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RESEARCH ARTICLE

IMPROVED HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY/MASS SPECTROSCOPY (HPLC/MS) METHOD FOR DETECTION OF ANTHRAQUINONES AND ANTIOXIDANT POTENTIAL DETERMINATION IN *ALOE SINKATANA*

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ABSTRACT

Objectives: Medicinal plants, either as an extract, pure compound or as a derivative, offer limitless opportunities for the discovery of new drugs. Sudan is a very rich source of medicinal plants which are used in the treatment of a wide range of diseases. *Aloe sinkatana*, has great potential to be developed as drug by pharmaceutical industries. The present study is undertaken to investigate the antioxidant potential of *Aloe sinkatana* by DPPH radical scavenging activity. In addition, the study also performed to explore the possibility of using HPLC-MS technique for the determination and analysis of *Aloe sinkatana.*

Methods: The extracts of *Aloe sinkatana* were analyzed for antioxidant activity by using DPPH free radical scavenging activity. The results indicated that the extracts showed a high effective free radical scavenging in the DPPH assay, also these extracts exhibited a noticeable antioxidant effect at low concentrations.

Results: During *in vitro* evaluation the antioxidant potential of methanolic extract was the highest, followed by aqueous extract in DPPH radical scavenging activity. So the methanolic extract of the plant, exhibited a great antioxidant effect at 50 µg/ml which may be attributed to high phenolic content. Therefore, methanolic extract to be a more active radical scavenger than aqueous extract. The HPLC-MS analysis had shown the methanolic extract of *Aloe sinkatana* to be rich in the major anthraquinones and their glucosides, which revealed 9 compounds, and also UV spectroscopy detected the presence of two flavonoids.

Conclusion: The results indicated that the extracts of *Aloe sinkatana* is a potential source of natural antioxidants or nutraceuticals with potential application to reduce oxidative stress with consequent health benefits. Due to stronger antioxidant potential and phytochemical composition, *Aloe sinkatana* could be proved as a valuable prospect in pharmaceutical formulations by taking part in the antioxidant defense system against generation of free radicals.

Keywords: *Aloe sinkatana*, anthraquinones, antioxidant, free radicals, HPLC/MS, medicinal plants.

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INTRODUCTION

Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs[1]. The study of plants used in traditional medicine requires the effective integration of information on chemical composition of extracts, pharmacological activities of isolated compounds, as well as indigenous knowledge of traditional healers. According to the World Health Organization, more than 80% of the world's people depend on traditional medicine for their primary healthcare needs. The beneficial medicinal effects of these plant materials typically result from the combinations of secondary products present in the plant making the medicinal actions of plants unique to particular plant species or groups[2]. Several studies have shown that plant derived

antioxidant secondary metabolites scavenge free radicals and modulate oxidative stress-related degenerative effects[3,4]. Free radicals have been beneficial in many diseases such as cancer, atherosclerosis, diabetes, neurodegenerative disorders and aging[5,6]. Previous research reports suggest that higher intake of antioxidant rich food is associated with decreased risk of degenerative diseases particularly cardiovascular diseases and cancer[7].

Anthraquinones are a group of phenolics and are widely distributed in many plant families such as *Fabaceae*, *Liliaceae*, *Rubiaceae* and *Rhamnaceae*[8]. Anthraquinones derivatives such as emodine, physcione, rhein and chrysophanol have been used as colorants in food, drugs and cosmetics. Nowadays, anthraquinones attracted attention especially related to their interesting biological properties. They are known for their different biological activities including antimicrobial[9], anticancer[10], antioxidant[11] and anti-inflammatory[12]. In this direction, it is suggested that consumption of anthraquinones-rich plants such as *Rhamnus* and *Frangula* species[13-15] can represent a valid intake way in order to benefit of their biological activities. From this point, the presence of anthraquinones is valuable as important criterion in the plants quality used for medicinal purposes. The recently revised family, Aloeacae, in the order Liliaceae, Liliflorae, was one of the widely distributed families of flowering plants. It consisted of 250 genera comprising 3700 species, mostly perennial herbs with rhizomes or bulbs[16]. When Dahlgren and Clifford (1982) made a major revision of superorders, orders and families within the monocotyledons, this family was sub-divided into several other families. Several free anthraquinones occur in roots and leaves of Aloe species. Aloe-emodin[16] is a typical leaf constituent and is wide spread in the genus. Chrysophanol[17] occurs both in roots and leaves[17,18], while aloe-emodin[18] has been reported only from leaves[19]. Two main types of anthraquinones are present in Aloe, these are 1.8 dihydroxyanthraquinnone e.g chrysophanol and 7-hydroxyl aloe–emodin[19].

