Comparative study of different extracts and TLC analysisof important medicinal plant *Aloevera*

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Abstract:
All medicinal plants are very important for humans. Mostly significant phytochemicalsare present in the plant. Several medicinal plants have many uses and more significant phytochemicals have been discovered.The market for medicinal plants in India stood at Rs. 34.4 billionUS$ in 2022 and is expected to increase at a CAGR of 13% to Rs. 61.5 billion US$ by 2027.In this investigation, a comparative studywas performed in three different solutionsethanol extract, water extractand methanol extract of *Aloe vera*whole plant analysis. Ethanol extract solution is better than water and methanol solution. Alkaloids, Glycosides, Carbohydrates and sugar, Flavonoids are highly present and saponins, tannins, phenolics, steroids and amino acids are present in ethanol solution. Glycosides and amino acids are absent in the methanol solution. Important phytochemical was separated to extract by thin-layer chromatography method. When standard rutin was tested with *Aloe vera*extract, it was found that the mobile phase traveled up to 6 cm while the rutin traveled 3.4 cm above baseline. With an Rf value of 0.56, the rutin spot was seen under long UV, short UV, and normal light. The Rf values of the sample extract spots were measured in long UV 0.56, short UV 0.56 and normal light 0.56 and 0.65.The qualitative analysis of *Aloe vera*plant extracts to screen and estimate various phytochemicals provides useful information for the pharmaceutical industry to manufacture drugs and underlines the importance of using these plant extracts in high-intensity research as they are vital to healthcare.

Abbreviation: Phytochemical, TLC,Alkaloids, Glycosides, Carbohydrate sugar, Flavonoids, saponins, tannins, phenolic, steroids and amino acid.

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1. Introduction:

Aloevera isasignificant medicinal plant with various active ingredients present in it. Over 430 species of genus *Aloe* finds all over the world and grows in hot climatic regions (Salehi B et. al. 2018). The shape of this plant is looks like a cactus. *Aloe vera*is one of the common plants which finds in India and reported various pharmacological activities including anti-inflammatory, immunomodulatory, antibacterial, antifungal, antiviral, antiproliferative, antidiabetic, laxative, wound healing, moisturizing, anti-aging and skin protection. Phytochemical screening is a method for determination of constituents present in the plant.

*Aloe vera*chemical content changes according on the environment, geography, growing conditions, plant age, and method of processing. Anthraquinones, phenolic chemicals

found in abundance in *Aloe vera* latex, have potent laxative properties i.e antibacterial, analgesic, & antiviral properties. *Aloe vera* gel appears to be a significant source of polysaccharides. Three-year-old *Aloe vera*was found to have significantly higher quantities of polysaccharides. Both mono and polysaccharides, such as the long-chain sugars comprising glucose, mannose, and glucomannans, are found in *Aloe vera*. Chemical testing has shown that the clear gel contains biological stimulators, vitamins, minerals, enzymes, proteins, and amino acids (Mabusela, W.T et al 1990).

2. Material and Method:

The present investigation was performed at the Govt. MLB Girls PG (Auto) Bhopal Madhya Pradesh India**.** This study focused on three key characteristics, namely**"Comparative study of three different extract solution ethanol, water-methanolof Important medicinal plant *Aloe vera*".**

2.1 Plant Collection:

Plant substances in the plant component (cell), a sample must be prepared before extraction. All the plant component is collected and preserved in a dried poly bag. *Aloe vera* plant parts were obtained from the MP forest area by Bhopal M.P. India in January 2023.*Aloe vera*plant parts were cleaned separately and washed separately with distilled water. After completing the cleaning and washing activity, the plant parts were collected in a separate beaker.*Aloe vera* plant parts were dried in the laboratory room. The plant parts of the *Aloe vera*are converted into powder formhomogenized instruments and stored inglass bottles until later use.

2.2 Identification of Plant:

The plant and plant part are identified by Govt. MLB Girls PG (Auto) Bhopal Madhya Pradesh India**.**

2.3 Plant Extract:

Plant extract is prepared in three different solutions.

2.4 Extract Drug value:

When the components of a drug cannot be easily estimated in any other way, extracting values of raw drugs are helpful for their evaluation. Additionally, these values reveal the makeup of a bulk drug's ingredients.

