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Medicinal importance, pharmacological activities, and analytical aspects of aloin: A concise report

Kanika Patel[1], Dinesh K. Patel[2*]

[1]G.L.A Institute of Pharmaceutical Research, Mathura – 281 406 (U.P.), India
[2]Department of Pharmaceutics, Indian Institute of Technology, Banaras Hindu University, Varanasi – 221005 (U.P.), India

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ABSTRACT

Natural products such as pure phytoconstituents and plant extracts offer limitless opportunities for the new drug development due to its unmatched chemical diversity. Plants play an important role in the medicinal preparations for both preventive and curative purpose. Some of the currently available drugs i.e. aspirin, digitalis, anti-malarial (quinine) and anti-cancer (vincristine, vinblastine) were derived from the plant sources. Aloin ($C_{21}H_{22}O_9$), a yellow colour compound is a mixture of two diastereoisomers, aloin A and aloin B. Aloin is an anthrone C-glucoside having molecular weight 418, and it is the main phytoconstituents of aloes. Aloin is used for various pharmacological purposes such as laxative agent. It is also used as ingredients of various laxative pharmaceutical preparations. So far, varieties of analytical methods have been developed for the estimation of aloin in aloes product, which are mainly based on HPLC and TLC techniques. In the present review, pharmacological activities and analytical aspects of aloin were highlighted along with some useful tissue culture techniques. This review could be helpful to the researcher for the investigation of new molecule from aloin in the future.

1. Introduction

Plants are used an excellent source of drugs, and some of the currently available drugs were derived from the plant sources. In the developing countries, plant and plant-derived products still play an important role in the medical health care system. Some of the modern medicine i.e. aspirin, anti-malarial, anti-cancer and digitalis have derived from the plant source. Plants and phytochemical have beneficial effect against microorganism, inflammation, cardiovascular diseases, blood disorders, cerebral disorders, immune system, oxidative stress, reproductive disorder and cancer chemotherapy. A large number of the drugs prescribed worldwide are derived from plants[1,2]. Medicinal plants are very ancient and only true natural medicines and they can use either directly or in the extract forms. The use of plants, parts of plants and isolated phytochemicals for the prevention and treatment of various ailments has been in practice from immemorial time[3,4]. More than 25% of the drugs prescribed worldwide are derived from plants and in India, about 80% of the rural population uses medicinal herbs or indigenous systems of medicine. About 2-3 decades ago, most of the drugs were obtained from herbal source[5]. Herbal medicines are gaining popularity both in developing and developed countries due to its fewer side effects. According to the World Health Organization (WHO) more than 21 000 plants are used for medicinal purposes in the world[1,6].

1.1. Overview of *Aloe vera*

Aloe vera (*A. vera*) belonging to family liliaceae is widely distribution in the tropical and subtropical regions of the world. Most of *Aloe* species are indigenous to Africa, but now have wide distribution in the tropical and subtropical regions of the world. The genus Aloe contains over 400 different species and *Aloe barbadensis* (*A. barbadensis*) Miller is considered to be the most biologically active. Traditionally, *A. vera* gel is used both, topically (treatment

*Corresponding author: Dinesh Kumar Patel, Department of Pharmaceutics, Indian Institute of Technology, Banaras Hindu University, Varanasi-221005 (U.P.), India.
E-mail: dkp.iitbhu@gmail.com
Tel: +91 9919192440

