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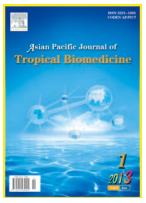

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In vitro antioxidant activities of *Solanum surattense* leaf extract

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PEER REVIEW

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Comments

In this study the effect of alcoholic leaf-extract of *S. surattense* on free radical scavenging activity were determined by *in vitro* model. *S. surattense* were used at different concentrations. And hydroxyl radical and hydrogen peroxide, inhibition of superoxide anion radical and 2, 2-diphenyl-1-picryl hydrazyl (DPPH) radical, total antioxidant activity and reducing ability were study. Nevertheless the data and the conclusion of this work are interesting.
(Details on Page 33)

ABSTRACT

Objective: To evaluate the antioxidant activity of alcoholic leaf-extract of *Solanum surattense* (Solanaceae) (*S. surattense*). **Methods:** Leaf extract were tested for *in vitro* free radical scavenging assays, such as hydroxyl radical and hydrogen peroxide, inhibition of superoxide anion radical and 2, 2-diphenyl-1-picryl hydrazyl radical (DPPH), total antioxidant activity and reducing ability. Further, total phenolic content of *S. surattense* was analyzed. **Results:** *S. surattense* extract effectively scavenged free radicals at all different concentrations and showed its potent antioxidant activity. Further, these effects were in a dose dependent manner. Results were compared to standard antioxidants such as butylated hydroxytoluene, ascorbic acid and α-tocopherol. **Conclusions:** *S. surattense* have strong antioxidant potential. Further the study validates the therapeutic benefits of the Indian system of medicine.

KEYWORDS
Solanum surattense, DPPH, Lipid peroxidation, Antioxidant

1. Introduction

The health promoting benefits of antioxidants of plants are thought to be resulted from their potential effects against the reactive oxygen/nitrogen species. Restriction on the use of synthetic antioxidants due to their possible undesirable effects on human health[1] has led to a growing interest in natural antioxidants of plant origin in recent years . Hence, the development of antioxidants from natural origin has attracted considerable attention and is thought to be a desirable development. Moreover, several studies have indicated that medicinal plants contain a wide variety of natural antioxidants

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such as phenolic acids, flavonoids and tannins, which possess antioxidant activity[2].

Solanum surattense (Solanaceae) (*S. surattense*) is commonly used in Indian traditional medicine for curing various ailments such as respiratory diseases, gonorrhoea, rheumatism, fever and asthma[3]. The plant is useful in fever, cough, asthma and pain in chest, being used in the form of decoction or an electuary. The fruit and leaf extract possess significant antihyperglycaemic activity[4-6]. There is an increasing interest in natural antioxidants, *e.g.* polyphenols, present in medicinal and dietary plants, which might help to prevent oxidative damage[7].

Hence, our aim was to assess the antioxidant and free radical scavenging activities of alcoholic leaf extract of *S. surattense* and to determine its total phenolic content.

2. Materials and methods

2.1. Chemicals

Chemicals like 1,1-Diphenyl-2-picryl-hydrazyl (DPPH), 2,2′-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid)-(ABTS), nicotinamide adenine dinucleotide (NADH), nitroblue tetrazolium (NBT) and phenazine methosulphate (PMS) were from Sigma (St. Louis, Missouri, USA). All other chemicals used were of analytical grade obtained from E. Merck and HIMEDIA, Mumbai, India.

2.2. Plant materials and extraction

Leaves of *S. surattense* were collected from local areas of Chidambaram, Tamil Nadu, India. The plant was botanically identified and authenticated in the Department of Botany, Annamalai University, Annamalainagar, Tamil Nadu, India and a voucher specimen (No. AU 189) was deposited at the herbarium of botany.

2.3. Preparation of leaf extract

The plant leaves were shade dried at room temperature of (32 ±2) °C and the dried leaves were ground into fine powder using pulverizer. The powdered part was sieved and kept in deep freezer until the time of use. One hundred grams of dry powder was suspended in 400 mL of ethanol for 72 h. The extract was filtered using a muslin cloth and concentrated at (40±5) °C.

