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PHYTOCHEMICAL & PHYSIOCHEMICAL SCREENING OF THE HERBAL DRUG ALOEVERA AND *CLITORIA TERNATEA* LEAVES EXTRACT

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Keywords:

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ABSTRACT: Egyptians referred to aloe vera, a botanical remedy, as "the plant of immortality." It helps with diabetes, hypertension, wound healing, and other medical conditions. *Aloe vera* gel extract and *Clitoria ternatea* watery, methanolic, and ethanolic extracts of plants was studied. A small sample of *Aloe vera* and *Clitoria ternatea's* ethanolic, methanolic, and watery extracts were put through a phytochemical test to check alkaloids, tannins, carbohydrates, saponins, phenols, and flavonoids, and steroid and glycosides, as well as a physical chemical test to check the extractive value and ash value. The results of the phytochemical examination revealed phenols, steroids, glycosides, alkaloids, saponins, tannins, flavonoids, and tannins were all present. The findings of the current research support the use of plant species as traditional medicine for the treatment of numerous illnesses since they show that ethanolic, methanolic, and watery extracts of *Aloe vera* and *Clitoria ternatea* contain bioactive chemicals that have significant medicinal properties. The plant leaf extracts of *Aloe vera* and *Clitoria ternatea* demonstrated an abundance of phytochemicals as secondary metabolites in the current research, and they may be exploited in the pharmaceutical industry to create powerful drugs. The findings from the investigations on the aforementioned plant provide support for its usage in conventional medicine to treat illnesses and diseases.

INTRODUCTION: In the form of Mesopotamian tablets and ancient Egyptian papyrus, aloe is recorded as being highly helpful in treating infections, laxatives, and skin disorders [1]. It is also known that Alexander invaded the Indian Ocean's Socotra Island in order to seize quantities of aloe, which he used to cure wounded troops [2]. Ayurveda, India's ancient medical system, states that it may be used internally as an antihelminthic, laxative, uterine stimulant, and haemorrhoid treatment.

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Psoriasis and eczema are treated externally with it, often in conjunction with licorice roots. In Arabian medicine, its gel is used as a headache reliever, cooling, laxative, disinfectant, anti-conjunctivitis, and wound healing agent [3].

There are more than 300 species in the genus Aloe. Most of these species are indigenous to South Africa, Madagascar, and Arabia. *A. barbadensis, A. ferox, A. perryi*, and *A. vera* are among the Aloe Arborescens species that have the highest therapeutic value [4]. Variable species have varied quantities of active chemicals [5]. The Xanthorrhoeaceae family includes this genus *A. vera* is a perennial plant with pea-green, succulent leaves that is arbores cent or shrubby. Long, meaty, triangular leaves with spikes on the margins make

up this plant. Transparent parenchymal meaty gel was extracted from the middle of the leaf. South Africa and South America are the original home of this plant. The only places where it is not grown are rain forests, deserts, and tundra. In the USA's southern Texas state, aloe is grown for commercial purposes. This plant takes four years to reach maturity and lives for around twelve years [6].

A lovely perennial climber with prominent blue or white blooms is called *Clitoria ternatea* Linn. It is a member of the Fabaceae family or is sometimes referred to as "shankhapuspi" and "butterfly pea". It has been used for centuries to cure a number of diseases [7, 8]. The plant is widespread throughout India, the Philippines, and Madagascar is examples of tropical Asia [9]. It is endemic to south-east Asia. In the Ayurvedic medical system, *C. ternatea* roots, seeds, and leaves are often employed. This plant's extracts have been employed they are used in the Ayurvedic "Medhya Rasayana" as a revitalising therapy for neurological issues and are claimed to sharpen the intellect [10]. To treat stomatitis, piles, female sterility, hematemesis, sleeplessness, epilepsy, psychosis, leucorrhea, and polyurea, the whole plant and seed preparations are utilized [11].

