



**EVALUATION OF ANTIOXIDANT AND FREE RADICAL
SCAVENGING POTENTIAL OF *STRYCHNOS POTATORUM* AN
INDIAN MEDICINAL HERB BY USING IN-VITRO RADICAL
SCAVENGING ASSAYS**

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ABSTRACT

Oxidative stress is considered to be one of the most significant factors that contribute to life threatening disease like myocardial infarction. From the recent research it was evident that medicinal plants with potential antioxidant activity will alleviate the condition of oxidative stress and its related pathology. The main aim of the present research work is to evaluate the antioxidant potential of aqueous, alcoholic and hydro alcoholic extracts of the plant *Strychnos potatorum* by in vitro DPPH, Nitric oxide, Hydrogen peroxide and ABTS radical scavenging assays. Epicarp of *Strychnos potatorum* Linn was extracted with water, Ethanol and mixture of Ethanol: water (6:4) (hydro-alcoholic extract) by using soxhlet extraction method. The respective extracts were screened for antioxidant activity by in vitro assay techniques. The results obtained from the study reveals that all the three extracts showed promising antioxidant activity in which the ethanol extract (EESP) projects highest significant activity followed by aqueous (AESP) and hydro-alcoholic (HAESP) extracts. In DPPH radical

scavenging assay EESP exhibit strong scavenging activity the equivalent percentage inhibition ranges from 25.96 to 81.04% followed by this AESP with inhibition of 22.03 to 79.25% and HAESP with percentage inhibition of 24.89 to 77.46%. Results of nitric oxide

radical assays shows that EESP shown higher percentage inhibition of 22.82 to 72.1% similarly AESP with 12.93 to 35.37% and HAESP with 5.18 to 44.22% inhibition. In hydrogen peroxide radical inhibition assay EESP contributes higher inhibition percentage of 16.68 to 74.98% whereas AESP with 6.12 to 60.57% and HAESP with 7.81 to 63.85%. Result of NO radical scavenging property reflects that EESP projects highest inhibition of 22.82 to 72.1% followed by this AESP with 12.93 to 35.57% and HAESP with 5.18 to 44.22%. Datas obtained from ABTS radical scavenging assay clearly reflects that EESP has higher percentage inhibition of 26.31 to 78.79% followed by this AESP with inhibition of 12.69 to 51.95% and HAESP with percentage inhibition of 10.68 to 67.17%. It was concluded from the results that EESP had shown the highest radical scavenging activity among the extractives when compare to that of the standard's. In future epicarp of *Strychnos potatorum* may be considered as a valuable natural antioxidant source for scavenging free radical. It may be developed as a potential plant based therapeutic moiety for treating diseases caused by free radicals.

KEYWORDS: Oxidative stress, Free radicals, *Strychnos potatorum*, Antioxidant activity, DPPH, Nitric oxide, Hydrogen peroxide, ABTS scavenging assays.

INTRODUCTION

Oxidative stress is the common phenomenon associated with pathology that causes damage in our body system due to the generation of free radicals. Inattention of these stresses could exacerbate free radical damage. It is reported^[1] that free radicals could damage DNA forming as a main product 8-oxo-guanine^[2] and it may lead to the incidence of cancer.^[3] Antioxidants generally scavenge free radicals, stabilize them from free radical damage and help in normalizing health disorders.^[4]

In acute myocardial infarction (MI), reactive oxygen species (ROS) are generated in the ischaemic myocardium especially after reperfusion. ROS directly injure the cell membrane and cause cell death. However, ROS also stimulate signal transduction to elaborate inflammatory cytokines, e.g. tumour necrosis factor-alpha (TNF-alpha), interleukin (IL)-1beta and -6, in the ischaemic region and surrounding myocardium as a host reaction. Inflammatory cytokines also regulate cell survival and cell death in the chain reaction with ROS. Both ROS and inflammatory cytokines are cardiodepressant mainly due to impairment of intracellular Ca (2+) homeostasis. Inflammatory cytokines stimulate apoptosis through a

TNF-alpha receptor/caspase pathway, whereas Ca(2+) overload induced by extensive ROS generation causes necrosis through enhanced permeability of the mitochondrial membrane.^[5]

Anti-oxidative therapy, mainly using natural and synthetic antioxidants, represents a reasonable therapeutic approach for the prevention and treatment of liver diseases due to the role of oxidative stress in contributing to initiation and progression of hepatic damage. However, although concept of anti-oxidative therapy has been raised for decades and intensive efforts have been paid, there is a long way to go for the application of antioxidants in CVD.