2.4.1 Preparation of EthanolExtract:

Take 5.085 gm extract sample and mix it with 90 % of 100 mL ethanol and vigorous shaking for the first six hours, the substance was left to stand for 18 hours. After that, it was quickly filtered while taking safety measures to prevent solvent loss.

2.4.2 Preparation of Water Extract:

Take 5.134 gm extract sample and mix it with 100 mL of chloroform water and vigorous shaking for the first six hours, the substance was left to stand for 18 hours. After that, it was quickly filtered while taking safety measures to prevent solvent loss.

2.4.3 Preparation of Methanol Soluble Extract:

Take 5.052 gm extract sample and mix it with 100 mL of methanol-water andvigorous shaking for the first six hours, the substance was left to stand for 18 hours. After that, it was quickly filtered while taking safety measures to prevent solvent loss.

5.: Phytochemical tests:

5.1 Test for alkaloids:

Take a small amount of *Aloe vera* extract, add it to 2 mL of 1% HCl, and heat it to check for the presence of alkaloids. Drangendroff's, Mayer's and Wagner's a were mixed with shaking and added sodium picrate solution.

Observation: Precipitation or turbidity was confirmation of the presence of alkaloids.

An orange color precipitate is shown after being treated with Drangendroff's reagent (1 ml) and a yellow color precipitate isshown after addingsodium picrate (1 ml).Wagner's reagent and sample extract (1 mL) do not produce a red-brown precipitate.

5.2 Test for saponins:

Take 2 g sample extract and 20 ml water was collected in 100 mL glass beaker and boiled.

Using membrane filters 5 ml of distilled water and 10 ml of the filtrate were quickly shaken.
Observation: The presence of saponins was revealed by the foam's appearance.

5.3 Tet for Glycosides:

(a) Legal test:

Take 01ml sample extract, pyridine solution and sodium nitroprusside solution (for used alkaline)
Observation: Pink color solution is changed to red color, that is indicated the presence of glycoside.

(b) Baliet test:

Add 1 mL of sodium picrate solution to 1 mL of the sample.
Observation: From yellow to orange.

(c) Keller-killiani test:

Take 01 gm of sample and 10 mL of 70% IPA for 02 minutes. The solution is filtered.
Take filtered 0.5ml of lead acetate solution and 05 mL of chloroform. The chloroform layer is parted by the evaporation dish and evaporation. After the cooled residue is collected and add 03 mL of glacial acid and 01-04 drops with the help of a dropper of 5% ferric chloride solution. Pour Carefully and slowly add 02 mL of concentrated H_2SO_4.
Observation: At the intersection of both liquids, a reddish-brown layer forms, and the higher film gradually turns bluish-green, deepening through time.

(d) Borntrager's test:

Take a sample and mix 0.1 to 0.4 mL dilute H_2SO_4. Test tubes are boiled and filtered in the solution with the use of chloroform. The solution is treated with 01 ml of ammonia.
Observation: The ammonia layer is showing red color.

5.4 Test for carbohydrates and sugars:

a) Fehling test:

Equal parts of two different regents the Fehling A and B were added with 2 mL of their solution sample extract solution and gently boiling.
Observation: As reducing sugar is present, brick-red precipitate forms at the test tube's bottom.

b) Benedicts Test:

Take 02mL sample and mix Benedicts Reagent. Heat for 02 minutes and cool.
Observation: A red precipitate is shown to form and sugar is present

c) The Molisch test:

Molisch's reagent of 2 mL was added to the sample extract and properly agitated. 02 ml of con. H2SO4 was added to the test tube.
Observation: As a result, a violet ring forms at the interphase, indicating the presence.

5.5 Tet for Tannins & Phenolic Compounds:

Take 01 mL sample extract in a glass baker and added a few amounts of lead acetate solution.
Results: If White precipitates appear. It is shown that tannins are present.

5.6 Test for flavonoids:

Take about 0.1 mL of ethanol extract and mix it with ammonia solution.
Results: The presence of flavonoids is shown by the appearance of fluorescence in ultraviolet and visible light.

5.7 Test for steroids:

Take a 1-gram extract sample,0.3 mL of chloroform, 03mL of acetic anhydride & 03mL of glacial acetic acid for the Libermann-Burchard test. Applied few drops of strong sulphuric acid and used in tap water to cool.
Observation: Its appearance of a bluish green tinge indicates the presence of flavonoids.

