ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 08, Issue, 02, pp.18922-18934, February, 2018

ORIGINAL RESEARCH ARTICLE OPEN ACCESS

INVESTIGATIONS ON BIOCHEMICAL COMPONENTS OF *ALOE FEROX* MILL LEAF EXTRACTS AND THEIR ANTIOXIDANT AND ANTI-INFLAMMATORY PROPERTIES

[1,*]Chandra Sekhar Singh, B., [2]Mulugeta Negara, [3]Alemu Lencho and [4]Roja Rani, A.

[1]Department of Plant Sciences, College of Agriculture and Veterinary Sciences, Ambo University, Ambo, Post Box, 19, Ethiopia
[2]College of Agriculture and Veterinary Sciences, Ambo University, Ambo, Post Box: 19, Ethiopia
[3]Department of Plant Sciences, College of Agriculture and Veterinary Sciences, Ambo University, Ambo, Post Box, 19, Ethiopia
[4]Department of Genetics and Biotechnology, Osmania University, Hyderabad, India

ARTICLE INFO

Article History:
Received 19th November, 2017
Received in revised form
25th December, 2017
Accepted 24th January, 2018
Published online 28th February, 2018

Key Words:
Aloe ferox,
Phytochemical screening,
Antioxidant,
Anti inflammatory
and Biochemical.

ABSTRACT

The present investigations were carried out to understand the phytochemical screening, antioxidant, anti inflammatory and biochemical properties of *Aloe ferox leaf* extract by using various organic solvents. The powdered leaf material was subjected to extraction of phytochemicals by soxhlet method with chloroform, ethyl acetate and methanol. The phytochemical result shows that the active compounds presence in high concentration, such as phytosterols, lactones, flavonoids, phenolic compounds, tannins and glycosides. This methanolic extract of *Aloe ferox* shows promising antioxidant DPPH radicals decreased in DPPH free radical scavenging assay and these extracts also shows more flavonoids than the other solvent extracts of *Aloe ferox* leaf, which also shows the reductive capabilities when compared with BHT. The methnol extract of *Aloe ferox* (AFME) shows significant inhibition against carrageenan-induced paw edema in the dose dependent method. *Aloe ferox* exhibits highest anti-inflammatory and analgesic activities in the highest dose (500 mg/kg) tested. This dose level exerted highest anti-inflammatory activity of 0.61% and 0.52% for carrageenan induced paw edema respectively. The analgesic activity was observed as 38% and 50% for the 250/500 mg/kg dose in phase 3 and 4, respectively and increase in the paw volume. The anti-inflammatory activity exerted by AFME suggests that it could affect by bradykinin, kinnin, prostaglandin and synthesis of lysozymes. The methanol leaf extracts were structural elucidation and isolated an anticancer compound of acemannan were analyzed by HPLC and ^{13}C NMR at the mobile phase of methanol: acetnitrile: water (in the ratio of 25:35:40) and provided a total of 6 peaks at a retention time 4.545 min. From the HPLC results, the highest peak was observed at retention of 563.778 mAU. ^{13}C-NMR results show a number of peaks in between δ 16.832 to 71.196 and δ 174.6 to 176.777 (indicating presence of aromatic rings), the highest peak was observed between 48.996 - 49.007.

Citation: Chandra Sekhar Singh, B., Mulugeta Negara, Alemu Lencho and Roja Rani, A., 2018. "Investigations on biochemical components of Aloe *ferox* Mill leaf extracts and their antioxidant and anti-inflammatory properties", *International Journal of Development Research*, 8, (02), 18922-18934

INTRODUCTION

Aloe ferox is one of the species of Aloe and widely distributed in South Africa. For centuries the leaf extracts of *Aloe ferox* has been used therapeutically for the treatment of many human diseases.

Corresponding author: **Chandra Sekhar Singh, B.,**
Department of Plant Sciences, College of Agriculture and Veterinary Sciences, Ambo University, Ambo, Post Box, 19, Ethiopia.

Medicinal plants have been used as medicines for long time in different forms of crude drugs. *Aloe ferox* is a potential crop in arid regions and it will remain beneficial as a household remedy. Fresh leaf gel can easily be prepared and applied to wounds. The *Aloe ferox* leaf extract revealed the presence of bio-active constituents which are known to exhibit medicinal as well as physiological activities. It may be an agent for treating oxidative stress related disease along with potential

application as a lead compounds for designing potent anti-inflammatory activity and they can be used for treatment of various diseases such as diabetes, antiviral, antifungal and anti-inflammatory.Since ancient days the use of active compounds from plant extracts which may serve as a source of synthesizing modern drugs with desirable properties involve in treatment of human diseases (Chakrapani *et al*. 2014). *Aloe ferox* Mill has one hundred and thirty biological active compounds and has more nutritional values than *Aloe vera*. It is commercially known as *Cape aloe*. Ethno pharmacological research findings showed that the genus of *A. ferox* and *Aloe vera* has been used in the treatment of human health diseases (Du Loots *et al*. 2007; Chen *et al*. 2012: Celestino *et al*. 2013). It has many medicinal values such as antibacterial, antifungal, antiviral, wound healing, antidiabetic, antitumor, cardiovascular diseases, neurodegeneration to anticancer property (Chandrasekhar *et al*. 2013). It was a local traditional medicine adopted by colonists at the Cape of Good Hope and it was the first exported product to Europe in the eighteenth century. A solid resin obtained from its leaf latex is soluble in alkalis, conc. acetic acid, glycerin, absolute alcohol and hot ethanol. It's part soluble in boiling water and it's much insoluble in ethyl ether. The major components are polysaccharides, cellulose, hemicellulose, glucomannans, mannose derivative, and acetylated compounds. *Aloe ferox* plant has a greater concentration of biologically active compounds (Choi and Chung 2003).

Its healing properties are much more powerful than those of the *Aloe vera* plant. Aloin or barbaloin is known as the main laxative component of Aloe preparations and it has generally been used as a key component for the quality control of pharmaceuticals containing Aloe. The barbaloin content of latex from different *Aloe species* was accessed by a number of methods and found to be between 10-25% on a dry weight basis of the latex and about 1% on a leaf dry weight basis. The leaf weight increases during winter and decrease in summer. Seasonal fluctuations have been attributed to water availability (Waller *et al*. 2004). The total gel content of active ingredients are affected by seasonal, climatic and soil variations (Ramachandra and Srinivasa Rao, 2008). The anthraquinone content is subjected to seasonal variation in Aloe. In present scenario, there has been an increasing interest in finding natural products, antioxidants, which may exhibit the human body from free radicals and retard the progress of many chronic diseases (Milady and Damak, 2008). The particular chemical mediators vary with the sort of inflammatory process. Some of the compounds are amines, histamine, serotonin, lipids and prostaglandins and small peptides such as Kinins (Lucas *et al*. 2006) has suggested that many anti-inflammatory drugs might exert a number of their effects by scavenging oxidants and decreasing formation of reactive oxygen species (ROS) by activated phagocytes. The inflammation has deferent phases and the 1st phase was caused by an increase in vascular permeability, second one by infiltrate of leucocytes and therefore the third one by tumor formation. Patel Pinal *et al* (2011) reported that the second phase of edema is useful and sensitive to steroidal and non-steroidal anti-inflammatory agent in clinical trials. Aloe resin B modulates melanogenesis via competitive inhibition of tyrosinase, thus showing promise as a pigmentation-altering agent for cosmetic or therapeutic applications (Mabusela *et al*. 1990). Medicinal plants led to the isolation of early drugs like cocaine, codeine, digitoxin, and antimalarial in drug discovery, in addition to that some other drugs like morphine are still in use (Samuelson, 2004; Newman *et al*. 2000). More recently, drug discovery techniques have been applied to the standardization of herbal drugs, to elucidate analytical marker compounds (Balunas and Kinghorn, 2005). Hence, medicinal plants have been playing an important role as a source of medicine for thousands of years. The World Health Organization (WHO) estimates that up to 80 percent of people still rely mainly on traditional remedies such as herbs for their medicines (Arun Kumar and Muthuselvam, 2009). The particular chemical mediators vary with the sort of inflammatory process and include like amines, histamine, serotonin, lipids and prostaglandins and small peptides such as Kinins (Cotran *et al*. 2001). Lucas *et al*. (2006) has reported that many anti-inflammatory drugs might exert a number of their effects by scavenging oxidants and decreasing formation of reactive oxygen species (ROS) by activated phagocytes. The anti-inflammatory activity exerted by *Aloe ferox* methanol extract (AFME) suggests that it could have affecting by bradykinin, kinnin, prostaglandin, and synthesis of lysozymes. The combination of antioxidants and inflammation stems from the recognition that, free radicals are produced during the inflammatory process by phagocytosing cells and generated as by-products of the oxidative degradation of arachidonic acid. From this it can be inferred that the anti-inflammatory activity of AFME may be due to its antioxidant activity. The present study reveals that the chemical constituents from *Aloe ferox* leaf extracts by using different organic solvents and its anti-oxidative, anti-inflammatory and biochemical properties. These pharmacological and biochemical investigations will be helpful in projecting this plant as a therapeutic target in inflammation and cancer research.

