Aloin Content Determination In*Aloe Vera* With Its Physicochemical Properties Along With Its Physicochemical And Phytochemical Evaluation In *Piper nigrum*

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Abstract-India has a vast diversity of herbs. Around 3,000 years before, these herbs were acknowledged and use as medicinal plants and helpful for treating people. New scientific research has established that some plants and herbs have presence of many active compounds and possess specific pharmacological properties. *Aloe vera* (ghritkumari) and *Piper nigrum* (black pepper or maricha) are the oldest plants which are used as a medicinal herb as well as in household. *Aloe vera* or Ghritkumari species are currently used by cosmetic and pharmaceutical industries. *Aloe vera* has antibacterial, antiviral, and antiseptic properties which help to treat skin-related problems. *Piper nigrum* is a species that is used in various medications since very old times. They have also been used as domestic medicine against various infections, a quality attributable to the existence of certain chemical moieties in them. Physicochemical analysis and bioactive compound evaluation of the medicinal plants in essence of*Aloe vera*and*Piper nigrum* was undertaken in this study.In physicochemical analysis of *aloe vera*, many parameters were tested against pulp extract and leave extract (i.e., pulp and leave); parameters are color, odour, taste, total solid, total ash value, total dissolved solid, specific gravity, P_H, refractive index. For aloin content evaluation, High-Performance Layer Chromatography techniques were used in the *Aloe vera* plant. In physicochemical analysis, many standardization parameters like alcohol soluble extractive values, water-soluble extractive values, loss on drying, total ash value, acid insoluble ash and total ash value of *P. nigrum* fruits were analysed. For phytochemical screening, following a 6-stage extraction process is performed, the extracts collected from*P.nigrum* were exposed to a number of preliminary biochemical and phytochemical tests. The presence of tannins, saponins, terpenoids, flavonoids, and alkaloids wastested in the chloroform and aqueous extract which is used in the following investigation. However, from the aggregation of all our results, we concluded that the presence of these phytochemicals and bioactive component in these medicinal plants indicates potential therapeutic properties for welfare of humans.

Keywords: Aloe vera, Piper nigrum, Aloin content evaluation, Phytochemical screening, HPLC.

Introduction

India is known for its ancient medicative systems-Ayurveda, Siddha, and Unani. Medical systems are found mentioned even within the ancient Vedas and different scriptures. The ayurvedic concept was regarded and evolved between 2500 and 500 BC in India. Understanding the essential fundamentals of Indian Ayurveda is to spend longer with nature and observe nature, the plants, and herbs. Every plant or herb features a specific quality and might be used to treat a multitude of ailments and diseases. Medicative plants like Aloe vera, black pepper, haldi, tulsi, elaichi, and adrak, satavri are unremarkably utilized in an exceeding form of Ayurvedic home remedies and are considered to be the foremost effective aid among fighting ailments related to throat and skin. As a rich source of nutrients, antibacterial and antioxidant properties, Ayurvedic herbs are non-toxic for humans so the product or remedies created using them are usually recommended for their high therapeutic value.

'Kumari' is also known as *Aloe vera* and it belongs to the family 'Liliaceae'. It is also known as Ghritkumari. The plant grows in a semi-wild state throughout the dried parts of India. The plant grows 30-60 cm in height, is perennial, with a short stem. The leaves are large, 40-50 cm long, thick, fleshy, lance-shaped, with a sharp apex and spiny margins. The whole plant is bitter and sweet in taste (rasa), sweet in the post-digestive effect (vipaka) and cold in potency (virya). The fresh gel or its mucilage or its solid extract is used for medicinal purposes. In inflammatory conditions, associated with pain and swelling, the external application of its leaf extract, is very beneficial. Ghritkumari is useful in number of diseases. In small doses, it is an effective appetizer, digestant, liver stimulant and in large doses, it works as an anthelmintic and purgative. It is very useful as a blood purifier, hence valuable in skin disease and jaundice due to viral hepatitis. Kumari is a valuable herb in the treatment of tumours also[1]. Now a days *Aloe vera*is used in allopathic,

