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PHYTOCHEMICAL AND PHARMACOLOGICAL SCREENING OF *Aloe vera* Linn.

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Abstract- *Aloe vera* is a perennial, drought-resisting, succulent plant belonging to the *Asphodelaceae* family which, historically has been used for a variety of medicinal purposes. It has a vast traditional role in indigenious system of medicine like ayurveda, siddha, unani and homoeopathy. Clinical evaluations have revealed that the pharmacological active ingredients are concentrated in both the gel and rind of the *Aloe vera* leaves. Bioactive compounds from *Aloe vera* are very effective in various treatments, such as burns, allergic reactions, rheumatoid arthritis, rheumatic fever, acid indigestion, ulcers, diabetes, skin diseases, dysentry, diarrohoea, piles and inflammatory conditions of the digestive system and other internal organs, including the stomach, small intestine, liver, kidney, and pancreas.
The present study aimed to evaluate the antidiabetic activity of *Aloe vera* ethanolic extract in an induced hyperglycemic and normal rats. In the induced hyperglycemic experiment twenty rats were divided into 4 groups. They were fasted for 18 hours and then injected intraperitoneally with 2 mg/kg Body weight (Bwt) of 50% glucose solution. The first group served as a control, the second was administered orally with 10 ml/kgBwt glibenclamid (hypoglycemic drug) and the other two groups were given100 and 500 mg/kgBwt of *Aloevera*extract. The plasma glucose level was determined after 1, 2 and 4 hours following glucose loading. In the normoglycemic experiment, fifteen rats were divided into three groups; one as control and the other two were given 100, 500 mg/kg Bwt *Aloe vera* extract orally. The extract was given for 6 days. Clinical signs, body weight and plasma glucose level were recorded. The results in the hyperglycemic experiment revealed highly significant decrease ($P < 0.01$) in plasma glucose in the group received 500 mg/kg Bwt of *Aloe vera* ethanolic extract. However, the reduction in plasma glucose level at a dose of 100 mg/kg Bwt *Aloe vera* extract and glibenclamide was similar. In the normoglycemic rats administration of *Aloe vera* extract caused diarrhea and reduction in body weight but no changes in the relative liver weight. The plasma glucose level was not affected by administration of *Aloe vera* ethanolic extract. There were no pathological changes occurred in tissues except an increased number of goblet cells in the intestinal mucosa.
Keywords- Aloevera, Pharmacological, Antimicrobial, Tannin, Antidiabetic.

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Introduction

Natural products are known to play an important role in Pharmaceutical biology. Plants have been an important source of medicine for thousands of years. Even today, the World Health Organization estimates that up to 80 percent of people still rely mainly on traditional medicines. In fact, many of the current drugs either mimic naturally occurring molecules or have structures that are fully or in part derived from natural motifs1. Natural antimicrobials can be derived from barks, stems, leaves, flowers and fruits of plants, various animal tissues or from microorganisms2. Genus *Aloe* is a perennial succulent herb have grown in temperate and subtropical parts of the world. This plant genus is originated in

Africa. The genus includes 200 or more species. Some of them are cultivated for the resinous latex contained in their thick, fleshy leaves. Since biblical times, aloe plants have figured among folkloric remedies as purgatives and as treatments for skin disorders. *Aloevera*is a member of liliaceae family. It is commonly called aloe, burn plant, lily of the desert, elephant's gall. *Alovevera* (L.) in synonym *A. brobadensis* is a cactus (leaves) like plant with green, dagger- shaped leaves that are fleshy, tapering, spiny, marginated and filled with a clear viscous gel3. The name, aloe, is derived from the Arabic "alloeh" or Hebrew "halal" meaning bitter shiny substance. Two types of exudates are secreted by aloe leaves. One is a bitter reddish-yellow juice contained in the pericyclic cells located under the strongly cutinized epidermis of the leaves. This "juice" has been generally used for laxative purposes and in dried form. Its bitterness is due to the presence of aloin, aloe-emodin and related compounds.