of wounds, minor burns, and skin irritations) and internally to treat constipation, coughs, ulcers, diabete, headaches, arthritis, immune-system deficiencies[7]. *A. vera* have good effect on stomach acid secretion, frostbite, inflammation, radiation burns, blood glucose levels, viral action, and to modulate the immune response[8]. Leaf exudates and mucilaginous gel of *A. vera* possesses anti-inflammatory, antifungal, antibacterial, anticancer, antioxidant, cytoprotective, antidiabetic and hypoglycaemic, cardiac stimulatory and immunomodulatory activities. It is used in gastric ulceration, remedy against a variety of skin disorders, promotes wound healing as well reducing edema and pain[7]. The mucilaginous gel is incorporated in various type of pharmaceutical formulation such as over-the-counter drugs, dietary supplements, and cosmetics as in ointments, creams, lotions and other preparations essentially for topical use[9]. *A. vera* has long been used for medicine, dietary supplements and cosmetic purposes. *Aloe* extracts have been used to treat inflammation, cancers and AIDS[10]. *A. vera* consists of high content of phenolic compounds, glycosides (aloins), 1,8-dihydroxyanthraquinone derivatives (aloe emodin). 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]. Of all the classes of compounds occurring in "Aloe drug", the anthrones aloin A and aloin B are most important one. Aloin A and aloin B, two diastereomeric C-glucoside of the aloe-emodin aglicone differing in the configuration at C-10 aglicone moiety, are mainly responsible of the above-mentioned purgative properties[11].

1.2. Overview of aloin

Aloe contains many active ingredients, but the best known is aloin (lo-glucopyranosyl-1,8-dihydroxy-3-(hydroxymethyl)- 9(lOH)-anthracenone). Aloin is a bitter-tasting yellow crystal, and it a C-glycoside derivative of an anthraquinone. Although the C-glycoside side chain of aloin is not easily hydrolyzed In vitro, orally administered aloin is known to be hydrolyzed by esterases secreted by intestinal microflora. Once the C-glycoside is hydrolyzed, it forms aloe-emodin anthrone, which is further auto-oxidized to the quinone aloeemodin[8]. Aloin is an anthraquinone glycoside. It has molecular weight 418, molecular formula $C_{21}H_{22}O_9$ and it's chemical structure and overview were shown in the Figure 1. The IUPAC name of aloin is 8-Dihydroxy-10-(β -D-glucopyranosyl)-3- hydroxymethyl) -9(10H)-anthracenone. It is yellow-brown compound estimated at levels from 0.1 to 0.66 % of leaf dry present in cells adjacent to the rind of the leaf in gel. It is used as laxative agent to maintain digestion system treating constipation by inducing bowel movements. Once ingested, it increases peristaltic contractions in the colon, and induces bowel movements[12]. Aloin, a mixture of two diastereoisomers, aloin A and aloin B, is an anthrone C-glucoside component of aloes. The other main aloe components are aloesin and aloeresin A, aloe-emodin, homonataloin and nataloe-emodin, aloinoside A and B, aloenin A and B, 4-hydroxyaloin and 5-hydroxyaloin[13]. The levels of aloin in aloe are highly variable and appear to depend on the species and strain of aloe as well as growing conditions. Aloin, which is localized in the outer rind of the aloe plant, has been reported to constitute up to 30% of the aloe plant's dried leaf exudates. Aloin occurs naturally as a mixture of diastereomers. Studies of aloin's biosynthesis indicate that aloin B is preferentially formed. Nonenzymatic conversion to aloin A, is thought to result in the mixture of aloin A and aloin B observed for naturally derived aloin. Aloin has been reported to be nonmutagenic using an *in vitro* assay. *In vivo* studies have shown that orally administered aloin is poorly absorbed but is metabolized by intestinal microflora to aloe emodin, which is readily absorbed[14].

2. Pharmacological activities of aloin

2.1. Effect of aloin on cancer

The effect of aloin on cell viability was examined by the use of Jurkat T cells. Treatment with aloin showed in a reduction in cell size, compromised membrane integrity, and loss of mitochondrial membrane potential in a dose-dependent manner. Additionally, treatment with aloin also showed in the alteration of the cell cycle. Loss of cell membrane integrity was preceded by a loss of mitochondrial membrane potential, suggesting a mitochondrial-dependent pathway for aloin-induced apoptosis[15]. Protective effect of aloin on inducible nitric oxide synthase (iNOS) and nuclear factor kappa B (NF- kappa B) synthesis of HaCat cells induced by ultraviolet B (UVB) irradiation was examined. NO generation and iNOS mRNA synthesis were markedly downregulated by pretreatment with aloin. The activation of NF- kappa B P65 was inhibited while the proliferation of HaCat cells was increased. Our results confirm that aloin treatment could inhibit NF- kappa B P65 activation, and downregulate iNOS mRNA expression and NO generation induced by UVB irradiation[16]. The cytotoxicity of a low MW fraction of A. barbadensis gel (LMWF) was determined. The toxic activity of LMWF was compared with that of sodium dodecyl sulfate, aloe-emodin and aloin using the chemiluminescence assay. Result showed that aloin was found to be of similar potency to these toxic substances[17]. The effects of 5 purified compounds including aloin from *A. vera* on human

