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Chemical and Antimicrobial Studies on the Skin of *Aloe indica* Leaves at Different Ages of Plants

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Abstract

A comparative study on mineral profile, water content, solid content and ash contents were done on the skin of the leaves at three different ages of *Aloe indica* plants. Fatty acid analysis and screening of anti-bacterial sensitivity against two bacteria were also carried out. Mineral profile was evaluated by Atomic absorption spectrophotometer and Flame photometer. Whereas the esterified fatty extract analysis was done by GC/MS spectrophotometer. Sodium, potassium, magnesium and calcium were found to be present as major minerals whereas zinc, copper, silver, phosphorous, chromium were found as trace elements. No arsenic was found to be present in any of the samples. The amount of different mineral contents exists in the order of 1 year< 2 years> 3 years. A comparative study of the mineral contents of the skin with the fillet was also done. Percent compositions of the fatty acid contents were found to be very different in different maturity skin extracts. Skin comprises higher proportion of solid and ash content and lower proportion of water content than the fillet. Leaves of 2 year ages plants contained higher proportion of solid content than both of the 3 year and 1 year ages plants leaves. The 80% ethanol extract of the skin showed antimicrobial sensitivity in the order of 3 years > 2 year l> 1year ages of plants.

Keywords: *Aloe indica*, Mineral contents, Fatty acid analysis, Antimicrobial sensitivity.

Introduction

Most dignified well discovered medicinal plant is *Aloe vera. A. vera* issues from numerous tropical and subtropical countries in the world [Encyclopedia 2004]. It practices plenteous in Bangladesh too. The diversity issue from Bangladesh is *A. indica*. It belongs to the family *Aloeaceae* and genus *Aloe. A* large number of researches have

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been carried out on different species of *Aloe vera* and most of the researches were performed on *Aloe barbadensis* (Xiao, 2000; Shupe, 2003 and Ni, 2004). *Aloe vera* is a biological vehicle in that it acts as a physical or physiological carrier for active biological agents but, also adds biological activity to the test agent no matter what the pharmacologic agent under consideration is. Therapeutic uses of *Aloe vera* have been reported in the medical literature for over 50 years, although it has been reported in the botanical and naturopathic literature for many more years. Recent study showed its use in the treatment of *arthritis, high cholesterol, hypertensive, interstitial cystitis, nonbacterial prostatitis, chronic pelvic pain, immune system disorders such as AIDS, and diabetes,* (Shelton, 1991; Strickland, 1994; Rajasekaran, 2005 and Rajasekaran, 2006). It is also effective as the antibacterial and anti-fungal agent (Hirata, 1983; Shupe, 2001). Studies and case reports provide support for the use of *Aloe vera* in the treatment of radiation ulcers and stasis ulcers in humans and burn (Crewe, 1939). Modern clinical medical use of *Aloe* began in the 1930s with reports of successful treatment of X-ray and radium burns (Lee, 1999). The physicochemical and biological properties of any plants and plant products greatly altered with the alteration of geographic state, circumstances under existing condition, tillage of land etc. A comparative study on three different Aloe species available in Bangladesh has been reported (Chowdhury, *et al*; 2004). Comparative study report on physicochemical properties on different maturity *Aloe indica* plants is not available in literature. But this is an important factor because physicochemical and biological attributes also largely depends on maturity of the plants (Winter, 1995).

We now report about some of the physicochemical and biological properties on three distinct ages *Aloe indica* leaves skin which were cultivated in Northern region of Bangladesh.

Materials and Methods

All the chemicals employed during the study were of analytical grade. Double distilled water was utilized to made ready solutions requisite. Sophisticated instrumentations were handled during carrying experiment. Mineral profiles were fixed by administering Atomic Absorption Flame Emission Spectrophotometer, SHIMADJU, AA-11, Japan, Atomic Absorption Spectrometer, Spectra AA-220, Varian, Australia and Flame Photometer, JENWAY, PFF-7, UK. Fatty acid analysis was done by GC/MS technique set into practice by using GC/MS Spectrophotometer (GC-17 Gas Chromatograph, coupled to a GC/MS/ QP5050A mass spectrometer, SHIMADJU, Japan).

