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Phytochemical study in ethanolic leaves extract of *Aloe vera* using Gas chromatography

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Article History:	ABSTRACT
Received on: 20.09.2018 Revised on: 25.02.2019 Accepted on: 28.02.2019 Keywords:	Medicinal plant <i>Aloe vera</i> are reported to have anti-cancer properties. In the present work, a phytochemical study in <i>Aloe vera</i> was evaluated qualitatively and further analysed using gas chromatography. <i>Aloe vera</i> leaves were washed, shade dried and homogenized to get a filtrate which was extracted in ethanol using Soxhlet apparatus. The ethanolic extract was subjected to various phytochemical tests and also evaluated using gas chromatography. As a result, the qualitative phytochemical analysis of <i>A.vera</i> ethanolic leaves extracts confirmed the presence of alkaloids, flavonoids, carbohydrates, proteins, saponins, phenols, terpenoids and phytosterols. Gas chromatographic analysis of <i>A.vera</i> ethanolic leaves extracts showed all these eight compounds. All these compounds are of pharmacological importance as they possess properties such as anti-cancer, anti-diabetic, analgesic, antibacterial, and antifungal activity. Future work is essential to explore its therapeutic applications.
<i>Aloe vera</i> , Anti-cancer activity, Flavonoids, Gas chromatogram, Tannins	



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INTRODUCTION

India has a conventional method in developing drugs from medicinal plants. Worldwide, herbal drugs are prescribed nowadays widely due to their effectiveness, minimal side and relatively low cost even when their biologically active compounds are unknown (Modak *et al.*, 2007). Increased investigations on plants phytochemical constituents are found to be efficient against various diseases as a source of human disease management (Kumar *et al.*, 2012). The World Health Organization (WHO)

declared that up to 80% of developing countries among the world's population uses locally available plant resources for their primary healthcare when compared to western pharmaceuticals. In addition, herbal drugs that have been isolated or derived from natural sources based on their use in traditional medicine are approved by various regulatory agencies (Butler, 2004).

Plant natural compounds extracted from different Indian plants have been reported to possess anti-cancer properties. Researches are being carried out throughout the world to find a lead compound which can block the production of cancer cells in humans. Phytochemicals such as flavonoids, terpenoids and steroids have received considerable attention due to their diverse pharmacological properties, which include cytotoxic and chemopreventive effects (Farombi *et al.*, 2011). The isolation of the vinca alkaloids, vinblastine and vincristine from the Madagascar periwinkle, *Catharanthus roseus* introduced a new era in the use of plant material as anticancer agents (Moudi *et al.*, 2013).

Aloe vera is one among such herbs that contain more than 200 biologically active substances. These plants are widely distributed around the world, especially in African and eastern European continent. Its leaves gel is used as a folk medicine for over 2000 years in China, India and Japan. This plant belongs to xerophyte with 99% water content and remaining one percent is solid content including water- and fat-soluble vitamins, minerals, enzymes, simple/complex polysaccharides, phenolic compounds, and organic acids (Feminia *et al.*, 1999). *A. vera* gel contains polysaccharides with glucose and mannose molecules in the form of linear chains. This chain contains more concentrated mannose molecules known as polymannans. It's attributed to the fact that the polysaccharides found in the inner leaf parenchymatous tissue are responsible for *aloe vera* medicinal properties (Ni *et al.*, 2004)

Aloe vera contains anthraquinone compound known as aloin is known for its remarkable chemoprotective activity. According to Hamiza *et al.* (2014), the research performed in cancer-induced Wistar rats' colon, aloin compound act as anticancer therapeutic which was effective against 1, 2-dimethylhydrazine-induced pre-neoplastic lesions of Wistar rats where aloin inhibited vascular endothelial growth factor (VEGF) secretion in cancerous cells. *Aloe-emodin* (AE) is another natural compound present in *Aloe vera* that found to possess anticancer functions (Lin *et al.*, 2011). On the basis of the above facts and information, the present work has been designed to evolve the strategy for the identification of such bioactive compounds isolated from medicinal plant *Aloe vera* against cancer with the objectives to analyse the phytochemical constituents of *A. vera* by the qualitative method and to quantify the phytochemical constituents of *A. vera* by gas chromatography.

MATERIALS AND METHODS

Extraction of plant materials

The plant was obtained from Mahadhanapuram, Karur district, Tamilnadu. It was taxonomically determined and verified by the rapinat herbarium and centre for molecular systematics, St Joseph College, Trichy, Tamil Nadu, India with number SR001. *Aloe vera* plant was washed, leaves were taken and dried under shade for seven days. They were then grounded well to powder. The powdered *aloe vera* sample (500g) was treated with 80% ethanol at 70°C using Soxhlet apparatus and continued the extraction for 24 h. The ethanolic extract was filtered and kept in a hot air oven at 40°C for 24 hours to evaporate the ethanol, and finally, a residue was obtained. The final residue was placed in an airtight container and

stored in a deep freezer for future use (Elgorashi *et al.*, 2004).

Preliminary qualitative phytochemical analysis

Preliminary qualitative phytochemical analysis of compounds such as alkaloids, steroids, triterpenoids, glycosides, carbohydrates, flavonoids, tannins, phlobatannins, antiquinones and saponins were carried out to identify the secondary metabolites present in the ethanolic leaves extract of *Aloe vera*. The methods were as described below, according to Harborne, (1998).

Test for Alkaloids

About 1.36g of mercuric chloride dissolved in distilled water and 5 g of potassium iodide were mixed together and made upto 100 ml with distilled water. To this mixture, 1 ml of the plant extract was added to form a pale precipitate that showed the presence of alkaloids.