The roots are beneficial in treating Leprosy, inflammation, leucoderma, bronchitis, asthma, pulmonary tuberculosis, ascites, fever, otalgia, hepatopathy, and as a cathartic. They are also bitter, cooling, laxative, intellectually stimulating, diuretic, anthelmintic, and tonic [12]. Snake bites and scorpion stings may also be treated using the root, stem, and flower [13]. Numerous pharmacological actions of *C. ternatea* have been shown, including anxiolytic, depressive, anticonvulsant, antistress [14], sedative [15], antipyretic, anti-inflammatory, analgesic [16, 17], anthelmintic[18], as well as antimicrobial properties [19]. *C. ternatea* extract has been demonstrated to enhance apical and basal dendritic branches, acetylcholine concentration, and acetyl cholinesterase activity in rats [20]. It has also been proven to improve learning capacity and memory. Numerous secondary metabolites are present in the plant, including kaempferol, clitorin (glucoside), taraxerol, and the lactone aparajitin [21]. Seeds include sistosterol, hexacosanal, and anthoxanthin [22]. The identification of novel sources of therapeutic and commercial relevance depends heavily on screening of medicinal plants for phytochemicals [23]. A phytochemical examination of a methanol extract of the roots of *Clitoria ternatea* was used to confirm the presence of tannins, resins, and other ingredients [24, 25, 26]. In the present study, phytochemical analysis was used to check *Clitoria ternatea* plant sections for the presence of alkaloids, tannins, glycosides, resins, steroids, saponins, flavonoids, and phenols. Additionally, total flavonoids, saponins, and phenols in the root extract as well as flavonoids in the shoot, flower, and seed extracts were quantitatively analysed. The current study's objective was to identify the phytochemical composition of these two important plants. *A. vera* and *C. ternatea*, as well as their potential to treat a variety of different diseases and ailments.

MATERIAL AND METHOD:

Plant Collection and Powder Preparation [27]: Plant leaves were collected from a nearby nursery and authenticated at ACME Research Solutions (Authentication Letter No. ACME/PA/11031). After drying under shade for at least 15 days, they were powdered and sieved to obtain smaller particles. The final powdered sample was stored in an airtight container awaiting further analysis. **Tables 1** and **2** show the extractive values for water-soluble, alcohol-soluble, and acid-soluble ash as well as their percentage in three solvents: ethanol, methanol, and water.

Physicochemical Parameters [28, 29, 30]:

Chemical Reagents: The following companies provided the chemicals for this study: Hi Media Laboratories Pvt. Ltd., SD Fine-Chem. Ltd., and SRL Pvt. Ltd. are all based in Mumbai, India. The investigation only used analytical-grade compounds.

Determination of Water-Soluble Extractive Value: To determine the 5 grams of a drug's water-soluble extractive value were collected and combined with 100 milliliters of water in a beaker, stirring thoroughly to prevent sedimentation. After drying and mixing had taken place, it was filtered using a 20mm filter before being dried on a Petriplate (whose weight had been noted beforehand). Finally, this dried formulation was heated at 105°C before measuring its air-dried amount by weighing the Petridish with the drug and comparing it against its initial weight.

Determination of Alcohol Soluble Extractive Value: The extractive value of a medication that is soluble in alcohol is an important indicator that tells you the quantity of chemical constituents present that can be extracted using alcohol. In this study, a 5g sample of the substance was stored for 18 hours after being combined with 100% alcohol in a flask with a cover to maximize extraction of its constituents into the alcohol. After drying, the sediment and supernatant were separated and their alcohol soluble extractive value determined by filtering with a 20 mm filter. Comparing different batches of drugs using this method provided valuable information about their quality; it allows users to compare chemical composition between batches for consistency in dosage forms.

Alcohol soluble extractive = Value (%w/w)

Determination of Ash Value: To calculate the ash value of plant material, one gram of dried drug was placed into a preheated crucible that had been heated to 100°C to prevent moisture absorption. Once evenly distributed within, it was then baked at 100°C until ash formed. After moisture had been lost, the drug-containing crucible was removed from the oven and Allowed to cool to room temperature. After that, its weight was used to calculate the drying and ash values.

Total ash (%w/w) = (weight of ash/weight of sample) × 100

Determination of Acid Insoluble Ash: To determine the acid insoluble ash weight of a drug, further investigation was necessary. To do this, a crucible filled with 2M hydrochloric acid (HCl) was heated for approximately 5 minutes, after which it was collected and dried at temperatures ranging from 500 to 600°C for around 30 minutes. Finally, both parts - drug plus crucible - were weighed together; using an extraction formula, we were able to calculate how much ash had been extracted.

Acid insoluble ash (%w/w) = (weight of ash/weight of sample) × 100

Determination of Water-soluble Ash: After being combined with 25mL of water and filtered using Whatman filter paper, the sample's ash was recovered. The residue was washed twice with reverse osmosis (RO) water to eliminate any impurities, and then collected as a supernatant. The insoluble material was dried at 400 – 500 °C for 10 hours, then weighed along with its water-soluble component.

Water soluble ash (%w/w) = (wt. of ash – wt. of insoluble ash/ wt. of sample) ×100

Loss on Drying: On a tarred petridish, the powdered drug was properly weighed at ten grams. Before being reweighed, it was dried at 105°C for an hour in a hot air oven. It was possible to calculate the initial and final weights and determine the loss during drying.