The other exudate is a transparent, slippery mucilage or gel produced by the thin-walled tubular cells in the inner central zone (parenchyma) of the leaf. The raw "gel" resembles colorless gelatin with hair-like connective matrices and is also sometimes called "juice." In antiquity, this mucilage was applied to inflamed skin and during the 20th century it was used on radiation burns. The bio active compounds are used as astringent, haemostatic, antidiabetic4,5, antiulcer, anti-septic6, antibacterial7. antiinflammatory, antioxidant and anticancer agent and also, effective in treating stomach ailments, gastrointestinal problems, skin diseases, constipation, radiation injury, wound healing, burns, dysentery, diarrhoea and in the treatment of skin diseases. Currently the plant is widely used in skin care, cosmetics and as nutraceuticals8. In the present study we focus on some of the phytochemical, pharmacological and traditional properties of Aloevera.

Materials and Methods

Collection of Plant Material- The plant of *Aloe vera* (leaves) was collected from in and around the area of Bidar, Karnataka. The plant part (leaves) was identified by a taxonomist in the Department of Botany, B.V.B. college of UG and PG Bidar, Karnataka INDIA.

Preparation of Plant Extract- The leaves of *Aloe vera* was air dried and crushed to small piece using Mortar and Pestle and powdered in an electric grinder. Twenty grams of powdered plant materials mixed with 100ml of various solvents (Distilled water, Ethanol and Acetone solution). The extracts preparations were done as previously described by Alade and Irobi [6]. The plant extracts were prepared by using soxhlet apparatus collected and stored in a vial for further studies.

Screening of Phytochemical and Pharmacological Activity- Phytochemical components were analyzed qualitatively and screened for pharmacological activities of Aloevera.

Disc Preparation- The 6mm (diameter) discs were prepared from whatmann No. 1 filter Paper the discs were sterilized by autoclave at 12°C.After the sterilization the moisture discs were dried on hot air oven at 50°C.Then various solvent extract discs and control discs were prepared.

Antibacterial and Antifungal Activity of *Aloe Vera*- The antibacterial and antifungal activity studies were carried out by disc diffusion technique [12]. The sterile nutrient agar plates and potato dextrose agar plates were prepared. The bacterial test organisms like *Escherichia coli, Staphylococcus aureus, Klebsiella pnemoniae, and Bacillus aereus* were spread over the nutrient agar plates by using separate sterile cotton buds. Then the fungal test organism like *Aspergillus niger, Aspergillus fumigates and Neurospora crassa* were spread over the potato dextrose agar plates After the microbial lawn preparation three different extracts of plant disc were placed on the organism inoculated plates with equal distance control discs were also prepared. All bacterial plates were incubated at 27°C for 24 hrs and fungal plates at 24°C for 72hrs. The diameter of the minimum zone of inhibition was measured in mm. For each test, three replicates were performed.

Drug- Glibenclamide, an oral hypoglycemic drug, belongs to the group sulphonylurea (second generation) was used.

Animals- Wister albino rats of either sex were used. They were housed in standard environmental condition of temperature, humidity and fed a fixed diet. They were left for a week as an adaptation period.