5.8 Test of Amino Acid:

The crude extract sample was added with a 0.2% solution of ninhydrin (02mL), and the mixture was then heated for a few minutes for stand.
Observation. A violet or pink color then appeared, indicating the presence of amino acids**.**

5.9 TLC analysis:

5.9.1 Plates:

They are used for thin-layer chromatography and are pre-made, chemically inert, and stable. On its surface, a thin layer of the stationary phase is applied. The uniform thickness and small particle size of the stationary phase on the plate.

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5.9.2 Chamber:

Plating is done in a chamber. It is responsible for maintaining an inside atmosphere that will promote the growth of spots. Also, it keeps the process completely dust-free and stops the solvent from evaporating.

5.9.3 Mobile phase

The mobile phase, or the portion of the reaction that moves, is made up of a solvent combination or a solvent. There shouldn't be any debris at this stage. As purity quality gets better, spots form more easily.

5.9.4 Filter paper sheet:

This must be inserted within the chamber. It is damp during the mobile phase.

5.9.5. Procedure:

The adsorption phenomenon provides the basis for thin-layer chromatography.In this type of chromatography, the mobile phase, which contains dissolved solutes, passes across the surface of the stationary phase. Each solvent extract underwent silica gel 60F254 based thin-layer chromatography (TLC), which was cut to a size of 7 x 6 cm (Merck) using regular household scissors. The plates were gently marked with a pencil. A 1-microliter sample volume was selected for the TLC application, which was spotted in glass capillaries. Along five tracks, the capillaries were placed 1 cm apart. The twin trough chamber used a toluene, ethyl acetate, and formic acid (5:4:1) solvent system along with other solvent systems (Patel et al., 2017). For development, mobile phase was pre-saturated for 20 minutes. Retention factor (Rf), which was computed for several samples, is a measure of how the active chemical moves. The generated thin layer chromatographic plates were examined in a TLC cabinet in the United States under normal light, short UV light (254 nm), and long UV light (365 nm) (Electronic India).

Detection and Calculation of R_f Value:

Rf= Distance traveled by solute/Distance traveled by the solvent

3.Result and discussion:

In this investigation, three different solution was used for *Aloe vera* hole plant analysis.

The ethanol plant extract of *Aloe vera* used in this study's phytochemical test revealed the presence of phytochemical (Refer to table No. 01 and Graph No.01). Vitamins, minerals, enzymes, carbohydrates, phenolic compounds, lignin, saponins, sterol, and amino acids are just a few of the nutrients found in the A. vera plant. Alkaloids, Glycosides, Carbohydrates and sugar, Flavonoids are highly present and saponins, tannins, phenolic, steroids and amino acid are present in phytochemical test. A. vera contains vitamins A, B1, B2, B6, B12, C, and E that the body is unable to produce on its own. Vitamins B complex and C are anticipated to be crucial in reducing stress and inflammation. This outcome is similar to the phenols, saponins, phytosterols, terpenoids, alkaloids, flavonoids, carbohydrates, proteins, and ethanolic extract of dried leaves that Karpagam et al. reported on in 2019.

In water extract of *Aloe vera* plant Alkaloids, Glycosides, Carbohydrates and sugar, Flavonoids and saponins, tannins, phenolic, steroids and amino acid are present show in table no. 01 and graph no.01and Glycosides. This result is similar to Dharajiya et al. 2017, studied on the fresh leaves of *Aloe vera* prepared by cold maceration. Alkaloids, saponins, sterols, flavonoids, and phenols are present in methanol, hexane, ethyl acetate, and aqueous solvents, respectively. Phenols, alkaloids, saponins, sterols, and flavonoids are present in methanol.

Methanol Extract of *Aloe vera* plant Alkaloids, Carbohydrates and sugar, Flavonoids and saponins, tannins, phenolic and steroids are present and Glycosides and amino acid are negative show in table no. 01 and graph no.01.