High-performance liquid chromatography (HPLC) is the usual technique for the determination of individual components in *Aloe vera* leaf extracts. There are already several examples of the use of HPLC for the analysis of aloin derivatives[20]. All of them have been developed using a particulate reverse phase column. ElSohly *et al.*,[21] have developed methods using both liquid chromatography/mass spectrometry (HPLC-MS) and liquid chromatography/photodiode array detection (HPLC/PDA) for the determination of aloe emodin and aloin A in aloe-based products. Rebecca *et al.*,[21] also developed methods based on HPLC-MS for the studies of the exudates of the plants. However, only few reports are available on the antioxidant activity evaluation of *Aloe sinkatana* extract. So, the present study is undertaken to investigate the antioxidant potential of *Aloe sinkatana* by DPPH radical scavenging activity. In addition, the study also performed to explore the possibility of using HPLC-MS technique for the determination and analysis of *Aloe sinkatana.*

MATERIALS AND METHODS

Plant Material

Leaves of *Aloe sinkat*ana were collected in Erkawiet (East of sudan) in March 2009. They were kindly identified by Dr. Aalyia Awad, botanist, a voucher specimen was deposited under the registration in the herbarium of the University of EL-Neelain. The leaves of the plant collected were separately dried at room temperature then finely ground with an electrical grinder.

Chemicals and Solvents

All chemicals and solvents used were HPLC grade. Laboratory and analytical grade chemicals, reagents, and solvents including; petrolum ether, chloroform, ethylacetate, butanol, methanol and 1,1-diphenyl-2-picrylhydrazyl (DPPH) , were from Merck India Ltd.

Extraction and Fractionation

Leaves (50gm) were extracted for 3 hours by using Soxhlet apparatus. The filtrate of this methanol extract was concentrated under reduced pressure until all the methanol had evaporated. The concentrate was redissolved in distilled water and lyophilized.

Determination of Antioxidant Activity (Scavenging Activity of DPPH Radical)

The DPPH free radical scavenging assay was carried out for the evaluation of the antioxidant activity. This assay measures the free radical scavenging capacity of the investigated extracts. DPPH is a molecule containing a stable free radical. In the presence of an antioxidant, which can donate an electron to DPPH, the purple colour typical for free DPPH radical decays, and the absorbance change at λ=517 nm is measured. This test provides information on the ability of a compound to donate a hydrogen atom, on the number of electrons a given molecule can donate, and on the mechanism of antioxidant action. The method was carried out as previously described by[22]. The methanolic and aqueous extracts were redissolved in methanol and 5% ethanol, respectively, and various concentrations (10, 50, 100, 500 and 1000 µg/ml) of each extract were used. Similar concentrations of ascorbic acid were used as positive control. The assay mixture contained in a total volume of 1 ml, 500 µl of the extract, 125 µl prepared DPPH (1 mM in methanol) and 375 µl solvent (methanol or 5% ethanol). After 30 min incubation at 25°C, the decrease in absorbance was measured at λ=517 nm. The radical scavenging activity was calculated from the equation:

$$\%\text{RSA} = \frac{\mathbf{A}\text{control} - \mathbf{A}\text{sample}}{\text{Abscontrol}} \text{X}100$$

Where, %RSA=% of radical scavenging activity, **A**control=Absorbance of control, **A**sample=Absorbance of sample

