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INVITRO ANTIOXIDANT ACTIVITY OF ALOE VERA GEL EXTRACT

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ABSTRACT

This study aimed to investigate the invitro antioxidant activity of hot water and ethanolic extract of A. vera, commonly known as Gwar Patha or Ghrit Kumari, rank second among the important edible medicinal plant in the world especially in Madhya Pradesh. Hot water and ethanolic extracts were prepared from A.vera and their antioxidant properties were studied by following DPPH, Ferrous ion chelating activity, FRAP Assays. Naturally occurring antioxidant compound (Flavonoid) was tested in the extracts by flavonoid concentration test. The results revealed that the hot water extract displayed better scavenging effects on DPPH 87.03% , Chelating activity 71.5% & Reducing power 0.59 followed by ethanol extract 69.90%, 67% & 0.48 respectively for all the above three tests. The present study showed that A. vera can be considered as good source of natural antioxidants that can be used as a possible food supplement with beneficial effect in health and also in the pharmaceutical industries.

INTRODUCTION

The chemical that stops or inhibits the build-up of free radicals in the human body is known as an antioxidant. The human body produces a vast number of reactive oxygen species (ROS) and reactive nitrogen species (RNS) as by-products of different metabolic processes. These are known as free radicals and make up a big group of reactive organic species. The human body continuously produces free radicals, which can be neutral or ionic substances with one or more unpaired electrons that can also live on their own. Hydroxyl radicals (OH-), superoxide radical anion (O2-), hydrogen peroxide (H2O2), singlet oxygen species (10_2) and nitric oxide (NO) are examples of ROS and RNS, respectively (1).

High ROS levels cause oxidative stress, which can have a number of harmful effects, such as activation or deactivation of enzymes, lipid peroxidation of cellular membranes, DNA breakage, altered lipid-protein interaction, and ultimately the promotion of mutations that start tumor progression (2,3). The detrimental effects that free radicals have on the body can be reduced if the equilibrium between their production and elimination is preserved.

Oxidative damage by free radicals and reactive oxygen species is the cause of many human diseases, including cancer, diabetes, gastritis, atherosclerosis, ischemia, central nervous system injury, and reperfusion injury of many tissues (4,5,6,7). The human body has several endogenous defence systems to shield cellular molecules from harm caused by oxygen radicals. Both enzymatic and non-enzymatic processes are part of these endogenous defensive mechanisms. Antioxidative enzymes such as glutathione peroxidase (GSH-Px), catalase (CAT), and superoxide dismutase (SOD) are part of the enzymatic defence system (8). These enzymes serve as our first line of defence against oxidative damage. Antioxidants such as glutathione, vitamin E, and vitamin C, among others, are part of the non-enzymatic defense system and help shield the body from the harm caused by oxidative stress. These natural defensive mechanisms, however, are not enough to fend off extreme oxidative stress, and an excess of reactive radicals can harm tissue. A number of synthetic antioxidants like butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), a-tocopherol, tert butylhydroquinone (TBHQ) and trolox are extensively used in non-food industries, but their use in the food industries is prohibited because they are alleged to be extremely carcinogenic (9). As a result, the development and utilization of more efficient antioxidants of natural derivation are preferred (10). For many years, traditional medicine has utilized herbs to treat a variety of illnesses and ailments. Numerous kinds of medicinal plants are excellent sources of phytochemicals with strong antioxidant capabilities (11). Aloe belongs to the group of raw materials rich in bioactive compounds showing health-promoting advantages. It contains e.g. polyphenols, anthranoids, and derivatives of pyron, saponins, steroids, fibre, salicylic acid, mineral components. The content of polyphenolic compounds is slightly higher in the leaf

skin than in the flower. Catechin prevails in the skin, while the flower contains more gentisic acid (López *et al*., 2013).

The perennial succulent plant Aloe vera L. (also known as Aloe barbadensis Miller) is a member of the Aloeaceae family, which is a subfamily of the Asphodelaceae [17]. A. vera is the most commonly recognized and utilized of the 400 Aloe species for a variety of therapeutic and aesthetic applications [18–20]. The plant is composed of rosette-shaped turgid green leaves that are linked at the stem. An inner transparent pulp (gel) and an outer green rind (skin) make up each leaf. Phenolic chemicals are abundant in the plant [18, 21–26]. Additionally, it contains a lot of aloe emodin, a 1, 8-dihydroxyanthraquinone derivative, and aloins, which are their glycosides and are used as cathartic [27–29]. *Aloe vera* leaf skin (AVLS) has been shown in numerous studies to offer a wide range of medicinal properties, such as purgative [30], antibacterial [31]. Aloe vera gel is the flesh of aloe vera leaf which is tasteless but functions as an antioxidant due to its flavonoid content. It is important to note that flavonoids have antioxidant properties which capture free radicals considered to be beneficial to human health [1]. The aim of the carried out research was an evaluation of the antioxidative activity of the aloe extract.