2.4. Hydroxyl radical scavenging assay

The hydroxyl radical scavenging activity of *S. surattense* was determined by the method of Halliwell *et al*[8]. In this assay, hydroxyl radicals are produced by the reduction of H_2O_2 by the transition metal (iron) in the presence of ascorbic acid. The generation of hydroxyl radical is detected by its ability to degrade deoxyribose to form products which forms a pink colour chromogen when heating with TBA. Addition of *S. surattense* competes with deoxyribose for hydroxyl radicals and diminishes the colour formation. The incubation mixture in a total volume of 1 mL contained 0.1 mL of buffer, varying volumes of *S. surattense* (50, 100, 150, 200 and 250 μg), 0.2 mL of 500 μmol/L ferric chloride, 0.1 mL of 1mM ascorbic acid, 0.1 mL of 1 mmol/L EDTA, 0.1 mL of 10 mmo/L hydrogen peroxide and 0.2 mL of 15 mmol/L 2-deoxyribose. The contents were mixed thoroughly and incubated at room temperature for 60 min. Then 1 mL of 1% TBA (1 g in 100 mL of 0.05 N NaOH) and 1 mL of 28% TCA were added. All the tubes were kept in a boiling water bath for 30 min. The absorbance of the supernatant was read in a spectrophotometer at 535 nm with reagent blank containing water in place of extract. The percentage of scavenging was determined. The efficiency of *S. surattense* was compared with various concentrations (50, 100, 150, 200 and 250 μg) of standard α-tocopherol (vitamin E). Decreased absorbance of the reaction mixture indicated increased hydroxyl radical scavenging activity. The percentage of scavenging was calculated as shown below:

$$\% \text{ Scavenging } [OH^{\cdot}] = \frac{A_0 - A_1}{A_0} \times 100$$

Where A_0 was the absorbance of the control, and A_1 was the absorbance in the presence of the sample of *S. surattense* or standard.

2.5. Scavenging of hydrogen peroxide

The ability of the *S. surattense* to scavenge H_2O_2 was determined according to the method of Ruch *et al*[9]. A solution of H_2O_2 (40 mmol/L) was prepared in phosphate buffer (pH 7.4). H_2O_2 concentration was determined spectrophotometrically from absorption at 230 nm in a spectrophotometer (SL 159, UV- Visible Spec, Elico, India). Extracts (50, 100, 150, 200 and 250 μg) in distilled water were added to a H_2O_2 solution (0.6 mL, 40 mmol/L). Absorbance of H_2O_2 at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without H_2O_2. The percentage of scavenging of H_2O_2 of *S. surattense* and α-tocopherol was calculated using the following equation:

$$\% \text{ Scavenging } [H_2O_2] = \frac{A_0 - A_1}{A_0} \times 100$$

2.6. Superoxide anion scavenging activity

Superoxide anion scavenging activity of *S. surattense* was determined by the method of Nishimiki *et al.*[10] with modifications. The assay was based on the oxidation of NADH by PMS to liberate PMS_{red}. PMS_{red} converted oxidized nitroblue tetrazolium (NBT_{oxi}) to the reduced form NBT_{red}, which formed a violet colour complex. In the PMS-NADH-NBT system, superoxide anion is derived from dissolved oxygen by PMS-NADH coupling reaction and reduces NBT. The colour

formation indicated the generation of superoxide anion, which was measured spectrophotometrically at 560 nm. A decrease in the formation of colour after addition of the antioxidant was a measure of its superoxide scavenging activity.