Collection of plant materials

Aloe indica leaves of three different maturity plants were collected from Natore,

Bangladesh. It was then cleaned, air dried, and preserved in a cold condition as early as possible.

Sample Preparation

Two types of sample were prepared for each of the three different ages of plants. For type-I, fillets from the clean Aloe leaves were disunited by hand filleting method. It was then crushed and concentrated at low temperature by using rotary vacuum evaporator at reduced pressure, then freeze dried and hoarded in deep freeze as type-I sample (Fillet). For type-II, leaves skin after disunion of the fillets as described above was collected crushed and air dried. The air dried skins were dried at 40^{O}C in an electric oven. Dried skins were then powdered and packed in cellophane packets and hoarded in a cold state for carrying experiments as type II sample (skin).

A. Proximate analysis

Water, solid and ash content of *A. indica* leaves fillet and skin of the leaves were estimated by using standard methods (AOAC, 1984).

B. Estimation of mineral concentrations

Mineral contents of the fillet and skin were resolved by the dry ashing order and fixed by administering Atomic Absorption Spectrophotometer and Flame photometer. Dry specimens (fillet and skin) of *Aloe indica* ascertained before were ashed disunitedly. And the weight of the ashes was enrolled. These samples were employed for mineral content fixation. 1:1 HNO_3 was summed up to each of the samples and heated at low flame to depart of carbonaceous matter left in the ash. To each sample after cooling 2-3 drops of concentrated HNO_3 was added to it to completely dissolve the sample. The sample was then shifted to a 100 ml volumetric flask and maked volume up to 100 marks with distilled water. These solutions were used as stock solution. A definite volume of solution from the stock solution was passed through the Atomic Adsorption Spectrophotometer and curve was observed. Comparing these curves with standard curves minerals were identified. If the value of any mineral was above the standard curve then the solution was diluted and again introduced into AAS. Similarly sodium and potassium content was determined by Flame photometer.

C. Fatty acid analysis

C.1. Extraction of fat from the skin of *Aloe indica* leaves

Fat was extracted from the powdered skin of *Aloe indica* leaves by hot (solvent) extraction method using Soxhlet extraction unit. n-Hexane was used as solvent. The extraction was continued for about 6 hrs. The extractives were collected, filtered and evaporated to dryness by using rotary vacuum evaporator. It was then freeze dried.

C.2. Saponification of fat

About 500 mg of extracted crude fat was taken into a round bottom flask and saponified with 80 ml of prepared alcoholic (methanolic) sodium hydroxide by refluxing it in a boiling water bath for about 1 hr. Extra base was used for neutralization of HCl.

C.3 Preparation of methyl ester of fatty acid (Giannelli, 1999; Hossain, 2003)

The above whole mass was then hydrolyzed by heating in a boiling water bath for about 1 hr. The solution was dried and then esterified with 20 ml BF3-Methanol complex by heating the mixture in a boiling water bath for about 40 mins. The methyl ester of the fatty acid was separated by partitioning it with n-hexane and water. The n-hexane soluble part was concentrated, dried and analyzed by GC/MS Spectrophotometer.

C.4. Identification of fatty acids

The fatty acid exists in the fat samples were identified by comparing the relative retention time and peak position of the sample chromatogram with the standard.

C.5. Relative percentage of fatty acids

Amount of fatty acid present in the fat sample is proportional to the area under peaks. So area under the peaks representing each compound was calculated and summed. The area under the peak was divided by total area under all peaks to obtain the proportion of each fatty acid of the compound.