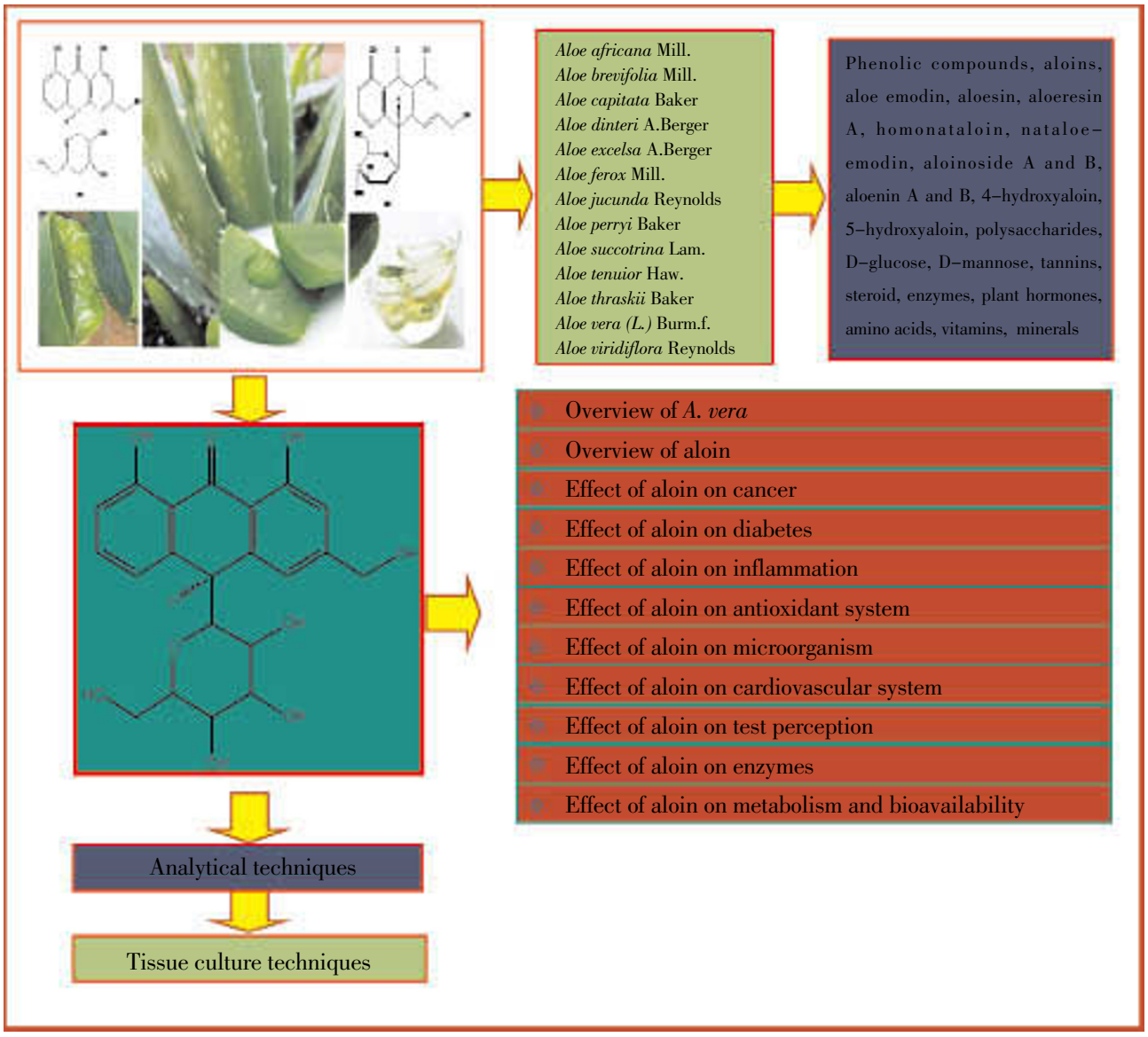

Figure 1. Chemical structure and overview of aloin.