MATERIALS AND METHODS

Collection of plant material: *Aloe ferox* seeds were purchased and authenticated from South African National Biodiversity Institute (SANBI), South Africa vide voucher no: SANB, South Africa: IQ.No:144/2011: IP.No.387/11. The germplasm was grown in research farm at Indian Immunologicals Ltd (IIL), Hyderabad. The two years old plants were used in this study and the leaves of the plant were made free from dust particles, dried under shade and powdered coarsely. 500 g of powdered leaves of *Aloe ferox* extractions were prepared by using different organic solvents. Then the powder was extracted using ethyl acetate, methanol and hexane as solvent (increasing order of polarity) by soxhlet apparatus. *Aloe ferox* was extracted continuously using soxhlet apparatus with methanol and ethyl acetate successively for about 48 hrs at 30°C. The extracts were concentrated under reduced pressure using rotary vacuum flash evaporator to get a constant volume. The concentrated extract was eluted with ethyl acetate, hexane and methanol in ascending order of polarity by performing column chromatography. The resultant extracts were used for this study.

Analysis of Phytochemical compounds: The phytochemical study was performed to identify the compounds like flavonoids, flavonols, alkaloids, saponins, total phenols, tannins, alkanes, pyrimidines, proanthocyanidins, fatty acids, indoles, phytosterols, aldehydes, dicarboxylic acids, ketones, organic acids and alcohols by some modified procedures of earlier studies (Shreya *et al*. 2013; Parmar *et al*. 2012).

The following tests were performed for phytochemical analysis:

Tests for Alkaloids

- ***Dragendorff's test:*** To the extract add 1ml of Dragendorff's reagent. An orange red colored precipitate indicates the presence of alkaloids.
- ***Wagner's test:*** To the extract, add 1 ml of Wagner's reagent is added. Reddish brown colored precipitate indicates the presence of alkaloids.
- ***Mayer's test:*** To the extract 1ml of Mayer's reagent is added. A dull white colored precipitate indicates the presence of alkaloids.
- ***Hager's test:*** To the extract add 3ml of Hager's reagent. Yellow colored precipitate indicates the presence of alkaloids.

Tests for Carbohydrates

- ***Molish test:*** To the extract, 1ml of α-naphthol solution was added and Conc. sulfuric acid was added along the sides of the test tube. Purple or Reddish violet color at the junction between the two liquids indicates the presence of carbohydrates.
- ***Fehling's test:*** To the extract, equal quantities of Fehling's solution A and B were added. Upon heating gently, a brick red precipitate indicates the presence of carbohydrates.
- ***Benedict's test:*** To the extract 5ml of Benedict's reagent, 8 drops of solution was added and mixed well. After that it was boiled for 2 minutes vigorously and cooled. Red precipitate indicates the presence of carbohydrates.

Tests for Proteins

- ***Biuret test:*** To the extract, 1ml of 40% sodium hydroxide and 2 drops of 1% copper sulphate solution was added. A violet color indicates the presence of proteins.
- ***Xanthoprotic test:*** To the extract, 1ml of Conc. Nitric acid (HNO_3) was added. When a white precipitate was formed, it is boiled and cooled. Then 20% of sodium hydroxide or ammonia was added. Orange color indicates the presence of aromatic amino acids.
- ***Lead Acetate test:*** To the extract, 1ml of lead acetate solution was added. A white precipitate indicates the presence of proteins.

Test for Amino acids

- ***Ninhydrin test:*** 2 drops of freshly prepared 0.2% ninhydrine reagent was added to the extract and heated. Development of blue color indicates the presence of proteins, peptides, amino acids.

Tests for Steroids

- ***Libbermann Burchard test:*** The extract was dissolved in 2ml chloroform in dry test tube. 10 drops of acetic anhydride and 2 drops of conc. sulphuric acid were added. The solution becomes red, and then blue and finally bluish green in color indicates the presence of steroids.
- ***Salkowaski test:*** The extract was dissolved in chloroform and equal volume of Sulfuric acid was added to it. Bluish red to cherry red color was observed in chloroform layer; whereas acid layer assumes marked green fluorescence indicates the presence of steroids.

Foam test for Saponnins

- The extract of 1 ml was diluted separately with 20 ml distilled water and shaken well in a graduated cylinder for 15 minutes where as1cm layer of foam indicates the presence of saponnins.

Test for Phenolic Compounds and Tannins

- Small quantities of alcoholic and aqueous extracts in water were tested for the presence of phenolic compounds and tannins with dilute ferric chloride solution (5%), 1% solution of gelatin containing 10% sodium chloride, 10% lead acetate and bromine solutions.

Keller-Killiani test for glycosides

- A total of 1 mL of glacial acetic acid, few drops of ferric chloride solution and conc. H_2SO_4 (Slowly through the sides of the test tube) were added to the extract. Appearance of reddish brown ring at the junction of the liquids indicated the presence of de-oxysugars.

Legal's test for lactones

- Sodium nitroprusside and pyridine added to the extract mixture. Then the mixture was treated with NaOH. Development of deep red colour indicated the presence of lactones.

Separation of compounds by column chromatography

The optimized gradient of solvents (methanol and ethylene acetate in 1:1 weight ratio) was used as mobile phase in silica gel adsorption column chromatography. The methanol extracted *Aloe ferox* leaf sample was adsorbed onto silica gel by grinding in a mortar and left for about 48 hours to dry. The column (diameter of 60 mm and a length of 48 cm) was packed with a solution of silica gel with n-butanol using the wet slurry method. The mesh size of silica gel adsorbent used was around $60 - 120\ \mu m$. This involves making ready a solution of silica gel, with n-butanol during this case, in a beaker and subsequently adding this unto the column till it is about three fourths filled. The solution was stirred for spreading and quickly added to the column before the gel settles. This method was accustomed to stop the tack of air bubbles. A ball of cotton was pushed into the column to settle the packed silica gel. A substantial amount of n-butanol: acetic acid: water (in the ratio of 4:1:1) was poured continuously into the column and allowed to drain but prevented from reaching the ball of cotton. The quantity collected was poured into the column. Periodically, a piece of rubber tubing was used to agitate the column to allow for the escape of trapped air bubbles. Concerning 16 fractions (20 ml each) are eluted and collected at 15 min interval in dry glass bottles. Elution was carried out with increasing polarity using chloroform, hexane, methanol and ethyl acetate. The elutes were collected around 200 ml from each fraction through column chromatography

separation (**Fig. 1** a). The fractions were collected and the resolutions of the compounds were checked on commercially available TLC plates. The fractions showing the same identity of the compounds (i.e., same Rf values) are mixed and then concentrated using rotavapor. The column fractions were once more tested with TLC chromatogram and the Rf values were determined (Tomer *et al*. 2009).

Extraction of compounds by Thin Layer Chromatography (TLC)

The Methanolic leaf extract of *Aloe ferox* obtained from column chromatography fractions were allowed to TLC experiment. 10 µL of extract was applied on silica gel plates. After drying, the plate was eluted with water-methanol-ethyl acetate (13:17:100 v/v). The plate was allowed to dry in air and observed for separation of compounds on it (Vanessa *et al*. 2013). The retention factor (Rf) was obtained and the plate was examined in ultraviolet radiation at 365 nm. It was used absolutely an aloin solution as reference standard. Resolutions of collected fractions were checked on commercially available TLC plates. The retention factor (Rf) is equal to the distance traveled by the plant extract/distance traveled by the solvent system.

Antioxidant activity (DPPH assay): The antioxidant activity of the *Aloe ferox* leaf extract was measured in terms of hydrogen-donating or radical scavenging ability by using the DPPH radical scavenging method (Milady and Damak, 2008). DPPH solution (1.5 ml) was incubated with 1.5 ml of *Aloe ferox* leaf extract fractions at various concentrations (0.01–1 mg).The reaction mixture was shaken well and kept in dark room for incubation at room temperature for 30 min. The control was prepared without any extract. The absorbance of the solution was measured at 517 nm against a blank (Shreya *et al*. 2013). The radical scavenging activity (RSA) was measured as a decrease in the absorbance of DPPH (1, 1-diphenyl, 2-picrylhydrazyl) and was calculated using the following equation:

$$\text{cavenging effects (\%)} = \frac{(1 - \text{absorbance of the test sample (517 nm)} \times 100}{\text{Absorbance of the control (517 nm)}}$$

Anti-inflammatory activity

Animals: Healthy SD (Sprague Dawley) adult male rats (150-200g) obtained from National Institute of Nutrition (NIN) Hyderabad, India. The animals were housed under standard laboratory conditions maintained at 25 ± 10^{o} C and under 12/12 h light/dark cycle and fed with standard pellet diet, water ad lib. The protocol was approved by the institutional animal ethics committee and by the animal regulatory body of the Indian Government (Registration No. 380/01/a/ CPCSEA) for this experiment. Drugs: Carrageenan (Sigma Chem.), Carboxy methyl cellulose (SD fine chem.) Ibuprofen (local market), were used during the experimental protocol.

