"PHYTOCHEMICAL INVESTIGATION OF ALOE BARBADENSIS MILLER ROOT EXTRACT"

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INTRODUCTION:

The importance of plants is known to us well. The plant kingdom is a treasure house of potential drugs and in the recent years there has been an increasing awareness about the importance of medicinal plants. Drugs from the plants are easily available, less expensive, safe, and efficient and rarely have side effects. The plants which have been selected for medicinal use over thousands of years constitute the most obvious choice of examining the current search for therapeutically effective new drugs such as anticancer drugs, antimicrobial drugs, antihepatotoxic compounds. According to World Health Organization (WHO), medicinal plants would be the best source to obtain variety of drugs. About 80% of individuals from developed countries use traditional medicines, which has compounds derived from medicinal plants.

Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas. A large number of phytochemicals belonging to several chemical classes have been shown to have inhibitory effects on all types of microorganisms in vitro. This can be derived from barks, leaves, flowers, roots, fruits, seeds. The phytochemical analysis test confirmed the presence of alkaloids, flavonoids, saponins, glycosides, fats, proteins, and phytosterols. In this method, aqueous and organic extracts are prepared from those plant samples that are the reservoir of secondary metabolites, such as leaves, stems, roots, or bark. Phytochemical screening of plant extracts revealed the presence of alkaloids, steroids, terpenoids and cardiac glycosides. Quantitative determination of total phenolics, total flavonoids, and various in vitro antioxidant activities (DPPH, ABTS and FRAP) of methanolic extract was carried out using colorimetric methods.*Aloe vera* (*Aloe barbadensis* Miller, family Xanthorrhoeaceae) is a perennial green herb with bright yellow tubular flowers that is extensively distributed in hot and dry areas of North Africa, the Middle East of Asia, the Southern Mediterranean, and the Canary Islands. *Aloe vera* derives from "Allaeh" (Arabic word that means "shining bitter substances") and "Vera" (Latin word that means "true").*Aloe vera* has been traditionally used to treat skin injuries (burns, cuts, insect bites, and eczemas) and digestive

problems because its anti-inflammatory, antimicrobial, and wound healing properties. Research on this medicinal plant has been aimed at validating traditional uses and deepening the mechanism of action, identifying the compounds responsible for these activities. The most investigated active compounds are aloe-emodin, aloin, aloesin, emodin, and acemannan. Likewise, new actions have been investigated for *Aloe vera* and its active compounds. *Aloe vera* contains more than 75 different compounds, including vitamins (vitamin A, C, E, and B12), enzymes (i.e., amylase, catalase, and peroxidase), minerals (i.e., zinc, copper, selenium, and calcium), sugars (monosaccharides such as mannose-6-phosphate and polysaccharides such as glucomannans), anthraquinones (aloin and emodin), fatty acids (i.e., lupeol and campesterol), hormones (auxins and gibberellins), and others (i.e., salicylic acid, lignin, and saponins).

ALOEVERA

Aloe vera is a succulent plant species of the genus *Aloe*. It is widely distributed, and is considered an invasive species in many world regions. An evergreen perennial, it originates from the Arabian Peninsula, but grows wild in tropical, semi-tropical, and arid climates around the world.[4] It is cultivated for commercial products, mainly as a topical treatment used over centuries. The species is attractive for decorative purposes, and succeeds indoors as a potted plant. It is used in many consumer products, including beverages, skin lotion, cosmetics, ointments or in the form of gel for minor burns and sunburns. There is little clinical evidence for the effectiveness or safety of *Aloe vera* extract as a cosmetic or topical drug, and oral ingestion has risk of toxicity.

ETYMOLOGY

The genus name *Aloe* is derived from the Arabic word *alloeh*, meaning "bitter and shiny substance" or from Hebrew. The specific epithet *vera* comes from *verus* meaning "true" in Latin.

Common names
Common names use *aloe* with a region of its distribution, such as *Chinese aloe*, *Cape aloe* or *Barbados* aloe.

Taxonomy
The species has several synonyms: *Aloe barbadensis* Mill., *Aloe indica* Royle, *Aloe perfoliata* var. and *Aloe vulgaris* Lam. Some literature identifies the white-spotted form of *Aloe vera* as *Aloe vera* var. *chinensis*; and the spotted form of *Aloe vera* may be conspecific with *A. Massawa*. The species was first described by Carl Linnaeus in 1753 as *Aloe perfoliata* var. *vera*, and was described again in 1768 by Nicolaas Laurens Burman as *Aloe vera* in *Flora Indica* on 6 April and by Philip Miller as *Aloe barbadensis* some ten days after Burman in the *Gardener's Dictionary*.[20]
Techniques based on DNA comparison suggest *Aloe vera* is relatively closely related to *Aloe perryi*, a species endemic to Yemen. Similar techniques, using chloroplast DNA sequence comparison and ISSR profiling have also suggested it is closely related to *Aloe forbesii*, *Aloe inermis*, *Aloe scobinifolia*, *Aloe sinkatana*, and *Aloe striata*. With the exception of the South African species *A. striata*, these *Aloe* species are native to Socotra (Yemen), Somalia, and Sudan. The lack of obvious natural populations of the species has led some authors to suggest *Aloe vera* may be of hybrid origin.