K562 leukaemia and on its multidrug resistant (MDR) variant, K562/R were studied in vitro. Only the aglycone, aloe emodin, produced reproducible antitumour effects, which, interestingly, were more pronounced in the MDR, P-glycoprotein overexpressing cell line[18].

2.2. Effect of aloin on diabetes

The effect of *A. vera* extract containing high concentration of polyphenols with known concentrations of aloin and aloe-emodin was administered orally for a period of 4 weeks to insulin resistant ICR mice. Results showed that the polyphenol-rich extract from *A. vera* was able to decrease significantly both body weight and blood glucose levels compared to the control group. Compared to the *A. vera* extract and pioglitazone treated mice control group showed highest glucose levels in the insulin tolerance curve test[19]. In another study, the protective effect of aloe component including aloin isolated from Aloe arborescens (*A. arborescens*) Miller on streptozotocin induced necrosis of B cells in the pancreatic islets of the mouse were examined to clarify its mechanism of action involved in anti-diabetic effects of aloin[20].

2.3. Effect of aloin on inflammation

Anti-inflammatory effects of aloin and aloe-emodin with other polyphenols were compared in the present investigation. Results demonstrate that aloe-emodin, dose-dependently inhibited inducible nitric oxide synthase (iNOS) mRNA expression and nitric oxide (NO) production. Aloin also suppressed the production of NO, although it did not suppress PGE2 production. The present results indicate that aloin and aloe-emodin possibly suppress the inflammatory responses by blocking iNOS and COX-2 mRNA expression[21].

2.4. Effect of aloin on antioxidant system

Antioxidant and prooxidant effects of aloin and aloe-emodin on free radical-induced DNA breaks were investigated. Aloin prevented OH-induced DNA breaks compared to the control group. Aloin at lower concentrations increased DNA damage indicating its prooxidant effect. The greater reducing activity of aloin at low concentrations, however, free radical-scavenging activity of aloin gradually predominated over its reducing power, resulting in the protection of DNA[10].

2.5. Effect of aloin on microorganism

Aloe ferox (*A. ferox*) and *Withania somnifera* (*W. somnifera*) are among the southern African plants commonly used for the treatment of sexual transmitted disease (STIs). Extracts from both species together with pure aloin were evaluated against six strains of *N. gonorrhoea* and nine strains of *C. albicans*. The extracts showed activity against *N. gonorrhoea* while pure aloin inhibited the growth of both microorganisms[22]. The antiplasmodial activity and toxicity data of 34 *Aloe* species and their main constituents including aloin were investigated against chloroquine-resistant *Plasmodium falciparum* (*P. falciparum*) strain. Homonataloin was a more potent inhibitor of parasite growth than aloin[23]. Aqueous extracts of *A. ferox* and *W. somnifera*, together with aloin were evaluated for antiviral activity against herpes simplex virus type 1 (HSV-1) *in vitro*. The aqueous extracts showed detectable activity against the virus in monolayers of the Vero African green monkey cell cultures, whereas aloin showed significant activity[24].

2.6. Effect of aloin on cardiovascular system

Hypotensive effects of aloeemodin, aloin A, elgonica dimer A and bisbenzopyran from *A. barbadensis* have been investigated. Aloe emodin has emerged as a potent hypotensive agent in current pharmacological investigations and caused falls in mean arterial blood pressure in rats[25].

2.7. Effect of aloin on test perception

Variation in human taste is a well-known phenomenon and mitter taste in humans is believed to be mediated by a family of 25 G protein-coupled receptors (hT2Rs, or TAS2Rs). Polymorphism in two hT2R genes results in different receptor activities and different taste sensitivities to three bitter molecules including aloin. The hT2R43 gene allele, which encodes a protein with tryptophan in position 35, makes people very sensitive to the bitterness of the natural plant compounds aloin and aristolochic acid[26].

