Research Article

Phytochemical screening and *in vitro* antioxidant activities of ethanolic gel extract of *Aloe adigratana* Reynolds

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ABSTRACT

Objective: *Aloe adigratana* Reynolds is an herb that belongs to the *Xanthorrhoeaceae* family. It is native species to Tigray region in Northern Ethiopia and locally known as ere, it is called the healing plant because of its wound and burn healing properties. The present study was carried out to perform the phytochemical screening with different organic solvents and evaluate the *in vitro* antioxidant activities of ethanolic extract of *A. adigratana* Reynolds gel. **Methods:** The present study was to assess the qualitative phytochemical analysis and *in vitro* antioxidants potential of ethanolic extract of *A. adigratana* Reynolds gel by 1,1-diphenyl-2-picrylhydrazyl, reducing power assay, hydroxyl radical, nitric oxide, and superoxide radical scavenging activities using standard methods. **Results:** Qualitative phytochemical screening revealed that ethanolic extract of *A. adigratana* Reynolds gel contained a maximum number of secondary metabolites such as flavonoids, tannins and phenolic compounds, and terpenoid then other solvent extracts; further, the *in vitro* antioxidant activity of *A. adigratana* Reynolds ethanolic gel extract showed significant superoxide radical and nitric oxide scavenging activity with 76.67% and 65.39% inhibition with half maximal inhibitory concentration = 36.94 µg/ml and 62.35 µg/ml, respectively. **Conclusion:** It can be concluded that ethanolic extract of *A. adigratana* Reynolds gel had significant antioxidant properties, due to the presence of polyphenolic compounds, and could serve as a potential source of natural antioxidants, for the development of therapeutic antioxidant drugs.

KEY WORDS: Phytochemical, *Aloe adigratana* Reynolds gel, Antioxidants, 1,1-diphenyl-2-picrylhydrazyl

INTRODUCTION

Oxidative stress plays a major role in the development of chronic and degenerative diseases such as cancer, arthritis, aging, autoimmune disorders, and cardiovascular and neurodegenerative diseases.[1] The human body has several mechanisms to counteract oxidative stress by producing antioxidants, which are either naturally produced *in situ* or externally supplied through foods and/or supplements. Endogenous and exogenous antioxidants act as free radical scavengers by preventing and repairing damages caused by ROS and therefore can enhance the immune system and lower the risk of cancer and degenerative diseases.[2] Antioxidants are the substances capable of stabilizing, or deactivating, free radicals before they damaging to the cell. Many synthetic antioxidants such as butylated hydroxytoluene, butylated hydroxyanisole, and tertiary butyl hydroquinone are used for the treatment of oxidative stress-related diseases, but long-term use of these synthetic antioxidants has been causing liver and kidney damage and mutagenesis. Therefore, there is a great tendency toward natural antioxidant sources such as plant derivatives. The plant-derived secondary metabolites mainly the phenolic and flavonoids can minimize the generation of reactive oxygen species and alleviate the chronic diseases caused by oxidative stress.

Aloe adigratana Reynolds was described in 1957 based on a plant grown in St. Petersburg (Leningrad) from seeds sent by Schimper from Tigray floristic region in Ethiopia. *A. adigratana* Reynolds (እሬት-*eret* in Amharic and ዕረ-*ere* in Tigrigna) belongs to *Xanthorrhoeaceae* family. *A. adigratana* Reynolds is native to Tigray region Northern part of Ethiopia and is grown in most subtropical and tropical regions. A recent study has shown that *A. adigratana*

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Reynolds leaves were used in cotton fabric industry to control *Staphyllococcus aureus* infection to cotton plants.[3] Methanol and water extracts of *A. adigratana* Reynolds leaves have shown the *in vivo* antimalarial activities in *Parasitaemia berghei*-infected mice.[4]

The objective of present study was to screen the phytochemical analysis of different solvent extracts of *A. adigratana* Reynolds gel and evaluate the *in vitro* antioxidant potential of ethanolic extract of *A. adigratana* Reynolds gel, to evaluate a relationship between the antioxidant activity and the phytochemical constituents.