PLANT PROFILE
Botanical name: *Aloe barbadensis miller*

Taxonomical classification:

- Kingdom: Plantae
- Subkingdom: Viridiplantae
- Division: Trcheophyta
- Class: Magnoliopsida
- Order: Asparagales
- Family: Xanthorrhoeaceae
- Genus: Aloe
- Species: vera

PLANT DESCRIPTION:

The botanical name of Aloe vera is Aloe barbadensis miller. It belongs to Asphodelaceae (Liliaceae) family, and is a shrubby or arborescent, perennial, xerophytic, succulent, pea-green color plant. The aloe plant has long (up to 20 inches long and 5 inches wide), triangular, fleshy leaves that have spikes along the edges. The fresh parenchymal gel from the center of the leaf is clear; this part is sometimes dried to form aloe vera concentrate or diluted with water to create aloe juice products. The sticky latex liquid is derived from the yellowish green pericyclic tubules that line the leaf (rind); this is the part that yields laxative anthraquinones. The flowers (not used medicinally) are yellow (Surjushe *et al.,* 2008).

Table:-Summary of the chemicals composition of *A. vera*

Class	**Compound**	**Properties**
Anthraquinones/ anthrones	Aloe-emodin, aloetic-acid, anthranol, barbaloin, isobarbaloin , emodin, ester of cinnamic acid.	Aloin and emodin acts as analgesics, antibacterials and antivirals.
Carbohydrates	Pure mannan, acetylated mannan, acetylated glucomannan, glucogalactomannan, galactan, galactogalacturan, arabinogalactan, galactoglucoarabinomannan, pectic substance, xylan, cellulose	A glycoprotein with antiallergic properties, called alprogen and novel anti-inflammatory compound.
Chromones	8-C-glusoly-(2'-O-cinnamoly) -7-O-methlyaloediol A, 8-C-glucosyl-(S)-aloesol, 8-C-glucosyl-7-O-methylaloediol A, 8-C-glucosyl-7-0-methylaloediol, 8-C-glucosyl-noreugenin, isoaloeresin D, isorabaichromone, neoalosin A	The novel anti-inflammatory commands .
Enzymes	Alkaline phosphatese, amylase,bradykinase, carboxypeptidase, catalase,cyclooxidase, cyclooxygenase,lipase, oxidase, phosphoenolpyruvate, carboxylase,	Bradykinase helps to reduce excessive inflammation when applied to the skin topically, while others help in the breakdown of sugars and fats.

	superoxide dismutase	
Inoraganic compounds	Calsium,chlorine, chromium, copper,iron,magnesium, manganese,potassium,phosphorous, sodium,Zinc	They are essential for the proper functioning of various enzymes systems in different metabolic pathways and few are antioxidants
Miscellaneous including organic comounds and lipids	Arachidonic acid, Y-linolenic acid, steroids(campestrol, cholesterol, Bsitosterol), triglycerides, triterpenoid, gibberillin, lignins,potassium sorbate,salicylic acid, uric acid	
Proteins	Lectins, lectin-like substance	It also contains salicylic acid that possesses anti-inflammatory and antibacterial properties. Lignin, an inert substance, when included in topical preparations, enhances penetrative effect of the other ingredients into skin. Saponins that are the soapy substances from about 3% of the gel and have cleansing and antiseptic properties.
Saccharides	Mannose, glucose, L-rhamnose, aldopentose	
Vitamins	Vitamin A, B12,C, E,choline and folic acid	Vitamin A, C and E are antioxidants and antioxidant neutralizes free radicals
Hormones	Auxins and gibberellins	That helps in wound healing and have anti inflammatiory action.