Approximately 50% of the drugs approved by the Food and Drug Administration (FDA) are phytogetic compounds or derivations. Natural compounds have been crucial in drug development.^[6,7] Morphine, vinblastine, vincristine, quinine, artemisinin, etoposide, teniposide, paclitaxel and camptothecin are examples of pharmaceuticals derived from natural compounds. Natural compounds have been a good source for developing new pharmaceuticals because of their vast diversity. This characteristic of natural compounds enables the synthesis of drugs that differ from other chemical compounds in terms of their complex structures and biological potency.^[8] Additionally, natural compounds are used for drug development and to identify and study targets and pathways involved in disease.^[9]

Medicinal plants are used as a source of drugs for the treatment of various human health disorders all over the world from ancient times to the present day. They are important natural wealth. They provide primary healthcare services to people from all walks of life. They serve as important therapeutic agents as well as important raw materials for the manufacture of traditional and modern medicines. A total of 250,000 species of flowering plants are referred to as medicinal plants. Plant derived natural products such as flavonoids, terpenes, alkaloids, carbohydrates and tannins have got considerable attention in recent years due to diverse pharmacological properties.^[10]

Strychnos potatorum is a medium-sized, glabrous tree. Stem is fluted and covered with black, thick, square to rectangular scales. Seeds are globose in shape. Population of nirmali is depleting fast due to self nongenerative mechanism in fruits. Fruit is a berry, black when ripe, globose, 12 cm in diameter, whitish, shining, with short addressed yellow silky hairs.^[11,12]

A dried seed was found to have diuretic and antidiarrheal activities. The seed powder was found to possess antidiabetic activity. Mannogalactans isolated from the seeds of *Strychnos potatorum* showed antihypercholesterolemic activity in experimental rats. Powdered stem bark mixed with lime juice given in cholera. The paste of seed is reported to be consumed internally along with little tender coconut milk in urinary disorder and retention of urine.^[13]

As per the literature research it was strongly evident that the plant *Strychnos potatorum* has wide range of pharmacological activity which includes Anti-diabetic^[14], Anti-inflammatory^[15,16], Anti-ulcerogenic^[17], Hepatoprotective^[18], Antioxidant activity, Anti-arthritis^[19], Anti-nociceptive^[20], Anti-pyretic, Anti-diarrheal^[21], Diuretic^[22] and Antimicrobial properties.^[23]

MATERIALS AND METHODS

Plant collection

Fruits of *Strychnos potatorum* was collected and were identified by expert taxonomist. Plant material were then washed separately with fresh water to remove dirt and other contaminants. Epicarp of the fruits was separated and were shade-dried for several days with occasional sun drying. The dried materials were ground into coarse powder by a grinding machine and the materials were stored at room temperature (RT) for future use.

Preparation of the extract

1 kg of coarse powdered epicarp of *Strychnos potatorum* was passed through a 60 No mesh sieve. Air dried powdered drug was extracted with the following solvents like Water, Ethanol and mixture of Ethanol: water (6:4) (hydro-alcoholic extract) by using soxhlet extraction. Then the extracts obtained such as aqueous extract of *Strychnos potatorum* (AESP), Ethanol extract of *Strychnos potatorum* (EESP) and Hydro-alcoholic extract of *Strychnos potatorum* (HAESP) was filtered, concentrated by rotary vacuum pump to get the solid mass.

DPPH radical scavenging assay

Free radical scavenging ability of the *Strychnos potatorum* extracts was evaluated by DPPH radical scavenging assay.^[24,25] The hydrogen atom donating ability of the plant extractives was determined by the decolorization of methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH produces violet/purple color in methanol solution and fades to shades of yellow color in the presence of antioxidants. A solution of 0.1 mM DPPH in methanol was prepared, and 2.4 mL of this solution was mixed with 1.6 mL of *Strychnos potatorum* extracts

in methanol at varying concentrations (10–100 µg/mL). The reaction mixture was vortexed thoroughly and left in the dark at RT for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid was used as reference. Percentage DPPH radical scavenging activity was calculated by the following equation:

$$\% \text{ DPPH radical scavenging activity} = \{(A_0 - A_1)/A_0\} \times 100$$

A_0 is the absorbance of the control, and A_1 is the absorbance of the extractives/standard. Then % of inhibition was plotted against concentration, and from the graph IC_{50} was calculated. The experiment was repeated three times at each concentration.