One millilitter of NBT (100 μmol of NBT in 100 mmol/L phosphate buffer, pH 7.4), 1 mL of NADH (468 μmol in 100 mmol/L phosphate buffer, pH 7.4) solution and varying volumes of *S. surattense* (50, 100, 150, 200 and 250 μg concentration) were mixed well. The reaction was started by the addition of 100 μL of PMS (60 μmol of 100 mmol/L phosphate buffer, pH 7.4). The reaction mixture was incubated at 30 °C for 15 min. The absorbance was measured at 560 nm in a spectrophotometer. Incubation without *S. surattense* was used as blank. Ascorbic acid was used as standard for comparison. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The percentage of scavenging was calculated as the previous equation.

2.7. DPPH radical scavenging activity

The radical scavenging activity of *S. surattense* against DPPH was determined spectrophotometrically by the method of Brand Williams *et al*[11]. DPPH reacts with an antioxidant compound that can donate hydrogen and it gets reduced. The change in colour (from deep violet to light yellow) was measured. DPPH is a stable free radical and accepts an electron, or hydrogen radical to become a stable diamagnetic molecule. The intensity of the yellow colour depends on the amount and nature of radical scavenger present.

A reaction mixture containing 1 mL of 0.1 mmol/L DPPH, various concentration of *S. surattense* (20, 40, 60, 80 and 100 μg) were made up to 3 mL using water. Then the tubes were incubated for 10 min. The formed yellow colour chromophore was measured at 517 nm. BHT was used as a standard for comparison.

2.8. Total antioxidant activity by ABTS radical cation decolorization assay

The total antioxidant activity of *S. surattense* was measured by the method of ABTS radical cation decolourisation assay[12]. The improved technique for the generation of $ABTS^{+}$ described here involves the direct production of the blue/green $ABTS^{+}$ chromophore through the reaction between $ABTS^{+}$ and potassium persulphate. Addition of *S. surattense* and other antioxidants compete with $ABTS^{+}$ diminish the color formation.

$ABTS^{+}$ was dissolved in water to a 7 mmol/L concentration. $ABTS^{+}$ was produced by reacting $ABTS^{+}$ stock solution with 2.45 mmol/L potassium persulphate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. Because $ABTS^{+}$ and potassium persulphate react stoichiometrically at a ratio of 1:0.5, this will result in incomplete oxidation of the $ABTS^{+}$. The incubation mixture in a total volume of 5 mL contained 0.54 mL of $ABTS^{+}$, 0.5 mL of 100 mmol/L phosphate buffer pH 7.4 and varying concentrations of *S. surattense* (20, 40, 80, 120 and 160 μg). The blank contains water in place of samples. The absorbance was read in a spectrophotometer at 734 nm and compared with standard BHT at various concentrations (20, 40, 80, 120 and 160 μg).

2.9. Reducing ability

The reducing power of *S. surattense* was determined by the method of Oyaizu[13]. Substances which have reduction potential react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric-ferrous complex that has an absorption maximum at 700 nm. Increase in the reduction of ferric to ferrous ion increases the absorbance indicating the reducing ability of *S. surattense*. Varying concentrations of *S. surattense* (50, 100, 150, 200, 250 and 300 μg) in double distilled water was mixed with 2.5 mL of phosphate buffer and 2.5 mL of potassium ferricyanide. The mixture was incubated at 50 °C for 20 min after which, 1.5 mL of TCA was added and centrifuged at 3 000 ×g for 10 min. From all the tubes, 0.5 mL of supernatant was mixed with 1 mL of distilled water and 0.5 mL of ferric chloride. The absorbance was measured at 700 nm in a spectrophotometer. α-tocopherol was used as a standard for comparison. Increased absorbance of the reaction mixture indicates increasing reducing power. Incubation with water in place of additives was used as the blank.

2.10. Total polyphenolic content

Total phenolics in the *S. surattense* were determined using Folin-Ciocalteau reagent by the method of Singleton and Rossi using gallic acid as the standard[14].

