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A study of phytochemicals, bioactives and *In vitro* antioxidant potential of *Aloe vera*

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Abstract
The overall goal of this work was to pursue antioxidant activity, phytochemical compounds, and total phenolic contents of *Aloe vera (L.)* extracts. The present protocol demonstrated that anthraquinone of *Aloe* extracts can be used as a plant pesticide. Phytochemical analysis showed the higher amount of flavonoids present in all the three extracts water, methanol and ethanol compared to other secondary metabolites. Total phenolic content were determined by using Folin Ciocalteu method in all the three extracts. The water extract of *Aloe* were detected highest phenolic compounds as 42.1±2.8 gallic acid equivalent (GAE) /g fractions. However the antioxidant activity was evaluated by using phosphomolybdenum assay. The antioxidant activity of methanol extract of *Aloe* showed higher phosphomolybdenum reduction as 84.7 mg Tannic acid equivalent (TAE)/g compared to water and ethanol extracts. Total anthraquinone content was evaluated by using UV spectrophotometer and demonstrated that anthraquinone has an effect to acts as a potential pesticide for plants.

Keywords: *Aloe vera (L.)*, antioxidant, anthraquinone, phytochemical and phenolic compound

Introduction
Natural products or plant products are an important source of medicine, especially traditional medicine in the form of pure compounds or homogenized extracts which provides opportunities for new drug discoveries (Thangaraj P 2016) [18]. In the past few years, phytochemicals have been extensively studied as active constituents of many drugs (Odeja O *et al*. 2015) [13]. There is a significant increase in the use of medicinal plants and their products due to the presence of bioactive compounds like flavonoids, alkaloids, tannins, anthraquinone, and natural antioxidants (Odeja O *et al*. 2015; Odeja O *et al*. 2016) [13, 14]. Antioxidants are substances that have the ability to scavenge free radicals and slow down or prevent tissue damage (Senguttuvan 2014) [15]. *Aloe L*. is one of the popular genera originated from the old world (Salehi B *et al*. 2018) [16]. *Aloe vera (L.) Burm f.* (Aloe barbadensis miller) is a tropical perennial succulent drought-resistant xerophyte species. Since centuries Aloe plants have been widely known and used as an herbal medicine for its curative and therapeutic properties. Today they account for over 75 active phytochemicals from the inner gel of the plant leaf (Nejatzadeh Barandozi F 2013) [12] and also contain several anthracenes and chromine derivatives such as aloin A, aloin B, anthraquinone and 7 hydroxyaloin (Choi J *et al*. 1996) [7]. Studies demonstrated that Synthetic insecticides are replaced by plant extracts as botanical pesticides for safer pest management (Amoabeng B.W *et al*. 2019) [3]. Studies have been reported that anthraquinones derived from natural source is used as agricultural pesticides and crop protectants (Shelagh T De Liberto2016) [17]
In this study, qualitative and quantification of phytochemical activity is determined and first attempt to understand the scope of anthraquinone from *Aloe vera (L.)* as a potential pesticide for plants in the future.

Materials and Methods
Collection of aloe leaves
The plant *Aloe vera (L.*barbadensis*)* collected from Rajasthan was identified and authenticated at the TNAU (Tamil Nadu Agricultural University) and further maintained in the Greenhouse at Genewin Biotech, Hosur (Aarthi Pugazhendhi & D. Satish Sekar 2017) [1]. Fresh leaves from this species produced from tissue culture technology were collected and washed thoroughly and air-dried.

Preparation of aloe extract
The dried, powdered plant sample was extracted sequentially with multiple solvents 80% ethanol, 80% methanol solution and water using Soxhlet apparatus (Thangaraj P 2016; Odeja O *et al*. 2016) [18, 14] at temperature 80 °C for 72 hours. Respective solvent extracts were collected under 24^0C to 28^0C temperature and maintained at 4^0C for further studies (Odeja O *et al*. 2015) [13].

Preliminary qualitative phytochemical analysis
The extracts were subjected to qualitative phytochemical screening for the identification of flavonoids, alkaloids, Phylobatannins, Reducing sugar, Steroids, Saponin, anthraquinones (Senguttuvan *et al*. 2014; NS & PSS 2018; Aiyegoro O. A. & Okoh A. I. 2010; Auwal *et al*. 2014) [15, 10, 6, 5]

Quantitative analysis of Phytochemicals
Quantification of total phenol content
The total phenolic content of plant *Aloe vera (L.*barbadensis) was estimated using Folin Ciocalteu reagent by the method of (Nasseri M A *et al*. 2019) [11]. Different solvent extracts of aloe plants were taken separately and for each 1 ml of extract 5 ml of Folin's reagent and sodium carbonate solution were added. The reaction mixture was shaken well and kept for 30 min. After incubation OD was recorded at 765 nm. The calibration curve was established using standard Gallic acid. The total phenolics is calculated from a prepared standard curve in terms of mg/g GAE of dry extract.