D. Screening for antimicrobial sensitivity

A comparative antimicrobial sensitivity of *Aloe indica* gel is reported (Agarry, 2005) but no works found on leaves skin. In the present study antimicrobial sensitivity of *A. indica* leaves skin extract (alcohol) was investigated against two pathogenic (organisms) bacteria, one is *Staphylococcus aureus* (gram + ive) and another is *Salmonella spp* (gram - ive) by using a agar diffusion method. The organisms were maintained on Nutrient Agar and were seeded on the Petri dish by pour plate methods using standard order. Muller Hinton Agar media was used through out the experiment. The standard porcelain beads were impregnated with the samples (*0% ethanol extracts of *A. indica* leaves skin at different ages of plants). In different concentrations propylene glycol was used as solvents and the beads were placed separately at different positions of the solidified agar medium. Locally available chloramphenicol (30 LI g/ml) was used as standard. The petri dishes were incubated at 37^{O}C for 24 hours and inhibition zones were measured. The result were calculated and compared with chloramphenicol. All the experiments were carried out aseptically (Begum, 1997). Produced zone of inhibition during this study are expressed in Table V.

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Results and Discussion

A comparative study on different parts of *Aloe indica* leaves fillet and skin revealed

that the 2 years ages plant leaves contained less amount of water in its fillets and skins than the 1 year and 3 years ages plant leaves. On the other hand the total solid content and ash contents were found to be maximum in 2 year ages plants leaves skin and fillets.

It is observed that the fillets of 1 year ages plant leaves contained water, intermediate between 2 year and 3 year ages of plants leaves.i.e. 1 year, 2 year and 3 year ages plant leaves fillets contained 98.67 %, 96.38 %, and 98.98 % of water respectively. Similarly 1 year, 2 years and 3 year ages plant leaves skin contained 89.60 %, 89.32 % and 89.57 % of water respectively. Correspondingly solid content obtained throughout the experiment was found to be for fresh fillets 1.68, 2.58 & 1.03 % and for fresh skin 10.40, 10.58 & 10.42 % in 1 year, 2 years and 3 year ages of plants respectively. Ash content of a substance indicates the amount of residue obtained by direct burning of all organic substances from that. It is assumed from the experiment that the inorganic constituents of a substance are non-volatile at the temperature at which the organic materials are burned. Results obtained throughout this study are shown in table I. It is observed that ash content obtained for leaves fillet are 0.28, 0.39 & 0.25 %; for leaves skin 1.78, 1.96 & 1.56 % respectively in 1, 2 and 3 years ages plants respectively.

A number of elements which are essentially required in small amounts for the body func tions are called minerals. Rajasekaran and co-workers reported about the presence of minerals in different species of Aloe vera (Rajasekaran, *et. al*., 2005). We now made a comparative research study on different minerals and trace elements content in different parts at three different maturity of *Aloe indica* plants leaves (Table II). Na, K, Ca and Mg were found to be the major minerals and among the three samples these minerals were present in highest amount in 2 year ages plant leaves fillet and skin. So these

Table I. Results of proximate analysis

Ages of plants	Types of sample	Moisture content g/100 g of fresh sample	Solid content g/100 g of fresh sample	Ash content g/100 g of fresh sample	Ash content g/100 g ofdry sample
1 year		98.50	1.50	0.28	18.67
2 year	Leaves fillet	97.42	2.58	0.61	23.64
3 year		98.90	1.10	0.25	22.00
1 year		89.60	10.40	1.78	17.11
2 year	Leaves skin	89.32	10.68	1.96	18.35
3 year		89.57	10.43	1.56	14.96