Hydroxyl radical scavenging activity

H_2O_2 is rapidly decomposed into oxygen and water and this may produce hydroxyl radicals (OH^*) that can initiate lipid peroxidation and cause DNA damage in the body. The ability of *Strychnos potatorum* extracts to scavenge hydrogen peroxide can be estimated.^[26] A solution of hydrogen peroxide (40 mM) is prepared in phosphate buffer (50 mM pH 7.4). The concentration of hydrogen peroxide is determined by absorption at 230 nm using a spectrophotometer. The extract of sample AESP, EESP and HAESP was prepared in different concentrations (10-100 µg/ml) is added to hydrogen peroxide and absorbance at 230 nm is determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. BHA was used as reference standard. The percentage of hydrogen peroxide scavenging is calculated as follows:

$$\% \text{ scavenged } (H_2O_2) = [(A_i - A_t)/A_i] \times 100$$

Where A_i is the absorbance of control and A_t is the absorbance of test.

Nitric Oxide radical scavenging assay

The concentrations of test samples AESP, EESP and HAESP are made into serial dilution from 10–100 µg/mL and the standard gallic acid.^[27] Griess reagent was prepared by mixing equal amounts of 1% sulphanilamide in 2.5% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride in 2.5% phosphoric acid immediately before use. A volume of 0.5 mL of 10 mM sodium nitroprusside in phosphate buffered saline was mixed with 1 mL of the different concentrations of the extracts (10–100 µg/mL) and incubated at 25°C for 180 mins. The test sample was mixed with an equal volume of freshly prepared Griess reagent. Control samples without the extracts but with an equal volume of buffer were prepared in a similar manner as was done for the test samples. The absorbance was measured at 546 nm. Gallic acid was used as the positive control. The percentage inhibition of the test

sample and standard was calculated and recorded. The percentage nitrite radical scavenging activity of the AESP, EESP, HAESP and gallic acid were calculated using the following formula:

percentage nitrite radical scavenging activity:

$$\text{nitric oxide scavenged (\%)} = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100,$$

where A_{control} = absorbance of control sample and A_{test} = absorbance in the presence of the samples extracts or standards.

ABTS radical scavenging assay

This assay carried out for the purpose of evaluating the anti-oxidant potential of the extracts AESP, EESP and HAESP against 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) or ABTS radicals.^[28] The ABTS reagent was prepared by mixing 5 mL of 7 mM ABTS with 88 μ L of 140 mM potassium persulfate. The mixture was then kept in the dark at room temperature for 16 h to allow free radical generation and was then diluted with water (1 : 44, v/v). To determine the scavenging activity, 100 μ L ABTS reagent was mixed with 100 μ L of test sample (10-100 μ g/ml) and was incubated at room temperature for 6 min. After incubation, the absorbance was measured 734 nm. Gallic acid with same concentrations of extract was measured following the same procedures described above and was used as positive controls. The antioxidant activity was calculated using the following equation: The ABTS scavenging effect was measured using the following formula:

$$\% \text{ ABTS radical scavenging activity} = \{(A_0 - A_1)/A_0\} \times 100$$

A_0 is the absorbance of the control, and A_1 is the absorbance of the extractives/standard. Then % of inhibition was plotted against concentration, and from the graph IC50 was calculated. The experiment was repeated three times at each concentration.

RESULTS

Effect of *Strychnos potatorum* on DPPH radical scavenging assay

The results of DPPH free radical scavenging potential of AESP, EESP, HAESP and standard ascorbic acid shows that among all extracts EESP possessed significantly higher level of radical scavenging activity at the concentration of 10 to 100 μ g/mL. EESP exhibit strong scavenging activity the equivalent percentage inhibition ranges from 25.96 to 81.04% followed by this AESP with inhibition of 22.03 to 79.25% and HAESP with percentage

inhibition of 24.89 to 77.46%. Whereas at the same concentration, the standard ascorbic acid exhibit inhibition of 43.86 to 94.67%. The IC₅₀ value of EESP, AESP and HAESP was 44.06 ± 12.19, 50.22±2.21 and 48.9 ± 1.7 µg/mL, respectively as shown in table 1. The IC₅₀ value of ascorbic acid (standard) was 17.96±2.77µg/mL. The results were tabulated in table 2 and represented in figure 1.