Experimental Procedure

Two experiments were carried out, one in rats with an induced hyperglycemia and the other in normal animals. In induced (hyperglycemic) experiment 20 rats, divided randomly into 4 groups, 5 rats each, were used. Group A was left as control, group B received glibenclamide hypoglycemic drug and groups C and D received different concentration of *Aloe vera* ethanolic extract. All animals were fasted for 18 hours. Then 50% glucose solution was administered intraperitoneally at a dose of 2 mg/kg Body weight (Bwt) to induce hyperglycemia to all groups except group A which was used as control. Immediately after using gastric tube group A received 20 ml/kg Bwt distilled water, group B given glibenclamide at a dose of 10 mg/kg Bwt. While groups C and D received 100 and 500 mg/kg Bwt *Aloe vera* ethanolic extract respectively. Plasma glucose level was determined at zero time then at 1, 2 and 4 hours after glucose loading according to Siest and Schiel [22] method. Blood was collected from the orbital plexus by capillary tubes into fluorinated test tubes under inhalation anesthesia using halothane according to Khana *et al*. [14]. The Blood was centrifuged at 3000 rpm for 5 minutes to separate plasma. In the second experiment, normal fifteen rats were randomly divided into three groups, 5 rats each. Group A was given 10 ml distilled water, groups B and C received 100 and 500 mg/kg Bwt of *Aloe vera* ethanolic extract orally for 6 days using gastric tube. Clinical signs and body weight were recorded. The plasma glucose level was determined at zero time and at the end of the experiment. Animals were slaughtered and liver weights were recorded. Slices of liver, kidney, spleen, gastrointestinal tract, pancreas and brain were fixed in 10% buffered formalin embedded in paraffin wax, sectioned.

Results and Discussion

The present study carried out on the *Aloe vera* revealed the presence of medicinal active constituents. The phytochemical active

compounds of *Aloe vera* were qualitatively analyzed and the results are presented in Table 1. In analysis of Tannin compounds brownish green colour developed to indicate the presence of Tannin. Similarly based on the presence or absence of colour change indicate positive and negative results are indicate. In this screening process Tannin, Saponin Flavonoids and Terpenoids gave positive results and phlobactanins and Steroids gave negative results.

Table 1- Phytochemical analysis of aleovera components Pharmacological Activities

Sl. No.	Phytochemical components	Results
1	Tannin	+
2	Phlobatannins	-
3	Saponin	+
4	Flavonoids	+
5	Steroids	-
6	Terpenoids	

+ Present, - Absent

Antibacterial Activity

Antibacterial activity of *Aloe vera* was analyzed against *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa* and Escherichia *coli.* The maximum antibacterial activities were observed in acetone extract (12±0.45, 20±0.35, 20±0.57, 15±0.38) other than aqueous extract (0.00, 9±0.54, 0.00, 0.00) and ethanol extract (7±0.38, 20±0.36, 15±0.53, 0.00). Among the three bacterial organisms maximum growth suppression was observed in *Streptococcus pyogenes* (20±0.35) and *Pseudomonas aeroginosa* (20±0.57) when compared with *Staphylococcus aerues* (12±0.45) and *Escherichia coli* (15±0.38). Results are presented in Table 3 and Fig. 1. Ferro *et al.* [15] have shown that *Aloe vera* leaf gel can inhibit the growth of the two Gram-positive bacteria *Shigella flexneri* and *Streptococcus progenes*. Specific plant compounds such as anthraquinones [13] and dihhydroxyanthraquinones as well as saponins [14] have been proposed to have direct antimicrobial activity.