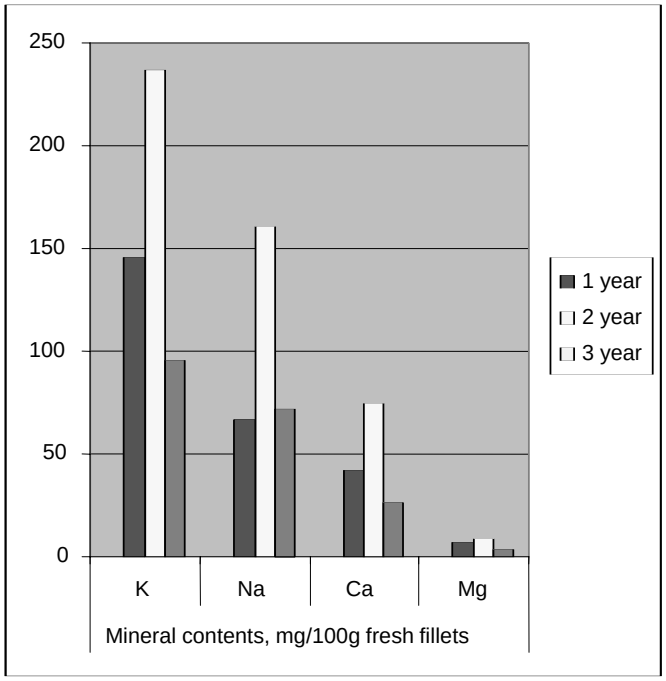
mineral exists in the order of 1 year< 2 years> 3 years. Zn, Cu, Ag, P and Cr were found as trace elements. None of the sample contained As in it (Table II and Graph 1 and 2).

A comparative study of different minerals content like, Na, K, Ca, Mg content of the skin with the fillets revealed that the skin contained a very good amount of the above

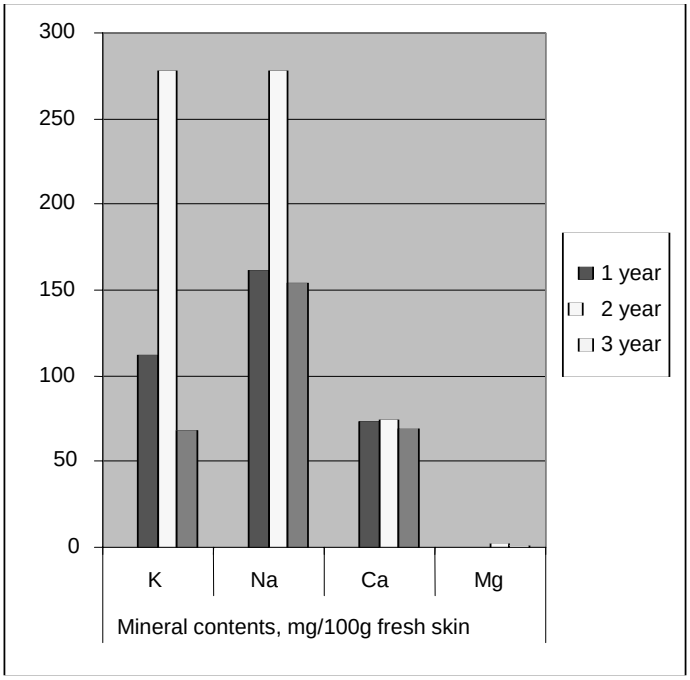
Table II. Mineral contents in mg/100g of *Aloe indica leaves* (fillets and skins) fresh wet basis

Ages of plants	K	Na	Ca	Mg	Ag	Zn	Cu	Cr	P	As
					Fillets					
1 year	146.00	66.53	42.40	6.90	ND	Trace	Trace	Trace	0.013	ND
2 year	236.60	160.50	74.50	8.90	ND	0.040	Trace	Trace	0.260	ND
3 year	96.00	71.70	26.60	3.90	0.56	Trace	Trace	Trace	0.070	ND
					Skins					ND
1 year	112.00	161.50	73.10	0.390	0.32	0.01	Trace	Trace	0.030	ND
2 year	278.00	277.80	74.40	1.96	0.05	Trace	Trace	Trace	0.014	ND
3 year	68.50	154.00	69.40	0.60	ND	Trace	Trace	Trace	0.026	ND

ND= Not detectable



Graph 1. Major mineral contents in mg/100 g of fresh fillets of *Aloe indica* leaves.



Graph 2. Major mineral contents, mg/100 g of fresh skin of *Aloe indica*. leaves.

Table III. Comparative relative percentage of different compounds in the fatty extracts of the skin of *Aloe indica* leaves which are present in all the three different ages' plants.