High Performance Liquid Chromatography HPLC/MS

The chromatographic apparatus consisted of a Model 616 pump, a Model 996 diode-array detector, a Model 717+ auto-sampler (all from Waters, Milford, MA, USA). An octadecyl silica glass cartridge column, Separon2 SGX C18 (15.3 mm I.D., 7 mm particle size) was used for the separation. The mobile phase contained 10% MeOH and 90% 5 mM aq. NH4OAc in water. For substituted anthraquinones, 40% MeOH±60% 5 mM aq. NH_4OAc as the mobile phase

was used, because in 10% MeOH±90% 5 mM aq. NH4OAc these compounds were strongly retained on the chromatographic column. The low rate for HPLC with UV detection was 1 ml/min. When the MS detection was applied, the low rate was reduced to 0.6 ml/min to enhance the electrospray response with optimum low rate after splitting. The post-column splitting 1:20 was used, so that 30 ml/min of eluent was introduced into the electrospray ion source. Samples were dissolved in the mobile phase and the injection volumes were 10 ml in all cases.

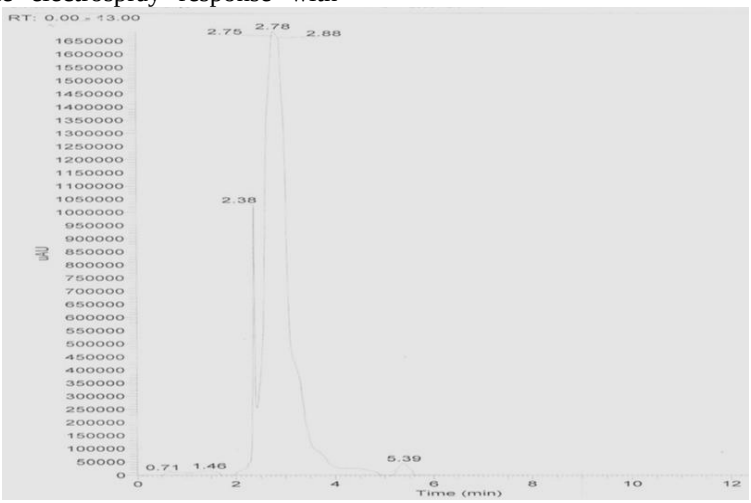

Figure 1: HPLC Spectrum of methanol extract of *Aloe sinkatana* leaf

RESULTS AND DISCUSSION

Various medicinal properties have been attributed to natural herbs. Extractive value useful for the evaluation of a crude drug and at the same time gives idea about the nature of the chemical constituents present, which is helpful for the estimation of specific constituents[23]. Medicinal plants constitute the main source of new pharmaceuticals and healthcare products. A whole range of plant derived dietary supplements, phytochemicals, and pro-vitamins that assist in maintaining good health and combating disease are now being described as functional foods and nutriceuticals. Plant- derived products are also increasingly accepted and used in the cosmetic industry. The roles of herbal plants in disease prevention and cure have been attributed, in part, to the antioxidant properties of their constituents of liposoluble and water soluble vitamins, and a wide range of amphipathic molecules, broadly termed phenolic compounds.

Table 1: Antioxidant activity of different extract of *Aloe sinkatana* leaf

Type of extract	Conc (mg/ml)	% Inhibition	IC50±SD (mg/ml0
Petrolum ether	5	48.0±0.02	-
Chloroform	5	36.3±0.03	-
Ethylacetate	5	40.8±0.02	-
Butanol	5	46.4±0.02	-
Methanol	5	51.9±0.01	3.5±0.01
Water	5	60.3±0.01	2.02±0.01