Table 1: Percentage inhibition of AESP, EESP, HAESP and standard on DPPH radical scavenging assay.

Concentration (µg/ml)	% Inhibition of AESP	% Inhibition of EESP	% Inhibition of HAESP	% Inhibition of Ascorbic Acid
10 µg/ml	22.03 ± 2.23	25.96 ± 8.99	24.89 ± 2.23	43.86 ± 1.55
20 µg/ml	35.62 ± 1.63	37.05 ± 4.06	35.62 ± 4.06	54.7 ± 1.76
40 µg/ml	45.99 ± 3.21	49.57 ± 9.12	45.63 ± 4.46	60.46 ± 0.58
60 µg/ml	55.29 ± 4.06	61.73 ± 9.25	59.58 ± 1.23	71.3 ± 2.34
80 µg/ml	67.09 ± 3.76	69.95 ± 6.46	66.38 ± 4.29	84.51 ± 0.58
100 µg/ml	79.25 ± 1.07	81.04 ± 3.7	77.46 ± 2.7	94.67 ± 1.17

Data are given as Mean ± SD (n=3)

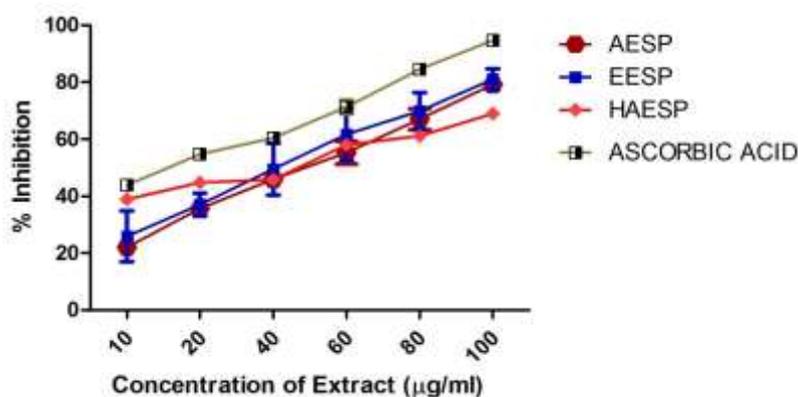


Figure 1: Percentage inhibition of AESP, EESP, HAESP and standard on DPPH radical scavenging assay.

Table 2: IC₅₀ Values for DPPH radical scavenging Assay by AESP, EESP, HAESP and standard.

Test Drug / Standard	IC ₅₀ Value DPPH Assay ± SD (µg/ml)
AESP	50.22 ± 2.21
EESP	44.06 ± 12.19
HAESP	48.9 ± 1.7
ASCORBIC ACID	17.96 ± 2.77

Data are given as Mean ± SD (n=3)

Effect of *Strychnos potatorum* on Hydroxyl radical scavenging assay

Data's obtained from hydrogen peroxide radical inhibition assay of AESP, EESP, HAESP and standard BHA exhibits that EESP had ranked first among all the three extracts screened for radical quenching property at the concentration of 10 to 100 μ g/mL. EESP contributes higher inhibition percentage of 16.68 to 74.98% whereas AESP with 6.12 to 60.57% and HAESP with 7.81 to 63.85%. Whereas at the same concentration, the standard BHA shown inhibition of 45.18 to 90.61 % as shown in table 3. The IC₅₀ value of EESP, AESP and HAESP was 58.48 \pm 1.41, 81.2 \pm 5.51 and 80.13 \pm 1.92 μ g/mL, respectively. The IC₅₀ value of BHA (standard) was 17.27 \pm 3.63 μ g/mL. The results were tabulated in table 4 and represented in figure 2.

Table 3: Percentage inhibition of AESP, EESP, HAESP and standard on Hydrogen Peroxide radical scavenging assay.