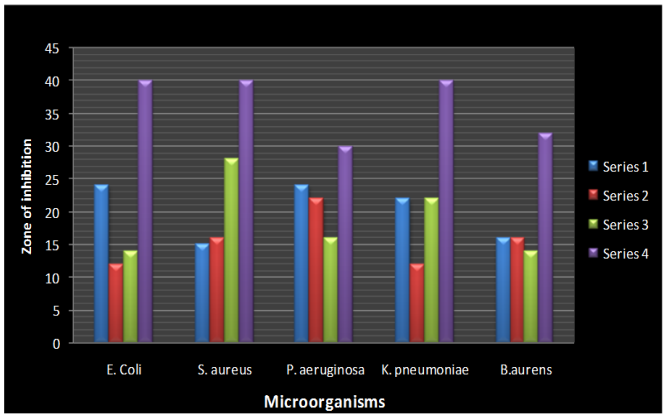

Fig. 1- Antibacterial activity

Antifungal Activity

Antifungal activity of *Aloe vera* was analyzed against *Aspergillus flavus* and *Aspergillus niger.* The maximum antifungal activities were observed in acetone extract disc (15±0.73 and 8±0.37) other than aqueous extract (0.00 and 0.00) and ethanol extract (11±0.53 and 10±0.32). Among the two fungal organisms maximum growth suppression was observed in *Aspergillus flavus* (15±0.73) than *Aspergillus niger* (8±0.37). Results are presented in Table 4 and Fig 2. Many previous studies such as that of Ferro *et al.,* [15] have focused on the antimicrobial activity of *Aloe vera* whole gel. Other reports have attempted to determine the antimicrobial activity of purified fractions such as anthraquinones [13] and saponins [14]. *Aloevera*extracts have been shown to inhibit the growth of fungi that cause tinea, however evidence for control beneath human skin remains to be established. For bacteria, inner -leaf gel from *Aloe vera* was shown to inhibit growth of *Streptococcus* and *Shigella* species in vitro. In contrast, *Aloe vera* extracts failed to show antibiotic properties against *Xanthomonas* species [15].

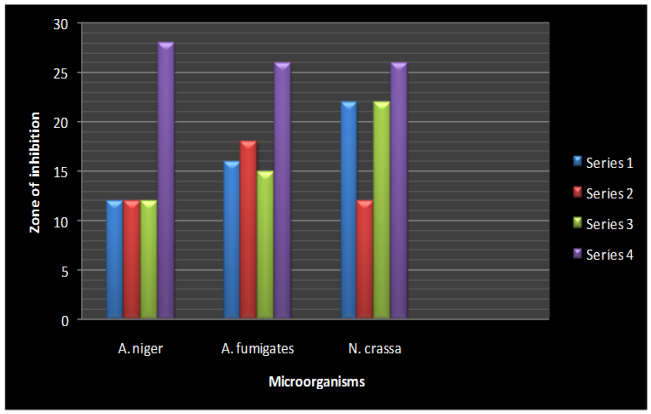

Fig. 2- Antifungal activity

Antidiabetic Activity

The data of the plasma glucose level of hyperglycemic rats treated with *Aloe vera* ethanolic extract are shown in Table- 3, Fig. 3. After one hour all groups showed an increase in the plasma glucose level compared to zero time. The increase varies from 51% in the control group to 43.7% in the group received glibenclamide. However in the groups received 100 and 500 mg/kg Bwt of *Aloe vera* ethanolic extract, the glucose level increased by 33.5 and 30.8% respectively. Two hours later the glucose level was reduced by 4.7% in the controls and 16.4% in the glibenclamide treated groups while in groups C and D the reduction was 12.7 and 23.1 respectively. After four hours the control reached the starting level i.e. a 37.5% reduction compared to the two hours level. The plasma glucose level in animals received glibenclamide and 100 mg/kg Bwt of *Aloe vera* ethanolic extract were still remained above the starting level and was before reduced by 11.5 and 5.8% compared to the two hours level respectively. Meanwhile a noticeable decrease in the plasma glucose level was recorded in rats receiving 500 mg/kg Bwt *Aloe vera* and almost reached the starting level. In the second experiment where the normal rats received *Aloe vera* ethanolic extract, there was watery diarrhea after two days till the end of the experiment and was more profound in the group received 500 mg/kgBwt of *Aloe vera* ethanolic extract. The body weight of the control rats increased by 5.6% while the groups received ethanolic extract of *Aloevera*decreased by12.2 and 20.8% at the dose of 100 and 500 mg/kgBwt respectively Table 4, Fig. 4. Meanwhile the relative livers weights were not significantly different between the controls

and the groups treated with *Aloe vera* ethanolic extract (Table 4). The blood glucose level in the groups of rats treated with ethanolic extract of *Aloe vera* were not significantly different from the the control. However, slight decrease in the blood glucose level in all the groups occurred. The reduction in the control was 1.4% and in the groups received 100 and 500 mg/kgBwt of *Aoe vera* ethanolic extract was 7.1 and 12% respectively Table- 5, Fig. 5. Histologically there was an increase of goblet cells in the mucosa of the intestine beside sloughing of epithelium. There were no pathological changes in the tissues examined in the control and treated animals.