Pharmacological activity

- **Healing properties:** Various researchers reported that the effective components for wound healing may be tannic acid and a type of polysaccharide. Other researcher have also reported that glucomannan, a mannose-rich polysaccharide and gibberellin a growth hormone interacts with growth factor receptors on the fibroblast therby stimulating its activity and proliferation which in turn significantly increase collagen synthesis after topical and oral Alov vera. Aloe gel not only increased collagen content of the wound but also changed collagen composition and increased the degree of collagen cross linking. Due to this, it accelerated wound contrcation and incresed the breaking strength of resulting scar tissue **(Heggers et al., 1996).** An increased synthesis of hyaluronic acid and dermatan sulfate in the granulation tissue of a healing wound following oral or topical teratment has been reported.
- **Immune modulation:** Immunostimulant and antiinflammatory (gel) ,In a case studies of 14 HIV-1+ patients who were prescribed 800 mg/day of acemannan, there was asignificant increase in the number of circulating monocyte and macrophages which mirrored clinical improvements. In a pilot study in HIV-infected persons acemannan increased the number of white blood cells and improved symptom. Aloe extracts also increased phagocytosis in asthmatic adults **(Visuthikosol et al., 1995)**.
- **Antimicrobial:** Aloe vera contains 6 antiseptic agents: Lupeol, salicyclic acid, urea nitrogen, cinnamonic acid, phenols and sulfur. They all have inhibitory action on fungi, bacteria and viruses. Acemannan acts alone and synergistically with azidothymidine (AZT)and acyclovir to block reproduction of Herpes and the AIDS virus, AntifungalAloe extract treatment of guinea pig feet that had been infected with Trichophyton mentagrophytes resulted in a 70% growth inhibition compared with untreated animals. In recent, atudies, a polysaccharide fraction has shown to inhibit the binding of benzopyrene to primary rat hepatocytes, therby preventing the formation of potentially cancer-initiating benzopyrene-DNAadducts. An induction of glutathione Stransferase and an inhibition of the tumorproting effects of phorbol myristic acetae has also been reported which suggest a possible benefit of using aloe gel in cancer chemoprevention **(Surjushe et al., 2008).**

- **Skin and mucus membranes:** In humans, aloe has been reported to accelerate healing from deep scrapes, frostbite, flash burns of the conjunctiva, and even canker sores. Only one study has had an opposite effect; that is, aloe-treated surgical wounds healing by secondary intention took longer to heal than comparison wound. Despite the conflicting research, some dentists and otolaryngologists use aloe gel to promote healing in injured tissues in the mouth, nose, sinuses and ea. Aloe gel has most often been used as a topical treatment for burn wounds. In a study of 27 adults with partial thickness burns, those treated with aloe healed an average of six days faster than those treated with Vaseline gauze **(Visuthikosol et al., 1995).**
- **Effects on skin exposure to UV and gamma radiation:** Aloe vera gel has been reported to have a protective effect against damage to skin. Exact role is not known , but following the administration of alove vera gel, an antioxidant protein, metallothionein, is generated in the skin which scavenges hydroxyl radicals and prevents suppression of superoxide dismutase and glutathione peroxidase in the skin. Its reduces the production and release of skin keratinocyte-derived immunosuppresive cytokines such as interlukin -10 and hence prevents UV-induced suppression of delayed type hypersensitivity **(Byeon et al., 1998).**
- **Anti-diabetic effects:** Several pre-clinical (in animal) and clinical (in human) trials showed a blood glucose lowering effects for Aloe vera gel preparations in different forms (e.g. juice or as constituents in bread etc.). In a study on streptozotocin- induced diabetic rats oral administration of Aloe vera gel (alcohol insoluble residue extract) significantly reduced the fasting blood glucose, herpatic transminases, plasma and tissue cholestrol, triglicerides, free fatty acids and phospholipids and in addition also significantly increased plasma insulin levels. The decresaed plasma levels of high density lipoprotein cholesterol and increased levels of low density lipoprotin cholestrol in the sterptozotocin-induces rats were restored to normal after teratment with gel extract **(Rajasekaran et al., 2006).**

- **Plant collection**

The medicinal plant *Aloe barbadensis* root(100 gm) were collected locally from Bhopal,M.P. After cleaning, plant parts were dried under shade at room temperature for 3 days and

Then in oven at 45°C till complete dryness. Dried plant parts were stored in air tight glass containers in dry and cool place to avoid contamination and deterioration.

Authentication of selected traditional plant - The roots of medicinal plant *Aloe barbadensis* was authenticated by a plant taxonomist in order to confirm its identity and purity.