Various concentrations of *S. surattense* (100, 200, 300, 400 and 500 μg) were mixed with 2 mL of 2% sodium carbonate. After 2 min, 0.1 mL of diluted Folins-phenol reagent (1:1 ratio with water) was added and incubated at room temperature for 30 min. The absorbance was measured in a spectrophotometer at 720 nm. Standard gallic acid of concentrations ranging from 10-50 μg and water blank were processed together. The total phenolic content in the extract was expressed as gallic acid equivalents:

$$\text{Total polyphenolic content} = \frac{\text{Test OD}}{\text{Standard OD}} \text{ [Standard concentration (μg)]}$$

2.11. Phytochemical analysis

The ethanolic extract analysis of *S. surattense* by Harborne method showed the presence of alkaloids, flavonoids, tannins, glycosides, triterpenoids and sterols in our laboratory[15].

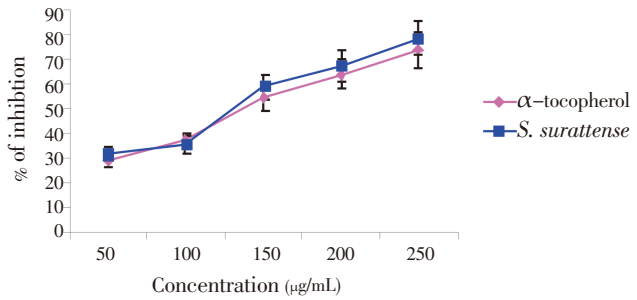
2.12. Statistical analysis

All the values were expressed as means±SD of six determinations.

3. Results

3.1. Inhibition of hydroxyl radical

The scavenging ability of *S. surattense* on OH^- is shown in Figure 1 and compared with α-tocopherol. Degradation of deoxyribose by OH^- released certain products, which upon heating with TBA under acid condition would yield a pink colour with maximum absorbance at 532 nm. *S. surattense* leaf-extract exerted inhibition of OH^- formation during incubation period and percentage of inhibition is higher than α-tocopherol at all concentrations.



Figrue 1. Hydroxyl radical scavenging ability of *S. surattense* leaf extract and α-tocopherol.

3.2. Scavenging of hydrogen peroxide

The scavenging ability of *S. surattense* on H_2O_2 is shown in Figure 2 and compared with α-tocopherol. *S. surattense* was capable of scavenging H_2O_2 in a dose-dependent manner and the scavenging activity was better than α-tocopherol at all concentrations.

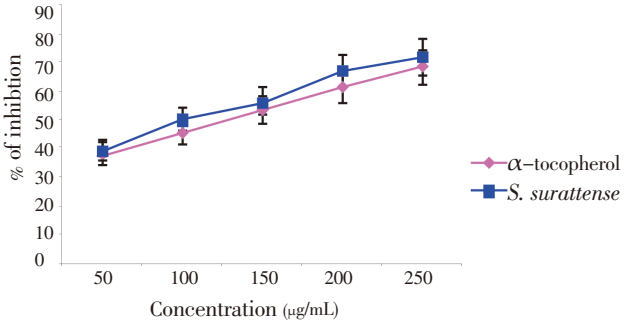


Figure 2. Scavenging of hydrogen peroxide by *S. surattense* leaf-extract and α-tocopherol.

3.3. Inhibition of superoxide anion radical

The superoxide anion scavenging ability of *S. surattense* has been presented in Figure 3. In the PMS-NADH-NBT system, superoxide anion was derived from dissolved oxygen by PMS-NADH coupling reaction and reduces NBT. The decrease of absorbance at 560 nm with antioxidants indicates the consumption of superoxide anion in the reaction mixture. The inhibition of $O_2^{\bullet -}$ was found to be concentration dependent and the percentage of inhibition by *S. surattense* was greater than ascorbic acid.