MATERIALS AND METHODS

Plant Material Collection

The leaves of *A. adigratana* Reynolds were collected from Tahtay Maychew Woreda, Akabi seat kebele, a central zone of Tigray region, Northern Ethiopia, in February 2017. The plant material was authenticated and specimen herbarium was deposited at Addis Ababa University, Biology Department, the National Herbarium of Ethiopia, and it was given the voucher specimen number of HB 001. The leaves were washed with tap water to remove dirts and impurities. The leaves were sliced across the width with sharp knife and the inner exposed surfaces revealed a transparent glutinous gel was obtained and was kept in the plastic tub, and allowed for air dried at 25°C in shade for 15 days and pulverized in mortar and pestle and stored in airtight container for further use.

Preparation of the Extracts

The powdered medicinal plant material was taken and subject to serial exhaustive solvent extraction methods, during these solvents of increasing polarity from a low polar (petroleum ether) to a high polar solvent (ethanol) to ensure that wide polarity range of compounds could be extract, during extraction solvents were diffuse in to the plant material and solubilize the phytocompounds with similar polarity.[5] For qualitative determination, the extracts were placed in pre-weighed flasks before drying. The remaining plant parts residues were extracted with other solvents sequentially.

Percentage Yield and Phytochemical Screening

The qualitative preliminary phytochemical analysis was carried out for petroleum ether, chloroform, ethyl acetatem and ethanol soluble fractions and carried out by the following methods as per the standard methods.[6,7]

Quantitative *In Vitro* Antioxidant Scavenging Assays

The ethanolic extract of *A. adigratana* Reynolds gel was analyzed for quantitatively *in vitro* antioxidant scavenging assays. The antioxidants activities were determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH),[8,9] reducing power,[10] hydroxyl radical,[11] nitric oxide,[12] and superoxide radical scavenging assay.[13] All the analyses were done in triplicates and average values were taken.

DPPH radical scavenging activity

About 4 ml of various concentrations of ethanolic extract of *A. adigratana* Reynolds gel and ascorbic acid (20, 40, 60, 80, and 100 µg/ml) were mixed with 1.0 ml of methanolic solution containing DPPH radicals, resulting in the final concentration of DPPH being 0.1 mM. The mixture was shaken strongly and left to stand for 30 min, and the absorbance was measured spectrophotometrically at 517 nm. Ascorbic acid was used as positive control and half maximal inhibitory concentration (IC_{50}) values were also calculated. Lower absorbance of the reaction mixtures indicates higher free radical scavenging activity.

The capability of scavenging the DPPH free radical was calculated using the following equation:

$$\%\,\text{of radical scavenging activity} = \frac{(\text{Abs control} - \text{Abs test})}{\text{Abs control}} \times 100$$

Where Abs control=Absorbance of DPPH solution (DPPH+methanol).

Abs test=absorbance of (DPPH solution+plant extracts or ascorbic acid solutions). All the tests were performed in triplicates and the results were averaged.

Reducing power activity

About 2.5 ml of various concentrations ethanolic extract of *A. adigratana* Reynolds gel and ascorbic acid (20, 40, 60, 80, and 100 µg/ml), 2.5 ml of 1% potassium ferricyanide, and 2.5 ml of 0.2 M sodium phosphate buffer were mixed and incubated at 50°C for 20 min, and reaction was completed by the addition of 2.5 ml of 10% (w/v) of trichloroacetic acid, followed by centrifugation at 3000 rpm for 10 min. 5.0 ml of the supernatant upper layer was mixed with 5.0 ml of deionized water and 1.0 ml of 0.1% ferric chloride. The absorbance was measured at 700 nm against blank that contained distilled water and phosphate buffer. Increased absorbance of the reaction mixtures indicates increased reducing power of the plant extract.

$$\%\,\text{of reducing power} = \frac{(\text{Abs control} - \text{Abs test})}{\text{Abs control}} \times 100$$

Where Abs control=Absorbance of potassium ferricyanide solution (potassium ferricyanide + sodium phosphate buffer).

Abs test=Absorbance of (potassium ferricyanide solution+plant extracts or ascorbic acid solutions). All the tests were performed in triplicates and the results were averaged.