- **Extraction Method**

Generally three methods are employed in the extraction of plant materials. (1) Maceration, (2) Percolation (3) Successive Soxhlet Extraction. Maceration and percolation may be employed in extraction of thermo labile constituents. Soxhlet extraction is rapid and continuous and may be employed in extraction, which cannot be done by either maceration or percolation method. Due to the various advantages offered by soxhlet extraction, this method was selected for present study **(Redfern *et al.*, 2014).**

Crude drug roots

⇩

Defatted with petroleum ether

⇩

Extract was collected and dried

⇩

Extraction with Ethanol

⇩

Extract was collected and dried

⇩

Calculated % yield

- **Solvent extraction of plant extract**

❖ **Preparation of the extract-**

Coarsely powdered plant parts (root) of *Aloe barbadensis* (100 gm) was then extracted by successive extraction using different organic solvents, defatted with petroleum ether (40-60^{0}C) and successively extracted with ethanol for 36 hrs using soxhlet apparatus. To ensure complete extraction each extract was evaporated to dryness under reduced pressure by rotary evaporator

and the resulted dried residue was stored in air-tight container for further use **(Redfern *et al.*, 2014).**.

Formula;

$$\% \, yield = \frac{\text{Actual yield}}{\text{Theoretical yield}} \text{x} 100$$

Figure : Soxhlet apparatus

- **Solubility determination of extract**

Solubility of the extract was determined in different solvents. The results are summarized in the result and discussion chapter.

- **Phytochemical.investigation**Experiment was performed to identify presence or absence of different phytoconstituents by detailed qualitative phytochemical analysis. The colour intensity or the precipitate formation was used as medical responses to tests. Following standard procedures were used **(Kokate *et al*., 2006).**

  - **Test for Carbohydrates**

- **Molisch'sTest:** The aqueous solution of the extract to 1 ml were mixed with few drops of Molish reagent (naphthol) and conc. H_2SO_4 (sulphuric acid) was added dropwise along the wall of the test tube. When two liquid mixes up, formation of purple colour ring at the junction occurs. It indicates the presence of carbohydrates.

- **Fehling's Test:** Equal amount of Fehling A and Fehling B solution were mixed (1ml each) and 2ml of aqueous solution of extract was added. Boil it for 5-10 minutes on water bath. Formation of reddish brown coloured precipitate due to cuprous oxide formation showsthe presence of reducing sugar.
- **Benedict's test:** In a test tube equal amount of Benedict's reagent and extract were mixed and heated for 5-10 minutes in the water bath. Depending on the amount of reducing sugar present in the test solution, appears green, yellow or red which shows the presences of reducing sugar.
- **Barfoed's Test:** In the aqueous solution of extract, 1 ml of Benedict solution was added and heated for boiling. In the presence of monosaccharides red colour indication was seen due to formation of cupric oxide.

▪ **Tests for Alkaloids**

- **Dragendorff'sTest:** 1 ml of extract was taken. Alcohol was mixed and was shaken well with little drops of acetic acid and Dragendroff's reagent. The presence of alkaloids indicates by the presence of an orange red precipitate.
- **Wagner's Test:**In acetic acid 1ml of extract was dissolved. Few drops of Wagner's reagent were added. The presence of alkaloids indicated the reddish-brown precipitate.
- **Mayer's Test:** 1 ml of extract was dissolved in acetic acid with a few drops of Mayer's reagent added to it. The presence of alkaloids was indicated by the formation of a dull white precipitate.
- **Hager's Test:** 1-2 ml of extract was dissolved in acetic acid. To it 3 ml of Hager's reagent was added; the formation of yellow precipitate is indicatedby the presence of alkaloids.**Test for Saponins**
- **Froth Test:** 1ml of extract was added in distilled water and shaken well. The presence of saponin was indicated by stable froth formation.

▪ **Test for Triterpenoids and Steroids**

- **Libermann-Burchard Test:** The extract was dissolved in chloroform. To it 1 ml of acetic acid and 1 ml of acetic anhydride were added, then heated on a water bath and subsequently cooled. Then added few drops of concentrated sulphuric acid along the sides of the test tube. Presence of steroids indicated by the appearance of bluish green colour.

- **Salkowski Test:** The extract was dissolved in chloroform and equal volume of concentrated sulphuric acid was added. The presence of steroids was indicated by the formation of bluish red to cherry red colour in chloroform layer and green fluorescence in the acid layer.

▪ **Test for Tannin and Phenolic Compounds**

- **Ferric Chloride Test:**Few amount of extract was dissolved in the distilled water. Add to it a few drops of dilute solution of ferric chloride. Formation of dark blue colour indicated the presence of tannins.
- **Gelatin Test:**Few amount of extract was dissolved in the distilled water.2ml of 1% gelatin solution containing 10% sodium chloride were added. Presence of phenolic compounds indicated by the development of white precipitate.
- **Lead Acetate Test:** Few amount of extract was dissolved in a test tube along with distilled water, to it few drops of lead acetate solution were added. Formation of white precipitate indicates presence of phenolic compounds.

▪ **Test for Flavonoids**

- **Shinoda's Test:** A few magnesium turnings and little drops of concentrated hydrochloric acid to 1 ml of extract in alcohol were added. It was heated on a water bath.when the formation of red to pink colour occurred , indicated the presence of flavonoids.