MATERIAL AND METHODS

Sample Collection and Processing

The *Aloe vera* plant was collected from Raipur district and final identification was done by Dr. P.K. Joshi, Principal Scientist, Centre of Excellence on MAPs and NTFP, IGKV, Raipur. The leaves of collected plant material were then processed *viz*., collection of gel by peeling off hard leave coating.

Preparation of Organic Extraction

The extracts were prepared by cold percolation method. The prepared mixtures were stirred using a sterile glass rod at 24 h interval. The extracts were filtered by Whatmann filter paper no 1(Dulger and Gonuz, 2004). The filtrates were then concentrated in water bath. After the extraction process was completed, the solvent was filtered and evaporated at 40^0C for 18h to dryness under reduced pressure by using vacuum pump. The residues obtained were stored in sterile bottle under refrigeration condition (4^0C) prior to use for further analysis.

Determination of Antioxidant Activity

Estimation of DPPH Assay: The DPPH• radical decoloration activity of the A. vera extracts was determined using the DPPH solution methanol, following the modification method of Sogi et al. [23]. A portion of the DPPH stock solution (0.24 g/100 mL methanol) was diluted into 10 parts methanol at 80% (4:1 ratio of methanol and water, respectively) so that the working solution obtained an absorbance of 1.10 ± 0.02 at 515 nm. 3 mL of the working solution of DPPH was mixed with 0.6 mL of blank, standard, or sample, kept in the dark for 20 minutes, and the absorbance was recorded at 515 nm. Methanol at 80% (control) was used to calculate the radical decoloration activity of a standard curve, which was prepared with trolox solution (50–250 µM, R2: 0.9905). Samples were analyzed in triplicate, and the results are expressed in units equivalent to trolox (ET), mM ET/g fresh weight (FW).

Estimation of Ferrous Ion Chelating Assay: Among the transition metals, iron is known as the most important lipid oxidation due to its high reactivity. The ferrous state of iron accelerates lipid oxidation by breaking down hydrogen and lipid peroxides to reactive free radicals. In the presence of chelating agents, the complex formation of ferrous and ferrozine was disrupted, resulting in a decrease in red color of the complex. Measurement of the color allows estimation of metal chelating activity of the coexisting chelator [17]. The chelating of ferrous ions by the extracts of *A. vera* was estimated by the method of Decker and Wetch with some modifications and EDTA was used as reference standard [18].

Estimation of Reducing Power (FRAP) Assay: The determination of ferric reducing antioxidant power or ferric reducing ability (FRAP assay) of the extracts was performed as described by Sogi *et al.* [23]. The different concentration of water and ethanolic extract of *A. vera* was prepared followed by stock preparation. Various concentrations of both extracts were subjected with phosphate buffer and $K_4[Fe(CN)_3]$. Mixture was incubated at

room temperature and TCA was added. It was centrifuged at 6000 rpm for 10 min. Supernatant was collected and ferric chloride was added to it. Absorbance of the reaction mixture in terms of reducing power was recorded, these were compared in terms of L- ascorbic acid.

RESULTS AND DISCUSSION

DPPH Assay: DPPH is a stable free radical and possesses a characteristics absorbance at 517 nm, which decreases significantly on exposure to radical scavengers by providing hydrogen atom to become stable diamagnetic molecule [22]. In this investigation, DPPH Scavenging activity of different extracts of *A. vera* were presented in Fig.1, where hot water extract of *A. vera* shown best scavenging capacity (87.03%). than ethanol extraction (69.90%) at a concentration of 1 mg/ml. However, the scavenging activity of standard BHT at the same concentration was 91.86%. Previously reported, the DPPH scavenging activity of buckwheat [23], buckwheat seed components [24], *A. vera* [25] and *Cassia* [26] fruit bodies were 74%, 66.2%, 77.2% and 51.92% respectively at a concentration of 20 mg/ml, whereas at a concentration of 1 mg/ml the radical scavenging activity of hot water extract of *A. vera* was 85% was much effective than cold water extract and methanol extract.

