



## Research Article

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### STUDIES ON ANTIMICROBIAL AND ANTHELMINTIC POTENTIAL OF ALOE

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#### ABSTRACT

Aloe is one of the ancient herbal medicinal plants used widely in the Indian subcontinent. The plant also finds therapeutic role in modern medicine. The present work was aimed to determine the antimicrobial activity of aloe. Successive soxhlet extraction was done and all the extracts were subjected to phytoanalytical studies viz. determination of phenolics, flavonoids, carbohydrates, proteins and ascorbic acid. The ethyl acetate extract was subjected to antimicrobial studies. The results demonstrated excellent antimicrobial potential of aloe extract as compared to tetracycline and amphotericin B. Thus it could be concluded that aloe is an integral source of antimicrobial compounds. However, further exhaustive studies are underway to determine compounds responsible for it.

**Keywords:** Aloe, antimicrobial, extracts, bacteria, fungi, phytoconstituents

#### INTRODUCTION

Discovery of antibiotics and their use in antimicrobial chemotherapy is one of the miracles in the field of therapeutics, and widely practiced to combat a number of microbial infections. Due to injudicious and irrational prescribing of antimicrobial agents, phenomenon of antimicrobial resistance against pathogens like *Streptococcus pneumonia*, *Staphylococcus aureus*, *Klebsiella*, *Neisseria*, *Enterococci* etc. has been developed<sup>1,2</sup>. Due to this phenomenon, many antibiotics became irrelevant for the treatment of such infections<sup>3</sup>.

Aloe is one of the oldest medicinal plants known to humans since prehistoric times. It is one of the traditionally used plant as folk remedies<sup>4-6</sup>. The plant extract is known to contain soothing and wound healing potential<sup>7</sup>. The present work was designed to evaluate antimicrobial potential of Aloe found in Mahakaushal region of M.P., India.

#### MATERIALS AND METHOD

##### Chemicals

Until otherwise specified all the chemicals were purchased from Central Drug House, India and were of analytical grade.

##### Worm Collection and Authentication

The earthworm *Eisonia fatida* (African type) were collected and authenticated from Madhya Pradesh Pashu Chikitsa Vishwavidyalaya, Jabalpur (M.P.), India. Piperazine citrate (10 mg/ml) was used as standard.

##### Test microorganisms

All the microorganisms were incubated at  $37 \pm 0.1^\circ\text{C}$ , for 24 h in Nutrient broth, *C. albicans* in YEPD broth at  $28 \pm 0.1^\circ\text{C}$  for 48 h.

##### Plant collection and extraction

Aloe was identified and authenticated by Dr. Ms. Indu Gupta, Retd. Professor, Department of Botany, Model Science College, Jabalpur, M.P., India. Aloe was washed and dried in shaded area for 7 days. The Aloe was

powdered and subjected to soxhlet extraction using solvents in eluotropic series viz. petroleum ether, toluene, chloroform, acetone, ethyl acetate, ethanol and finally with water for 20 cycles for each solvent. All the extracts were lyophilised and stored at  $4^\circ\text{C}$  till use.

##### Phytochemical analysis

Phytochemical analysis of various extracts was done by following standard method<sup>8</sup>.

##### Phytoanalytical studies

##### Estimation of Total phenolics

The total phenolics in the extracts were estimated by spectrophotometric assay<sup>9</sup>. 1 ml of the sample (concentration 1 mg/ml) was mixed with 1 ml of Folin and Ciocalteu's phenol reagent. After 3 minutes, 1 ml of saturated sodium carbonate solution was added to the mixture and it was adjusted to 10 ml with distilled water. The reaction was kept in the dark for 90 minutes, after which the absorbance was read at 725 nm. Gallic acid was used for constructing the standard curve and the results were expressed as  $\mu\text{g}$  of gallic acid equivalents/mg of extract (GAEs).

##### Determination of total flavonoids

Flavonoid contents in the extracts were determined by spectrophotometric method<sup>9</sup>. The (250  $\mu\text{l}$ ) extract (concentration 1 mg/ml) was mixed with 1.25 ml of distilled water and 75  $\mu\text{l}$  of a 5%  $\text{NaNO}_2$  solution. After 5 minutes, 150  $\mu\text{l}$  of 10%  $\text{AlCl}_3$  solution was added. After 6 minutes, 500  $\mu\text{l}$  of 1 M NaOH and 275  $\mu\text{l}$  of distilled water were added to prepare the mixture. The solution was mixed well and the absorbance was read at 510 nm. ( $\pm$ )-Catechin was used to calculate the standard curve and the results were expressed as  $\mu\text{g}$  of ( $\pm$ )-catechin equivalents (CEs) per mg of the extract.