No	Compounds	A_1	A_2	A_3
1	Methyl laurate	0.39	0.89	0.08
2	Methyl myristate	1.03	1.90	0.20
3	Methyl palmitate	11.21	17.73	2.29
4	Methyl margarate	0.54	1.95	0.14
5	Methyl lenoleate	19.81	10.85	1.65
6	Methyl isostearate	0.52	0.60	1.13
7	Diethyl phthalate	2.86	0.06	0.10
8	(2E) 2- Undecenyl pentanoate	1.42	1.95	1.16
9	Heptadecyl hexanoate	0.54	0.87	0.42
10	Linolenic acid	19.81	26.11	3.24
11	Methyl stearate	1.68	2.38	0.53
12	Heneicosane	0.33	0.19	0.19
13	Methyl ecosanoate	0.70	0.58	0.81
14	Methyl heneicosanoate	1.39	2.67	1.91

A_1, A_2, and A_3 =skin of Aloe vera leaves of 1, 2 and 3 year ages plants respectively.

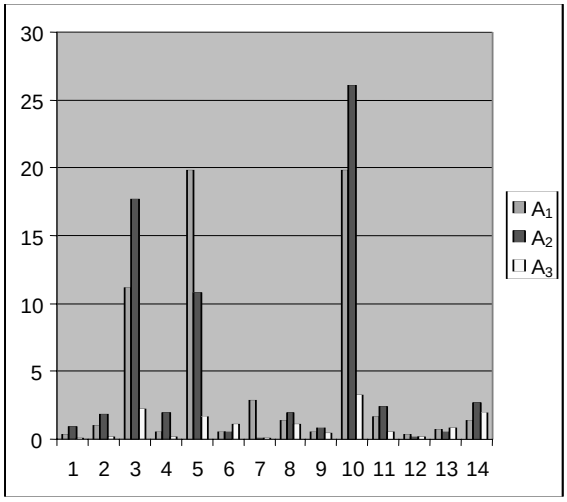
Table IV. Relative % of different compounds in the fatty extracts of the skin of *Aloe indica* leaves which are not present in all the three different ages' plants.

No	Compounds	A_1	A_2	A_3
1	Methyl (7E) -7- hexadecanoate	0.31	0.14	.....
2	Isooctyl phthalate	43.43	.....	78.70
3	Methyl heptadecenoate	0.29	.....	.....
4	Oleyl alcohol	0.42	0.48	.....
5	13- Heptadecyn-1-ol	0.42	.....	0.19
6	Tetratetracotane	0.45	.....	.....
7	Nonacosane	0.67	.....	0.53
8	Eicosane	0.37	.....	.....
9	Cyclododecanol	.....	.....	0.28
10	n-Decylmethyl phosphonofluoridate continued	.....	.....	0.18
11	4-Dioxaspiro (4,50 decane	.....	0.47	0.23
12	Hexadecane	.....	0.27	0.12
13	0.19 Nonadecane	.....	0.19	0.20
14	Thunbergol	.....	.....	3.04
15	Methyl heptacosanoate	.....	.....	0.68
16	Chol-5-ene-3,24-diol	.....	.....	1.20
17	Methyl azelaaldehydate	.....	0.23	.....
18	Methyl (5E) -5-octadecenoate	.....	0.15	.....
19	1,11 Undecenediol	.....	0.18	.....
20	1,4,6,6 tetramethyl-1-cyclohexene	.....	0.56	.....
21	13-heptadecyn-1-ol	.....	0.75	.....
22	Octyl methyl phosphonofluoridate	.....	0.38	.....
23	Oxotetrahydro-2-furancarboxilic acid	.....	0.15	.....
24	Methyl 14-methyl hexadecanoate	.....	0.22	.....
25	Cyclohexanol	.....	0.18	.....
26	Methyl (10E) , 10 nonadecenoate	.....	0.11	.....
27	Methyl nonadecenoate	.....	0.06	.....
28	Methyl 5 (4,8,2 trimethytridecyl) dihydro-2-(3H) furanone 5	.....	0.13	.....
29	Methyl isoheptadecanoate	.....	0.16	.....
30	Tritetracontane	.....	0.75	.....
31	Diisooctyl phthalate	.....	22.08	.....
32	Methyl pentadecanoate	.....	0.24	.....