Table 2- Anti bacterial and Anti fungal activity

Sl. No.	Micro-organisms	Petroleum Ether (40mg/ml)	Chloroform (40mg/ml)	Ethanol (40mg/ml)	Strepto Mycin	Flucona Zole
1	*E.coli*	24	12	14	40	---
2	*S.aureus*	15	16	28	40	---
3	*P.aeruginosa*	24	22	16	30	---
4	*K.pnemoniae*	22	12	22	40	---
5	*B. aereus*	16	16	14	22	---
6	*A.niger*	12	12	12	---	20
7	*A.fumigates*	16	18	15	---	26
8	*N.crassa*	22	12	22	---	26

Discussion

This study has revealed the presence of many secondary metabolites in the leaves of *Aloe vera*. It has the further confirmed that the plant extracts could be used for the treatment of various infections including skin transmitted infections and dental caries. The results lend credence to the folkloric use if this plant in treating microbial infection and shows that *Aloe vera* could be exploited for new potent antimicrobial agents.

In the present study the effects of ethanolic extract of *Aloe vera* on blood glucose level was performed in an induced hyperglycemic and normal rats to determine whether the extract has hypoglycemic properties. Administration of *Aloe vera* ethanolic extract to rats loaded with glucose caused significant drop in plasma glucose level. The drop was dose dependant. On the other hand when similar doses administered to normal rats no significant drop in plasma glucose level occurred. These findings suggested that the hypoglycemic effect of *Aloe vera* extract was induced only in hyperglycemic rats but not in normoglycemic animals. This is similar to the action of biguanid group of hypoglycemic drugs that do not cause hypoglycemia in normal subjects even when taken in excessive dose [8]. This groups of drug originally derived from the *plant* Galega officinalis. Its mechanism of action is by inhibition of hepatic glucose production and increase of muscle glucose uptake. However, Blumental *et al*. [4] reported that *Aloe vera* contained high calcium level. Abu Amra [1] stated that calcium stimulate the beta cells of langerhans that lead to an increase in insulin and liver glycogen levels. Shane and Whorter [20] stated that *Aloe vera* gel obtained from the inner portion of the leaves contain glycomannan which may account for its hypoglycemic effect. Ajabnoor [2] reported that the hypoglycemic effect of *Aloe vera* was mediated through the simulation and release of insulin from beta-cells of the pancreas. However, Ghannan *et al*. [7] stated that caution should be made when using *Aloe vera* in diabetic patients. The histological investigation in our study showed no morphological changes in the pancreas of rats treated with *Aloe vera.* This may indicate that the effect of *Aloe vera* ethanolic extract on glucose level may either be due to increased carbohydrate utilization or enhancement of glucose uptake by muscles rather than increased activity of pancreatic B. cells. The *Aloe vera* extract has an effect on the gastrointestinal tract as evident by diarrhea. This is in agreement with Ishii and Tanizawa [13] and Wendle [24] who suggested that the *Aloe* gel may be a contaminant with latex during isolation and this could lead to diarrhea. Brusick and Mengs [5] reported presence of anthraquinone glycosides in *Aloe* latex, reached the colon mostly undigested, resulted in more frequent stools with softer consistency. The increase in goblet cells in the gastrointestinal tract is in harmony with Kim and Kacew findings who stated that the plant stimulates mucus secretion. In our findings the body weight gain was reduced in rats treated with *Aloevera*, this may be attributed to the diarrhea. In contrast to Helal *et al*. [11] and Takaka *et al*. [23] who reported that treatment of *Aloe vera* on alloxan hyperglycemia improved the reduced on the body weights.

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