##### Determination of total carbohydrate

Total carbohydrate contents were estimated by Anthrone method<sup>10</sup>. Glucose was used to calculate the standard curve and the results were expressed as  $\mu\text{g}$  of glucose equivalents per mg of extract.

**Determination of total protein**

Total proteins were estimated by Lowry's method<sup>11</sup>. Bovine serum albumin was used to calculate the standard curve and the results were expressed as  $\mu\text{g}$  of bovine serum albumin equivalents per mg of extract.

**Determination of ascorbic acid content**

One mg of various extract was treated with 4.0 ml of 10% trichloroacetic acid and centrifuged for 20 minutes at 3500 rpm and 0.5 ml of supernatant was mixed with 0.1 ml DTC reagent (2, 4-Dinitrophenylhydrazine-thiourea-copper sulphate reagent). The tubes were incubated at 37°C for 3 hours. Ice cold 65%  $\text{H}_2\text{SO}_4$  (0.75 ml) was added and the tubes were allowed to stand at room temperature for an extended 30 minutes. The colour developed was read at 520 nm<sup>12, 13</sup>. Ascorbic acid was used to calculate the standard curve and the results were expressed as  $\mu\text{g}$  of ascorbic acid equivalents per mg of the extract.

**Evaluation of Antimicrobial Activity**

Nutrient Agar and YEPD Agar (20 ml) were poured into each sterilized Petri dish (10 X 100 mm diameter) after swabbing cultures (100 $\mu\text{l}$ ) of bacteria, yeast and distributing medium in Petri dishes homogeneously. For the investigation of the antibacterial and anti- candidal

activity, the dried extracts were dissolved in distilled water to a final concentration of 20% and sterilized by filtration through a 0.22 $\mu\text{m}$  membrane filter<sup>14,15</sup>. Each sample (100  $\mu\text{l}$ ) was filled into the wells of agar plates directly. Plates injected with the fungal cultures were incubated at 28 °C for 48 h, and the bacteria were incubated at 37 °C for 24 h. At the end of the incubation period, inhibition zones formed on the medium were evaluated in mm. Studies were performed in triplicate and the inhibition zones were compared with those of reference discs. Amphotericin B (10 $\mu\text{g}$ ) and tetracycline (30 $\mu\text{g}$ ) were taken as reference. (Figure 1 and 2)

**Evaluation of Anthelmintic activity**

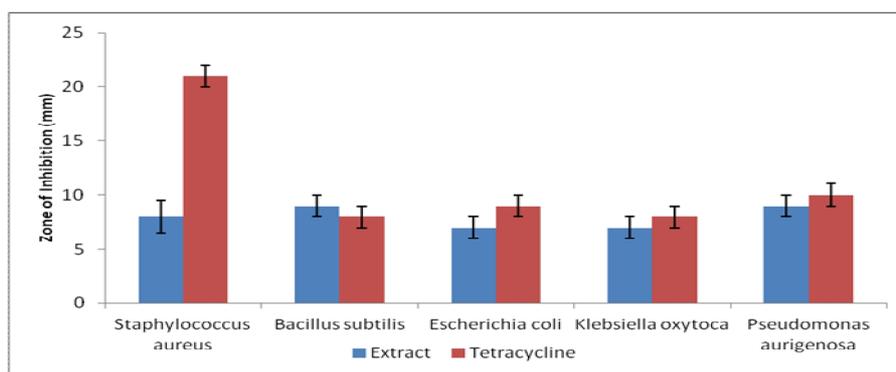
The anthelmintic assay was carried out as per the reported method of Ajaiyeoba *et al.*,<sup>16</sup> with necessary modifications. Different concentrations of extracts (25, 50, and 100 mg/ml in distilled water) were prepared, and three worms (same type) were placed in it. Time for paralysis was noted when no movement of any sort could be observed. Time for death of worms was recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50°C). (Figure 3)