Test for Flavonoids

To 1ml of the plant extract, 1ml of 10% lead acetate was added to give yellow precipitate that favours the presence of flavonoids.

Test for steroids

To 1ml of the plant extract, about 2ml of acetic anhydride and sulphuric acid were added to form bluish-green colour that confirmed the presence of steroids.

Test for glycosides

To 1ml of plant extract, about 1 ml of glacial acetic acid containing 1 drop of ferric chloride solution were dissolved and was then under layered with 1ml of concentrated sulphuric acid. A brown ring formed at the junction indicated the presence of a deoxy sugar.

Test for reducing sugars

To 1ml of the plant extract, about 0.5 ml of each of Fehling's reagent A and B were added and heated in a water bath for 10 min. A red brick precipitate formed indicates a reducing sugar.

Test for anthraquinones

About 1 ml of plant extract was added to 5 ml of chloroform. The extract was mixed and filtered. The filtrate was shaken with an equal volume of 10% of ammonia solution to form violet or red colour that indicates the presence of anthraquinones.

Test for Saponins

To about 1ml of plant extract, a drop of sodium bicarbonate was added, mixed well for 3 min to

form a honeycomb-like froth that showed the presence of saponins.

Test for Tannins

The green colour precipitate was formed when about 1ml of the plant extract was stirred with 2ml of distilled water and few drops of FeCl₃, which indicated the presence of tannins.

Gas chromatography Analysis

Ethanollic extract of *Aloe vera* was performed by the GC technique (Abdel-Aal *et al.*, 2012). The GC-analysis was carried out using a Clarus 500 Perkin-Elmer (Auto System XL) Gas Chromatograph equipped, coupled to a mass detector where the instrument set to a temperature of 110°C respectively for 2 min. The oven temperature then raised up to 280°C, with an increase of 5°C/min, and maintained for 9 min. Injection port temperature ensured at 250°C. Helium flow rate as 1 ml per min with the ionization voltage was about 70 eV. The samples were injected in split mode as a 10:1 ratio. Mass Spectral scan range was set at 45-450 (mhz). The chemical components were identified by Gas Chromatography.

RESULTS AND DISCUSSION

Phytochemical screening was investigated in confirming the presence of different phytochemicals from ethanolic leaves extract of the *A.vera*. The qualitative phytochemical analysis of ethanolic *A.vera* leaves extract revealed the presence of alkaloids, flavonoids, carbohydrates, proteins, saponins, phenols, terpenoids, phytosterols in test plant extracts, as shown in Figure 1.



Figure 1: Results for qualitative phytochemical analysis of Aloe vera

These phytochemicals are involved in plant defense mechanism against various microorganisms with steroids involved in anti-bacterial properties, flavonoids with anti-oxidant function and tannins are known to be involved in the anti-cancer action (Patel *et al.*, 2012). Phenolic compounds are involved in scavenging free

radicals with their reducing properties. In addition, flavonoids also prevent oxidative cell damage. The results are in line with the previous results of Wintola *et al.* (2011).

GC- Analysis

The phytochemical compounds present in the *A.vera* ethanolic extract was determined by GC analysis. In total, eight compounds were analysed from the ethanol extract of the *A.vera* by GC chromatogram, as shown in Figure 2. Chromatogram with their retention time (RT), concentration (%) present in the extract was presented.

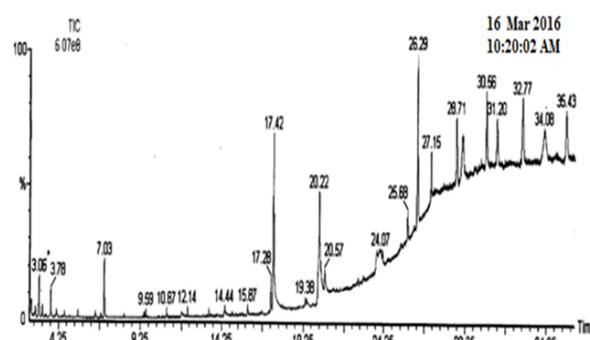


Figure 2: Gas chromatogram showing the peak symmetry of various phytochemicals

All these compounds have pharmacological properties with anti-inflammatory and antioxidant activity. The result confirmed the presence of such phytochemicals in *A. vera*, which might be important for its anticancer activity. The results further indicate that this medicinal plant would be studied completely for its beneficial effects which could be utilized to create a healthy environment. Other previous reports available on this green plant emphasize their effectiveness as chemotherapeutants (Surjushe *et al.*, 2008; Shelton *et al.*, 1991). A true fact about the phytochemicals is that apart from its role as therapeutic agents, they also have great value in incorporating new sources of phytochemical compounds for the synthesis of complex chemical substances.

Gas chromatography (GC) is used for a direct test of compounds existing in medicinal plants. Nowadays, GC-MS studies have been employed in rising amount for analysis of such medicinal plants as this study is efficient and proved to be a valuable method for the determination of non-polar components, volatile essential oil, fatty acids, lipids and alkaloids (Kalt *et al.*, 2014). The present study results are in accordance with previous studies.

CONCLUSION

These studies have proven that the *A.vera* ethanolic leaves extract contain some active ingredients with the potential of being anticancer

agents. Further work should be carried out on the characterization of specific antioxidant and anticancer components of *A.vera* and evaluation of their therapeutic significance in the prevention of diseases induced by oxidative stress.

Conflict of interest: None declared

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