homeopathic and ayurvedic medication systems. Not only as medicine now have people also used it as a food. Ghritkumari contains all beneficial vitamins, minerals, enzymes, amino acids, as well as it has natural sugar. It has various Bioactive compounds with emollient, purgative, anti-microbial, antioxidant, antihelminthic, antifungal, antiseptic, anti-inflammatory and cosmetic values for health care. *Aloin is an anthraquinone-C-glycoside present in Aloe vera. This compound is extremely variable among different species and highly depends on the growing conditions of the plants. Currently, HPLC analysis requires aloin extraction by procedures using various solvents. The most commonly used solvents are methanol; Methanol is the most frequently used solvent for the extractionofanthraquinones[2].

Piper nigrum (P. nigrum) (black pepper) is a precious medicinal plant that belongs to the own circle of relatives Piperaceae. Black pepper is a critical and maximum generally used spice and appeared as "the king of spices'' amongst diverse spices. *P.nigrum* is grown in diverse tropical areas like India, Indonesia, and Brazil.*P.nigrum* is also known as "kali mirch" (Hindi and Urdu), "usana" (Sanskrit), "Golmorich" (Bengali), "Black pepper" (English). Black pepper is likewise used as a medicine, a preservative, and a flavouring agent in perfumery. Marica one of the oldest names of P.nigrum consists of the fully mature dried fruit of Pipper nigrum linn. a climber, cultivated from north Konkan Kerala and also in Assam. Marica was held in high esteem by ancient sages of India. It is one of the herbs mentioned in allayurvedic scriptures. Marica is one of the ingredients of 'TRIKATU' – three pungent {sunthi (Zingiberofficinale), marica and pippali (Pipperlongum)} which alleviates cold, asthma, and body fats, it also improves the taste sensation, reduces flatulence and anorexia, and also is diaphoretic. Marica is used both, internally as well as externally. In the form of an external paste with sesame oil, it is beneficial in the skin diseases like scabies, leukoderma. The paste application helps in reducing the swelling and the pain. In case of tooth decay and aches, the marica powder is used for brushing the teeth. The lively thing in Piper nigrum is the alkaloid piperine. It promotes digestion with the aid of using stimulating the secretion of the digestive enzymes from the pancreas. In addition to this, it additionally obstructs in vitro oxidation with the aid of using extinguishing loose radicals at the side of the inhibition of enzymatic biotransformation of medication withinside the liver. Apart from these, pepper is used to alleviate pain, flu, colds, chills, rheumatism, fever, and muscularaches[1].The objectives of the research were physicochemical analysis of Aloe vera and itsaloin content evaluation through HPLC (High-Performance Liquid Chromatography); along with physicochemical and phytochemical screening of *Piper nigrum.*

Material and Methods

Aloe vera

Plant Material: Fresh leaves of aloe vera is collected from Himalaya drug company, Dehradun, India from which the pulp and leaves were separated and covered in aluminium foil and kept in air tight jar for further use in refrigerator.

Piper nigrum

Plant Material: Fresh dried fruit of black pepper was collected from Himalaya drug company, Dehradun, India. The collected black pepper was cleaned properly under running tap water to make them free from soil and dust and then kept in hot air oven at 60 degree Celsius for 24 hours.

Crude Extraction: 20 grams of coarse powder of black pepper is soaked in 100 ml of Chloroform, and aqueous at room temperature for 3 days inside an Iodine Flask with occasional shaking. The solvents were then filtered using Whatman ™ no.1 filter paper and concentrated using a water bath. The extracts were then kept in glass bottles for further usage.

Identification Test

The individual sample was subjected to the physicochemical and qualitative phytochemical screening. Phytochemical tests were carried out adoptingstandard procedures (Trease et.al 1983, Kokate et.al 1997, Hegde et.al 2010). All of the reagents were made by adopting standard procedures (Indian Pharmacopoeia 2014).

Aloe vera

Physicochemical Analysis Total Solid[3]

Weigh accurately or measure an accurate quantity of the substance under examination stated in the individual monograph, place in a tared dish, evaporate at as low a temp as possible until the solvent is removed and heat on a water-bath until the residue is apparently dry.