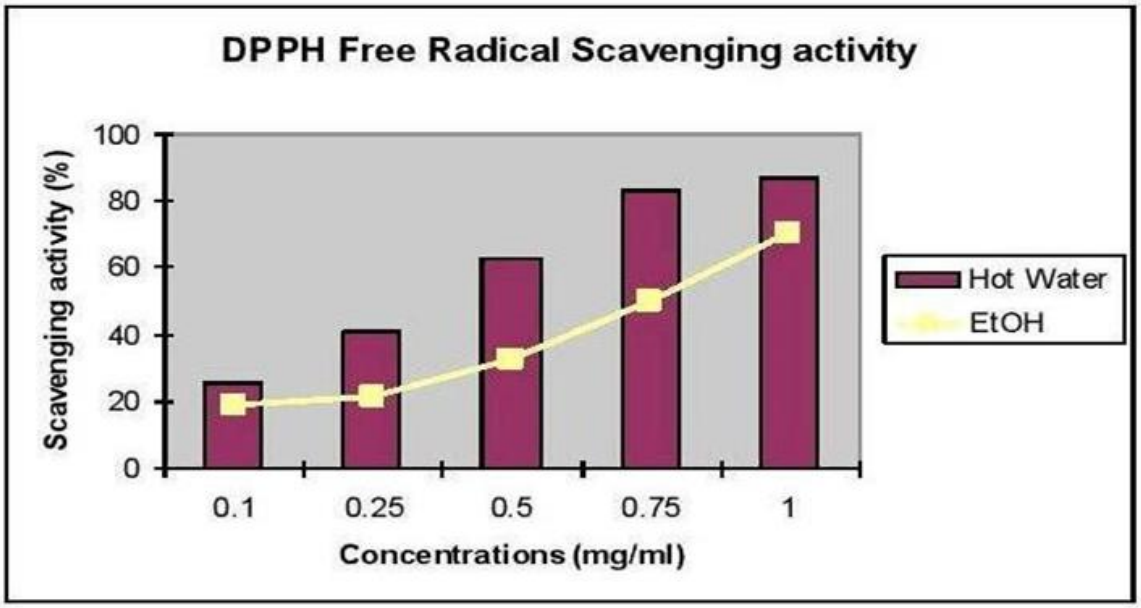

Fig. 1. DPPH Free Radical Scavenging Activity of hot water and ethanolic extract of *A. vera*

Ferrous Ion Chelating Assay: In the chelating assay, both extracts of *A. vera* and standard compound interfered with the formation of ferrous and ferrozine complex suggesting that it has chelating activity. The metal binding capacity of *A. vera* extracts and standard antioxidant was determined by assessing their ability to compare with ferrozine for the ferrous ions [17]. As shown in Fig.2, the formation of ferrozine-Fe^{2+} complex was not completed in the presence of *A. vera* extracts and standard. However, chelating activity of Hot Water and Ethanolic extract with concentration of 1.0 mg/ml (71.5% and 67%) was almost same than that of EDTA (73.2%). In earlier works, at 20 mg/ml extract of *A. vera* showed good anti-fungal activity [23], and PLA2 inhibition and antioxidant activities [24] by chelates ferrous ion by 39.5-42.6% and 45.8% respectively. Hence, the *A. vera* extract can be considered as a good chelator of ferrous ion.