Carrageenan-Induced Paw Edema method

Experimental design: Rats were divided into 4 groups; each group consists of 6 rats. Group I: Treated with 0.2 ml of 2% CMC daily for 1 week. Group II: Treated with Ibuprofen (intraperitoneally) 10 mg/kg body weight (b.w) daily for 1 week. Group III: Treated with methanolic extract of *Aloe ferox* leaves at the dose of 250 mg/kg body weight daily for 1 week. Group IV: Treated with methanolic extract of *Aloe ferox* leaves at the dose of 500 mg/kg body weight daily for 1 week. Acute inflammation was induced in all groups by injecting 0.1 ml of 1% (w/v) carrageenan into the sub-plantar region of the right hind paw of the rats (Venkatesa *et al*. 2001). On the 7th day, paw volume was measured 1 h prior to carrageenan injection using a plethysmometer from 0 to 3 h and after the carrageenan injection, the increase in the paw volume was measured and percentage inhibition was calculated.

$$\text{Percentage of inhibitition } = 100\,(1 - \frac{V_t}{V_c})$$

Where V_t is edema volume in test / standard compound V_c is edema volume in control (Mulla *et al*. 2010; Manjit *et al*. 2010).

Statistical analysis

The values were analyzed with the analysis of variance (one way ANOVA) to determine the significance of difference within the experimental groups. Methanolic extract of *Aloe ferox* leaves produced significant $P < 0.05$ analgesic activity at all test doses when compared to that control.

Chemical analysis

The NMR (Nuclear magnetic resonance: Bruker type AV 300 at 300 MHZ) technique was used to study the physical, chemical and biological properties of methanolic and ethylene acetate *Aloe fexox* plant extract and recorded ^{13}C-NMR spectra and δ values. The result graph was compared with the reference chart and possible functional group present in the plant were determined. The solvent used was $CDCL_3$ (Silverstein *et al*. 2005; Patra *et al*. 2012). ^{13}C NMR will be much sensitive than ^{1}H NMR because the^{13}C nuclei make up approximately 1% of the carbon nuclei on earth. Usually Organic compounds contain carbon, but, the C-12 nucleus does not have a nuclear spin, Because of that ^{13}C nucleus does due to the presence of an unpaired neucarbon-1tron. The ^{13}C, splits the carbon-13 peaks and poorer signal to noise ratio because of spin to spin coupling between a ^{13}C nucleus and the nuclei of ^{1}H atoms bonded to the ^{13}C. Each nonequivalent ^{13}C shows a different signal. A ^{13}C signal is split by the ^{1}H bonded to it according to the (n + 1) rule. It is very difficult to calculate there is often significant overlap between splitting patterns and signals. The usual operation of a ^{13}C-NMR spectrometer is a proton-decoupled mode and Coupling constants at 100-250 Hz.

The chemical compound analysis of the methanol and ethyl acetate extracts of *Aloe ferox* leaf was performed by using high performance liquid chromatography (HPLC). The HPLC system consists of BioSep- SEC- s2000 (**Fig. 1c**) prominence liquid chromatography pump (P680), SPD-20A prominence UV-PDA detector, autosampler ASI-100 injector fitted with 50 µl capacity fixed loop, chromatographic oven (TCC-100) and a photodiode array detector (PAD100) all from Agilent company, USA. The column used was Phenomenex luna 5 µ C_{18} (2) 100A (250 mm × 4.6 mm) at 28^{o}C temperature. The output signals were monitored and processed using chemstation software. The mobile phase optimized for the analysis was ammonium acetate and flow rate was 1 ml/minute and detection wave length was set at 232 nm (Patra *et al*. 2012).

The run time of the method was 4.545 minute and analytes were separated at the retention time 563.778mAU. The highest peak was also seen at the retention time. ^{18}C- HPLC Profile: Column: WATERS HR C18 300X3.9MM 6U; Mobile Phase: 50% Methanol in water; detection: 210 nm; flow rate: 1.0 ml/min. Desirable compounds were not identified in ^{18}C-HPLC.

RESULTS AND DISCUSSION

Phytochemical screening of Aloe ferox leaf extracts

Medicinal plants are used for the treatment of various diseases due to the presence of different phytochemicals. Extraction of components from plant material is depends on the polarity. The concentrations of compounds extracted in the different solvent extracts were significantly different from each other. Phenols, flavonoids, alkaloids, tannins, lactones, glycosides, saponins and ketones contents were found in both ethyl acetate and methanol extracts of *Aloe ferox*. These compounds aloin, aloe-emodin, and aloesin may be a contributing factor towards its antioxidant activity. Absence of flavonols and proanthocyanidins were observed in the methanol extracts when compared with the ethyl acetate extract. Phytosterols, fatty acids, indoles, alkanes, pyrimidines, organic acids, aldehydes, dicarboxylicacids and alcohols were present in methanol extract but absence in ethyl acetate extract. Phytochemicals and their quantities identified include various phenolic acids or polyphenols (Herraiz and Galisteo, 2004). The exudate of *Aloe ferox* contains 15–40% of anthrone 10-C-glucosides (anthraquinone derivatives) such as hydroxyaloins and aloin. Aloin is a mixture of the stereoisomers aloin A (barbaloin and aloin B isobarbaloin). Furthermore, the exudate contains the pyrone derivative aloenin and glucosylated 2-acetonyl-7-hydroxy-5-methylchromones. (e.g. aloesone, furoaloesone, aloe resin a, aloe resin B (aloesin) and aloeresin). *Aloe ferox* also contains glycosylated feroxidin (a tetralin) and feralolide (a dihydroisocoumarin). Aloin is a compound which is an inactive and responsible for laxative properties when it becomes an active aloe emodin anthrone by Eubacterium sp. Aloe may play a role in colorectal cancer in rodents because they have tumorigenic and genotoxic potential. The phytochemical screening results were shown in Table 1.

The chemical composition studies have been analyzed by few researchers on *Aloe vera* only, but the research related to *Aloe ferox* is very limited. Vogler and Ernst (1999) reviewed the phytochemical investigations and listed 75 potentially active constituents including vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acid and amino acids. The phytochemical analysis of *Aloe ferox* leaf extract revealed the presence of flavonoids, flavonols, alkanes, alkaloids, aldehydes, ketones, alcohols, total phenols, saponins, tannins, phytosterols, proanthocyanidins, fatty acids, indoles, pyrimidines, organic acids and dicarboxylic acids. The plants are used for the treatment of various diseases due to the presence of phytochemicals. The major leaf exudate compounds identified were aloeresin, aloesin, aloin A and aloin B, aloinoside B (Viljoen *et al*., 2001). The major (25-40%) constituent of aloe is the Hydroxyanthraquino derivatives aloin (barbaloin, a mixture of aloin A and B, the diastereoisomeric 10-C glucosides of Aloe-emodin anthrone) and 7-hydroxyaloin isomers.

Other constituents present in minor quantities include Aloe-emodin, chrysophanol, derivatives aloresin B (aloesin, upto 30%) with its p-coumaryl derivative aloeresins A and C and the aglycone aloesone Dagne *et al*., (2000). Seven of the eight essential amino acids required by human body are also present in aloe gel Chauhan *et al*., (2007) were reported. The concentrations of compounds extracted in different solvent extracts were significantly different from each other (Fig. 1b). It gives an indication that solvents possess different extracting capacity for each compound. Phenols, flavonoids, alkaloids, saponins and ketones contents were found in both ethyl acetate and methanol extracts of *Aloe ferox*. These compounds aloin, aloe-emodin, aloesin, Isobarbaloin and acemannan may be a contributing factor toward its antioxidant and anti cancer activity (Fig. 2 a-e). Extraction of components from plant material depends on the polarity. Absence of flavonols, proanthocyanidins and tannins were observed in the methanol extracts against ethyl acetate. Phytosterols, fatty acids, indoles, alkanes, pyrimidines, organic acids, aldehydes, dicarboxylicacids and alcohols were absent in ethyl acetate where as they are present in methanol extract.

Antioxidant activity

The DPPH scavenging potential of the whole leaf extracts of *Aloe ferox* was depends on the concentration of the extracts.