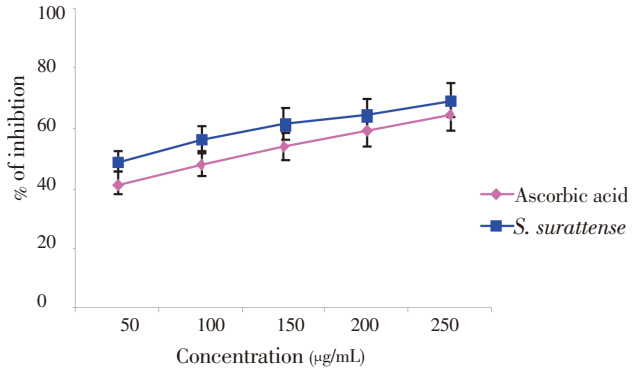


Figure 3. Inhibition of superoxide anion radical by *S. surattense* leaf-extract and ascorbic acid.

3.4. Inhibition of DPPH radical

The scavenging ability of *S. surattense* on DPPH is shown in Figure 4 and compared with that of BHT. The scavenging effect of extract and standard on the DPPH radical was expressed as percentage inhibition. *S. surattense* exhibited effective antioxidant activity and was better than BHT.

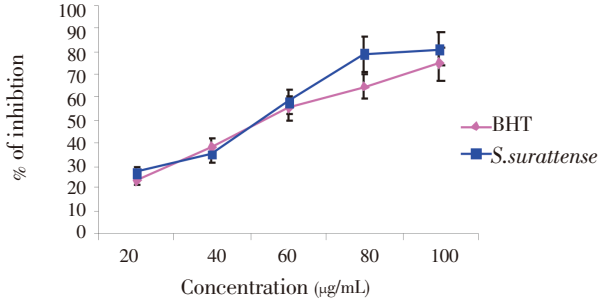


Figure 4. Inhibition of DPPH radical by *S. surattense* leaf extract and BHT.

3.5. Total antioxidant activity-ABTS radical cation decolourization assay

Total antioxidant activity of *S. surattense* was assessed by measuring the reduction of the ABTS radical cation as the percentage of inhibition at 734 nm. The effect of various concentrations of extract (from 20 to 160 μg) on $ABTS^{\bullet +}$ radical is shown in Figure 5. *S. surattense* exhibited effective antioxidant activity. The inhibition was found to be concentration dependent and the antioxidant activity was better than the standard BHT.

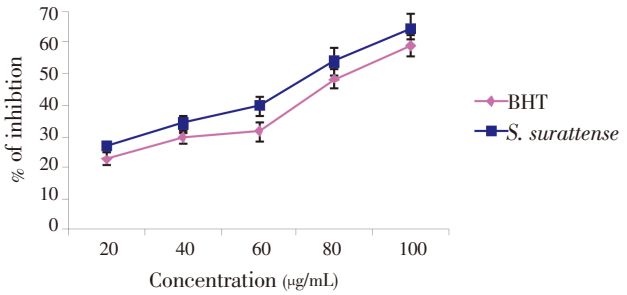


Figure 5. Total antioxidant activity of *S. surattense* leaf extract by ABTS radical cation decolourization assay.

3.6. Reducing ability

Figure 6 shows the reductive capabilities of *S. surattense* and α-tocopherol. The reducing power of *S. surattense* increased concentration dependently and it showed higher reducing power than α-tocopherol.

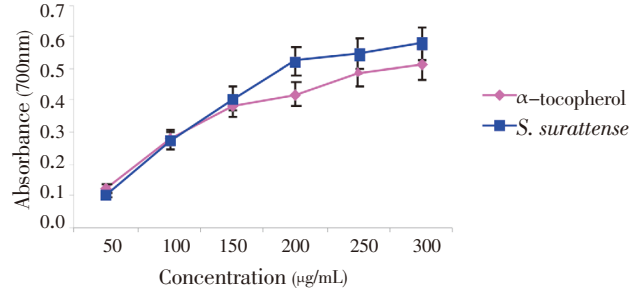


Figure 6. Reducing ability of *S. surattense* leaf extract and α-tocopherol.

