacterial cultures were obtained from the culture collections of the Srinivasa Ramanujan Centre, SASTRA University, Kumbakonam, Thanjavur, Tamil Nadu, India. The organisms were as follows: *Klebsiella pneumoniae, Staphylococcus aureus, Streptococcus pneumoniae*, *Shigella dysenteriae, Salmonella typhi* and *Bacillus* spp. The bacterial cultures were maintained on nutrient agar slants. They were sub cultured twice in a month and subsequently stored at 4°C.

2.5. Preparation of McFarland Nephelometer standard

Mcfarland tube number 0.5 was prepared by mixing 9.95ml of 1% sulphuric acid in Mueller Hinton broth (MHB) and 0.05% ml of 1% Barium Chloride in distilled water in order to estimate bacterial density. The tube was sealed and used for comparison of bacterial suspension with standard *(Saeed and Tariq, 2007)*.

2.6. Preparation and standardization of inoculum

Few colonies from pure culture of each test organism were transferred to 5ml of MHB. The broth was incubated at 35-37°C for 18-24 hours. The turbidity of the culture was checked with 0.5 McFarland Nephelometer standards to get 150 x 106 cfu/ml, within 15-20 minutes *(Saeed and Tariq, 2007).*

2.7. Minimum Inhibitory Concentration (MIC) of ethanolic extract by disk diffusion test

Antibacterial activity of the ethanol extract and essential oil was carried out by disc diffusion method. Mueller Hinton (Hi Media Laboratories) agar plates were prepared aseptically. Sterile antimicrobial discs of 6mm diameter (Hi Media Laboratories) were loaded aseptically with 15µl of varying concentrations of the ethanol extract (5µg/ml, 10µg/ml, 15µg/ml and 20µg/ml) or essential oil (5µl/ml, 10µl/ml, 15µl/ml and 20µl/ml) and left for 15 minutes for excess pre-diffusion of extracts/ oil. The inoculum suspension of each bacterial strain was swabbed on the entire surface of the Mueller-Hinton agar plates and the sterile discs impregnated with extract or oil was placed. Discs saturated with 10µl/ml of chloramphenicol (Hi Media Laboratories) were used as positive control. A 15µl aliquot of 1% Dimethyl Sulfoxide (DMSO) was used as negative control. Tests were performed in duplicate. The plates were incubated at 37ºC for 24 hours. After incubation, the plates were observed for zone of inhibition around the disc. The diameter of inhibition zones was measured *(Nanasombat and Lohasupthawee, 2005).*

2.8. Minimum Inhibitory Concentration (MIC) of essential oil by TTC staining method

120 µl of bacterial culture was added into separate sterile eppendorf tubes. 10 µl of various concentrations of the oil (2, 4, 6, 8 and 10µl) from the stock concentration (10µl/ml) were added to the broth cultures. The tubes were incubated at 37ºC for 24 hours. 20 µl of 0.5% TTC (Triphenyl Tetrazolium Chloride) aqueous solution was added to detect the presence of live cells. MIC was defined as the lowest concentration of oil that inhibited visible growth as indicated by TTC staining. Alive cells turn red on addition of TTC whereas white solution indicates no growth. Negative and positive control was also performed using 1% DMSO and 10µl/ml of chloramphenicol *(Sartoratto et al., 2004).*

3. RESULTS

3.1. Minimum Inhibitory Concentration (MIC) of ethanolic extract and essential oil by disk diffusion method

The data pertaining to the antimicrobial potential of the ethanolic extracts and essential oils of *Aloe vera* and *Opuntia dillenni* is presented in *Table 1 and 2.* The ethanol extract of *Aloe vera* had a very strong inhibitory effect on most of the pathogens tested. MIC of its ethanol extract was determined as 5µg/ml for Bacillus spp. and *Klebsiella pneumonia* whereas for *Staphylococcus aureus* and *Streptococcus pneumoniae*, it was 10µg/ml and for *Salmonella typhi* and *Shigella dysenteriae*, it was 15µg/ml.

It was observed that ethanol extract of *Opuntia dillenni* was effective against *Staphylococcus aureus* and *Klebsiella pneumoniae.* It had no inhibitory effect on *Bacillus spp*., *Shigella dysenteriae, Salmonella typhi* and *Streptococcus pneumoniae* as evident from absence of zone of inhibition. The MIC of the extract against *Staphylococcus aureus* and *Klebsiella pneumoniae* was determined as 10µg/ml.