Evaluation of antioxidant activity

The total antioxidant potential of the extracts was assessed spectrophotometrically by the phosphomolybdenum assay (Afsar T *et al*. 2018) [4]. About 1 ml of each sample extract (0.5 mg ml^{-1}) was added with a 3 ml phosphomolybdenum reagent solution. The blank solution was prepared only with a reagent solution without extract. The sample extracts and blank were incubated at 95°C for 2½ h. After incubation absorbance of the extracts was recorded at 695 nm against a blank solution. The total antioxidant potential evaluated as the optical density of the sample at 695 nm. Higher the antioxidant activity higher the absorbance. Total antioxidant capacity is expressed in terms of TAE (Wan C *et al*. 2011) [20].

Isolation and quantification of anthraquinone from aloe

Aloe peel powder was soaked in 60% ethanol overnight. The supernatant and residue were separated by centrifuge at 4000 rpm. After that filtrate was subjected to reduced pressure distillation to remove ethanol and water. A colloidal aloe extract was obtained. 20% sulfuric acid and chloroform was added to that extract and then was refluxed. After chloroform got dehydrated, a yellowish-brown colloidal part was obtained as a crude extract of anthraquinone (Tan Z *et al*. 2012) [19].

Determination of total anthraquinone content

Total anthraquinone content was determined using a UV spectrophotometer. The calibration curve is established by analyzing standard samples with a known amount of dihydroxyanthraquinone (Gibson M.R &Schwarting A.E. 1947) [8]. The maximum OD measured at 220 nm. Thus, LOD (limit of detection) of total anthraquinone concentration was obtained using

$$DL=3.3\sigma/S$$

Where,
DL-Limit of Deviation
σ-Standard deviation
S-Slope of calibration curve respectively.

Results

Preliminary qualitative phytochemical analysis

In this study phytochemical investigation of different solvent aloe extracts observed various bio actives like phylobatannins, reducing sugar, terpenoids, flavonoids, alkaloids, steroids, and Saponins (Table 1). However, from the above table, all secondary metabolites were detected in all three ethanol, methanol, and water extracts. Compared to the amount of all other phytochemicals, flavonoids showed a higher amount in all three extracts. Whereas other phytochemicals showed in moderate quantity.

Table 1: Preliminary phytochemical screening of aqueous, ethanolic and methanolic extracts of aloe leaf

Phytochemicals	Water extract	Ethanolic extract	Methanolic extract
Phylobatannins	+++	+++	++
Reducing sugar	++	++	++
Terpenoids	++	+++	+++
Flavonoids	++++	++++	++++
Alkaloids	+++	++	+++
Steroids	++	+++	++
Saponins	+++	++	++

++++: highly present, +++: moderatelypresent, ++: low

Total phenolic content

Phenolic compound with redox properties responsible for antioxidant activity, as a basis phenolic content was measured using the Folin-Ciocalteu reagents in each extract. The results were derived from calibration curve y = 0.118x - 0.005, R^2= 0.9861 at 765 nm of gallic acid (0-250 µg/ml) and the total phenolic content is expressed in terms of gallic acid equivalents per gram (GAE/g) dry plant material (Fig 1 and Fig 2)

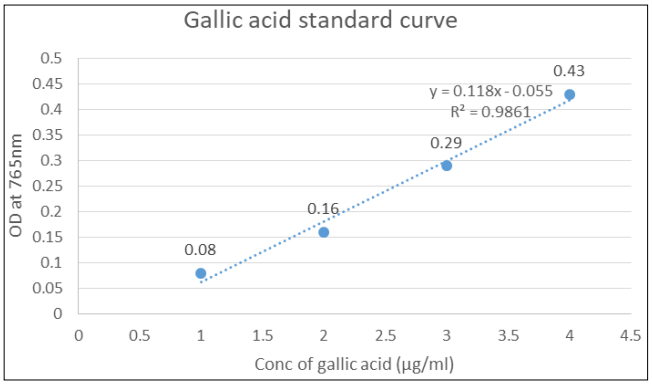

Fig 1: Gallic acid standard curve

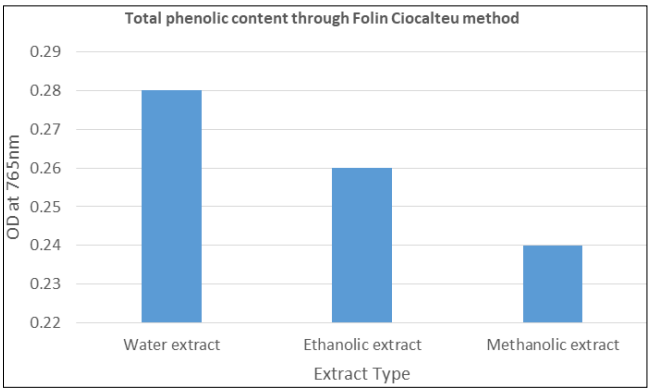

Fig 2: Total phenolic content

The phenol compounds in ethanol and methanol extracts ranged 37.7±2.9 mg in GAE/g and 39.2±3.0 mg in GAE/g respectively. The phenol content in aqueous extract ranged 42.1±2.8 GAE/g (Table 2). This result suggests that plant components can be polar or non-polar in nature and relies on the extraction procedure and solvents used (Aryal S *et al*. 2019) [2].