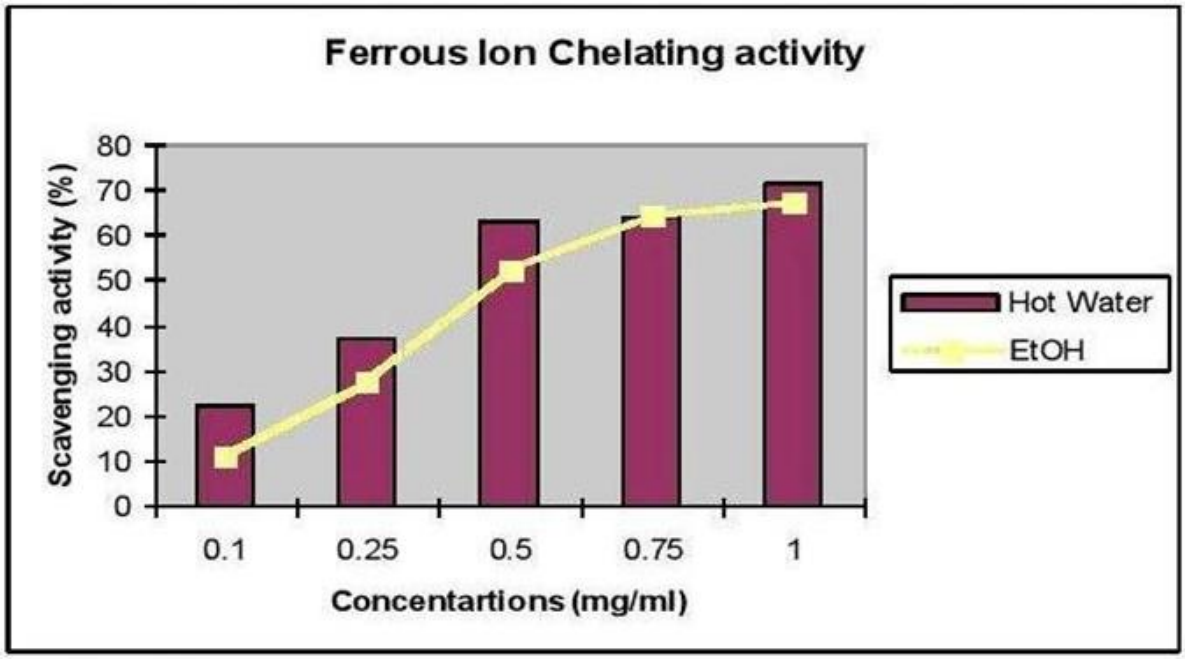

Fig. 2. Ferrous Ion Chelating Activity of hot water and ethanolic extract of *A. vera*

Reducing Power (FRAP) Assay : The result revealed that reducing power of hot water and ethanolic extracts of *A. vera* increased with an increase in concentration and was 0.59 and 0.48 in 1mg/ml of sample respectively (Fig. 3). Hot water extract displayed some higher reducing power as compared to Ethanolic at the same concentration. The reducing power of both the extract of *A. vera* was compared with that of L-ascorbic acid (1.03). It has been reported that reducing power was associated with antioxidant activity. In a related work Kumar *et al.* reported that the EC50 value for the hot water tannin extract of some medicinal plant was 1.36 mg/ml. apparetly the reducing power of *Aloe* was better than *Cassia* [26].

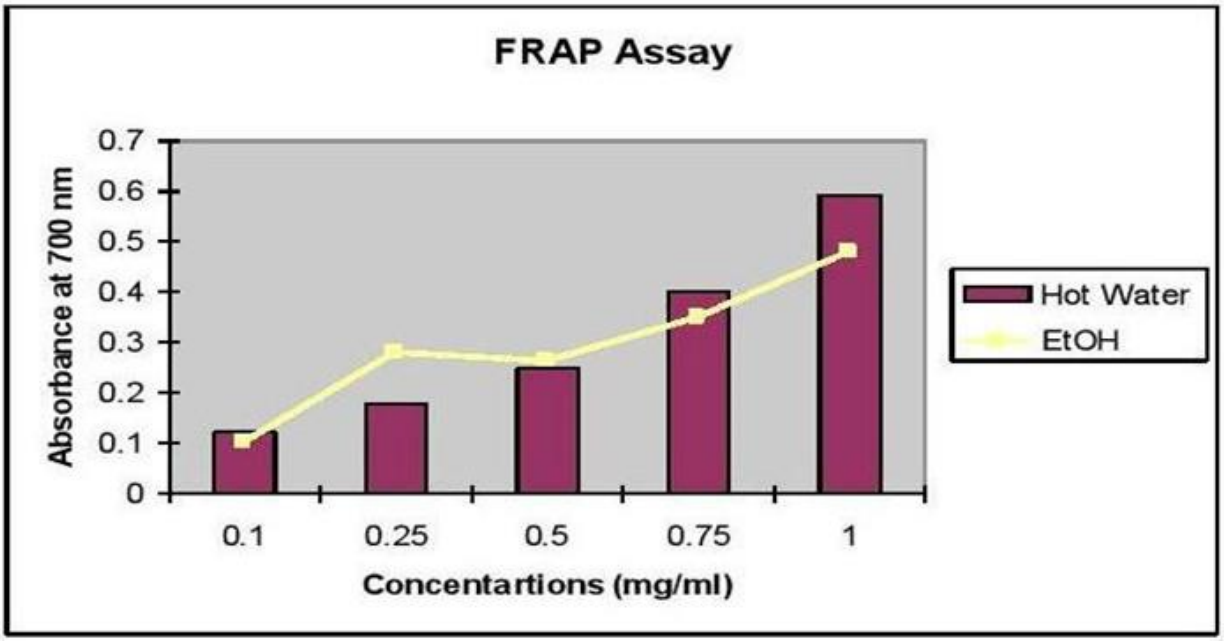

Fig. 3. FRAP (Reducing Power) Assay of hot water and ethanolic extract of *A. vera.*

CONCLUSION

A. vera is an edible mushroom although nutritional and most of the medicinal properties of the mushroom are well known but antioxidant activity of test fungus compounds have yet to be determined. In this study, hot water and ethanol extraction take into the account and it infer from result that hot water is more suitable solvent to obtain antioxidant metabolite of *A. vera*; is possibly due to the high levels of flavonoid compounds (which is confirmed by phytochemical analysis of extract data not given here) and biological activity of test fungus can be directly co-related with the chemical constituents present in it. The results showed that *A. vera* could be a good source of natural antioxidants that can be used as a possible food supplement with beneficial effect in health and also in the pharmaceutical industry.

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Conflict of Interest

The authors declare none.

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