Phytochemical Screening for Aloe Vera and *Clitoria ternatea* [31, 32, 33, 34]:

Test for Alkaloids: To test for alkaloids, a mixture of 10 mL ammoniacal chloroform and 5 mL chloroform was added to 5 grammes of the powdered material. After extraction and filtering, a few drops of 0.5 M sulphuric acid were added to the filtrate which was vigorously shaken until a creamish-white precipitate appeared.

Test for Carbohydrates: To test for carbohydrates, a diluted leaf extract was placed into a test tube before adding 2 ml each of Fehling-A and Fehling-B solutions. The mixture was then heated for several minutes, looking for the appearance of a brick-red colour, indicative of carbohydrates present. This test is commonly known as Fehling's test and it has become widely used to detect reducing sugars in biological samples. This reaction takes place when copper ions in Fehling's solution are reduced by reducing sugars, forming a brick-red precipitate of cuprous oxide. This method can be useful for qualitative detection of carbohydrates; its intensity provides an estimate of the concentration of reducing sugars present.

Test for Flavonoids: Testing for flavonoids in macerated samples was done using ethyl acetate. A quantity of 0.5g of the macerated sample was added to several ml of ethyl acetate and heated over boiling water; filtering it and shaking with 1ml 1% aluminum chloride solution produced a yellow hue which indicates flavonoids present.

Test for Tannins: A test was conducted to detect the presence of tannins in a plant sample. Two grams of ground sample were mixed with 5mL of

45% ethanol and boiled for 5 minutes; then allowed to cool before filtering. 1 mL of filtrate had a few drops of a lead acetate solution added; when this formed a gelatinous precipitate it indicated there were tannins present. This qualitative method for detection involves creating an intricate complex between tannins and lead ions - something of interest because of possible pharmaceutical benefits including antioxidant, anti-inflammatory, or anticancer qualities. The presence of tannins is especially noteworthy due to their potential medicinal applications such as antioxidant, anticancer properties.

Test for Steroids: A sample was tested for steroids using a methanol extract, mixed with 2ml each of acetic anhydride and sulfuric acid and carefully added along the sides of a test tube. Color change in this mixture was observed, with violet or blue-green changes considered positive indications that steroids had been present.

Test for Phenols: Phenols are organic compounds commonly found in plants and known to possess antioxidant properties. To assess the presence of phenols in a methanol extract of leaves, a colorimetric test was performed. A few drops of extract were 2.1 ml of ferric chloride solution was added after being combined with distilled water and gently heated. If there is green or blue coloration present, then there must be phenols present. This test has become popular as an efficient and quick way to detect phenolic compounds present in plant extracts which have diverse biological activities as well as potential applications such as medicine and other industries.

Test for Glycosides: We added 0.6 mL of the methanol extract to a solution containing 1.1 mL of glacial acetic acid and traces of ferric chloride to check for glycosides. The last addition was 1 mL of pure sulfuric acid. A reddish-brown color ring at the junction between both layers was taken as proof positive of glycoside presence; furthermore, the upper layer turned into an unidentified bluish-green hue.

Test for Saponins: A test was conducted to detect saponins in a methanol extract. To do this, 0.5g of extract was dissolved in 3 drops of olive oil, 5 ml of distilled water, and rapidly agitated to examine any persistent froth development; if an emulsion developed, saponin presence could be established.

RESULTS:

TABLE 1: EXTRACTIVE VALUES

Sr. no.	Weight of plant material	Solvent	Extract Color *Clitoria ternatea*	Extract Color- *Aloe vera*	*Clitoria ternatea* leaves - Results (%)	*Aloe vera* Results (%)
1	2	Methanol	Light Green	Light Brown	15.02	7.89
2	2	Ethanol	Light Green	Light Brown	8.05	15.69
3	2	Water	Green	Light Brown	12.21	28.56

FIG. 1: *ALOE VERA* EXTRACT (ETHANOL, METHANOL, AND WATER)

FIG. 2: *CLITORIA TERNATEA* EXTRACT (ETHANOL, METHANOL, AND WATER)

TABLE 2: ASH VALUES

Sr. no.	Ash Values	*Clitoria ternatea* -Results %	Aloe Vera- Results (%)
1	Ash Value	12.12	6.98
2	Acid insoluble Ash Value	7.05	1.12
3	Water Soluble Ash Value	4.11	2.99