2.8. Effect of aloin on enzymes

For the treatment of hyperpigmentation disorders and cosmetic additives, the effects of aloin on tyrosinase enzyme were investigated in the present investigation. Tyrosinase activity was estimated by measuring the oxidation rate of L-dopa. Aloin had different levels of inhibition of tyrosinase and the inhibitory rate of aloin is higher than that of hydroquinone[27]. The effects of *A. barbadensis* gel and aloin on the activity of microbial and human metalloproteinases have been investigated. Aloins inhibit Clostridium histolyticum collagenase (ChC) reversibly and noncompetitively. aloins are also effective inhibitors of stimulated granulocyte matrix metalloproteinases (MMPs). The structural resemblances between aloins and the tetracyclines, suggest that the inhibitory effects of aloins are via an interaction between the carbonyl group at C9 and an adjacent hydroxyl group of anthrone (C1 or C8) at the secondary binding site of the enzyme[9].

2.9. Effect of aloin on metabolism and bioavailability

The intestinal uptake and metabolism of aloin was investigated using intestinal absorption model. The Caco-2 cell monolayer and the everted gut sac were incubated with aloin. The basolateral appearance of test compounds and their glucuronosyl or sulfated forms were quantified using HPLC. The aloin was absorbed as glucuronidated or sulfated form. These results suggest that a significant amount is transformed during absorption[28]. The bioavailability and tissue distribution of aloin was investigated in the rat model. The levels of aloin and its conjugates were measured in plasma, tissues, and urine. These results suggest that aloin is absorbed and reaches a peak plasma level within 1-1.5 h after the administration and a significant portion is possibly metabolized or is excreted in feces[29]. Effect of aloin on blood alcohol level was investigated using female Sprague Dawley rats. Increased the rates of blood alcohol elimination and the disappearance of alcohol from the body were found with aloin treatment. Pretreatments with aloin, resulted in a significantly decreased blood alcohol AUC and an increase in the rate of ethanol disappearance[8]. To assess the digestive stability and absorption of aloin and other aloe compound, *In vitro* digestion model coupled with caco-2 cell were used in the present investigation. Aloin and other compounds were transported into both apical and basolateral compartments after 1 h incubation in caco-2 cell. The involvement of several transporter proteins for aloin was also examined in this investigation[30].

3. Analytical aspects of aloin

A semiquantitative thin-layer chromatographic (TLC) method for aloin in aloe-based products was validated. This TLC method may be ideal for routine analysis of commercial products containing *A. vera* because of simple, sensitive, specific, rapid, and cheap method [31]. Determination of aloin in *Aloe* sp. by reversed-phase high performance liquid chromatography was developed. Aloin was successfully separated on a discovery column (4.6 mm × 250 mm, 5 mm) using methanol-water (50:50 v/v) as mobile phase at a flow rate 1.0 mL/min and detected at 354 nm[32]. High-speed countercurrent chromatography (HSCCC) has been used for the preparative separation of mixture of two diastereoisomers, aloin A and aloin B, combined with preseparation on silica gel chromatography. Three solvent systems composed of chloroform-methanol-water (4:2:3), ethyl acetate-methanol-water (5:1:5), and butanol-ethyl acetate-water (1:3:4) have been used in this methods[33]. Liquid chromatography/mass spectrometry (LC/MS) and LC with diode array detection (DAD) in the UV range (LC/UV) were developed for the determination of low levels of the anthraquinones aloin-A in aloe-based products. The on-column sensitivities were 0.25 ng by LC/UV and 0.01 ng by LC/MS for aloin-A. The methods are simple and sensitive and therefore, these are suitable for the determination of anthraquinones in various aloe-based products[34]. Transmission electron microscopy and HPLC were used to study the cell ultrastructure and aloin content of *A. vera* exposed to natural light or grown under shade. HPLC analysis showed that plants grown under the shade had a lower aloin concentration in leaves than those exposed to natural light[35]. A simple and accurate reverse phase HPLC method has been developed for the determination of aloin in *A. vera*. The method involves separation of aloin using methanol-water (1:1) as mobile phase using UV detector[36]. For the determination of low levels of the anthraquinones aloin A in aloe products, gas chromatography/mass spectrometry (GC/MS) was developed. Trimethyl silyl (TMS) derivatives of these analytes in the presence of Chrysophanol used as internal standard in this method. This method could be used to analyze several aloe based commercial products[37].