OH - radical scavenging assay

The reaction mixture (3 ml) contained 1 ml $FeSO_4$ (1.5 mM), 0.7 ml hydrogen peroxide (6 mM), 0.3 ml sodium salicylate (20 mM), and various concentrations of (20, 40, 60, 80, and 100 µg/ml) of ethanolic extract *A. adigratana* Reynolds gel and ascorbic acid allowed for incubation for 1 hour at 37°C, after incubation, the absorbance of the hydroxylated salicylate complex was measured at 562 nm. The hydroxyl radical scavenging activity of the extract is reported as % inhibition of deoxyribose degradation is calculated using the following equation:

$$\%\text{ of radical scavenging activity}=\frac{(\text{Abs control}-\text{Abs test})}{\text{Abs control}}\times 100$$

Where Abs control is the absorbance of the control reaction ($FeSO_4$+hydrogen peroxide+sodium salicylate), and Abs test is the absorbance in the presence of all of the extract samples ($FeSO_4$+hydrogen peroxide+sodium salicylate+ plant extract or ascorbic acid). All the tests were performed in triplicates and the results were averaged.

Nitric oxide scavenging activity

Various concentrations (20, 40, 60, 80, and 100 µg/ml) of ethanolic extract of *A. adigratana* Reynolds gel and ascorbic acid were mixed with 2.5 ml of sodium nitroprusside and made up to 3.0 ml with PBS. Then, the mixture was incubated for 15 minutes at 25°C. After incubation, 0.5 ml of the reaction mixture was removed and 0.5 ml of Griess reagent was added. Then, the absorbance was measured at 546 nm. Decreased absorbance of reaction mixture indicated increased nitric oxide scavenging activity.

The percentage of inhibition was calculated by comparing the results of the test with those of controls not treated with the extract, as per the following formula:

$$\%\text{ of radical scavenging activity}=\frac{(\text{Abs control}-\text{Abs test})}{\text{Abs control}}\times 100$$

Where Abs control is the absorbance of the control reaction (sodium nitroprusside + PBS+ Griess reagent), and Abs test is the absorbance in the presence of all of the extract samples (sodium nitroprusside+PBS+griess reagent+plant extract or ascorbic acid standard). All the tests were performed in triplicates and the results were averaged.

Superoxide radical activity

The reaction mixture consists of 20 µl of varying concentration of (20, 40, 60, 80, and100 µg/ml) ethanolic extract of *A. adigratana* Reynolds gel and ascorbic acid, to which 0.2 ml of EDTA, 0.1 ml of nitro blue tetrazolium, and 0.05 ml of riboflavin were added and the reaction was started by adding 2.25 ml of phosphate buffer to reaction mixture, and control tubes were set up without the extract. Similarly, the activity of the standard ascorbic acid was also carried out, allowed for incubated at 25°C for 5 min, and the absorbance at 560 nm was measured against blank. Decreased absorbance of reaction mixture indicated increased superoxide anion scavenging activity.

The percentage inhibition was calculated by comparing with the optical density of the control tubes.

$$\%\text{ of radical scavenging activity}=\frac{(\text{Abs control}-\text{Abs test})}{\text{Abs control}}\times 100$$

Where Abs control is the absorbance of the control reaction (EDTA+ nitro blue tetrazolium+riboflavin), and Abs test is the absorbance in the presence of all of the extract samples (EDTA+nitro blue tetrazolium+riboflavin+plant extract or ascorbic acid standard). All the tests were performed in triplicates and the results were averaged.

Statistical Analysis

All experiments were performed in triplicate (n = 3) and results were expressed as mean ± SD. Statistical analysis and IC_{50} values were calculated using the computer program Excel 2015. The simplest estimate of IC_{50} was to plot x–y and fits the data with a straight line (linear regression). IC_{50} value is then estimated using the following formula:

$$y=a*x+b$$

$$IC_{50}=\frac{(0.5-b)}{a}$$

Where IC_{50}=Half-maximal inhibitory concentration.

Results AND DISCUSSION

Percentage Yield of *A. adigratana* Reynolds Gel in Different Solvent Extractions

As showed in Table 1, the highest percentage of yield was obtained in ethanol extract (580 mg/100 g), followed by petroleum ether (186 mg/100 g), chloroform (134 mg/100 g), and ethyl acetate extract (120 mg/100 g) in *A. adigratana* Reynolds gel. Saritha *et al.*[14] reported that the percentage yield of extractive values of crude 95% ethanol extracts of *Aloe vera* gel was 154.1 mg/100 g. Then, the results of the present study were parallel with the results of other.