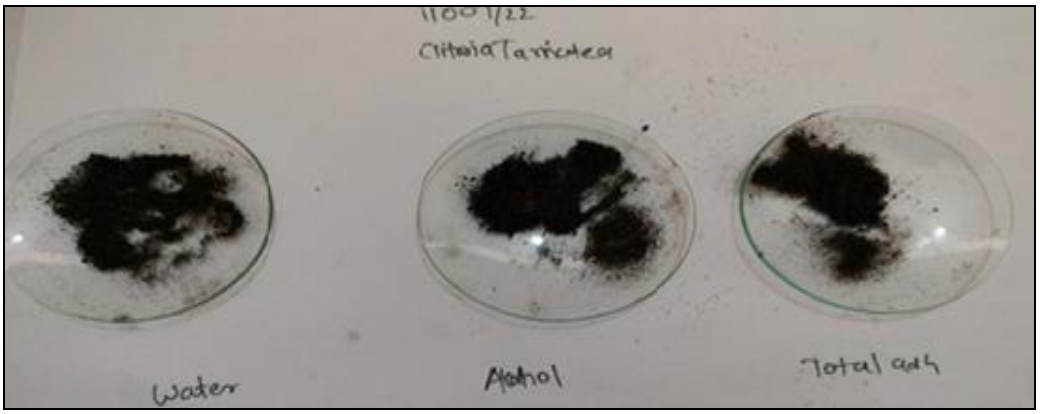

FIG. 3: *CLITORIA TERNATEA* ASH VALUE

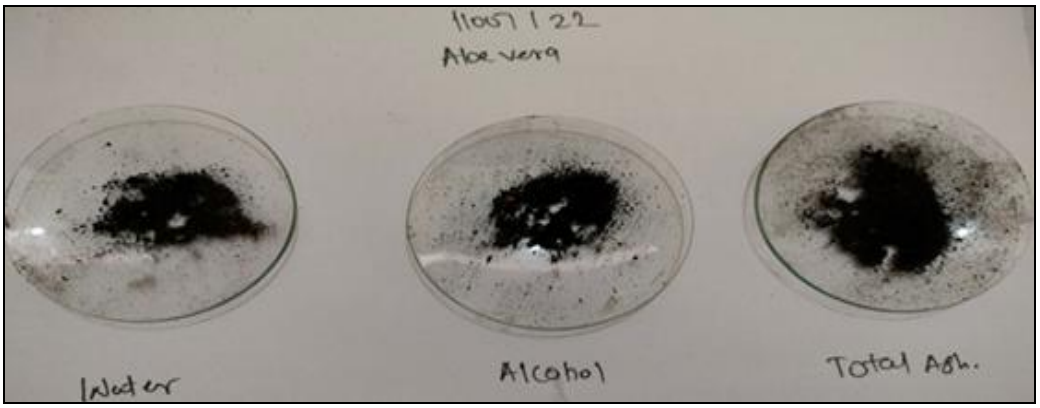

FIG. 4: *ALOE VERA* ASH VALUE

TABLE 3: PERCENTAGE YIELD (*CLITORIA TERNATEA* LEAVES AND ALOE VERA)

Sr. no.	Solvents	*Clitoria ternatea* -Yield % (V/W)	Aloe Vera -Yield % (V/W)
1	water	4.01	5.02
2	ethanol	2.20	8.25
3	methanol	3.06	7.98

TABLE 4: PHYTOCHEMICAL SCREENING (*CLITORIA TERNATEA* LEAVES)

Sr. no.	Test	Solvents		
		Methanol	Ethanol	Water
1	Alkaloids	Positive	Positive	Positive
2	Carbohydrates	Positive	Positive	Positive
3	Saponins	Positive	Negative	Negative
4	Glycosides	Negative	Negative	Positive
5	Steroids	Negative	Negative	Positive
6	Flavonoids	Positive	Positive	Positive
7	Tannins	Positive	Positive	Positive

TABLE 5: PHYTOCHEMICAL SCREENING (*ALOE VERA*)

Sr. no.	Test	Solvents		
		Methanol	Ethanol	Water
1	Alkaloids	Positive	Positive	Positive
2	Carbohydrates	Positive	Positive	Positive
3	Saponins	Positive	Positive	Positive
4	Glycosides	Negative	Positive	Negative
5	Steroids	Negative	Negative	Negative
6	Flavonoids	Negative	Positive	Positive
7	Tannins	Negative	Negative	Positive

DISCUSSION: The existence or absence of tannins, alkaloids, and saponins, flavonoids, glycosides, steroid, and carbohydrates were checked during the phytochemicals screening of the extract.

Alkaloids were detected, according to a Mayer's reagents test. The test for the presence of alkaloids in both plant leaves came out positive, as shown by the pale precipitate that resulted **Table 4** & **5**.