A_1, A_2, and A_3 =skin of Aloe vera leaves of 1 year ages plant, 2 year ages plant and 3 year ages of plants respectively.

minerals. Na and K content in 2 year ages plants leaf skin was found to be more than that of fillet. Ca content among the three skin samples were also found to be in good amount though the Mg content was not good in comparison of the fillet.

The esterified fatty extract compositions of *A. Indica* leaves skin obtained through out the experiment is presented in Table III and IV. Total numbers of compounds found to be present are 23, 36 and 26 in 1 year, 2 year and 3 year plants skin respectively. Only 14 compounds were found to be present in each of the three samples of esterified fatty extracts (A_1, A_2, and A_3). 9 of them were found to be present in maximum amount in sample A_2. The percent composition of the-compounds in the fatty extracts was also observed different in the skin at different ages of plant leaves. The 2 years ages plant skin contained lenoleic acid, 26.11%, palmitic acid, 17.73% and lenoliate, 10.85% as major components whereas 3 years ages plant skin contained isooctyl phthalate, 78.70% as major components and the other components were present less than 4%. It was very interesting to observed that 1 year and 3years ages plants skin contained 43.43% and 78.70% isooctyl phthalate respectively whereas 2 year ages plant leaves skin contained no isooctyl phthalate in it (Table IV). Lenoleic acid present in 1, 2 and 3 year ages plants leave skin were found to be 19.81, 26.11 and 3.24% respectively. Whereas palmitate was present as 11.21, 17.73 and 2.29 % respectively in 1, 2 and 3 year ages plant leaves skin respectively (Table III, Graph 3).

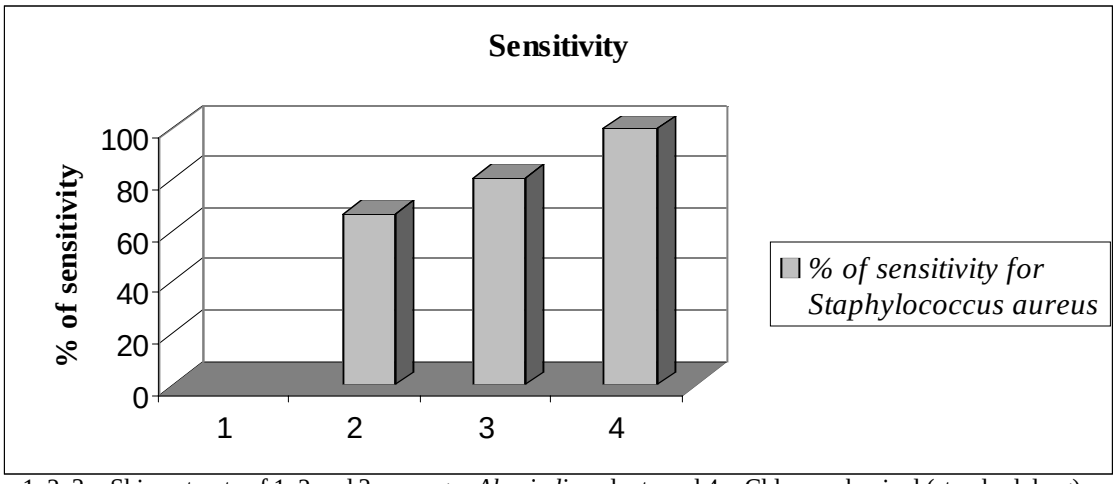


A_1, A_2, and A_3 = 1, 2 and 3 year ages plants leaves, 1-14=Compounds 1-14 of Table III.

Graph 3. Comparative relative percentage of different compounds in the esterified fatty extracts which were present in the skin among the three different ages of *Aloe indica* leaves.