The antioxidant effect of phenolic compounds is mainly due to their redox properties, and as a result of various possible mechanisms, which allow them to act as reducing agents, hydrogen donators, free radical scavengers, singlet oxygen quenchers, and/or metal chelators (transition- metal- chelating activity)[24]. The extracts of *Aloe sinkatana* were analyzed for antioxidant activity by using DPPH free radical scavenging activity. The anthraquinone derivatives, such as aloe-emodin, emodin, rhein, chrysophanol, and physcion, are reported to possess antiangiogenic activity, by preventing blood vessel formation in zebra-fish embryos. The anticancer effect of aloe-emodin has been established in two human cancer cell lines, Hep G2 and Hep 3B. Aloe-emodin inhibited cell proliferation and induced apoptosis in both examined cell lines by different antiproliferative mechanisms. In current study all the extracts showed a high effective free radical scavenging in the DPPH assay (Table 1). These extracts exhibited a noticeable antioxidant effect at low concentrations. So the methanolic extract of the plant, exhibited a great antioxidant effect at 50 µg/ml (Table 1). The results were in agreement with those of who found the methanolic extract to be a more active radical scavenger than aqueous extract. The HPLC analysis had shown the methanolic extract of *Aloe sinkatana* to be rich in the major anthraquinones and their glucosides, which reveled 9 compounds, and also UV spectroscopy detected the presence of two flavonoids. Plant phenolic compounds especially flavonoids are currently of growing interest owning to their supposed properties in promoting health (anti-oxidants)[24]. Flavonoids have been demonstrated to have anti-inflammatory, antiallergenic, anti-viral, anti-aging, and anti-carcinogenic activity[24]. The broad therapeutic effects of flavonoids can be largely attributed to their antioxidant properties. In addition to an antioxidant effect, flavonoid compounds may exert protection against heart disease through the inhibition of cyclooxygenase and lipoxygenase activities in platelets and macrophages[24]. Anthraquinones are structurally built from an anthracene ring (tricyclic aromatic) with a keto group each on carbon atom nine and ten. In plants, anthraquinones are found in a wide range of species[11]. The effects of anthraquinones and anthrones are very diverse[24]. Anthraquinones and anthrones are very reactive and have a broad

pharmacological activities including, potent anticancer, antidiabetic, antimicrobial, antiinflammatory, and cathartic properties as well as its cardio-, hepato-, and neuroprotective qualities. Anthraquinones and xanthones contain an aromatic core that serves as a scaffold for the attachment of diverse functional groups, resulting in a wide variety of molecules with distinct biological and biochemical characteristics[24].

CONCLUSION

Antioxidant rich plant extracts serves as sources of nutraceuticals that alleviate the oxidative stress and therefore prevent or slow down the degenerative diseases. An effort has been made to explore the antioxidant properties of commercial available herbal extracts. Current results show that medicinal plants can be promising sources of natural products with potential antioxidant activity. The results indicated that the extracts of *Aloe sinkatana* is a potential source of natural antioxidants or nutraceuticals with potential application to reduce oxidative stress with consequent health benefits.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHOR'S CONTRIBUTION

The manuscript was carried out, written, and approved in collaboration with all authors.