▪ **Test for Glycosides**

- **Borntragers Test:** Dilute sulphuric acid was added to 3 ml of test solution dilute sulfuric acid was added. It was boiled for 5 minutes and then filtrate was obtained. To the cold filtrate, equal amount of benzene or chloroform was added to the cold filtrate and shaken well. Separation of organic solvent layer was obtained and then ammonia was added to it. Presence of anthraquinone glycosides was indicating, the formation of pink to red colour in ammonical layer.
- **Keller Killiani Test:** 2 ml of test solution added in a test tube, 3 ml of glacial acetic acid and 1 drop of 5%ferric chloride. Add carefully 0.5 ml of concentrated sulphuric acid. The presences of Cardiac glycosides were indicated by the formation of blue colour in the acetic acid layer.

- **Test for fats and oils**

- **Solubility test**

- To 2-3 ml of alcoholic solution of extract, addfew ml. of chloroform and solubility was observed.
- To 2-3 ml of alcoholic solution of extract. Add few ml. of 90% ethanol and solubility was observed.
- **Quantitative Phytochemical Estimation**

- **TPC**

Folin-Ciocalteu method was used for the quantitative estimation of Total Phenolic Content (TPC). As a reference standard Gallic acid was used in different concentrations (10-100 g/ml) in ethanol. Test sample of every extract was prepared in ethanol (100 g/ml) or solvent of about similar polarity.0.5 ml of different concentrations of Gallic acid/test sample was added to 2 ml of Folin-Ciocalteu reagent followed by 4 ml sodium carbonate solution. Incubate the reaction mixture at room temperature. Allow to hold for 30 minutes with intermittent shaking. By using Ultra-Violet (UV) Spectrophotometer at wavelength 765 nm taking ethanol as blank the absorbance was recorded. To find out suitable line of regression Standard curve of different concentrations of Gallic acid was prepared. The TPC was obtained when the calibration curve of Gallic acid and expressed as mg Gallic acid equivalent (GAE)/g extract or g GAE/mg extract **(Ahmed et al., 2015).**

- **TFC**

By using colorimetric assay, quantitative estimation of Total Flavonoid Content (TFC) was determined. The reference standard used for the estimation and plotting of calibration curve is Rutin. Different concentrations of rutin (10-100g/ml) were prepared in ethanol. Test sample of every extract was prepared in ethanol (100 g/ml) or solvent of about similar polarity.0.5 ml of the diluted sample solution was dissolved with 2 ml of distilled water followed by 0.15 ml $NaNO_2$ solution. After 6 minutes, 0.15 ml $AlCl_3$ solution was added and allowed to hold for 6 minutes. After that, 2 ml NaOH solution was added to the reaction mixture and allowed to hold for 15 minutes. UV Spectrophotometer was used for recording the absorbance at 510 nm. Absorbance of the test samples was measured by line of regression of standard curve of rutin. TFC is expressed as Rutin equivalent (RE), mg RE/g extract or g RE/mg extract **(Ahmed et al., 2015)**.

➢ ***In-vitro*** **Antioxidant Assay**

- **DPPH**

In accordance with above reaction, antioxidant reacts with DPPH that is stable free radical and is reduced to DPPHH. Meanwhile the absorbance's decreased from the DPPH radical to the DPPH-H form. The degree of discoloration specifies the scavenging capability of the hydrogen-donating power of the antioxidant compounds or extracts. To assess the scavenging competence on DPPH, every extract (5-20mg/ml) in water and ethanol was mixed with 1 ml of ethanolic solution containing DPPH radicals (0.2mM).Vigorously, the mixture was shaken and kept for 30 mins in the dark room and absorbance was taken at 517nm against blank **(Nicklisch *et al.*, 2015).**

- **RESULT AND DISCUSSION**

Percentage Yield

In phytochemical extraction the percentage yield is very crucial in order to determine the standard efficiency of extraction for a specific plant, various sections of the same plant or different solvents used. The yield of extracts received from the *Aloe barbadensis* is shown in Table

Table – Percentage Yield of crude extracts of *Aloe barbadensis* extract

Extract	**Yield**
Pet ether	1.072%
Ethanol	4.768%

Solubility determination of extract

Table : Solubility

S.No.	**Solvent**	**Solubility of Extract**
1.	Water	Soluble
2.	Ethanol	Soluble
3.	Methanol	Soluble
4.	Pet ether	Insoluble
5.	DMSO	Soluble