Essential oil extracted from *Aloe vera* showed maximum inhibitory effect on *Streptococcus pneumoniae* at MIC of 5µl/ml and on *Staphylococcus aureus* with MIC of 10µl/ml. MIC for *Bacillus spp*., were determined as 15µl/ml and no effect was observed on the growth of *Klebsiella pneumonia, Shigella dysenteriae* and *Salmonella typhi.*

Essential oil extracted from *Opuntia dillenni* had only a slight inhibitory effect on *Staphylococcus aureus*, *Streptococcus pneumoniae* and Bacillus spp., whereas it didn't inhibit the growth of *Klebsiella pneumoniae*, *Shigella dysenteriae* and *Salmonella typhi*. MIC for the *Staphylococcus aureus* was determined as 10µl/ml, 5µl/ml for *Streptococcus* species and 15µl/ml for Bacillus spp.

3.2. Minimum Inhibitory Concentration (MIC) of essential oil by TTC staining

A new modified method was used to determine MIC of essential oil of *Aloe vera* and *Opuntia dillenni* against bacterial pathogens. This method incorporates the use of TTC (Triphenyl Tetrazolium Chloride) solution which indicates the presence or absence of organism on addition of oil. From Fig.1, it can be interpreted that oil of *Aloe vera* was inhibitory against *Staphylococcus aureus, Streptococcus pneumoniae*, *Bacillus* spp. and *Salmonella typhi* were inhibited at lower concentration itself. The growth of *Shigella dysenteriae* and *Klebsiella pneumoniae* were inhibited only at 8µl/ml and 10µl/ml respectively.

It was found that essential oil of *Opuntia dillenni* was highly effective against *Staphylococcus aureus, Streptococcus pneumoniae* and *Klebsiella pneumoniae* even at a low concentration of 2µl/ml. The oil was also effective against *Salmonella typhi* at 4µl/ml, but it didn't inhibit the growth of *Bacillus* spp. and *Shigella dysenteriae* until a higher concentration of oil (10µl/ml) was added *(Figure 1)*. The inhibition of growth in this method is indicated by the absence of development of red color on addition of TTC after incubation of culture with oil. Alive cells turn red whereas white color indicates growth inhibition.

4. DISCUSSION

The potentiality of *Opuntia dillenii* and *Aloe vera* were tested for susceptibility against *Staphylococcus aureus*, *Streptococcus* species, *Bacillus* species, *Klebsiella* species, *Salmonella* species and *Shigella* species. Compared to *Opuntia dillenii*, *Aloe vera* showed highest degree of antibacterial activity. Leaves and roots of many species in *Aloe* family have been used in many traditional treatments. The leaf sap of this genus is widely used by traditional healers and indigenous for the treatment of wounds, burns, rashes, itches, cracked lips and cracked skin. Antimicrobial effect of the crude extract of both the plants was studied in attempts to validate the use by traditional healers in the use of the latex and gel exudates for various medicinal ailments *(Coopoosamy and Magwa, 2007).* Mexicans have used *Opuntia* spp. leaves and fruits for their medicinal benefits, such as for treating arteriosclerosis, diabetes, gastritis, and hyperglycemia and ethanol extract of the stem of *Opuntia ficus - indica* var. *saboten* (OFS) was assessed to determine the mechanism(s) of its antioxidant activity *(Jeong-Chae Lee, 2002). Beena et al. (2009)* also reported the use of essential oil of *Zanthoxylum rhetsa* against human pathogens Although work in essential oils of the plants have been reported, work on essential oil of *Aloe vera* and *Opuntia dillenni* are scanty. Reports are available on the use of several plants by-products, which possess antimicrobial properties, on several pathogenic bacteria and fungi. Modified method of *Sartoratto et al. (2004)* using TTC to stain bacterial cells was found to be effective in MIC determination. Data from the literature as well as our results reveal the great potential of *Aloe vera* and *Opuntia dillenni* for therapeutic treatment, in spite of the fact that they have not been completely investigated. Therefore, more studies need to be conducted to search for new compounds from them. However before being used in new therapeutic treatments, the extracts should be studied for their toxicity *in vivo*.