Table 1. Phytochemical constituents of ethyl acetate and methanol extract of *Aloe ferox*

S. No	Phytochemical compounds	Ethyl acetate extract	Methanol extract
1	Total phenols	+	+
2	Flavonoids	+	+
3	Flavonols	+	_
4	Proanthocyanidins	+	_
5	Tannins	+	+
6	Alkaloids	+	+
7	Saponins	+	+
8	Phytosterols	+	+
9	Fatty acids	_	+
10	Indoles	_	+
11	Alkanes	_	+
12	Pyrimidines	_	+
13	Organic acids	_	+
14	Aldehydes	_	+
15	Dicarboxylic acids	_	+
16	Ketones	+	+
17	Glycosides	+	+
18	Alcohols	_	+
19	Lactones	+	+

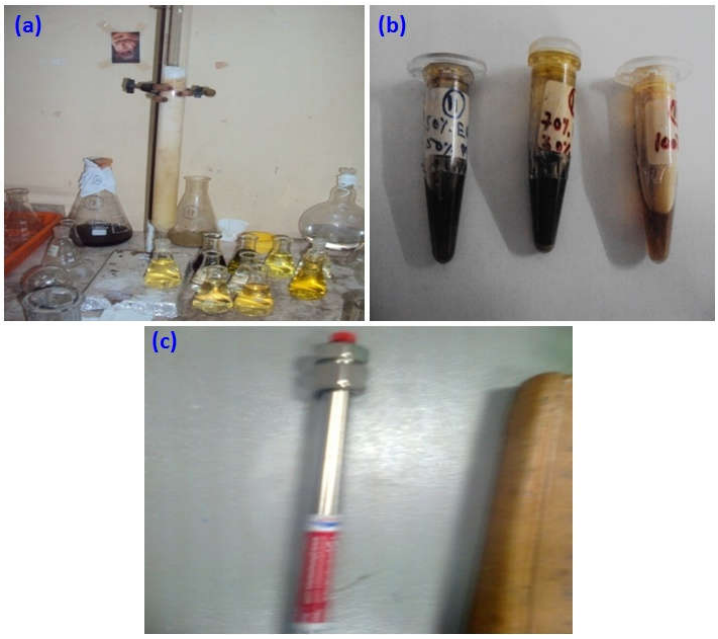

Fig. 1. (a to c) (a) Column chromatography extractions, (b) Concentrated solvent extracts with different concentrations, (C) PLC column: (Bio Sep - SEC- s2000)

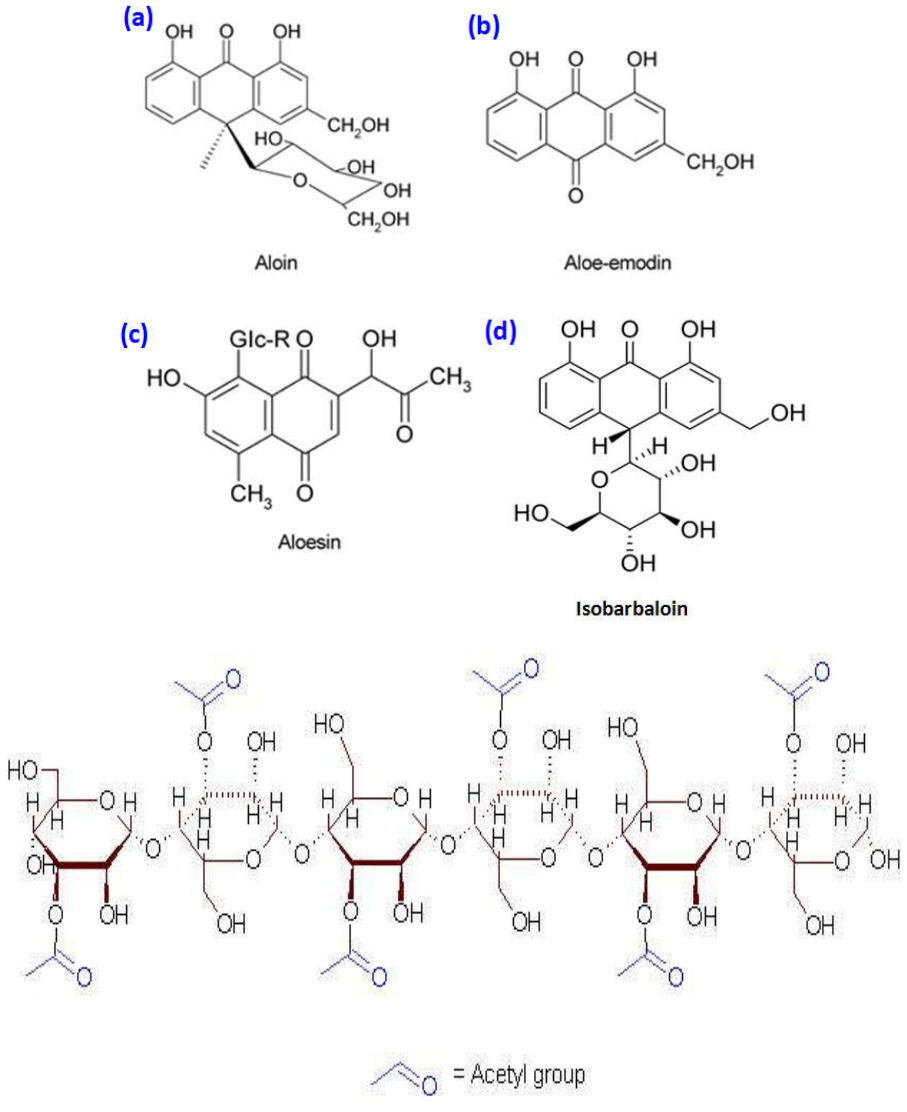

Fig 2 (e): Chemical structure of Acemannan

The percentage inhibition of DPPH by the various solvent extracts and the standard drugs was recorded in decreasing order of BHT > methanol > ethyl acetate. The percentage inhibition of BHT was significantly different from other extracts. The ethyl acetate extract showed the least inhibition. DPPH solution exhibited a deep purple color with a maximum absorption at 517 nm induced by antioxidants. Once this purple color disappears it assumed that an antioxidant is present in the medium and molecules of antioxidants will quench DPPH free radicals and convert them to a colorless product, ensuing to a decrease in absorbance at 517 nm and also the result of antioxidants on DPPH radical scavenging was shown owing to their hydrogen-donating ability.

The radical scavenging activity of values of methanol and ethyl acetate fractions are presented in the Table 2; results are expressed as the ratio percentage of sample absorbance decrease and the absorbance of DPPH solution in the absence of an extract at 517 nm.

Table 2. *Aloe ferox* leaf ethyl acetate and methanol extracts fractions

Concentration (µg/ml)	Absorbance	
	Aloe ferox ethyl acetate extract	*Aloe ferox* methanol extract
10	0.02	0.06
100	0.14	0.23
200	0.25	0.41
500	0.38	0.73

The radical scavenging activity percentage was proportional to the concentration of the extract. The presence of antioxidant polyphenols, alkaloids and indoles in the *A. ferox* leaf gel showed antioxidant capacity as confirmed by ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC) analyses (Fig. 3).

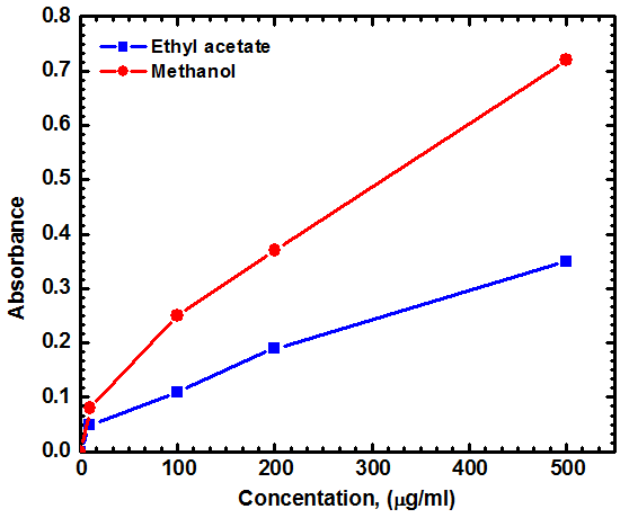

Fig. 3. 1, 1-diphenyl, 2-picrylhydrazyl DPPH radical scavenging activity of the whole leaf extract of *Aloe ferox* obtained using different solvents (red line indicates methanol extract, blue line indicates ethyl acetate extract)

Based on both the methods, the result shows that the flavonoid polyphenols to contribute to the majority of the total polyphenol content. Due to its phytochemical composition *Aloe ferox* leaf gel may show promise in alleviating symptoms associated with or prevention of cardiovascular diseases and diabetes (O'Brien *et al*. 2011).