Transfer to an oven and dry to constant weight at105 degree, unless otherwise stated in the mono graph.Owing to the hygroscopic nature of certain residues, it may be necessary to use dishes provided with well-fitting covers and to cool in a desiccators and weigh the dish. Calculated the

content of extractable matter in mg per g of air-dried material.

Total Ash Value[3]

Heat a silica crucible to red heat for 30 mins, allow to cool in a desiccator and weigh.

Weigh the accurately about 2g of the substance under examination and evenly distribute it in the crucible.

Dry at 100 degree to 150 degree for 1 hr and ignite to constant weight in a muffle furnace at 600 degree.

Allow the crucible to cool in a desiccator after each ignition.

Calculate the percentage of ash on the dried basis.

Refractive Index[3]

The Abbe refractometer is convenient for the most measurements of refractive index but other refractometer of equal or greater accuracy may be used.

Place the sample on the refractometer and spread it evenly, set the sodium light.

And check the refractive index from the eye piece.

Write refractive value of sample.

P_HValue[3]

The P_H value of a solution is determined potentiometrically by means of glass electrode, a reference electrode and a P_H meter either of the digital or analogue type.

Calibrate the apparatus using buffer solution D as the primary standard, and buffer solution A for adjusting the meter to read the appropriate $P_H$value.

Dip the glass electrode into sample and wait for 5 min.

Take the reading.

Specific Gravity[3]

Select a scrupulously clean, dry pycnometer that previously has been calibrated by determining its weight.

Fill the pycnometer with the sample and clean the excess liquid from the pycnometer.

Weigh the pycnometer with sample and subtract the tare weight of the pycnometer from the filled weight.

Total Dissolved Solid[3]

5gm sample is weighed and dissolved in 75ml water and place the beaker in the magnetic stirrer at 700- 1000c for 10 min.

Make up the sample in 100 ml and the filter it with Whatman filter paper 1.

Place the sample in petri dish and place it in water bath for 1 hr and then place the petri dish to hot air oven for complete moisture free.

Weight the petri dish and calculate the dissolved solid in it.

Quantification AloinContent

Extract purification: Three different samples of the aloe vera plant were taken i.e the pulp, the peel and the whole leaf. All the three samples were homogenizedin a blender separately and the resultant samples were subjected to HPLC to determine the aloin content in the sample.

Sample preparation: For all the 3 sample, approx 250mg sample weighed and mix with 50ml of methanol. Then sonicate the solution for proper mixing. And through nylon filter fill the vial of the HPLC tray.

Chromatographic condition: Samples were analyzed in Shimadzer LC 20A0 liquid chromatograph system with SPD-M20AuV detector in isocratic mode. The elution was carried out with gradient solvent system with flow rate 1 ml/min at 40°C temperature. The mobile phase consisted of acetonitrile (22%) and water (78%) (v/v) basis and phosphate buffer. The sample was injected at 20 µl. Deuterium (D2) Lamp Beckmann was used at wavelength 220 nm for detection of aloin.

Aloin content evaluation through HPLC Chemicals

Acetonitrile (HPLC grade), Ortho-phosphoric Acid (0.1%), Methanol (Chromatography Grade), Ultrapure Distilled water (Filtered with 0.22-micron filter), Aloin standard (52.3 % Pure), aloe vera sample (pulp, leave, leave + pulp).

Chromatographic System and instrument

A Shimadzu® LC-2010CHT HPLC system was used for analysis. With a spectrophotometric detector set at 420 nm, a column oven (set at 40 ̊C), a reverse-phase c18 column (250mm × 4.6 mm). The mobile phase was a 50:50 (v/v) mixture of acetonitrile and 0.1% ortho-phosphoric acid at the flowrate of 1 ml per minute.

Standard and Working Solution

Standard solution: 50.3mg of Aloin was dissolved in methanol in a 50 ml volumetric flask to a final concentration of 1.0 mg per ml and sonicated for 10 minutes in an ultrasound bath and completed to the final volume.