Scanning electron microscopy was used to analyse the structure of the stoma and cuticle of leaves, while HPLC was used to evaluate aloin content of 3 *Aloe* species i.e *A. vera*, *A. arborescens* and *A. saponaria*. Aloin content was highest in *A. arborescens*, followed by *Aloe vera* and *A. saponaria*[38]. Mucilage of fresh juice of *A. microdonta* leaves was precipitated from the leaf juice by addition of ethanol and the supernatant chromatographed on Amberlite XAD-2. The resulting fraction contained anthraquinone derivatives. Using flash chromatography on silica gel followed by preparative TLC, aloin A and 2 new compounds were isolated from *A. microdonta*[39]. A HPLC method was developed for simultaneous determination of aloin, and other chemical in "KANGXIN" tablets. The separation was achieved on a C18 column, a diode array detector at 260 nm and a mobile phase composed of acetonitrile and water under the gradient elution were used in the present investigation[40]. The effects of shading on the anthraquinone content of *A. vera* were studied using HPLC method. Shading treatment significantly decreased anthraquinone content. The aloin contents of aloe grown under shaded conditions decreased in comparison with the control[41]. The chemical composition of *A. arborescens* Mill. and its change by biostimulation (cold stress) were studied. Changes in the chemical composition of *A. arborescens* due to biostimulation for 5-10 d increased the antiradical and biostimulating activity of the raw material[42]. Aloin and polysaccharide present in extracts of *A. arborescens* Miller were formulated into a binary solution to protect eyes from bacterial infection and ultraviolet radiation (UVR). The UVR absorption spectrum and physical properties of the product was examined. The binary solution exhibited three absorption peaks in the UVA, B and C regions, respectively. Such UV absorption capability was attributed to the phenolic chromophores pertaining to aloin[43]. RAPD analysis yielded 2 distinct clusters of non-bitter and bitter types of *A. vera* used as vegetable in arid parts of Rajasthan. Based on aloin content and RAPD analysis, it can be concluded that non-bitter type of *A. vera* is a different ecotype that can be used as a vegetable[44]. Anatomy, histochemistry, fluorescent microscope and phytochemistry, was used to study the leaf structure, contents of chlorophyll, carotenoids, aloin, and the characteristics of production and storage of aloin in different leaf ages of *A. arborescens*. The content of aloin gradually reduced from the top to bottom leaves with further growth development[45]. The anthrone-C-glucosyls aloin A and B, 5-hydroxyaloin A, 10-hydroxyaloin A and B were separated by micellar electrokinetic capillary chromatography (MECC) with sodium dodecyl sulfate in borate buffer pH 9 within less than 7 min[46]. The dried flowers from *A. vera* (L.) Burm. f. were analysed by means of HPLC-DAD and HPLC-MS/MS, verifying the presence of the different phytoconstituents including aloin in the *A. vera*[47].

A multi-technique approach was chosen to assess their quality and authenticity of nine products, obtained from leading international suppliers, and was compared with fresh *A. vera* gel. Authenticity was evaluated by nuclear magnetic resonance spectrometry (^{1}H NMR). An HPLC-UV method was set up to verify the absence of hydroxyanthracene derivatives (aloin and aloin-related compounds). Aloin A was found to be present at concentrations from trace levels