The activity was carried out in triplicate and the results were expressed as mean values ± standard deviation. The concentration of extract providing 50% inhibition (EC50%) was calculated from the graph of scavenging effect percentage against the extract concentration. α-tocopherol and butylated hydroxyl toluene (BHT) were used as standards. Because, the presence of the polyphenols, alkaloids and indoles of the *A. ferox* leaf extract shows antioxidant capacity as confirmed by DPPH assay. The strong antioxidant activity of plant extract exhibited by the presence of phenolic compounds, which have direct antioxidant properties due to the presence of hydroxyl groups, which act as hydrogen donor. Also, they are found to be effective in scavenging free radicals as a result of their redox properties that allow them to act as reducing agents. The potent water soluble antioxidants, flavonoids are hydroxylated phenolics which help in radical scavenging and prevention of oxidative cell damage and they have reported to possess strong antioxidant activities. The concentration was low in the whole leaf extracts of *Aloe ferox*. But, methanol extracts has more flavonoids than the other solvent extracts during this study. *Aloe ferox* whole leaf extracts showed the reductive capabilities when compared with BHT. The reducing power of the extracts was concentration dependent and the antioxidant activities in all the solvents used were comparable with BHT. DPPH is a relatively stable free radical scavenger that converts the unpaired electrons to paired ones by hydrogen proton donation. Scavenging of DPPH radical during this study indicates the potency of the plant extracts in donating hydrogen proton to the lone pair electron of the radicals.

The antioxidant activity of plant extract exhibited by the presence of phenolic compounds, which have direct antioxidant properties due to the presence of hydroxyl groups acts as hydrogen donor. Also, they are found to be effective in scavenging free radicals as a result of their redox properties that allow them to act as reducing agents. The potent water soluble antioxidants and flavonoids are hydroxylated phenolics which help in radical scavenging and prevention of oxidative cell damage and they have reported to possess strong antioxidant activities. The concentration was low in the whole leaf extracts of *Aloe ferox*. But, methanol extracts has more flavonoids than the other solvent extracts during this study. *A. ferox* whole leaf extracts showed the reductive capabilities when compared with BHT. The reducing power of the extracts was concentration dependent and the antioxidant activities in all the solvents used were comparable with BHT. DPPH is a relatively stable free radical scavenger that converts the unpaired electrons to paired ones by hydrogen proton donation. Scavenging of DPPH radical during this study indicates the potency of the plant extracts in donating hydrogen proton to the lone pair electron of the radicals. As a result of the inhibition was more at a higher concentration in all the solvent extracts, it can be suggested that the plant extracts contains compounds capable of donating protons to the free radicals and it has proven the effectiveness of the extracts in an exceedingly concentration-dependent manner. The present study shows the scavenging activity of the leaf extracts of *A. ferox* in methanol and ethyl acetate. Among the ethyl acetate and methanol extractions of *Aloe ferox,* the methanol extract showed the highest antioxidant activity when evaluated by DPPH and reducing power method. The observed results suggest further analyses to confirm its prophylactic effect in the treatment of free radical mediated diseases. Most antioxidant activities rely upon the amount of the phytochemicals present in the plants.

The claimed therapeutic reputation needs to be verified in an exceedingly scientific manner. In this study *A. ferox* traditionally used for different health disorders was studied for their *in-vitro* antioxidant and anti-inflammatory activities. The anti-inflammatory activity of this plant material has not been reported till now in the literature. Reactive Oxygen Species (ROS) generated endogenously or exogenously are associated with the pathogenesis of various diseases such as diabetes, cancer, arthritis, atherosclerosis and aging process (Hajimahmoodi *et al*. 2008). The Inflammation process is a complex and ROS play an important role in the pathogenesis of inflammatory diseases. Thus antioxidants which can scavenge ROS are expected to improve these disorders. Many literatures have correlated the protein denaturation activity and autoimmune diseases and many studies on antioxidants have proved that oxidative stress has great importance in generation of autoimmune bodies responsible for autoimmune diseases. Since most of plants have poly phenolic compounds that has a sensible reducing and singlet oxygen quenching effect on free radicals. Literature suggest that, the anti-denaturation property of BSA was due to the presence of two interesting binding sites in the aromatic tyrosine rich and aliphatic threonine and lysine residue regions of the BSA (Williams *et al*. 2008). They have also reported that therapeutic molecules could be activating the tyrosine motif rich receptor dually with many studies on antioxidants have proved that oxidative stress has great importance in generation of autoimmune bodies responsible for autoimmune diseases. Most of the plants have poly phenolic compounds which have a good reducing, singlet oxygen quenching effect on free radicals.

Therefore this study *in vitro* anti-denaturation of Bovine serum albumin and reducing antioxidant activity was evaluated. The results were assumed that all plant species have moderate to significant antioxidant and anti-denaturation activity. It is well known that, phenolics constitute one of the major groups of compounds antioxidants (Cakiri *et al*. 2003). The abundance of plant extracts in polyphenol content should also explain the antioxidant activity results perhaps it could be the possible reason for antidenaturation and reducing antioxidant activity.

Anti - Inflammatory activity

The study shows the scavenging activity of the leaf extracts of *A. ferox* in methanol and ethyl acetate used for different health disorders was studied for their *in-vitro* antioxidant and anti-inflammatory activities. The antioxidants were analyzed using bovine serum albumin and reducing antioxidant activity method. Among the ethyl acetate and methanol extractions of *Aloe ferox,* the methanol extract showed the highest antioxidant activity when evaluated by the DPPH and reducing power method. The observed results will helpful for further analyses to confirm its prophylactic effect in the treatment of free radical mediated diseases. Most antioxidant activities rely upon the amount of the phytochemicals present in the plants. The results have clearly showed that, *Aloe ferox* plant had moderate to significant antioxidant and anti-denaturation activity. It was observed that *Aloe ferox* methanol extract (AFME) showed significant inhibition against carrageenan-induced paw edema in the dose dependent manner (Table 3).

Table 3. *Aloe ferox* leaf extract with Carrageenan Induced Paw Edema in Rats anti-inflammatory activity of *Aloe ferox*, leave values expressed as Mean ± SD; Number of animals in each group: 6

Group	Treatment	Mean paw volume (ml)				edema inhibition (%)
		0 hr	1 hr	2 hr	3 hr	
I	2% CMC	0.23 ± 0.02	0.37 ± 0.02	0.71± 0.02	1.01 ± 0.03	-
II	Ibuprofen 10 mg/kg	0.14 ± 0.01	0.18 ± 0.01	0.23 ± 0.02	0.28 ± 0.02	75%
III	AFME 250 mg/kg	0.18 ± 0.02	0.32 ± 0.02	0.40 ± 0.02	0.61 ± 0.02	38%
IV	AFME 500 mg/kg	0.17 ± 0.02[b]	0.29 ± 0.02[b]	0.41 ± 0.01[b]	0.52 ± 0.01[b]	50%

P< 0.01*: a, P< 0.001**: b and P< 0.0001***: Among them methanolic extract was more potent than the ethyl acetate extract.