Concentration (μ g/ml)	% Inhibition of AESP	% Inhibition of EESP	% Inhibition of HAESP	% Inhibition of BHA
10 μ g/ml	6.122 \pm 1.56	16.68 \pm 3.64	7.819 \pm 0.17	45.18 \pm 2.30
20 μ g/ml	18.19 \pm 1.69	29.09 \pm 1.09	15.93 \pm 1.91	54.68 \pm 3.93
40 μ g/ml	27.88 \pm 4.60	41.57 \pm 0.493	24.45 \pm 4.19	64.02 \pm 3.59
60 μ g/ml	38.44 \pm 5.72	49.03 \pm 3.51	40.59 \pm 2.18	70.96 \pm 2.20
80 μ g/ml	48.84 \pm 4.85	63.44 \pm 1.27	46.09 \pm 4.13	84.95 \pm 2.98
100 μ g/ml	60.57 \pm 3.01	74.98 \pm 1.05	63.85 \pm 0.51	90.61 \pm 1.06

Data are given as Mean \pm SD (n=3)

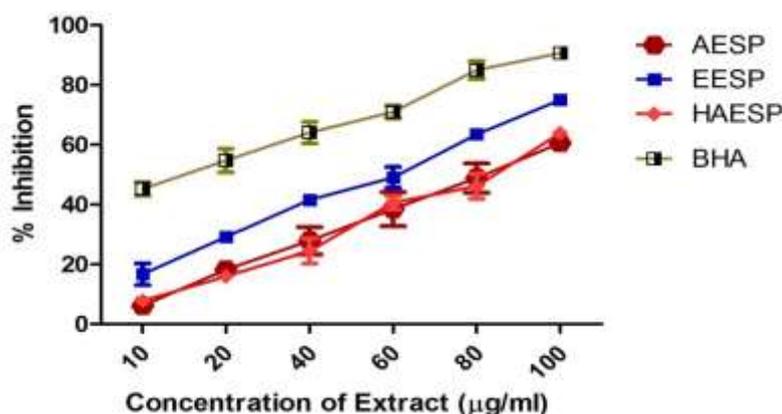


Figure 2: Percentage inhibition of AESP, EESP, HAESP and standard on Hydrogen Peroxide radical scavenging assay.

Table 4: IC₅₀ Values for Hydrogen Peroxide radical scavenging assay by AESP, EESP, HAESP and standard.

Test Drug / Standard	IC ₅₀ Value Hydrogen Peroxide Assay ± SD (µg /ml)
AESP	81.2 ± 5.51
EESP	58.48 ± 1.41
HAESP	80.13 ± 1.92
BHA	17.27 ± 3.635

Data are given as Mean ± SD (n=3)

Effect of *Strychnos potatorum* on Nitric oxide radical scavenging assay

Results of NO radical scavenging activity of AESP, EESP, HAESP and standard Gallic acid shows that EESP had revealed highest percentage inhibition activity among other extracts screened for radical scavenging property at the concentration of 10 to 100µg/mL. EESP projects highest inhibition of 22.82 to 72.1% followed by this AESP with 12.93 to 35.57% and HAESP with 5.18 to 44.22%. Whereas at the same concentration, the standard gallic acid shown inhibition of 42.35 to 87.66% as shown in table 5. The IC₅₀ value of EESP, AESP and HAESP was 54.46±2.59, 159.9±8.16 and 110.9±1.87 µg/mL, respectively. The IC₅₀ value of gallic acid (standard) was 15.97±8.25 µg/mL. The results were tabulated in table 6 and represented in figure 3.

Table 5: Percentage inhibition of AESP, EESP, HAESP and standard on Nitric Oxide radical scavenging assay.

Concentration (µg/ml)	% Inhibition of AESP	% Inhibition of EESP	% Inhibition of HAESP	% Inhibition of Gallic Acid
10 µg/ml	12.93 ± 4.28	22.82 ± 6.61	5.186 ± 2.61	42.35 ± 2.58
20 µg/ml	21.1 ± 3.96	32.86 ± 4.43	11.89 ± 2.50	55.76 ± 3.45
40 µg/ml	24.61± 2.74	43.68 ± 1.61	23.61 ± 2.23	63.65 ± 3.53
60 µg/ml	28.56 ± 1.37	56.05 ± 4.4	29.77 ± 1.02	70.49 ± 7.16
80 µg/ml	32.18 ± 2.54	64.79 ± 6.12	36.41 ± 2.32	78.52 ± 8.32
100 µg/ml	35.37 ± 2.32	72.1 ± 4.55	44.22 ± 0.64	87.66 ± 2.16