Table V. The zone of inhibition against two pathogenic bacteria.

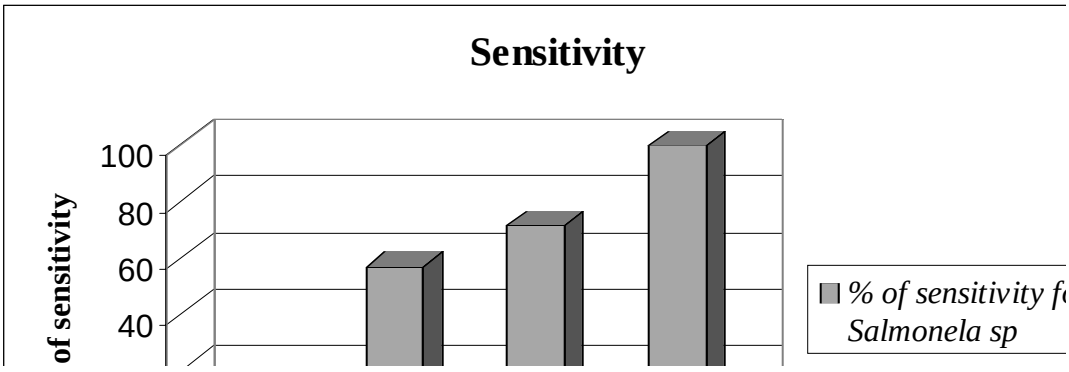
Ages of plants	*Aloe* vera leaves skin extract with 80% ethanol	Concentration	Zone of inhibition	% of sensitivity
		Staphylococcus aureus		
1 year	Same	20 mg/ml	Resistant	Resistant
2 year	Same	"	1.05	66.60
3 year	Same	"	1.70	80.95
Chloramphe nicol	Standard drug	30 ?g/ml	2.1	100
		Salmonella spp		
1 year	Same	20 mg/ml	Resistant	Resistant
2 year	Same	"	1.2	57.14
3 year	Same	"	1.5	71.43
Chloramphe nicol	Standard drug	30 mg/ml	2.1	100



1, 2, 3 = Skin extracts of 1, 2 and 3 year ages *Aloe indica* plants and 4 = Chloramphenicol (standard drug)

Graph 4. Antibiotic activity of the skin extracts of *Aloe indica* leaves against *Staphylococcus aureus.*

From antimicrobial sensitivity investigation (Table V and Graph 4 & 5), it was observed that extracts of 1 year ages leaves skin extract (80% ethanol) showed no activity. Whereas 2 year and 3 year ages plants skin extract s showed significant sensitivity 66.60 and 80.95 % respectively against *Staphylococcus aureus* and 57.14 and 71.43 % against Salmonella spp respectively. The antimicrobial sensitivity of the skin extracts can therefore be arranged in the order of 1 year ages plants < 2years ages plants < 3 years ages plants against both the bacteria. So *Aloe indica* leaf skin extract of 3 year ages plants is better as antibacterial agents.



1, 2, 3 = Skin extracts of 1, 2 and 3 year ages *Aloe indica* plants and 4 = Chloramphenicol (standard drug)

Graph 5. Antibiotic activity of the skin extracts of *Aloe indica* leaves against *Salmonella spp.*

Conclusion

The people of our country are used to take the fillets of the leaves of this plant (*Aloe indica*) only and the skins are leaved as waste. The results of our present study revealed that the skin of *Aloe indica* leaves contained a very good amount of minerals like Na, K, Ca and Mg. The fatty extract ofthe skin contained a good percentage of different essential fatty acids. The skin extracts also showed antimicrobial sensitivity against two bacteria. The 2 year ages plants skin contained the maximum amount of minerals and it also contained maximum-number of compounds in its fatty extract. For antibacterial activity 3 year age's plants skin is better. So it can be suggested that the skin of *Aloe indica* leaves can be used as a good source of some of the useful nutrient minerals and lipid components and its extract (80% EtOH) might be a potential source of antibacterial agent.

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