to 16 mg/kg[48]. Three species of *Aloe* from Bangladesh (*A. vera*, *A. indica* and *Aloe* sp.) were compared for their growth performance, aloin content and laxative properties in rats. *A. indica* was superior in terms of rapid growth and aloe powder production compared to the other two species. Aloin content was found to be highest in the *A. vera*[49]. For the analysis of extracts of *Aloe* plants, a method was developed with a laboratory assembled nano-LC system coupled with a UV detector, followed by an IT-mass spectrometer. With a step gradient mode of acetonitrile/H_2O mixtures and employing a capillary column packed with C18 leads to the separation of aloin, 5-hydroxyaloin and 7-hydroxyaloin. The optimized nano-LC-MS method was validated for the quantification of aloin[50]. Chemical characterization of *A. vera* gel was carried out for assaying various nutrients present in the gel. The skin and filet fraction contained approximately 90%-96% of water. Proteins, aloin, fibers, chrollophyl and soluble sugar in the *A. vera* were also characterized[51]. Analytical HPLC-MS studies of the exudate of *A. secundiflora* have revealed a mixture of phenolic compounds mainly anthrones (aloenin, aloenin B, isobarbaloin, barbaloin and other aloin derivatives), chromones and phenylpyrones with a low content of polysaccharides and aliphatic compounds[52]. Thirteen phenolic compounds from *A. barbadensis* and *A. arborescens* were analysed by HPLC using a reverse-phase column eluted with a methanol:water gradient, and detected by UV at 293 nm. aloin A and B with other compounds were identified and quantified in these samples[53].

4. Tissue culture techniques

Cultures were established from seed, root, leaf and embryo explants of aloe on N6 and MS media. Callus induction and plant regeneration from root and embryo explants were successful. The main drug components (aloesin, aloeresin A and C, and aloin A and B) were not detected in the callus but were present in the regenerated plants[54]. Axillary shoots, with 6-7 leaves were selected from the field-grown plants. One leaf and stem were separated from each shoot and portions of each were cultured on supplemented MS basal medium. When the medium was supplemented with 3% glucose and 10% alpha modification of Eagle's Medium instead of the sucrose, callus formation was enhanced but the protein and aloin concentrations in the callus were lower than in that produced on medium with 3% sucrose[55]. In a pot experiment, young plants with 7 leaves of *A. barbadensis* were cultured in nutrient solutions containing nitrogen. Shoot fresh weight and total biomass significantly increased with nitrogen content. Variation in aloin content was similar to that of ascorbic acid[56]. Efficient plant regeneration in *A. barbadensis* Mill. was achieved using callus derived from shoot meristem. The rate of shoot bud regeneration was dependent on the concentration of hormones in the nutrient media. The *in vitro* derived plantlets were hardened in the nethouse with 75% light and successfully established in soil[57].

5. Discussion

Phytochemicals are compounds that occur naturally in plants and they are responsinle for different color, flavor and smell of plants. They also form part of a plant's natural defense mechanism against diseases. China and India are the leading countries who use medicinal plants and their remedies date back to at least 7 000 years. According to the WHO 80% of the World's population uses traditional medicine for their primary health concern. Knowledge of traditional medicinal plants plays an important role in the development of new drugs. A large number of drugs prescribed worldwide are derived directly or indirectly from natural sources[58]. According to the World Health Organization (WHO) reports, about 80% of the world's population in 2001 used herbal medicine for their health aspect[59]. *A. vera* commonly known as the bitter aloe is a polymorphic species and found throughout world. It play an important role in cosmetic formulations and food supplements. *A. vera* have antioxidant, antimicrobial, antiinflammatory, anticancer and antimalarial activities. *A. vera* contains aloin with some other phytoconstituents mainly responsible for its pharmacological activities. Aloin plays an important role in the defense mechanisms of the plants. It is also used in the alcoholic beverages due to its bitter principle[60]. Aloin have been used for the treatment of various types of disorders and pharmacologically this unique phytoconstituents have been scientifically validated for its anticancer, antiinflamatory, antioxidant and antimicrobial activities. Positive effect of aloin on cardiovascular system, metabolic system and other enzyme system were also evaluated and scientifically validated. From the available literature data, it was concluded that aloin have valuable pharmacological activities and could play an important role in the development of new drug for the treatment of various disorders. In the present review, data were collected for aloin in reference to the pharmacological activities, tissue culture techniques, and analytical techniques. These data could be helpful to the scientist of the natural product chemistry and other allied science for the development of new molecule entities from aloin in the future.

Conflict of interest statement

The authors report no conflict of interest.

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