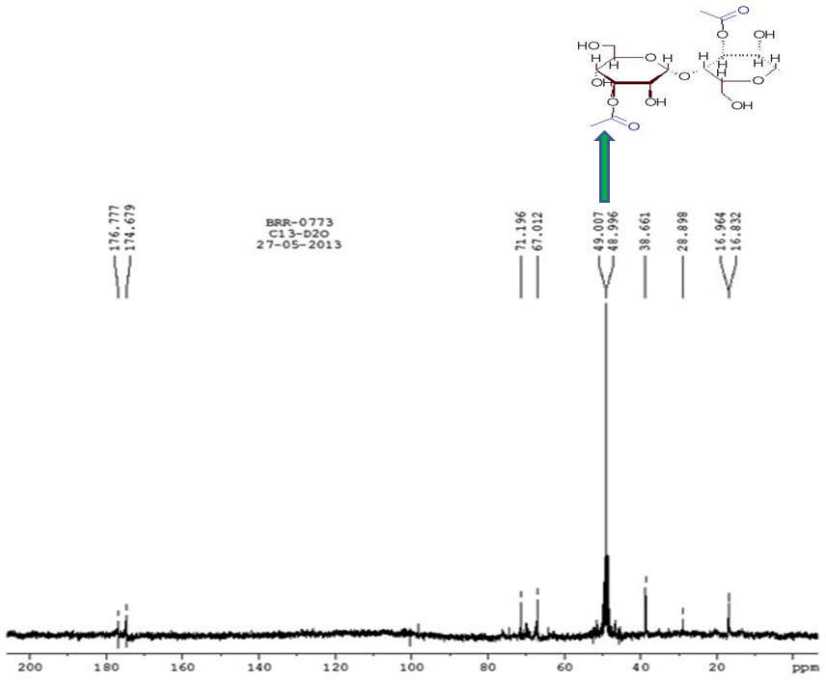

Fig. 4. Eluted compound in *Aloe ferox* by NMR ^{13}C

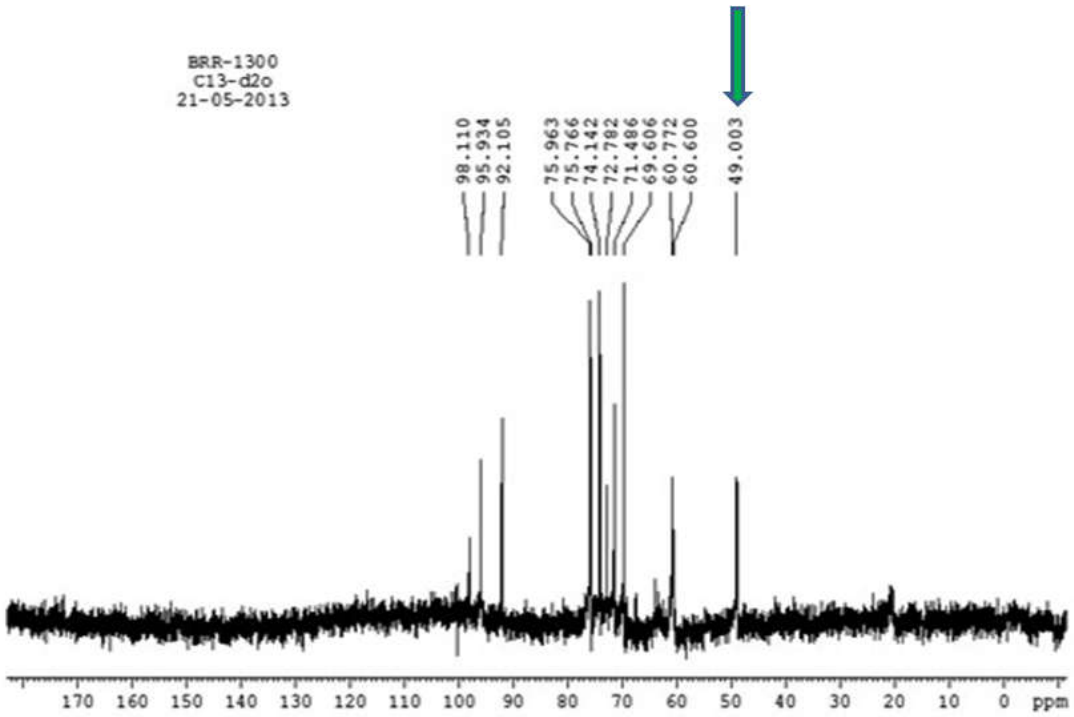

Fig. 5. Standard compound of NMR^{13}C

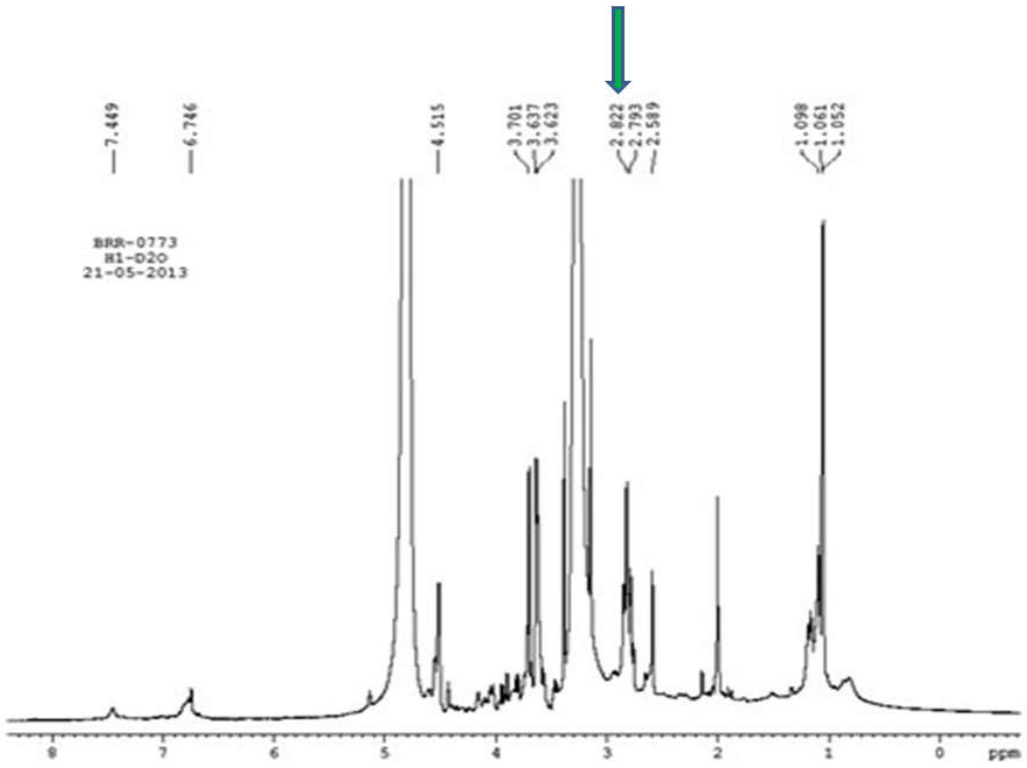

Fig. 6. Eluted compound in *Aloe ferox* by ^{1}H NMR

This response tendency of the extract in carrageenan-induced paw edema resulted sensible anti-inflammatory properties of the methanolic extract. This anti-inflammatory effect of AFME leaves may be because of flavonoids and earlier reports shows that number of flavonoids possessed anti-inflammatory activity. The presence of flavonoids might be responsible for the anti-inflammatory activity in methanolic extract. From the *in vitro* findings, it was clear that both methanolic and ethyl acetate extracts of *A. ferox* leaves showed good antioxidant and anti-denaturation activities.

Among them methanolic extract was more potent than the ethyl acetate extract. Many chemical compounds have been identified and separated from different medicinal plants with the help of several biophysical techniques like chromatography, NMR (Nishant and Anil, 2012) and mass spectroscopy. Analytical HPLC and ^{13}C- NMR spectroscopy was undertaken to assess the various components present in the *Aloe ferox* leaf extract. From the results, the anti-inflammatory activity of AFME may be due to its antioxidant activity.