Test Solution

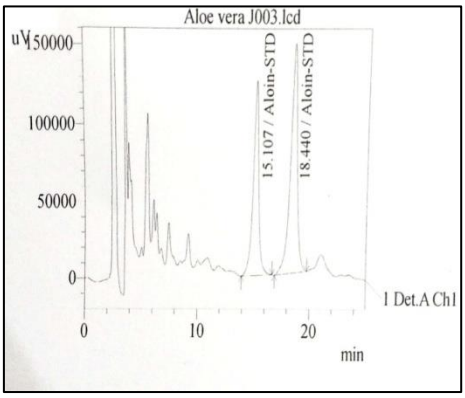
Test Solution was prepared by adding 500 mg of sample in 50 ml of methanol in a volumetric flask.

The solution is then placed in ultra sound bath for mixing the sample.

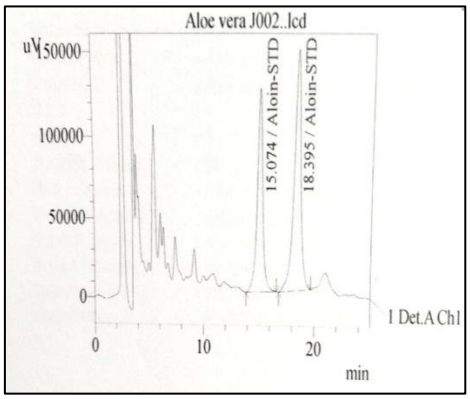
Procedure

All the solutions (Test, Standard, and Methanol blank) were then placed in the system. A method was designed to take 22% solvent as acrylonitrile and 78% ortho-phosphoric acid with 1 cycle being 25 minutes long, 7 cycles were run in total with first cycles of methanol blank, second, third and fourth of Standard and fifth, sixth and seventh of the samples. The following graphs were obtained:

Graph of Aloin Standard



Graph 1-Standard aloin-1



Graph 2-Standard aloin-2

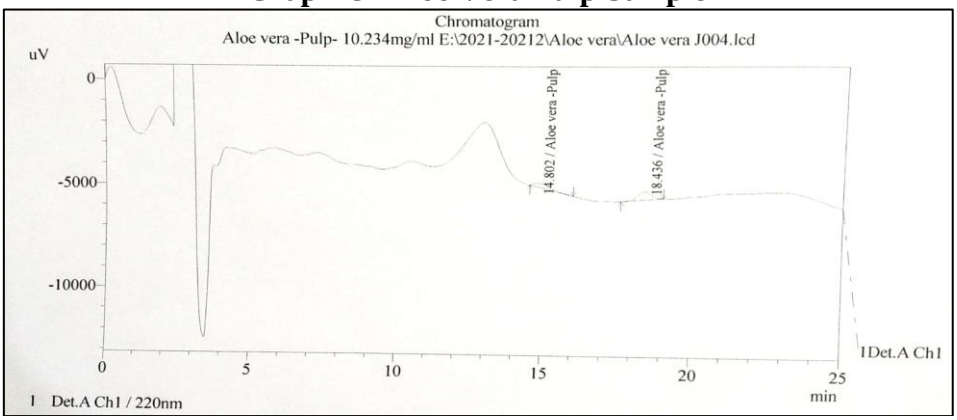
Aloin standard-1

Title	Sample name	Sample id	Ret. time	Average	Height	Conc.
Aloe vera j002	Aloin std-1	Aloin std-1	15.074	4261976	124576	0.00
Aloe vera j003	Aloin std-1	Aloin std-1	15.107	43317756	126131	0.00
Average			15.091	4289866	125354	0.00
%RSD			0.153	0.919	0.877	0.00
Maximum			15.107	4317756	126131	0.00
Minimum			15.074	4261976	124576	0.00
Standard deviation			0.023	39442	1100	0.00

Aloin standard-2

Title	Sample name	Sample id	Ret. time	Ave.	Height	Conc.
Aloe vera j002	Aloin std-1	Aloin std-1	18.395	6139179	145926	0.00
Aloe vera j003	Aloin std-1	Aloin std-1	18.440	6214683	147655	0.00
Average			18.418	6176931	146791	0.00
%RSD			0.174	0.864	0.833	0.00
Maximum			18.440	6214683	147655	0.00
Minimum			18.395	6139179	145926	0.00
Standard deviation			0.031	53390	1223	0.00