REFERENCES

1. Magbool FF, Elnima EI, Shayoub ME, Hussein SO. Phytochemical and antimicrobial screening of stem bark and leaves extract from *Ficus sycomorus*. World J Pharm Med Res 2017; 3(11): 234-239.
2. Magbool FF. *Quercus infectoria* galls: phytomedicine promising for treating candidiasis–a review. World J Pharm Res 2017; 6:17.
3. Ames B N, Shigenaga M K and Hagen T M, Acadamy of Sciences of the United States of America 1993; 90:7915-7922.
4. Joseph J A, *et al*. Reversals of age-related declines in neuronal signal transduction, cognitive, and motor behavioral deficits with blueberry, spinach, or strawberry dietary supplementation. J Neurosci 1999; 19(18): 8114-8121. *https://doi.org/10.1523/JNEUROSCI.19-18-08114.1999*
5. Halliwell B and Gutteridge J M, Oxford: Oxford University Press, 1999.
6. Yu B P. Cellular defenses against damage from reactive oxygen species. Physiological Rev 1994; 76: 139-162. *https://doi.org/10.1152/physrev.1994.74.1.139*
7. Thatte U, Bagadey S, Dahanukar S. Modulation of programmed cell death by medicinal plants. Mole Cellular Biochem 2000; 46: 199-214. PMID: 10726985.
8. Locatelli M, Genovese S, Carlucci G, *et al*. Development and application of high-performance liquid chromatography for the study of two new oxyprenylated anthraquinones produced by *Rhamnus* species. J Chromatogr A 2014; 1225:113–20. *https://doi.org/10.1016/j.chroma.2011.12.085*
9. Ifesan BO, Hamtasin C, Mahabusarakam W, *et al*. Assessment of antistaphylococcal activity of partially purified fractions and pure compounds from *Eleutherine americana*. J Food Protect 2009; 72:354–9. *http://dx.doi.org/10.4315/0362-028x-72.2.354*
10. Chen SH, Lin KY, Chang CC, *et al*. Aloe-emodin-induced apoptosis in human gastric carcinoma cells. Food Chem Toxicol 2007; 45:2296–303. *https://doi.org/10.1016/j.fct.2007.06.005*
11. Iizuka A, Iijima OT, Kondo K, *et al*. Evaluation of Rhubarb using antioxidative activity as an index of pharmacological usefulness. J Ethnopharmacol 2004; 91:89–94. *https://doi.org/10.1016/j.jep.2003.11.021*
12. Choi RJ, Ngoc TM, Bae K, *et al*. Anti-inflammatory properties of anthraquinones and their relationship with the regulation of Pglycoprotein function and expression. Eur J Pharm Sci 2013; 48:272–81. *https://doi.org/10.1016/j.ejps.2012.10.027*
13. Locatelli M, Epifano F, Genovese S, *et al*. Anthraquinone profile, antioxidant and antimicrobial properties of bark extracts of *Rhamnus catharticus* and *R. orbiculatus*. Nat Prod Commun 2011; 6:1275–80. *https://doi.org/10.1177/1934578X1100600917*
14. Kremer D, Kosalec I, Locatelli M, *et al*. Anthraquinone profiles, antioxidant and antimicrobial properties of *Frangula rupestris* (Scop.) Schur and *Frangula alnus* Mill. bark. Food Chem 2012; 131:1174–80. *https://doi.org/10.1016/j.foodchem.2011.09.094*
15. Kosalec I, Kremer D, Locatelli M, *et al*. Anthraquinone profile, antioxidant and antimicrobial activity of bark extracts of *Rhamnus alaternus*, *R fallax*, *R intermedia* and *R pumila*. Food Chem 2013; 136:335–41. *https://doi.org/10.1016/j.foodchem.2012.08.026*
16. Hutchinson, J. (1973). The Families of Flowering Plants, 3rd edition. Clarendon pess, Oxford.
17. Yagi A, Makino K, Nishioka I. Studies on the constituents of *Aloe saponaria* HAW. III. The structures of phenol glucosides. Chem Pharm Bull 1977; 25. *https://doi.org/10.1248/cpb.26.1111*
18. Dagne E, Alemu M. Constituents of the leaves of four Aloe species from Ethiopia. Bull Chem Soc Ethiop 1991; 5: 87-91.
19. Conner J M, Gray AI, Reynolds T, Waterman PB. Anthraceme and chromone derivatives in the exudates of *Aloe rabaiensis*. Phytochemistry 1989; 28(12): 3551-3553. *https://doi.org/10.1016/0031-9422(89)80392-9*
20. ElSohly MA, Gul W, Avula B, Khan IA. Determination of the anthraquinones Aloe-emodin and Aloin-A by liquid chromatography with mass spectrometric and diode array detection. J AOAC Int 2007; 90(1):28–42. *https://doi.org/10.1093/jaoac/90.1.28*
21. Rebecca W, Kayser O, Hagels H, Zessin KH, Madundo M, Gamba N. The phytochemical profile and identification of main phenolic compounds from the leaf exudate of *Aloe secundiflora* by high-performance liquid chromatography mass spectroscopy. Phytochemical Analysis 2003; 14(2):83–86. *https://doi.org/10.1002/pca.682*
22. Brand WW, Cuvelier HE, Berset C. Use of a free radical method to evaluate antioxidant activity. Food Sci Technol 1995; 82:25-30. *https://doi.org/10.1016/S0023-6438(95)80008-5*
23. Magbool FF, Elnima EI, Shayoub ME, Hamedelniel EI, Alhassan MS. Pharmacognostic, physicochemical standardization and phytochemical analysis of *Quercus infecoria* galls. American J Res Comm 2018; 6(10): 1-17.
24. Magbool FF, Elnima EI, Shayoub ME, Hussein SO. Preliminary phytochemical screening of *Quercus infectoria* galls. World J Pharmacy Pharmaceuti Sci 2018; 7(1): 2018.