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Phytochemical standardization of *Aloe vera* extract by HPTLC techniques

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ABSTRACT

Objective: To examine the phytochemical parameters of *Aloe vera* (*A. vera*) L. which can be used as a tool for its standardization. **Methods:** The phytochemical analysis, solubility test, heavy metal analysis, antimicrobial study and quantitative analysis of gallic acid and berberine by HPTLC method were included in present study. **Results:** Phytochemical analysis revealed the presence of alkaloid, carbohydrate, tannin, steroid, triterpenoid and glycoside. Total flavonoid and phenol content was found to be 1.9% and 13.11%. Concentartion of lead, arsenic, mercury and cadmium was found to be under the limit. Total bacterial count, yeast and moulds contents were found to be under the limit whereas *Escherichia coli (E. coli)* and *salmonella* was found to be absent in the extract. Quantitative analysis through HPTLC revealed the presence of 2.74% and 0.543% w/w of berberine and gallic acid. **Conclusions:** The results indicate that the plant extract are rich in berberine and gallic acid implying their importance to human health. This investigation could be used as source of standard parameters which can play an important role in its standardization.

1. Introduction

Medicinal plants are very ancient and only true natural medicines useful in several ways for the treatment of different diseases. They can be used directly or in extracted forms for the management of various ailments due to presence of various phytochemicals[1]. For the prevention and treatment of various health ailments, plants and isolated phytochemicals have been used from time immemorial. A large number of phyto drugs prescribed worldwide are derived directly or indirectly from natural sources. A large number of African and Asian populations use traditional medicines for their primary healthcare[2]. About 2-3 decades ago most of the drugs were of herbal origin. A variety of reasons strengthen why people like to use natural medicines such as fewer side effects on human health and cost effective. Herbal medications gain popularity due to a perception that there is a lower incidence of adverse reaction to plant preparation compound to synthetic pharmaceuticals[3].

Aloe vera (A. vera) L. is a cactus-like perennial plant belonging to family liliaceae, widely distribution in the tropical and subtropical regions of the world have been chosen in the present study[4]. Most of *Aloe* species are indigenous to Africa, but now have wide distribution in the tropical and subtropical regions of the world. They are grown in warm climates, both as wild and cultivated plants, in countries in southern, eastern and northern Africa, India and in China[5]. The genus *Aloe* contains over 400 different species with Aloe barbadensis Miller (*A. vera*), *Aloe aborescens* and, *Aloe chinensis* being the most popular. *Aloe barbadensis* Miller is considered to be the most biologically active[6]. Traditionally, *A. vera* gel is used both, topically (treatment of wounds, minor burns, and skin irritations) and internally to treat constipation, coughs, ulcers, diabete, headaches, arthritis, immune-system deficiencies[6]. The earliest recorded pharmacological usage was recorded in ancient Sumeria about 1750 B.C. where it was considered as an excellent treatment for stomach irritations and nausea [5]. *A. vera* consists of high content of phenolic compounds, glycosides (aloins), 1,8-dihydroxyanthraquinone derivatives (aloe emodin), beta-1,4 acetylated mannan, mannose-

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phosphate and alprogen glucoprotein. *A. vera* has been used throughout history in folk medicine as valuable ingredient for the food, pharmaceutical and cosmetic industries[4]. Fresh aloe juice from the inner leaf parenchyma contains 96% water, polysaccharides consisting mainly of D-glucose and D-mannose, tannins, steroid, enzymes, plant hormones, amino acids, vitamins and minerals[7]. Leaf exudates and mucilaginous gel of *A. vera* possesses anti-inflammatory, antifungal, antibacterial, anticancer, antioxidant, cytoprotective, cardiac stimulatory and immunomodulatory activities. It is used to protect against gastric ulceration, remedy against a variety of skin disorders, promotes wound healing as well reducing edema and pain too. It has also been shown to have antidiabetic and hypoglycaemic properties and cardiac stimulatory activity[3,4]. Adulteration represents a major concern for the *A. vera* market, mostly because of the high cost of the raw materials. Historically, the most common substance used to adulterate aloe gel powder is maltodextrin[6]. Development of various novel analytical techniques for the analysis of medicinally active phytoconstituents in the herbal has become an important step of herbal drug standardization. The overall objective has been to develop a novel qualitative and quantitative technique, which can help the quantification of different constituents of *A. vera* herbal extract.

2. Material and methods

2.1. Chemicals and reagents

Crude *A. vera* herbal extract was procured from Garlico Herbal Concentrate (M.P.). HPTLC precoated plates Silica Gel Merck 60F254 was used as a stationary phase. Berberine, gallic acid and rutin were used as a marker compound. All chemicals were of highest purity and were purchased from Sigma Pvt. Ltd and Rankem New Delhi *etc*.