- **Preliminary Phytochemical study**

Table : Phytochemical testing of extract

S. No.	Experiment	Presence or absence of phytochemical test	
		Pet. Ether extract	**Ethanolic extract**
1.	**Alkaloids**		
1.1	Dragendroff's test	Present	Present
1.2	Mayer's reagent test	Present	Present
1.3	Wagner's reagent test	Present	Present
1.3	Hager's reagent test	Present	Present
2.	**Glycoside**		
2.1	Borntrager test	Absent	Present
2.2	Legal's test	Absent	Present
2.3	Killer-Killiani test	Absent	Present
3.	**Carbohydrates**		
3.1	Molish's test	Absent	Present
3.2	Fehling's test	Absent	Present
3.3	Benedict's test	Absent	Present
3.4	Barfoed's test	Absent	Present
4.	**Proteins and Amino Acids**		
4.1	Biuret test	Present	Present
5.	**Flavonoids**		
5.1	Alkaline reagent test	Present	Present
5.2	Lead Acetate test	Present	Present
6.	**Tannin and Phenolic Compounds**		
6.1	Ferric Chloride test	Absent	Present
7.	**Saponin**		
7.1	Foam test	Present	Present
8.	**Test for Triterpenoids and Steroids**		
8.1	Salkowski's test	Absent	Present
8.2	Libbermann-Burchard's test	Absent	Present

The results of the phytochemical studies revealed that the lead acetate test gave a pink or red coloration of the solution that indicated the presence of flavonoids. There is dark blue or greenish gray coloration of the solution indicated the presence of tannins in the drug. Characteristic observation for steroids was found and the dark pink or red colour of the solution indicated the presence of terpenoids. Yellow or reddish brown precipitation indicated the presence of alkaloids. The solution pink to red indicates the presence of glycosides. Layer of foam formation indicates the absence of saponins. If the response is average then it indicates the presence in moderate quantity. If there is no response then negative.

Quantitative Analysis

Preliminary phytochemical testing of crude extracts confirmed the presence of phenolics and flavonoids in plant material. To estimate their amount total phenolic (TPC) and total flavonoid content (TFC) assays were performed.

- **Total Phenolic content (TPC) estimation**

Table Standard table for Gallic acid

S. No.	Concentration (µg/ml)	Absorbance
1.	20	0.146±0.002
2.	40	0.168±0.002
3.	60	0.189±0.2211
4.	80	0.212±0.001
5.	100	0.254±0.0015

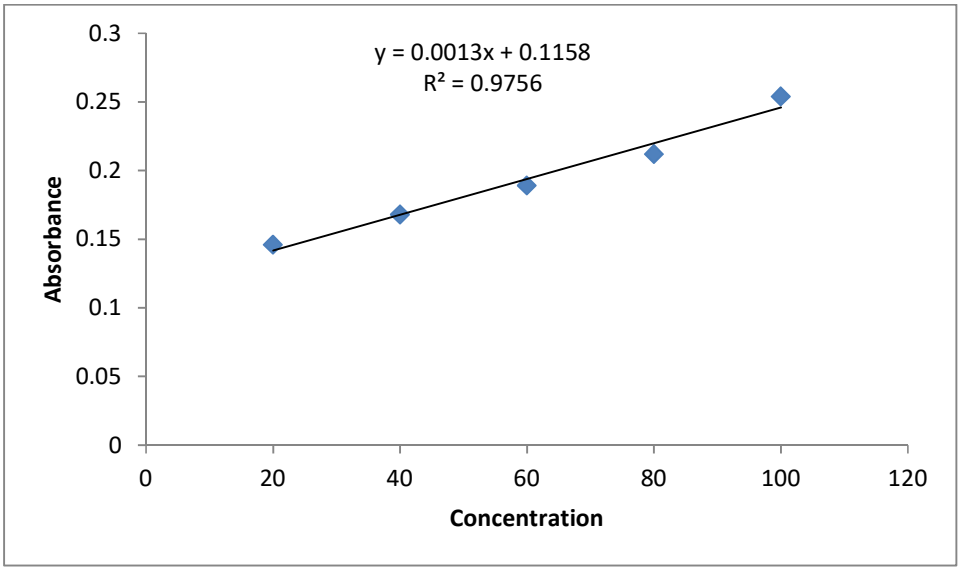

Figure : Graph represent standard curve of Gallic acid

- **Total Phenolic Content in extract**

Table : Total Phenolic Content

S.No	Absorbance	TPC in mg/gm equivalent of Gallic Acid
1	0.215	97 mg/gm
2	0.210	
3	0.213	

Table -Total Phenolic Content of extract *Aloe barbadensis*

Extracts	Total Phenolic content (mg/gm equivalent of Gallic acid)
Ethanol	97

- **Total Flavonoids content (TFC) estimation**

Table: Standard table for Rutin

S. No.	Concentration (µg/ml)	Absorbance
1.	20	0.139±0.002
2.	40	0.165±0.004
3.	60	0.199±0.003
4.	80	0.238±0.004
5.	100	0.285±0.003