3.7. Total phenolic content

The total phenolic content of *S. surattense* was estimated, since phenolics may significantly contribute to its overall antioxidant activity. The phenolic content of the extract was 46.7 mg gallic acid equivalents.

4. Discussion

Free radicals and other reactive oxygen species including superoxide anion radicals, hydroxyl radicals and hydrogen peroxide are highly reactive and potentially damaging transient chemical species formed in aerobic life. They are well recognized for playing a dual role as both deleterious and beneficial species, since they can be either harmful or beneficial to living system. In the recent past, several herbal drugs, which have free radical scavenging potential, have gained importance in treating chronic diseases[16,17].

Among the oxygen radicals, hydroxyl radicals are the most reactive and induce severe damage to the adjacent biomolecules. It can abstract hydrogen atoms from biological molecules, including thiols, leading to the formation of sulfur radicals capable to combine with oxygen to generate oxysulfur radicals, a number of which damage biological molecules. Hydroxyl radicals are produced *in vivo* by Fenton-type reactions, in which transition metals (*e.g.* iron) reduce hydrogen peroxide. Reducing agents such as ascorbic acid can accelerate $OH^{\cdot}$ formation by reducing Fe^{3+} ions to Fe^{2+}[18]. The result of this study shows the effect of the extracts on the iron (II)-dependent deoxyribose damage. The scavenging ability of *S. surattense* on $OH^{\cdot}$ was compared with α-tocopherol. *S. surattense* exhibited more pronounced hydroxyl radical scavenging activity compared to α-tocopherol in a dose-dependent manner. In $OH^{\cdot}$ radical scavenging assay the 50% inhibitory concentration (IC_{50}) value of the extract was 154.03 μg/mL. Phytochemical studies of *S. surattense* leaf extract revealed the presence of various bioactive compounds such as alkaloids, flavonoids, tannins, glycosides, triterpenoids and sterols, which may be acting synergistically. The phenolic compounds have direct antioxidative activity due to their hydroxyl groups and were found to play an important role in stabilizing lipid peroxidation[19,20]. Tanins have a characteristic feature of metal chelation and also act through their redox property and hydrogen donating potential[21].

H_2O_2 itself is not very reactive, but it may be toxic to cell since it may give rise to hydroxyl radicals in cells. *S. surattense* was capable of scavenging H_2O_2 in a dose-dependent manner and the scavenging activity was better than α-tocopherol at all concentrations. In H_2O_2 scavenging assay the IC_{50} value of the extract was 147.23 μg/mL. Scavenging of H_2O_2 by *S. surattense* may be attributed to their phytochemicals such as flavonoids, alkaloids, phenolics *etc.* which could donate electrons to H_2O_2, thus neutralizing it to water. Many authors also have correlated antioxidant activity with their polyphenolic or phenolic contents[22].

Superoxide radical is known to be very harmful to cellular components as a precursor of more reactive oxidative species, such as singlet oxygen and hydroxyl radicals. Furthermore, superoxide radical is considered to play an important role in the peroxidation of lipids. Therefore, studying the scavenging effects of *S. surattense* on superoxide radicals is one of the most important ways of clarifying the mechanism of antioxidant activity. The inhibition of O^{2-} was found to be concentration dependent and the percentage of inhibition was greater than ascorbic acid at all concentrations studied. The IC_{50} value of the extract was 145.22 μg/mL. (This sentence should be put in the part of Results) In H_2O_2 scavenging assay the IC_{50} value of the extract was 147.23 μg/mL. These results indicated that *S. surattense* had a notable effect in scavenging superoxide radicals. Inhibition of superoxide generation by *S. surattense* may be due to the presence of phytochemicals such as flavonoids, alkaloids and phenolics[23].