2.2. Phytochemical methods

Preliminary phytochemical screening for alkaloids, steroids, carbohydrates, tannins, fixed oils, proteins, triterpenoids, deoxysugar, flavonoid and glycosides were carried out according to the official procedures to know the presence of different phytoconstituents in the *A. vera* extract[8,9]. Thin layer chromatography (TLC) profile of the *A. vera* extract was carried out to conferm the presence of different phytoconstituents. In TLC, spots were developed in the different solvent system of varying polarity. Further developed spots were identified bye different spraying reagents. Solubility, loss on drying, heavy metal and microbiological analysis were also performed in the present investigation according to the IP, 1996 and WHO guidelines[10-12]. Total phenol and flavonoid content were also determined in the *A. vera* extract according to the standard official procedure[13,14]. Aluminum chloride colorimetric method was used for the total flavonoid determination using rutin as a standard. Gallic acid was used for the determination of total phenol content. Different combination of solvent system of varying polarity has been used for the optimization of solvent system in high performance thin layer chromatography (HPTLC) methods. Gallic acid and berberine were used as a standard marker compound at different concentration in methanol for quantitative analysis in the present investigation.

3. Results

Phytochemical analysis of the *A. vera* extract showed that alkaloid, saponin, tannin, flavonoid, steroid, glycoside, protein and amino acid were present. TLC analysis showed seven spots R_f (0.21, 0.48, 0.57, 0.96) in ethyl acetate: methanol: H_2O (81:11:8), solvent system and flavonoid was found to be present. For the total flavonoid and phenol content determination, rutin and gallic acid were used as

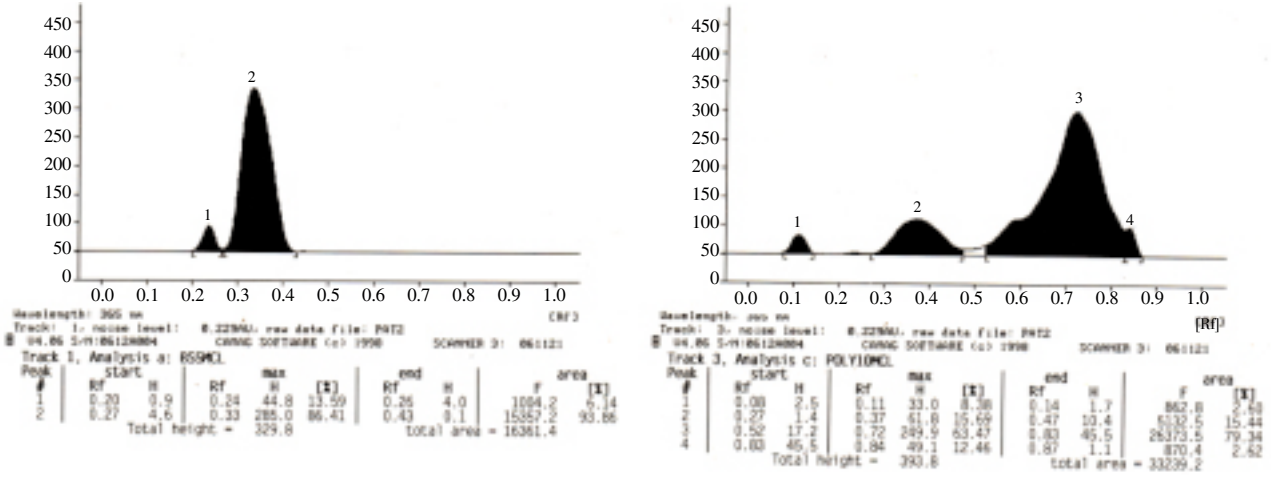

Figure 1. HPTLC chromatogram of standard berberine marker compound and *A. vera* extract.