Table 2: Total phenolic content through Folin Ciocalteu method

Extract type	Absorbance at 765nm	Total phenolic content mg in GAE/g
Water extract	0.28	42.1±2.8
Ethanolic extract	0.26	39.2±3.0
Methanolic extract	0.24	37.7±2.9

Total antioxidant potential

Total antioxidant capacity is obtained through Phosphomolybdenum assay (Kasangana P B *et al*. 2015) [9] for each extract. In the presence of extracts, Molybednum (VI) is reduced to Molybednum (V) and forms green color Phosphomolybdenum (V) complex which shows maximum absorbance at 695 nm (Wan C *et al*. 2011) [20]. Antioxidant capacity is expressed in terms of the Tannic acid equivalent (TAE) in mg/g of the extract. The result is derived from the

standard Curve y = 0.0016x - 0.052, R^2 = 0.9834 (Fig 3 and Fig 4) Where,

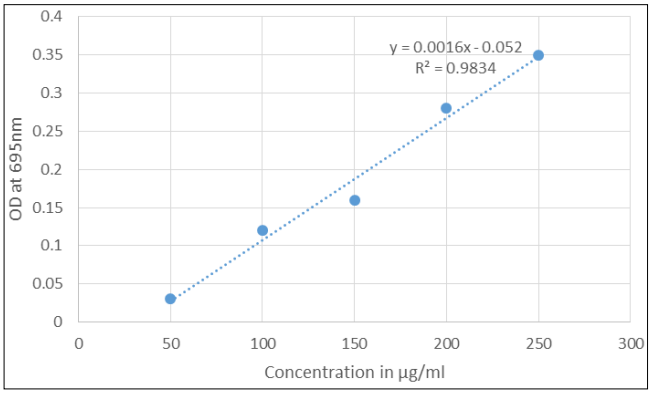

Fig 3: Tannic acid standard curve

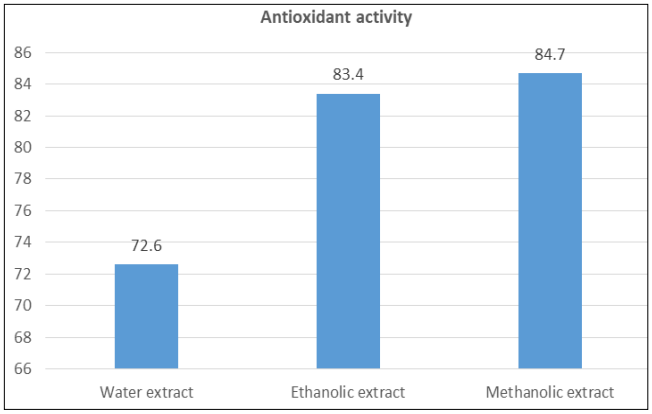

Fig 4: Antioxidant potential

X is the concentration of sample and A (y) being the absorbance of a sample Overall, the methanolic extract (84.7 mg TA/g extract) showed higher antioxidant potential than ethanol (83.4 mg TA/g extract) and water extract (72.6 mg TA/g extract) (Table 3).

Table 3: Antioxidant activities of water, ethanolic, and methanolic extract

Extract	Absorbance at 695nm	Phosphomolybdenum (mg TA/g extract)
Water extract	5.63	72.6
Ethanolic extract	6.54	83.4
Methanolic extract	6.64	84.7

Conclusion

This study revealed that the presence of phytochemicals in *Aloe vera (L.)* plant extract. In *Aloe* ethanol extract, the amount of flavonoids exceeds the amount of all the other phytochemicals and also detected that the more amount of phenolic compounds was observed in water extract than the ethanolic extract and further less quantities detected in methanolic extracts. This study demonstrated that the antioxidant activity of *Aloe vera (L.)* showed significant effect on all the water, ethanolic and methanolic extracts. Anthraquinone was successfully isolated from *Aloe vera (L.).* UV spectrophotometer is a widely used method to evaluate the total anthraquinone content. It was found that the anthraquinone has an effect to acts as a potential pesticide. Therefore, this research has opened a new door for using anthraquinone as a potential pesticide for plants.

Ethical statement

This paper is the original work that has not been previously published elsewhere. The paper is not currently being considered for publication elsewhere. Paper reflects the own research and analyses in a truthful and complete manner.

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