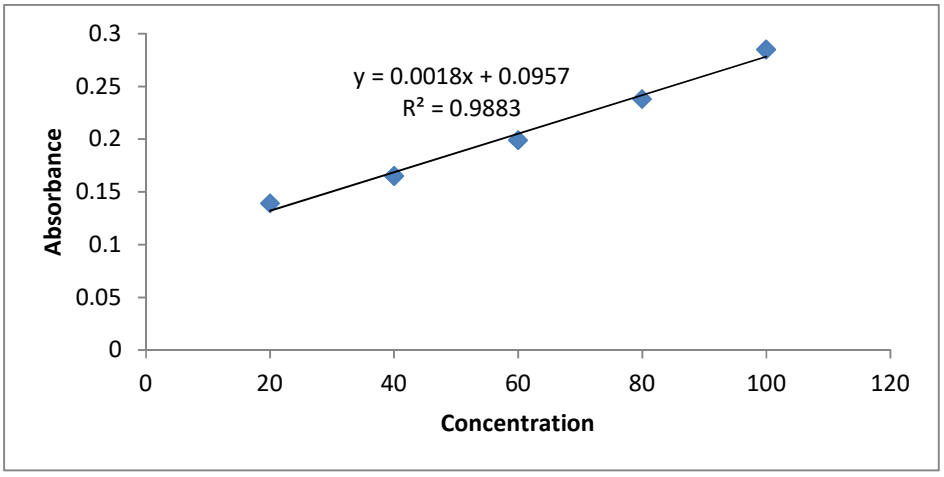

Figure : Graph represent standard curve of Rutin

Total Flavonoid Content in extract

Table: Total Flavonoid Content

S.No	**Absorbance**	**TFC in (mg/gm) equivalent of Rutin**
1	0.204	89 mg/gm
2	0.208	
3	0.201	

Table: **Total Flavonoid Content of extract *Aloe barbadensis***

Extracts	Total Flavonoid content (mg/gm equivalent of Gallic acid)
Ethanol	89.00

- ***In vitro* Antioxidant Assays**

In the present investigation, the in vitro anti-oxidant activity of extracts of *Aloe barbadensis* was evaluated by DPPH radical scavenging activity. The results are summarized in Tables

DPPH 1, 1- diphenyl-2-picryl hydrazyl Assay

Table - DPPH radical scavenging activity of Std. Ascorbic acid

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.467	51.04
40	0.412	56.81
60	0.328	65.61
80	0.274	71.27
100	0.132	86.16
Control	0.954	
IC50 22.81		

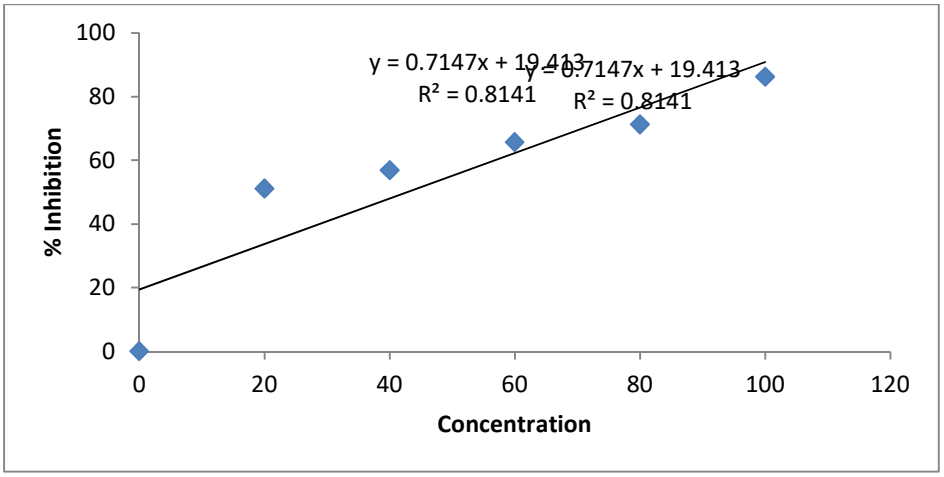

Figure : - DPPH radical scavenging activity of Std. Ascorbic acid

Table- DPPH radical scavenging activity of aqueous extract of *Aloe barbadensis*

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.486	46.29
40	0.458	49.39
60	0.425	53.03
80	0.365	59.66
100	0.325	64.08
Control	0.905	
IC50		**40.48**