Graph Of Aloe Vera Pulp Sample



Graph-3Aloe vera Pulp graph

Peak table

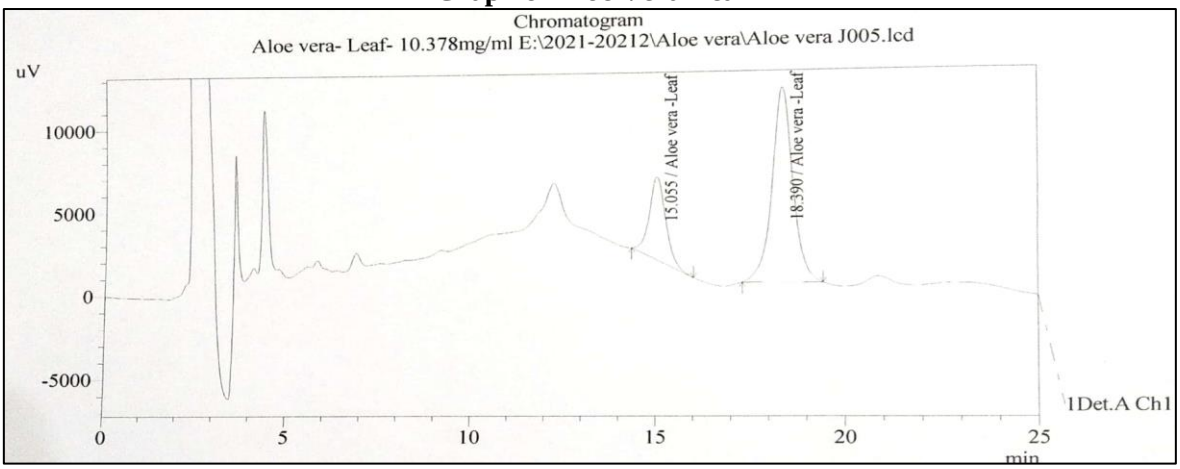
Peak#	Name	Ret. Time	Area	Height	Area%	Height
1	Aloe vera- pulp	14.802	8040	185	38.970	31.684
2	Aloe vera- pulp	18.436	12591	398	61.030	68.316
Total			20631	583	100.00	100.00

Calculation

$$\frac{\text{Area of Sample}}{\text{Area of Standard}} \text{ X } \frac{\text{Concentration of Sample}}{\text{Concentration of Standard}} \text{ X Purity of standard}$$

$$\frac{20631}{10466797} \text{X} \frac{11006}{10.234} 52.3 = 0.010\%$$

Graph of Aloe Vera Leaf



Graph 4-Aloe vera leaf graph

Peak table

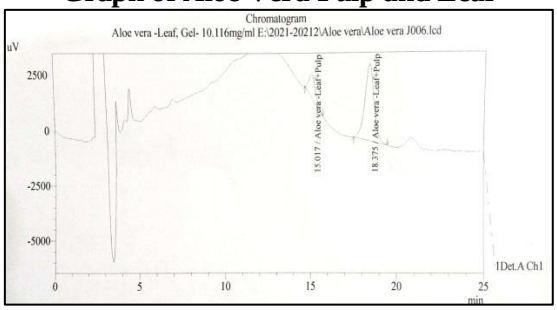
Peak#	Name	Ret. Time	Area	Height	Area%	Height
1	Aloe vera- leaf	15.055	152168	4949	25.739	30.048
2	Aloe vera- leaf	18.390	439026	11522	74.261	69.952
Total			591194	16472	100.00	100.00