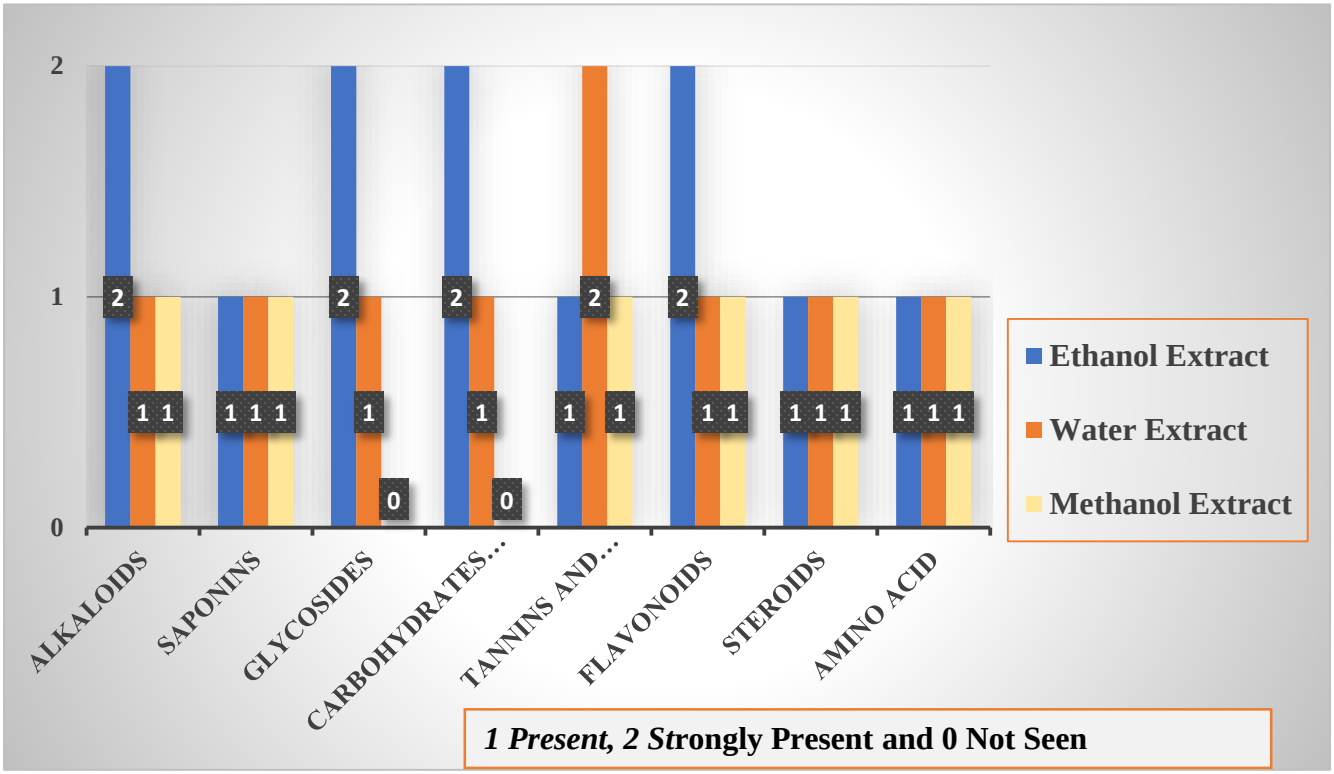
One of the best chromatographic methods for separating chemical mixtures is thin-layer layer chromatography. On the silica plate, chromatogram bands form, which are measured and quantified using the retention factor formula. Toluene, ethyl acetate, and formic acid

(5:4:1) were used as the mobile phase for extract separation. When standard rutin was tested with *Aloe vera* extract, it was found that the mobile phase traveled up to 6 cm while the rutin traveled 3.4 cm above baseline. With an Rf value of 0.56, the rutin spot was seen under long UV, short UV, and normal light. The Rf values of the sample extract spots were measured in long UV 0.56, short UV 0.56 and normal light 0.56 and 0.65.

Table No.01 Phytochemical analysis results of differentextracts.