Table 1: Antibacterial activity of ethanol extract and essential oil of *Aloe vera* on human bacterial pathogens by disk diffusion method

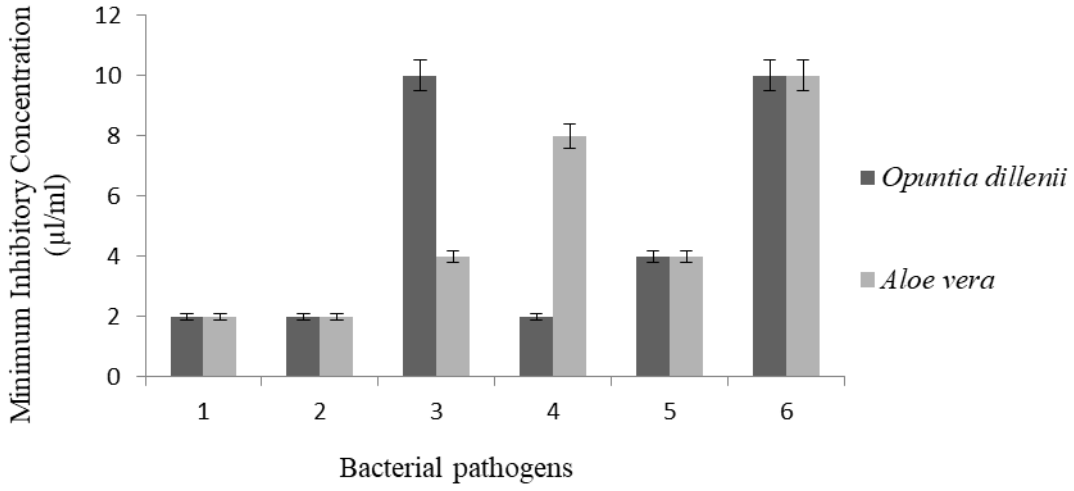
Test organism	Zone of inhibition (mm) in different concentration									
	Ethanolic extract (mg/ml)				Essential oil (ml/ml)				Chloramphenicol (10µl/ml)	Dimethyl Sulfoxide (1% DMSO)
	5	10	15	20	5	10	15	20		
Staphylococcus aureus	-	+	+	+	-	+	++	++	++	-
Streptococcus pneumoniae	-	+	+	+	+	+	+	+	+	-
Bacillus spp.	+	+	+	+	-	-	+	+	++	-
Klebsiella pneumonia	+	+	+	+	-	-	-	-	++	-
Salmonella typhi	-	-	+	+	-	-	-	-	+	-
Shigella dysenteriae	-	-	+	+	-	-	-	-	++	-

Key: (+ +) Inhibition zone between 11 to 15 mm (+) Inhibition zone between 6 to 10 mm (-) No inhibition

Table 2: Antibacterial activity of ethanol extract and essential oil of *Aloe vera* on human bacterial pathogens by disk diffusion method

Test organism	Zone of inhibition (mm) in different concentration									
	Ethanolic extract (mg/ml)				Essential oil (ml/ml)				Chloramphenicol (10µl/ml)	Dimethyl Sulfoxide (1% DMSO)
	5	10	15	20	5	10	15	20		
Staphylococcus aureus	-	+	+	+	-	+	++	++	++	-
Streptococcus pneumoniae	-	-	-	-	+	+	+	+	+	-
Bacillus spp.	-	-	-	-	-	-	+	+	++	-
Klebsiella pneumonia	-	+	+	+	-	-	-	-	++	-
Salmonella typhi	-	-	-	-	-	-	-	-	+	-
Shigella dysenteriae	-	-	-	-	-	-	-	-	++	-

Key: (+ +) Inhibition zone between 11 to 15 mm (+) Inhibition zone between 6 to 10 mm (-) No inhibition



Key: (1) *Staphylococcus aureus;* (2) *Streptococcus pneumonia*e; (3) *Bacillus* spp.; (4) *Klebsiella pneumoniae*; (5) *Salmonella typhi*; (6) *Shigella dysenteriae*

Figure 1: Minimum Inhibitory Concentration (MIC) of essential oil of *Aloe vera* and *Opuntia dillenni* on human bacterial pathogens

5. CONCLUSION

The present work corroborated with the ancient practice of using medicinal plants for treatment of various diseases. It was observed that the ethanol extract and essential oil of *Aloe vera* and *Opuntia dillenni* showed excellent antibacterial activity against all the human pathogens tested. Antibacterial activity of ethanolic extract and essential oil of plants revealed strong activity against important human pathogens. Minimum Inhibitory Concentration (MIC) of *Aloe vera* and *Opuntia dillenni* essential oil was low against the human pathogens. A new method viz., TTC staining comparatively used less was found to be very effective in determining MIC of essential oil. The composition of oil of *Aloe vera* and *Opuntia dillenni* extracts can be analyzed using Gas Chromatography - Mass Spectrometry (GC-MS).