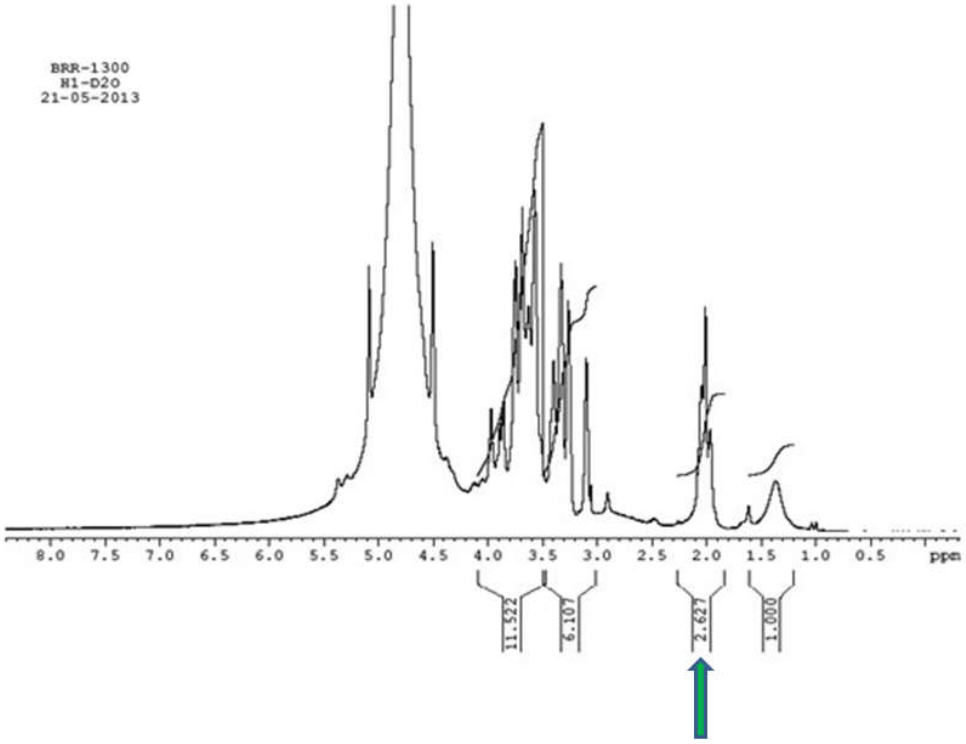

Fig. 7. Standard compound of ^{1}H NMR

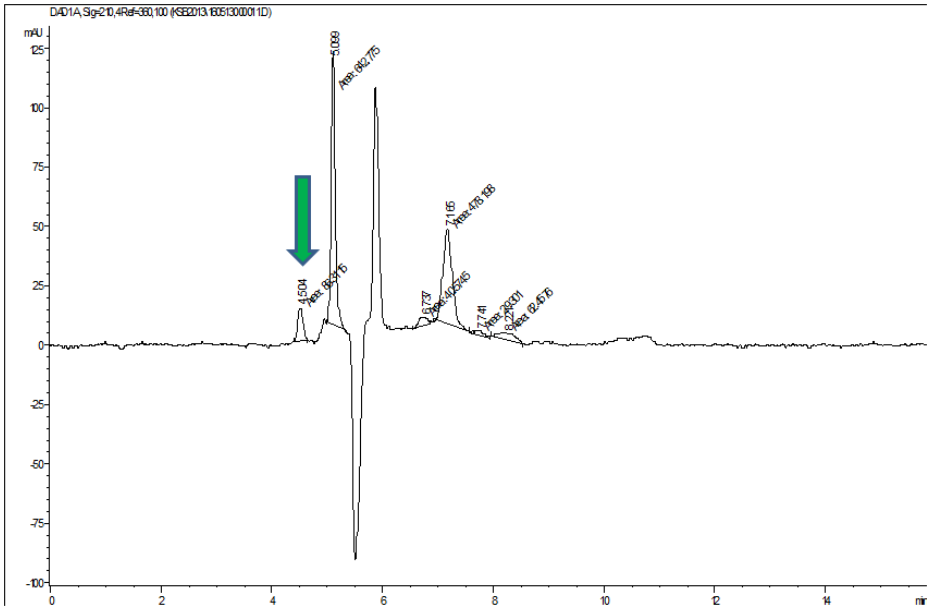

Fig. 8. HPLC analysis of *Aloe ferox* leaf extract

Hence, the anti denaturation of Bovine serum albumin and reducing antioxidant activity was evaluated. The results have clearly showed that all plant species have moderate to significant antioxidant and anti-denaturation activity. It was observed that *Aloe ferox* methanol extract (AFME) showed significant inhibition against carrageenan-induced paw edema in the dose dependent manner. From the *in vitro* findings, it was clear that both the methanolic and ethyl acetate extracts of *A. ferox* leaves showed good antioxidant and anti-denaturation activities. Among them methanolic extract was more potent than the ethyl acetate extract. These compounds aloin, aloe-emodin and aloesin may be a contributing factor towards its antioxidant activity as well as acemannan towards anti cancer activity.

Extraction of components from plant material depends on the polarity. Absence of flavonols, proanthocyanidins and tannins were observed in the methanol extracts against ethyl acetate. Phytosterols, fatty acids, indoles, alkanes, pyrimidines, organic acids, aldehydes, dicarboxylicacids and alcohols were absent in ethyl acetate where as present in methanol extract.

For chemical screening, HPLC coupled with NMR provides a great deal of preliminary information about the content and nature of constituents found in the active extracts. With all these results, we can conclude that *Aloe ferox* leaf extract can be used as a source of safe and natural antioxidant as well as anti-inflammation compound.

Chemical analysis in *Aloe ferox*

In the present scenario, there is an urgent and continuous need of exploration and development of affordable, effective new plant based drugs with better bioactive potential and least side effects. The traditional medicine is an important source of potentially useful new compounds for the development of chemotherapeutic agents. So, that the biological active compounds and extracts extracted by plant species (Essawi and Srour, 2000). The major active components of Aloe gel include minerals, aminoacids, poly phenols and poly saccharides, glucose and mannose are the main polysaccharides in the gel. The gel has been reported to possess various biological activities such as hepato protective and hematopoitic activity (Min-cheol Kang *et al*., 2014).n aloe gel includes campesterol, sterols, β-Sitosterol and Lupeol. Cancer continues to represent the largest cause of mortality in the world and claims over 6 million lives every year. Plant herbs were used as folk and traditional medicine and have been accepted currently as one of the source of cancer chemo prevention and drug discovery development.

The induction of oxidative stress has been linked with several types of cancers. Currently used anti cancer agents eg. doxorubicin, daunorubicin, mitomycin C, etoposide, cisplatin arsenic trioxide, ionizing radiation and photodynamic therapy depend exclusively or in part on the production of ROS for cytotoxicity, sensitivity of tumour cells to oxidative stress or apoptosis may affect treatment success. Treatment of (Ehrlich Ascite Carcinoma Cell) EACC tumors with *Aloe vera* leaf active principles isolated *via* supercritical carbondioxide extraction active principles resulted in a significant elevation in activity of key antioxidant enzymes (SOD, GST, tGPx, and LDH). Naveena *et al*., (2011) proved that ethanolic extract of *Aloe vera* was effective against Ehrlic acites carcinoma (EAC) tumor in mice which exhibited antitumor effect by modulating lipid peroxidation and augmenting antioxidant defense system in EAC bearing mice. ^{13}C-NMR analysis of the methanol extract of leaf of *Aloe ferox* showed a number of peaks. These signals confirmed the presence of aromatic ring indicating towards the occurrence of possible chemical compounds respectively. Identified the compound peaks and eluted from the injected compound by ^{13}C NMR – *Aloe ferox* – between - 48.996 – 49.001 at 50 ppm (Fig. 4) Compared with Standard compound - between 49.003 at 50 ppm (Fig. 5). Identified the compound peaks and eluted from the injected compound by ^{1}H NMR- *Aloe ferox* between 2.793 – 2.822 at 2.9 ppm (Fig 6) Compared with Standard compound - between 2.627 at 2.0 ppm (Fig. 7).

HPLC analysis of methanol extract of leaf of *Aloe ferox* was carried out with the mobile phase methanol: acetonitrile: water in the ratio 25:35:40 give total of 6 peaks at retention time 4.545 min (Fig. 8). The highest peak was seen at the retention time 563.778 mAU. ^{13}C-NMR analysis of the methanol extract of leaf of *Aloe ferox* showed a number of peaks in between δ 16.832 to 71.196 and δ 174.6 to 176.777 (indicating presence of aromatic rings). The highest peak was observed between 48.996 – 49.007. These signals confirmed the presence of aromatic ring indicating towards the occurrence of possible chemical compounds respectively. The HPLC analysis revealed the presence of polysaccharide acemannan *Aloe ferox* gel. Acemannan (Fig 2 d.) is an anticancer agent derived from *Aloe ferox* leaf gel extract.

Molecular formula of acemannan compound: $C_{64}H_{97}NO_{49}$; molecular weight of acemannan compound: 1664.43. The study stated that the methanol and ethyl acetate leaf extracts of *Aloe ferox* contain a variety of phytochemical compounds having potential antioxidant and anti-inflammatory activities. Aloin, aloe-emodin aloesin and acemannaan are extracted from the results of methanol and ethyl acetate mixture. Aloesin was isolated in a large proportion from the methanolic extract. From *in vitro* studies, the antioxidant and anti-inflammatory activities of *Aloe ferox* are attributed to its free radical scavenging activity due to the presence of flavonoids and phenolic compounds. For chemical screening, HPLC coupled with NMR provides a great deal of preliminary information about the content and nature of constituents found in the active extracts. With all these results, we can conclude that *Aloe ferox* leaf extract can be used as a source of safe and natural antioxidant as well as anti-inflammation compound and aloesin has much biological activities including anti-inflammatory activity and acemannan has potential anti-cancer agent.

Conclusion

The phytochemical analysis of *Aloe ferox* leaf extract revealed the presence of flavonoids, flavonols, alkanes, alkaloids, aldehydes, ketones, alcohols, total phenols, saponins, tannins, phytosterols, proanthocyanidins, fatty acids, indoles, pyrimidines, organic acids and dicarboxylic acids. These compounds aloin, aloe-emodin and aloesin may be a contributing factor towards its antioxidant activity as a source of natural antioxdants and acemannan towards anti cancer activity. *Aloe ferox* methanol extract (AFME) showed significant inhibition against carrageenan-induced paw edema in the dose dependent manner. We can conclude that *Aloe ferox* leaf extract can be used as a source of safe and natural antioxidant, anti-inflammation as well as anti cancer compound. This study proposing its potential application as a lead compounds for designing potent anti-inflammatory activity and they can be used for treatment of various diseases such as cancer, diabetes, anti viral, anti-fungal, anti inflammatory and immuno-modulator. Further pharmacological and biochemical investigations will clearly elucidate the mechanism of action of compound and will be helpful in projecting this plant as a therapeutic target in cancer and inflammation research. *Aloe ferox* has great potential to be used for gel industry and it has a bright future.

Funding

his work was supported by the Osmania University & Indian Institute of Chemical Technology, Hyderabad, India [grant no: UGC-CEPEPA]

Conflict of Interest

The authors have declared that no competing interests exist.

Acknowledgement

The author's thanks Department of Genetics and Biotechnology, Osmania University, Hyderabad for providing the facilities to carry out the research work and excellence guidance was rendered by Dr. Prabhakar Rao T, Technical Officer and Dr. S. Chandra Sekhar, Director, Indian Institute of Chemical Technology (IICT), Hyderabad, India during this investigation.

REFERENCES

Arun kumar, S. and Muthu selvam, M. 2009. Analysis of phytochem. Constituents and anti microbial activities of *Aloe vera* (L.) against clinical pathogens. World Jrnl. of Agric. Sci, 5: 572-576.

Balunas, M. J. and A, D., KInghorn. 2005. Drug discovery from Medicinal plants. Life Sciences. 78: 431- 441.

Cakir, A., Mavi, A., Yildirim, A., Duru, M, E., Harmandar, M., Kazaz, C., 2003. Isolation and Characterization of antioxidant phenolic compounds from the aerial parts of *Hypericum hyssopifolium* (L.) by activity guided fractionation. Jrnl. of Ethnopharmcol, 87: 73-83.