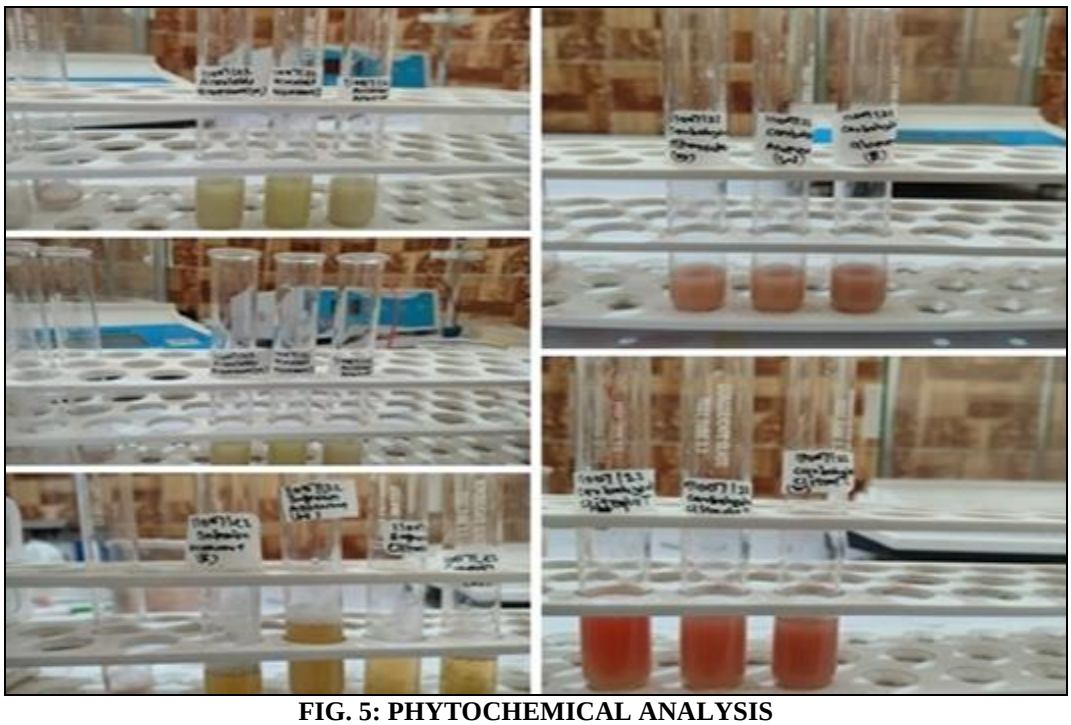

FIG. 5: PHYTOCHEMICAL ANALYSIS

By performing a froth test, the presence of saponins was identified; the formation of the presence of saponins was indicated by a steady, enduring foam in methanolic, ethanolic and watery extracts of *Aloe vera* and methanolic extracts of *C. ternatea*; thus, the test for saponins were positive except for the ethanolic and watery extract of *C. ternatea* In the fehling's A and B solution, the formation of a brick-red color, indicative of carbohydrates present in methanolic and ethanolic and watery extract of *Aloe vera* but beside the methanolic extract, the carbohydrate present in ethanolic and watery

extract of *Clitoria ternatea*. Yellow precipitate from the lead acetate test showed that tannins were present in the *Aloe vera* water extract but in the *Aloe vera* ethanolic and methanolic extract tannins was not present. While in the *Clitoria ternatea* methanolic, ethanolic and watery extract, tannins are present. No colour change was observed by steroid test, indicating the absence of steroid except watery extract of *C. ternatea* Bluish. A ferric chloride test was used to determine if phenols were present. The formation of a bluish colour indicated the existence of phenols. Yellow colours appearing meant that the test for flavonoids was successful. Flavonoids are present in all type of solvent extract of *Aloe vera* and *C. ternatea* but in the methanolic extract of *Aloe vera* this is absent. Only in the watery extract of *Clitoria ternatea* and the ethanolic extract of *Aloe vera* glycosides are present. The physicochemical screening results, Extractive value and Ash value are shown in **Table 1 & 2.**

CONCLUSION: The leaves of *A. vera* and *C. ternatea* have been subjected to pharmacognosy-anatomical, physicochemical, and preliminary phytochemical analyses, which have indicated the existence of phytoconstituents such alkaloids, steroids, carbohydrate, flavonoids, glycosides, saponins, phenoles and tannins. The study of phytochemicals revealed important details about the various phytoconstituents found in plants, assisting future researchers in choosing an extract for additional research on isolating the active ingredient. It also provided insight into the various phytochemicals that have been discovered to have a variety of activities.

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