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CONFLICT OF INTEREST STATEMENT:

The author declares no conflict of interest in this research work.

REFERENCES

Beena J, Reddy JL, Shafi PM (2009). Evaluation of antibacterial activity of Zanthoxylum rhetsa seed essential oil. Int. J. Agri Environ. & Biotech. 2 (4):455-457.

Coopoosamy RM, Magwa ML (2007). Traditional use, antibacterial activity and antifungal activity of crude extract of Aloe excelsa. African Journal of Biotechnology. 6 (20): 2406-2410.

Jeong-Chae L, Hak-Ryul K, Ju Kim, Yong-Suk J. (2002). Antioxidant property of an ethanol extract of the stem of Opuntia ficus-indica var. saboten. Journal of Agric. Food Chem. 50:6490-6496.

Jothi KR, Vijayalakshmi K, Tamilarasi L, Ezhilarasi B. (2014). Antibacterial Activity of leaf extracts of Aloe vera, Ocimum sanctum and Sesbania grandiflora against the Gram Positive Bacteria. Asian Journal of Biomedical and Pharmaceutical Sciences. 04 (35): 60-63.

Kedarnath, Kamble KM, Vishwanath BC, Patil CS (2013). Antimicrobial activity of Aloe vera leaf extract. International Journal of Applied Biology and Pharmaceutical Technology. 4(4): 286-290.

Mbajiuka CS, Obeagu EI, Okwandu GE. (2014). Antimicrobial effects of Aloe vera on some human pathogens. Int. J. Curr. Microbiol. App. Sci. 3 (3): 1022-1028.

Nanasombat S, Lohasupthawee P (2005). Antibacterial activity of crude ethanolic extracts and essential oils of spices against Salmonellae and other enterobacteria. KMITL Sci. Tech. J. 5 (3):527-538.

Oghenemaro EF, Johnson J, Itohan IM, Richard SO and Michael O. (2018). Antimicrobial activity of Aloe vera gel and honey against bacteria isolates from wound aspirates. Int. J. Pharm. Sci. & Res. 9(11): 4890-93.

Rajendran A, Narayanan V, Gnanavel I (2007). Evaluation of therapeutic efficacy of Aloe vera sap in diabetes and treating wounds and inflammation in animals. Journal of Applied Sciences Research. 3(11): 1434-1436.

Reza S, Vafa BR, Ashrafali RK, Amirhossein S , Hassan R, Vahid RA. (2019). Opuntia dillenii: A Forgotten Plant with Promising Pharmacological Properties. Journal of Pharmacopuncture. 22 (1):016-027.

Saeed S, Tariq P (2007). Antibacterial activities of Emblica officinalis and Coriandrum sativum against gram negative urinary pathogens. Pak. J. Pharm Sci. 20(1):32-35.

Saket K, Dan S J, Rajesh S. (2017). Evaluating Antimicrobial activity of Aloe vera Plant Extract in Human Life. Biomed J Sci & Tech Res. 1(7):1854-56.

Sartoratto A, Machado ALM, Delarmelina C, Duarte GMT, Rehder VLG (2004). Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil. Brazilian Journal of Microbiology. 35:275-280.

Souza LKH, Oliveria CMAD, Ferri PH, Oilveria Jr GGD, Souza Jr AHD, Fatima OD, Fernandes L, Silva MDRR (2003). Antimicrobial activity of Hyptis ovalifolia towards dermatophytes. Mem. Inst. Oswaldo Cruz Rio De Janerio. 98(7):963-965.

Vaijayanthimala J, Prasad RN, Pugalendi KV (2001). Antifungal activity of oil. Indian Journal of Microbiology. 41: 325-326.

Zeb MA, Sajid M, Rahman TU, Khattak KF, Halim M, et al. (2015) Phytochemical Screening and Antibacterial Activity of Opuntia dillenii and Onosma bracteatum. J Microbiol Exp 2(7): 00074.