Qualitative Phytochemical Analysis of *A. adigratana* Reynolds Gel

As showed in Table 2, the preliminary phytochemical analysis of different organic solvent extract of *A. adigratana* Reynolds gel revealed that the maximum number of secondary metabolites such as flavonoids, saponins, steroids, tannins and phenolic compounds, alkaloids, terpenoids, and cardic glycosides presents in ethanolic extracts of *A. adigratana* Reynolds gel. All these constituents are reported to exhibit strong antioxidant scavenging activity for the radicals involved in the lipid peroxidation.[15] Sirisha *et al*.[16] also reported that 80% ethanolic extract of *Aloe vera* leaf contained maximum secondary metabolites such as flavonoids, saponins, terpenoids, steroids, and polyphenolic, but like alkaloids, tannins, and cardic glycosides showed negative. Then, the results of the present study are coexisting with the results of other.

Quantitative *In Vitro* Antioxidant Assays

Based on the chemical reactions or reaction mechanisms, antioxidant methods are divided into two different categories, which are hydrogen atom transfer (HAT)-based assay and single electron transfer (SET)-based assay. SET and HAT reactions together used for evaluating the antioxidant activities of ethanolic extract of *A. adigratana* Reynolds gel were determined by DPPH, reducing power, hydroxyl radical, nitric oxide radical, and superoxide radical scavenging *in vitro* assay.

DPPH radical scavenging activity

The results of DPPH radical scavenging activity of the ethanolic extract of *A. adigratana* Reynolds gel and the standard ascorbic acid were shown in Figure 1.

Table 1: The percentage yield of *A. adigratana* Reynolds gel in different solvents

Solvent	% yield mg/100 g
Petroleum ether	186
Chloroform	134
Ethyl acetate	120
Ethanol	580

A. adigratana: Aloe adigratana

The percentage of inhibitory activity of free radical by 50% had been used widely as a parameter to measure antioxidant activity. In this study, both ethanolic extract and standard ascorbic acid significantly of reduced the DPPH radical with increasing concentrations. The percentage inhibition of the DPPH radical by the ethanolic extract of *A. adigratana* Reynolds gel and ascorbic acid standard at 100 µg/ml was 55% and 79% while the IC_{50} values were 82.62 µg/ml and 33.7 µg/ml, respectively, and the color changed from purple to yellow. Sirisha *et al*.,[16] reported that 80% ethanolic extract of *Aloe vera* leaf exhibited maximum inhibition 94.39% and its IC_{50} value was 30 µg/ml at 100 µg/ml concentration.

Reducing power activity

The antioxidant activity of ethanolic extract of *A. adigratana* Reynolds gel was determined by measuring its ability to transform $Fe3^{+}$ to $Fe2^{+}$. The reducing power was confirmed by the changes of yellow color of the test solution to various shades of green and blue depending on the concentration of the plant extract. As shown in Figure 2, the reducing power of the ethanolic extract and the standard ascorbic acid increased with an increase in concentration and the OD at 700 nm of ethanolic extract of *A. adigratana* Reynolds gel increased in a dose-dependent manner from 0.05 at 20 µg/ml to 0.098 at 100 µg/ml. The reducing power is a reflection of antioxidant activity.[17] Compounds with reducing power can donate electron and thus reduce the intermediates of

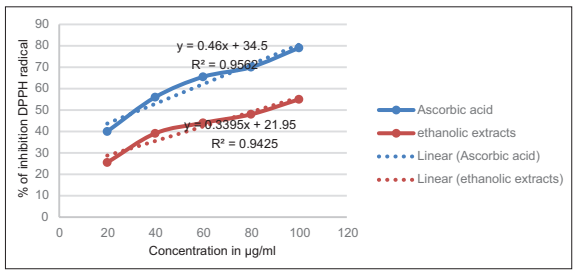

Figure 1: 1,1-diphenyl-2-picryl hydrazyl radical scavenging activity ethanolic extract of *Aloe adigratana* Reynolds gel compared to that of standard ascorbic acid. Each value is expressed as mean ± standard deviation ($n = 3$)

Table: 2: Qualitative phytochemical analysis of different organic solvent crude extracts of *A. adigratana* Reynolds gel

Phytoconstituents	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Ethanol extract
Alkaloids	+	--	--	+
Flavonoids	--	+	--	+
Tannins	--	+	--	+
Glycosides	+	--	+	+
Terpenoids	--	+	+	+
Saponins	--	--	+	--
Steroids	+	+	--	+
Carbohydrates	+	+	--	+
Fixed oils and fats	+	--	--	--
Amino acids and proteins	+	+	+	+

+: Present. --:Absent. *A. adigratana: Aloe adigratana*

lipid peroxidation and act as primary and secondary antioxidants.[18] Sirisha *et al*.[16] is also reported that 80% ethanolic extract of *Aloe vera* leaf exhibited the maximum absorbance of 0.62 ± 0.005 at 100 µg/ml concentration. Then, the result of the current analysis was also concurrent with the results of others.