Calculation

$$\frac{\text{Area of Sample}}{\text{Area of Standard}} \text{ X } \frac{\text{Concentration of Sample}}{\text{Concentration of Standard}} \text{ X Purity of standard}$$

$$\frac{591194}{10466797} \text{X} \frac{1.006}{10.234} 52.3 = 0.286\%$$

Graph of Aloe Vera Pulp and Leaf



Graph 5-Aloe vera pulp and leaf graph

Peak table-

Peak#	Name	Ret. Time	Area	Height	Area%	Height
1	Aloe vera- leaf and pulp	15.017	28142	1020	18.232	22.871
2	Aloe vera- leaf and pulp	18.375	126215	3441	81.768	77.129
Total			154358	4461	100.00	100.00

Calculation

$$\frac{\text{Area of Sample}}{\text{Area of Standard}} \text{ X } \frac{\text{Concentration of Sample}}{\text{Concentration of Standard}} \text{ X Purity of standard}$$

$$\frac{154358}{10466797} \text{X} \frac{1.006}{10.234} 52.3 = 0.0766\%$$

Piper nigrum

Physicochemical Analysis

Determination of foreign organic matter[3]

To determine the foreign matter, content material was spread on a thin layer on a A4 sheet.

Inspect the sample with the eye or with the use of a 6x lens and separate the foreign organic matter manually as completely as possible.

Weigh and determine the percentage of foreign organic matter from the weight of the drug taken.

Loss on Drying[3]

LOD is the loss of mass expressed as percent w/w.

To estimate the LOD, 1gm of the air-dried crude drug is accurately weighed in a flat dried weighing bottle and then dried to constant mass, the final weight is noted and the LOD calculated by formula.

$$\text{LOD}=\frac{FinalWt.}{IntialWt.}\text{x}100$$

Determination of Total Ash Value[4]

For determination of total ash 1gm of the ground air dried material, accurately weighed, in a crucible (usually silica or platinum) and spread in an even layer

It is then ignited by gradually increasing the heat to 500-600 degree Celsius until it is white, indicating the absence of carbon.

The residue is allowed to cool in a desiccator for 30 minutes, and weighed without delay. The content of total ash is calculated in mg per g of air-dried material.

Determination of Acid Insoluble Ash[4]

Boil the ash obtained in total ash determination for 5mins with 25ml of 2M hydrochloric acid

Collect the insoluble matter in an ashless filter paper.

Wash with hot water and ignite to constant weight.

The content of acid insoluble ash is calculated in mg per g of air-dried material.

Determination of Alcohol Soluble Extractive[4]

Macerate 5g of the air-dried drug, coarsely powdered, with 100ml of ethanol of the specified strength in a closed flask for 24 hours, shaking frequently during 6 hours and allowing to stand for 18 hours.

Filter rapidly, taking precaution against loss of solvent, evaporate 25ml of the filtrate to dryness in a tared flat bottomed shallow dish and dry at 105 degrees Celsius, to constant weight and weigh.

Calculated the content of extractable matter in mg per g of air-dried material.

Determination of Water-SolubleExtractive[4]

For determination of number of active constituents extracted with water from a given amount of sample.

Similar procedure was adopted as described above for total alcohol extractive except replacing ethanol by water.

Calculated the content of extractable matter in mg per g of air-dried material.

Phtochemical Screening[5]

Test for Tannins

2ml of each extract was added separately to 4 ml of water and a few drops of 0.1% $FeCl_3$were added to the extracts to form a blue coloured solution.

Test for Terpenoids

Salkowaski Test

5ml of extracts was taken in different test tubes.

To each of them 2ml of chloroform was added, along with it 3ml of concentrated sulphuric acid was added slowly to form a layer.

Test for Saponins
1ml of the extract was added to 20ml of distilled water in a test tube and was shaken vigorously for 15minutes.
Formation of the foamy layer indicated the presence of saponins.

Test for Cardiac Glycosides
Keller-Killani Test
2ml of glacial acetic acid was added to 5 ml of extracts containing a drop of $FeCl_3$solution followed by the addition of 1ml of concentrated sulphuric acid.
A greenish ring may form just above ring and gradually spreads throughout this layer.

Test for Flavonoids
Aqueous filtrate along with concentrated sulphuric acid was taken in a test tube;
5ml of dilute ammonia solution was added.
To all other filtrates few drops of 1% aluminium solution was added.
The presence of flavonoids was indicated by the development of yellow colour.