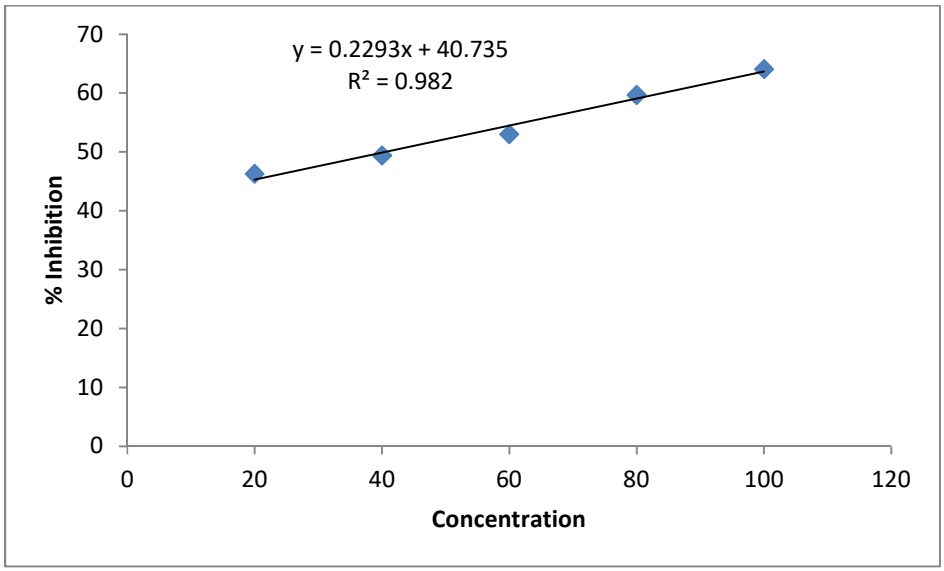

Figure: Graph represents the Percentage Inhibition *Vs* Concentration of extract of *Aloe barbadensis*

DISCUSSION

Aloe barbadensis Miller, usually referred to Aloe vera, has existed around since the dawn of time. It is a North African plant that has a variety of medicinal properties. This work was a contribution to studying the physio-chemical characteristics, the photochemical content, and the biological properties of Aloe Barbadensis Miller, a plant collected from a local garden located in the small town of Berriane (region of Ghardaïa, Algeria). The antioxidant activity was determined utilizing the DPPH method, and the solid medium diffusion method was employed to assess the antimicrobial potential against human pathogenic bacteria a gram positive (S. aureus) bacterial strain, two-gram negative bacteria (E. coli and P. aeruginosa), and one yeast (Candida albicans) and fungi. The phytochemical analysis demonstrated the existence of different phytochemical substances such as phenol, flavonoid, tannin, alkaloid, saponin, and terpenoid in aloe vera root extract. Furthermore, the antibacterial test of Aloe vera root extracts revealed that the gram-negative bacteria E. coli sensibility compared with the two other bacteria (S. aureus and P. aeruginosa), which showed resistance, and that this extract showed antifungal efficiency against the fungi. In addition, the aloe vera extract was given an antioxidant effect according to the DPPH free radical method. The findings of this study allowed us to confirm that Aloe vera's biological activity is principally due to the existence of various phytochemical compounds with biological activities, like phenolic compounds. According to these obtained results, A. barbadensis root extract can be utilized to treat certain diseases by providing a natural biological active component.

CONCLUSION

The plant sample kingdom represents a rich storehouse of organic compounds, many of which have been used for medicinal purposes and could serve as lead for the development of novel agents having good efficacy in various pathological disorders.

The fresh root of Aloe barbadensis was extracted. These bio-active components like carbohydrates, glycosides, flavonoids, alkaloids, triterpenoids and phenolic components are present in the ethanol extract Aloe barbadensis. They exhibit potential effect on anti-ulcer activity.

The results of the phytochemical studies revealed that the lead acetate test gave a pink or red coloration of the solution that indicated the presence of flavonoids. There is dark blue or greenish gray coloration of the solution indicated the presence of tannins in the drug. Characteristic observation for steroids was found and the dark pink or red colour of the solution indicated the presence of terpenoids. Yellow or reddish-brown precipitation indicated the presence of alkaloids. The solution pink to red indicates the presence of glycosides. Layer of foam formation indicates the absence of saponins. If the response is average then it indicates the presence in moderate quantity. If there is no response then negative.

Preliminary phytochemical testing of crude extracts confirm the presence of phenolic and flavonoids in plant materials. Total phenolic and flavonoids content was found to 86% and 97%. In this study the invitro antioxidant activity of extracts of *aloe barbadensis* was evaluated by DPPH radical scavenging activity and extract of aloe barbadensis give 40.48% antioxidant properties.

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