The DPPH radicals were widely used to investigate the scavenging activity of some natural compounds. Figure 4 shows the results of scavenging DPPH radical ability of *S. surattense* at various concentrations in comparison with same doses of BHT. In DPPH scavenging assay the IC_{50}

value of the extract was 55.62 μg/mL. *S. surattense* showed dose-dependent DPPH radicals scavenging activity. The decrease in absorbance of DPPH caused by antioxidants is due to the reaction between antioxidant molecules and radical, which results in the scavenging of the radical by hydrogen donation. Similar results have been reported by many authors[24,25].

Total antioxidant activity of *S. surattense* was determined by ABTS radical cation decolourization assay by measuring the reduction of the radical cation as the percentage inhibition. *S. surattense* (from 20 to 160 μg) exhibited effective antioxidant activity at all doses. The scavenging effect of *S. surattense* and BHT was observed to be linear increase in ABTS radical scavenging activity with increasing concentration. The inhibition was found to be concentration dependent and BHT. In ABTS scavenging assay the IC_{50} value of the extract was 89.28 μg/mL. The antioxidant activity of *S. surattense* might be attributed to the presence of phytochemicals such as flavonoids and phenolic compounds. Flavonoids possess a broad spectrum of chemical and biological activities including radical scavenging properties[26]. The antioxidative activity of *S. surattense* may be due to the reduction of hydroperoxides, inactivation of free radicals, or combination both.

The reducing power of a compound may serve as a significant indicator of its potential antioxidant activity[27]. In The reducing power increased with the increasing amount of extract. The reducing capacity of plant may serve as a significant indicator of its potential antioxidant activity. Seddik *et al.* reported that the reducing power of tannins prevents liver injury by inhibiting the formation of lipid peroxides[28]. The reducing power of *S. surattense* increased with increasing amount of sample.

Phenolic compounds are known as powerful chain breaking antioxidants. The phenolic compounds may contribute directly to antioxidative action. In the *S. surattense* 46.7 mg gallic acid equivalents/g of phenols were detected. The phenolic compounds may contribute directly to the antioxidative action[29]. The result indicates a strong association between antioxidative activities and phenolic compound, suggesting that phenolic compounds are probably responsible for the antioxidative activities of *S. surattense*. Phenolic compounds are also effective hydrogen donors, which makes them good antioxidants[30,31]. Thus, the therapeutic properties of *S. surattense* may be possibly attributed to the phenolic compounds present.

S. surattense confirms its potent *in vivo* antioxidant activity in STZ-diabetic rats[32]. The reported activity may be due to the presence of the phytochemicals and their free radical scavenging ability.

Determination of the natural antioxidant compounds of plant extracts will help to develop new drug candidates for antioxidant therapy[33]. The present study proved potent antioxidant activity of *S. surattense* and can be used as accessible source of natural antioxidants or nutraceuticals with potential application to reduce oxidative stress with consequent health benefits.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

The goal of this study was to evaluate the *in vitro* antioxidant activities of *S. surattense* leaf extract. The authors show that *S. surattense* leaf extract has antioxidant activities. The findings are novel and the paper shows some promising results.

Research frontiers

Studies indicated that *S. surattense* extract effectively scavenged free radicals and, proving its potent antioxidant activity. Results were compared to standard antioxidants.

Related reports

Sridevi *et al.* (2011) reported that *S. surattense* leaf extract has antioxidant potential in streptozotocin-diabetic rats.

Innovations and breakthroughs

Data's shows that *S. surattense* alcoholic leaf extract has antioxidant activities (*In vitro* model).

Applications

S. surattense leaf extract has antioxidant properties. This extract may be used for cardiovascular diseases after completing necessary trials.

Peer review

In this study the effect of alcoholic leaf-extract of *S. surattense* on free radical scavenging activity were determined by *in vitro* model. *S. surattense* were used at different concentrations, and scavenging activity of hydroxyl radical and hydrogen peroxide, inhibition of superoxide anion radical and 2, 2-diphenyl-1-picryl hydrazyl (DPPH) radical, total antioxidant activity and reducing ability were studied. Nevertheless, the data and the conclusion of this work are interesting.

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