Test for Alkaloids
Mayer's Test
Aloe vera Pulp ExtractAloe Vera Leave Extract
1ml of the extracts and 10ml of acid alcohol were boiled and filtered.
To 5ml of filtrates, 2ml of dilute NH_3 and 5ml of $CHCL_3$ were added and shaken to extract the alkaloidal base.
The chloroform layer was extracted with 10ml of acetic acid.
Positive test with Mayer's reagent gives a cream-coloured solution.

Test for Phenolic Compounds[6]
Ferric chloride test
Extract was diluted to 5ml of distilled water and filtered.
To the filtrate 5% of ferric chloride was added.
Dark green colour indicates the presence of phenolic compounds.

Results and Discussion

Aloe vera
Physicochemical analysis
Physical and chemical test are performed on Aloe vera. 2 samples of Aloe vera are undergone for physicochemical analysis, 1 Aloe vera pulp and 2 Aloe vera leave.

Table-1 Physicochemical analysis of Aloe vera pulp extract vera leave extract

Parameter	Experiment Result
Colour	Offwhite
Odour	Characteristic
Taste	Bitter
Total Solid	1.54%
Total Dissolved Solid	0.704%
Total Ash Value	0.1267%
Refractive Index	1.334
P_H Value	4.48
Specific Gravity	Not Applicable

Table 2-Physicochemical analysis of Aloe Aloin content

Parameter	Experiment Result
Colour	Green
Odour	Characteristic
Taste	Bitter
Total Solid	1.933%
Total Dissolved Solid	0.976%
Total Ash Value	0.2612%
Refractive Index	1.334
P_HValue	4.58
Specific Gravity	1.009g/ml

Through the graph the aloin content iscalculated in pulp, leaf and pulp, leaf sample. Results are as followed:

Table-3 Aloin content

Aloe Vera Sample	Amount Percent
Pulp	0.0101%
Leaf	0.286%
Pulp And Leaf	0.0766%

Piper nigrum
Physicochemical analyses Physical and chemical test are performed on blackpepper. Black pepper sample completely stand on standardized parameters. Result is as followed:

Table-4 Physicochemical analysis of black pepper

Parameters	Experiment Yeild	Standard Report
Foreign Matter (%)	0.132%	Not More Than 2.0%
Loss On Drying (%)	10.86%	Not More Than 12.0%
Total Ash Value (%)	4.72%	Not More Than 5%
Acid Insoluble Ash (%)	0.28%	Not More Than 0.5%
Alcohol Extractive (%)	8.736%	Not Less Than 6%
Water Extractive (%)	7.752%	Not Less Than 6%

Phytochemical analyses

The presence of active phytochemical constituents such as tannins, saponins, terpenoids, flavonoidsand othersare the principal reasons for a plant to exhibit medicinal activity. Flavonoids are well knownantioxidants and cardiac glycosides exhibit positive as well as negative effects on the heart. Saponins act as anti-feed ants in plants and likewisetannins shield the plants from phytophagousinsects and herbivores. Terpenoidsprovideplants their phytoalexin property. All these Phyto-active compounds in conjunction elevate the therapeutic, commercial and economic values ofsuch plants.The results forall the phytochemicalscreenings aredisplayed inTables-2for black pepper.

Table-5 Phytochemical analysis of black pepper

Test	Chloroform Extract	Aqueous Extract
Tannins	Absent	Present
Saponins	Absent	Present
Terpenoids	Present	Present
Cardiac Glycosides	Absent	Absent
Flavonoids	Present	Absent
Alkaloids	Absent	Absent
Phenolic Compounds	Absent	Absent

Conclusion

From the above research we can conclude that *Aloe vera* is a herb which have optimum amount of Aloin in it, which can either be used to make antibiotic derivatives or can be used as skin care product. *Piper nigrum*isa herb rich in phytochemicals and use for many disease internally and externally. Phytochemical present in piper nigrum are tannins, saponins, terpenoid, and flavonoids which are helpful for therapeutics and used as safe substitute to many of the chemical drugs which are present in market presently. This medicinal plant gives future scope for creating drug without side effect on humans.

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