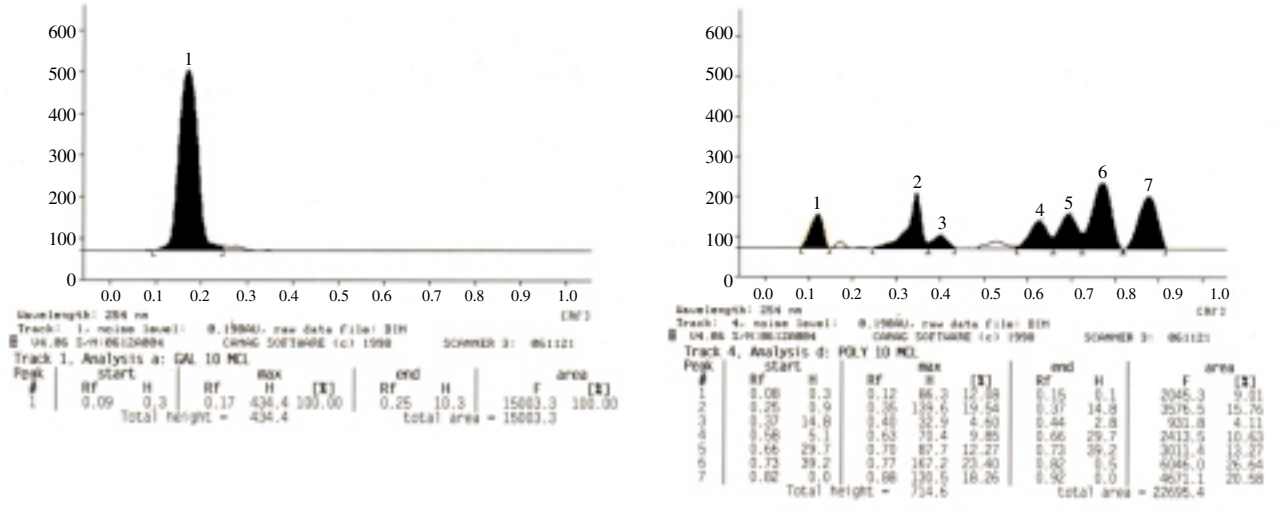

Figure 2. HPTLC chromatogram of standard gallic acid marker compound and *A. vera* extract.

a standard and the total flavonoid and phenol content of *A. vera* extract was found to be 1.9% and 13.11%. Loss on drying was 5.41%, where as solubily in water was 81.1% and total ash was found to be 7.12%. Heavy metal analysis showed that the level of lead, arsenic, mercury and cadmium complie the standard level. Microbiological assay showed that total bacterial count, yeast and moulds contents were found to be under the limit whereas *Escherichia coli (E. coli)* and *salmonella* was found to be absent in the extract. For quantitative analysis through HPTLC techniques, optimization of solvent system was done using combination of solvent system of varying polarity. n-Propanol: formic acid : water (90: 1: 9) was found to be suitable for quantitative analysis of berberine and toluene: ethyl acetate: formic acid (7:5:1) for gallic acid through HPTLC techniques. The content of berberine and gallic acid in *A. vera* extract were found to be 2.74% and 0.543% w/w.

4. Discussion

Phytoconstituents such as alkaloids, flavonoids, tannins, phenols, saponins, and several other aromatic compounds in the plants serve a defense mechanism against prediction by many microorganisms, insects and other herbivores[15]. These bioactive compounds are known to act by different mechanism. Tannins bind to proline rich proteins and interfere with the protein synthesis. Flavonoids are hydroxylated phenolic substance known to be synthesized by plants in response to microbial infection and found to be effective against a wide array of microorganisms in in vitro study. Coumarins are also known act against gram positive bacteria and it is produced in carrots in response to fungal infection which could be attributed to its antimicrobial activity. Antimicrobial property of saponin is due to its ability to cause leakage of proteins and certain enzymes from the cell. Steroids have been reported to have antibacterial properties, the correlation between membrane lipids and sensitivity for steroidal compound indicates the mechanism in which steroids specifically associate with membrane lipid and exerts its action by causing leakages from liposomes[15]. Heavy metals are important environmental pollutants and many of them are toxic even at very low concentrations. Heavy metal accumulations in edible plants are commonly reported by scientist to alert the public on potential risks of toxicity effects.Heavy metals enter the biological cycle through the roots and leaves of plants and are enriched in various plant organs. They can directly affect plant growth and an excess dietary intake of contaminated plants could also be dangerous for the health of humans and animals[5].

Several epidemiological studies suggest that plants rich in antioxidants play a protective role in health and against diseases and their consumption lowered risk of cancer, heart disease, hypertension and stroke. The curative properties of medicinal plants are due to the presence of different phytoconstituents such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols *etc*[16]. Tannins are known to be useful in the treatment of inflamed or ulcerated tissues, cancer, mild anti-septics. Flavonoids are considered as potential antioxidants and have protective action against allergies, inflammation, free radical, platelet aggregation, microbes, ulcers, hepatoxins, viruses and tumor[17-20]. Saponins showed potential anti-inflammatory, coagulant, antidiabetic, antioxidant, aldose reductase inhibitory activity and cholesterol binding properties[21-24]. Quality evaluation of herbal preparation is a fundamental requirement of industry and other organization dealing with ayurvedic and herbal products. The growing use of botanicals needs to develop standards of quality and manufacture. According to WHO guidelines, an herbal product needs to be standardized with respect to safety before releasing it into the market[25]. HPTLC is an inexpensive method for separation, qualitative identification, or semi-quantitative analysis of samples and it can be used to solve many qualitative and quantitative

analytical problems in a wide range of fields, including medicine, pharmaceuticals, chemistry, biochemistry, food analysis, toxicology and environmental analysis[26].

From the above findings, we can interpretate that the *A. vera* extract contained considerable amount of phenol, flavonoids, berberine and gallic acid. In future this study will be helpful for the quantitative determination of phytoconstituents in *A. vera*.

Conflict of interest statement

The authors report no conflict of interest.

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