**Table 1: Details of phytochemical screening**

Constituents	Petroleum Ether Extract	Toluene Extract	Chloroform Extract	Acetone Extract	Ethyl Acetate Extract	Ethanol Extract	Water Extract
Alkaloids	-	-	-	-	+	+	+
Glycosides	-	-	-	-	+	+	+
Tannins	-	-	-	-	-	-	+
Resins	-	-	-	-	-	-	-
Carbohydrates	-	-	-	-	-	+	+
Fats	+	+	+	+	-	-	-
Proteins	-	-	-	-	-	+	+
Sterols	+	+	+	+	-	-	+
Anthocyanins	-	-	-	+	+	+	+

**Table 2: Phytoanalytical studies of aloe extract**

Constituents	Phenolic content (GAEs)	Flavonoid content (CEs)	Total carbohydrate ( $\mu\text{g}$ glucose eq/ mg)	Total proteins (BSA eq/mg)	Ascorbic acid content (mg)
Petroleum Ether Extract	-	-	-	-	-
Toluene Extract	-	-	-	-	-
Chloroform Extract	-	-	-	-	-
Acetone Extract	-	79.54 $\pm$ 2.45	-	-	-
Ethyl Acetate Extract	102.23 $\pm$ 1.78	106.39 $\pm$ 2.47	42.32 $\pm$ 3.55	59.32 $\pm$ 2.54	49.54 $\pm$ 2.36
Ethanol Extract	126.42 $\pm$ 2.34	121.63 $\pm$ 1.57	89.86 $\pm$ 1.47	79.15 $\pm$ 2.75	56.84 $\pm$ 3.18
Water Extract	139.18 $\pm$ 3.56	152.38 $\pm$ 3.37	98.34 $\pm$ 3.75	85.24 $\pm$ 1.54	63.71 $\pm$ 2.38

**Figure 1: Zone of inhibition produced by extract of aloe on bacteria**

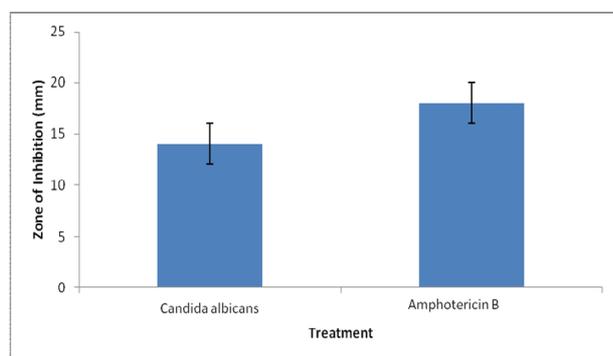


Figure 2: Zone of inhibition produced by extract of Aloe on *Candida albicans*

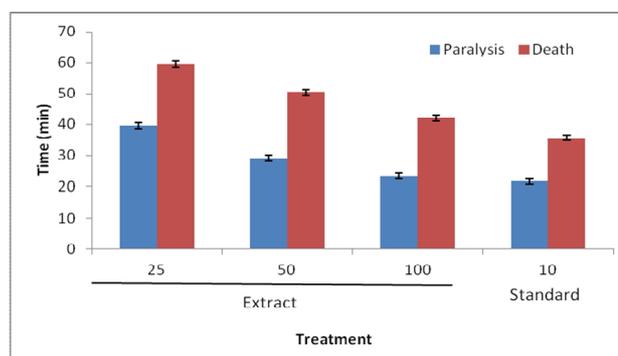


Figure 3: Anthelmintic activity of aloe extract

## RESULTS AND DISCUSSION

Results of phytochemical screening revealed that ethyl acetate extract, ethanol extract and aqueous extract contained a number of phytochemicals. The extracts also contained a number of phytochemicals like alkaloids, glycosides, tannins, phenolics and flavonoids (Table 1 and 2). Anti-bacterial effect of extract (100µg/ml) was screened against pathogens. After incubation, anti-bacterial activity of the extract was significantly compared with standard. Similar comparative results were obtained with anthelmintic studies

Aloe is one of the traditionally used herbal remedy in treatment of variety of disorders and used to treat sub-mucosal fibrosis<sup>17</sup> and ischemic conditions<sup>18</sup>. In the current studies, aloe extracts effectively inhibited the growth of pathogenic bacteria. Extract also caused paralysis and death of worms indicating its anthelmintic potential.

Thus it can be concluded that aloe is a rich source of antimicrobial and anthelmintic active principles.

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