Hydroxyl radical scavenging assay

As shown in Figure 3, the results of hydroxyl radical scavenging activity of both the ethanolic extract of *A. adigratana* Reynolds gel and the standard at varied concentration exhibited concentration-dependent OH scavenging activity were found to be maximum at a concentration of 100 µg/ml with inhibition of 58.33% in case of ethanolic extract and 79% in case of standard ascorbic acid, and the IC_{50} values of the ethanolic extract and the standard were found to be 59.99 µg/ml and 34.66 µg/ml, respectively.

The hydroxyl radical is an extremely reactive in biological systems and has been implicated as highly damaging species in free radical pathology and capable of damaging biomolecules of the living cells. These radicals combine with nucleotides in DNA and cause strand breakage leading to carcinogenesis, mutagenesis, and cytotoxicity.[19] In this study, as shown in Figure 3, the ethanolic extract of *A. adigratana* Reynolds gel was found to scavenge OH radicals significantly 58.3% at 100 µg/ml concentration with IC_{50} = 59.99 µg/ml in dose-dependent manner and protect the DNA, protein, and lipid from damage due to the presence of proton-donating antioxidants in the ethanolic gel extract of *A. adigratana* Reynolds. Rajamanikandan *et al*.[20] reported that the ethanolic extract of *Mollugo nudicaulis* showed a significant dose-dependent hydroxyl radical scavenging activity and it reached up to 73.84% at the concentration of 2.5 mg/ml. However, Vitamin C which was used as a positive control showed better radical scavenging effect 90% at the concentration of 2.5 mg/ml. Sirisha *et al*. [16] reported that 80% ethanolic extract of *Aloe vera* leaf exhibited maximum inhibition was 71.6% and its IC_{50} = 40 µg/ml. Then, the results of the present study also matched with the result of others.

Nitric oxide radical scavenging assay

The scavenging activity of the ethanolic extract against nitric oxide released by sodium nitroprusside was investigated and the result was showed in Figure 4, and the percentage of inhibitory activity of the ethanolic extract and ascorbic acid standard was 65.39% and 85.39% at 100 µg/ml while the IC_{50} values were 54.37 µg/ml and 23.15 µg/ml, respectively. Nitric oxide radical is a free radical produced in mammalian cells, involved in the regulation of various physiological process including neurotransmission, vascular homeostasis, antimicrobial, and antitumor activities. However, excess production of NO is associated with

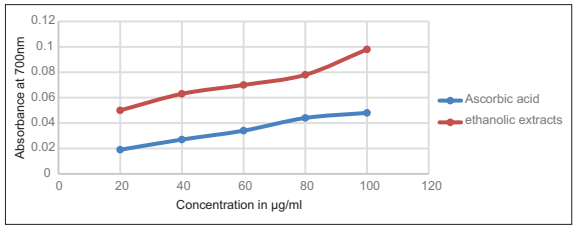

Figure 2: Reducing power activity of ethanolic extract of *Aloe adigratana* Reynolds gel compared to that of standard ascorbic acid. Each value is expressed as mean ± standard deviation (n = 3)

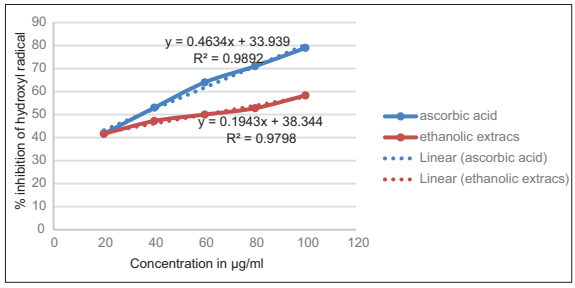

Figure 3: Hydroxyl radical scavenging activity of ethanolic extract of *Aloe adigratana* Reynolds gel compared to that of standard ascorbic acid. Each value is expressed as mean ± standard deviation (n = 3)