Celestino, VR., HM., Maranhao., C F. Vasconcelos, CR, Lima., GC., Medeiros, AV., Araujo, AG., Wanderley, 2013. Acute toxicity and laxative activity of *Aloe ferox* resin (L.) Revista Brasileira de Farmacognosia, 23: 2: 279-283.

Chakrapani. P., Nirmala, B R., Chandra, S, S, B., Arunjyothi, B., Prem, K., Venkatesh, K., Anupalli, R R., 2014. Comparative studies on antibacterial activity of Patchouli (*Pogostemon cablin* (Blanco) Benth) and Geranium (*Pelargonium graveolens*) aromatic medicinal plants. Afr. J. Biotecnol, 13; 23: 2379-2384

Chandra Sekhar, S,B., Rama Rao, B., Adhrvana Chari, M., Chakrapani, P., Lath,a T., Venkatesh, K., Arun Jyothi, B., Amareswari, P., Roja Rani, A, 2013. Cultivation, Phytochemical studies, biological activities and medicinal uses of *Aloe ferox* grand father of aloes an important medicinal plant. International Journal of Pharmacology, 9:7: 405- 415.

Chauhan, O,P., Raju, P,S., Khanum, F., Bawa, A,S., 2007, *Aloe vera* Therapeutic and food applications. Indian Food Industry, 26: 3: 43- 51.

Chen, W., Van, Wyk, BE., Vermaak, I., Viljoen, A,M., 2012. Cape Aloes - A Review of Phytochemistry, Pharmacology and commercialization of *Aloe ferox* Phytochem. Letters, 5:1-12.

Choi, S. and Chung, M, H. 2003. A review on the relationship between *Aloe vera* components and their biologic effects. Semin. Integr. Med, 1: 53-62.

Cotran, R, S., Kumar, V., Collins, T., 2001. Robbin's Pathological Basis of Disease. 6th Edn, WB Saunders, Philadelphia. 51.

Dagne E, Bisrat D, Viljoen A and Van Wyk BE. 2000. Chemistry of *Aloe species*. Curr. Org. Chem. 4: 1055-1078.

Du Loots, T, V., Westhuizen, F, H., Botes, L. 2007. *Aloe ferox* leaf gel Phytochem. Content, Antioxidant capacity and possible health benefits. Jrnl.of Agric. Food Chem.17: 6891-6896.

Essawi, T., Srour, M., 2000. Screening of some Palestinian medicinal plants for antibacterial activity. J. Ethanopharmacol. 46: 343-349.

Hajimahmoodi, M., Sadeghi, N., Jannat, B., Oveisi, M, R., Madani, S., Kiayi, M. 2008. Anti oxidant activity reducing power and total phenolic content of Iranian olive cultivar. J. of Biol. Sci. 8: 779 - 783.

Herraiz, T., J. Galisteo. 2004. Endogenous and dietary indoles: A class of antioxidants and radical scavengers in the ABTS assay, Free Radic. Res, 38: 323-331.

Lucas, SM., Roth well, NJ., Gibson, RM. 2006. The role of Inflammation in CNS injury and disease. Br. Journal of Pharmacology, 147: 232- 240.

Mabusela, WT., Stephen, AM., Botha, MC. 1990. Carbohydrate polymers from *Aloe ferox* leaves. Phytochemistry, 29: 3555 - 3558.

Manjit, S., Vijender, K., Ishpinder, S., Vinod G, Ajudhia, NK. 2010. Anti-inflammatory activity of aqueous extract of *Mirabilis jalapa* L. Leaves. Pharmacognosy Res. 2: 6: 364 - 367.

Miladi S, M Damak. 2008. *In vitro* antioxidant activities of *Aloe vera* leaf skin extracts. Journal of Chemical Society. Tunisia.10: 101-109.

Min Chul Kho, Yun Jung Lee, Jeong Dan Cha, Kyung Min Choi, Dae Gill Kang and Ho Sub Lee.2014.Gastrodia elata Ameliorates High-Fructose Diet-Induced Lipid Metabolism and Endothelial Dysfunction.Evidence-Based Complementary and Alternative MedicineVolume 2014, Article ID 101624, 10 pages.

Mulla, WA., Kuchekar, SB., Thora,t VS., Chopade, AR., Kuchekar, BS. 2010. Anti-Oxidant,Antinocieptive and anti-inflammatory activities of ethanolic extract of leaves of *Alocasia indica* (Schott.) J. Young Pharm, 2: 2: 137-143.

Naveena, Bharath BK and Selva S. 2011. Anti tumor activity of *Aloe vera* against Ehrlich Ascites Carcinoma (EAC) in Swiss albino mice. International journal of pharma and biosciences. 2: 400- 409.

Newman, DJ., GM. Cragg., KM, Sander. 2000. The Influence of Natural Products upon Drug Discovery. Natural Product Reports. 17: 215-234.

Nishant, KO., Anil, K. 2012. Profile of aqueous extract of different chromatographic fractions of *Aloe barbadensis* Mill. *Asian pacific* Jrnl. of Tropical Disease. 104 -108.

O'Brien, C., van Wyk, BE., van Heerden, FR. 2011. Physical and chemical characteristics of *Aloe ferox* leaf gel. South Afr. J. Bot. 77: 988 - 995.

Parmar P., Shashank, B., Suresh, D., Aanchal, J. 2012. Phytochemical studies of the secondary metabolites of *Ziziphus mauritiana* Lam. leaves. Journal of Current Pharmaceutical Research, 4:3.

Patel Pinal, D., Patel Natvarlal, J., Patel Dharmik, D., Patel Rakesh, K.2011. *In Vivo* evaluation of *Pleurotus Sajorcaju* Mycelium extract for anti- inflammatory activity Pharmacology, 2: 784-789.

Patra, JK., Gouda. S., Sahoo, SK., Thatoi, HN.2012. Chromatography separation. ^{1}H NMR and bio autography screening of methanol extract of *Excoecaria agallocha* L. from Bhitarkanika, India. Asian Pacific Jrnl. of Tropical Biomedicine. 50- 56.

Ramachandra, CT., Srinivasa Rao P., 2008. Processing of *Aloe vera* leaf gel: a review. Amer. J. Agri. Bio. Sci. 3: 2: 502-510.

Samuelsson, G., 2004. Drugs of Natural Origin: A Textbook of Pharmacognosy, 5th Edn., Swedish Pharm Press. Stockholm

Shreya, M., Arpita, P., Animesh, S., Suchismita, R., Arpita, M., Tapasi Das, M., Shrabani, P., Koushik, Dilip Kumar N. 2013. Analysis of phytochemical profile of *Terminalia arjuna* bark extract with antioxidative and antimicrobial properties. Asian Pac J. of Trop Biomed, 3: 12:960-966.

Silverstein, R M., Webster, FX., Kiemle, DJ. 2005. Proton NMR spectrometry. Spectrometric identification of organic compounds. USA: John Wiley and Sons, Inc. Int. Res.J. of Pharmacy.127- 204.

Tomer. K., Singh, V., Sethiya, N K., Singh, H P., Kumar, M., Chandra D. 2009. Isolation and Characterization of New Lanosteroid from ethanolic extracts of *Eclipta alba* Linn. Jrnl. Pharm Res,10: 2.

Vanessa, RLC., Hélida, MLM., Carlos, FBV, Cristiano, R L., Giovanna, CRM, Alice, VA, Almir G W, 2013. Acute toxicity and laxative activity of *Aloe ferox* resin. *Revista Brasileira de* Farmacognosia Brazilian Jrnl. of Pharmacognosy, 23: 2

Venkatesa, PR., Adira,j M., Shanmuga, PP. 2001. Synthesis, analgesic and anti-inflammatory evaluation of substituted 4-piperidones. Indian Drugs, 38: 156- 156.

Viljoen, AM., Van Wyk, BV., Newton, LE., 2001. The occurrence and taxonomic distribution of the anthrones aloin, aloinoside and microdotin in Aloe. Biochem. Syst. Ecol. 29:53-67.

Vogler, BK., Ernst E. 1999. *Aloe vera*: a systematic review of its clinical effectiveness. Br. J. Gen. Pract, 49: 823 - 828.

Waller, TA., Pelley, RP., Strickland, FM. 2004. Industrial processing and quality control of *Aloe barbadensis* gel. Reynolds (ed.) *Aloes*. The genus *Aloe*. CRC Press, 139-205.

Williams, LAD., Connar, AO., Latore, l., Dennis, O., Ringer, S., Whittaker JA, Conard J, Vogler B, Rosner H, Kraus, W. 2008. The *in vitro* anti denaturation effects induced by natural products and non steroidal compounds in heat treated bovine serum albumin is proposed as a screening assay for the detection of antiinflammatory compounds, without the use of animals, in the early stages of the drug discovery process. West Indian Medicinal Journal, 57: 327-331.

****