Test Name	Results in extractscrude indifferent soluble		
	Ethanol Extract	Water Extract	Methanol Extract
Alkaloids	2	1	1
Saponins	1	1	1
Glycosides	2	1	0
Carbohydrates and sugars	2	1	0
Tannins and phenolic compounds	1	2	1
Flavonoids	2	1	1
Steroids	1	1	1
Amino Acid	1	1	1

1 Present, 2 Strongly Present and 0 Not Seen

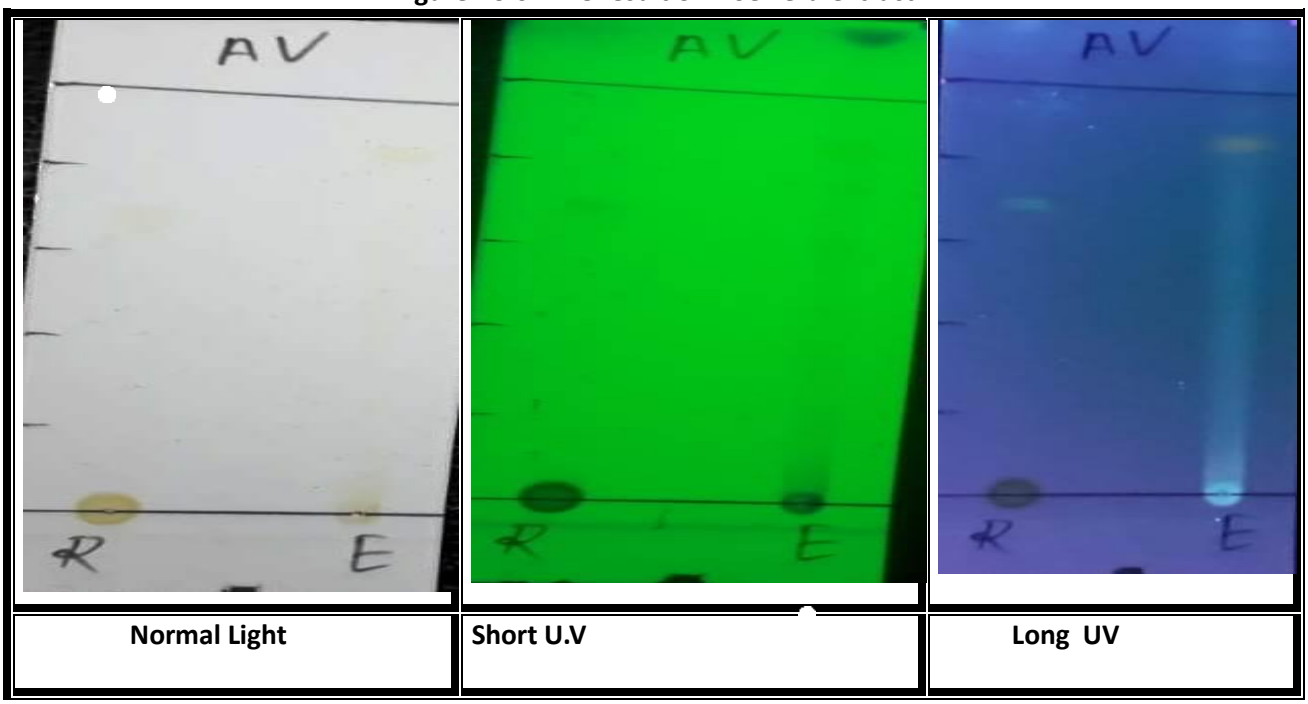


Graph No. 01 Phytochemical analysis results of different extract.

Table 02: Identification of phytoconstituents (Rutin) by TLC of ethanol extract sample of *Aloe vera:*

S. No.	**Mobile phase** Toluene: Ethyl acetate: Formic acid (5:4:0.5, v/v/v)	Spot distance	***Rf* value**
1.	**(Rutin)** Dis. travel by mobile phase= 6cm No. of spot at long UV=1 No. of spot at short UV = 1 No. of spot at normal light=1	 3.4 3.4 3.4	 Long-0.56 Short-0.56 Normal- 056
2.	**(*Aloe vera*)** Dis. travel by mobile phase= 6cm No. of spot at long UV = 1 No. of spot at short UV = 1 No. of spot at normal light= 2	 3.4 3.4 3.4, 3.9	 Long-0.56 Short-0.56 Normal- 0.56, 0.65

Figure no.01 TLC result of *Aloe vera* extract



Normal Light	**Short U.V**	**Long UV**

Conclusion:

In a recent study, a successful fast methodology for the synthesis of phytochemicals from whole-plant extracts of *Aloe vera* was developed.The current research focuses on the diverse phytochemical composition of the herb, whole plant component of *Aloe vera*, which makes it a popular choice for folk medicine and also requires consideration as a source for alternative medicine.

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