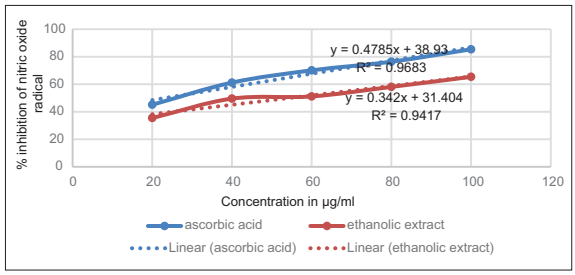

Figure 4: Nitric oxide scavenging activity of ethanolic extract of *Aloe adigratana* Reynolds gel compared to that of standard ascorbic acid. Each value is expressed as mean ± standard deviation (n = 3)

several diseases. It would be interesting to develop potent and selective inhibitors of NO for potential therapeutic use.[21] In the present study, as shown in Figure 4, the ethanolic extract of *A. adigratana* Reynolds gel exhibited potent nitric oxide radical scavenging activity of 65.39% at 100 µg/ml with IC_{50} value of 54.37 ± 1.81 µg/ml. Madhusudhhanan *et al*.[22] reported that alcoholic extract of *N. alba* flowers showed maximum inhibition was 47.25% at 125 µg/ml concentration.

Superoxide radical scavenging activity

Superoxide anion radical scavenging activity of ethanolic extract of *A. adigratana* Reynolds gel was shown in Figure 5, the decrease of absorbance indicates the consumption of superoxide anion in the reaction mixture by the ethanolic extract, and the maximum scavenging activity with value of 76.67%

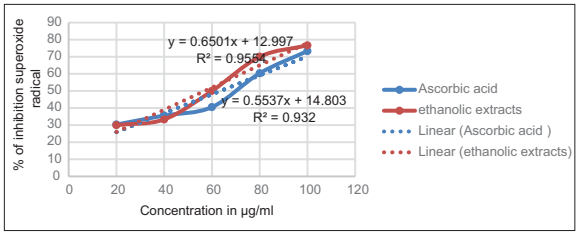

Figure 5: Superoxide radical scavenging activity of ethanolic extract of *Aloe adigratana* Reynolds gel compared to that of standard ascorbic acid

at 100 µg/ml compared with standard ascorbic acid value 73.33% at 100 µg/ml. The IC_{50} value of the standard was 63.57 µg/ml while that of the ethanolic extract was 56.92 µg/ml.

Superoxide anion radical is a weak oxidant produced during various biological reactions are highly toxic.[23] Superoxide anion radical is known as an initial radical and plays an important role in the formation of other reactive oxygen species, such as hydrogen peroxide or singlet oxygen.[24] As shown in Figure 5, the ethanolic extract of *A. adigratana* Reynolds gel scavenged the superoxide anion more effectively compared with other *in vitro* assays, at 100 µg/ml, its showed the 76.67% with IC_{50} value of 56.92 µg/ml which is attributed by the presence of phenolic groups that could donate electrons neutralizing superoxide anion. Sujith *et al*.[25] reported that 70% ethanolic extract of *Aloe vera* leaf exhibited that maximum inhibition was 26.35% and its IC_{50} value was 270.90 µg/ml at 100 µg/ml concentration. The results of the present study emphasized that the ethanolic gel extract of *A. adigratana* Reynolds showed significant scavenging activities against superoxide and NO free radicals may be due to the presence of an appreciable amount of bioactive secondary metabolites such as polyphenols, flavonoid, and terpenoid. Hence, these finding provide scientific evidence to support the traditional use of *A. adigratana* Reynolds for treatment of oxidative stress-related disease and indicate ethanolic extract of *A. adigratana* Reynolds a promising potential for the development antioxidant drug.

CONCLUSION

The antioxidative properties of the plants have become a great interest due to their possible uses as natural antioxidant drugs to replace synthetic drugs. The results obtained in the present study have shown that ethanolic gel extract of *A. adigratana* Reynolds has a high content of bioactive secondary metabolites such as flavonoids, terpenoids, steroids, polyphenolic, alkaloids, tannins, and cardic glycosides without any toxicity and shown potential antioxidant activity. Although the medicinal importance of the *A. adigratana* Reynolds gel is well known, its therapeutic importance for the development of pharmaceutical active compounds is not thoroughly worked out. This present study attempted to fill the gap of the medicinal importance of *A. adigratana* Reynolds gel in traditional and modern medical system.

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