Data are given as Mean ± SD (n=3)

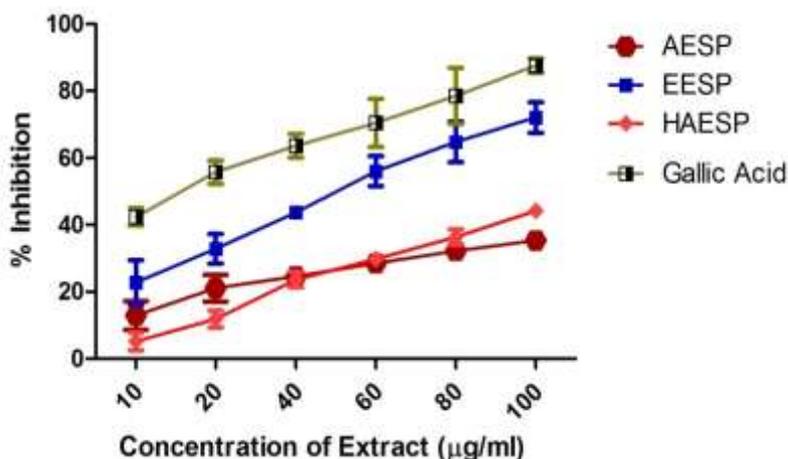


Figure 3: Percentage inhibition of AESP, EESP, HAESP and standard on Nitric Oxide radical scavenging assay.

Table 6: IC₅₀ Values for Nitric Oxide radical scavenging assay by AESP, EESP, HAESP and standard.

Test Drug / Standard	IC ₅₀ Value Nitric Oxide radical scavenging assay ± SD (µg /ml)
AESP	159.9 ± 8.168
EESP	54.46 ± 2.591
HAESP	110.9 ± 1.875
GALLIC ACID	15.97 ± 8.252

Data are given as Mean ± SD (n=3)

Effect of *Strychnos potatorum* on ABTS radical scavenging assay

From the results of ABTS radical scavenging assay it was clear that EESP possess significantly higher level of percentage inhibition when compare to AESP and HAESP at the concentration of 10 to 100µg/mL. Percentage inhibition by EESP was 26.31 to 78.79% followed by this AESP with inhibition of 12.69 to 51.95% and HAESP with percentage inhibition of 10.68 to 67.17%. Whereas at the same concentration, the standard gallic acid shown inhibition of 41.8 to 89.81% as shown in table 7. The value of IC₅₀ EESP, AESP and HAESP was 41.82±12.6, 92±3.79 and 69.11±6.38 µg/mL, respectively. The IC₅₀ value of gallic acid (standard) was 18.87±2.52µg/mL. The results were tabulated in table 8 and represented in figure 4.

Table 7: Percentage inhibition of AESP, EESP, HAESP and standard on ABTS radical scavenging assay.

Concentration ($\mu\text{g/ml}$)	% Inhibition of AESP	% Inhibition of EESP	% Inhibition of HAESP	% Inhibition of Gallic Acid
10 $\mu\text{g/ml}$	12.69 \pm 1.83	26.31 \pm 11.17	10.68 \pm 1.83	41.8 \pm 2.68
20 $\mu\text{g/ml}$	20.7 \pm 4.33	40.73 \pm 5.92	19.9 \pm 3.02	52.28 \pm 3.09
40 $\mu\text{g/ml}$	29.11 \pm 3.18	51.55 \pm 10.9	29.11 \pm 4.33	64.12 \pm 3.65
60 $\mu\text{g/ml}$	37.93 \pm 1.83	63.97 \pm 6.69	49.55 \pm 3.18	70.88 \pm 1.54
80 $\mu\text{g/ml}$	45.14 \pm 1.36	65.57 \pm 7.04	57.96 \pm 4.33	80.01 \pm 2.11
100 $\mu\text{g/ml}$	51.95 \pm 1.20	78.79 \pm 2.50	67.17 \pm 6.05	89.81 \pm 4.79

Data are given as Mean \pm SD (n=3)

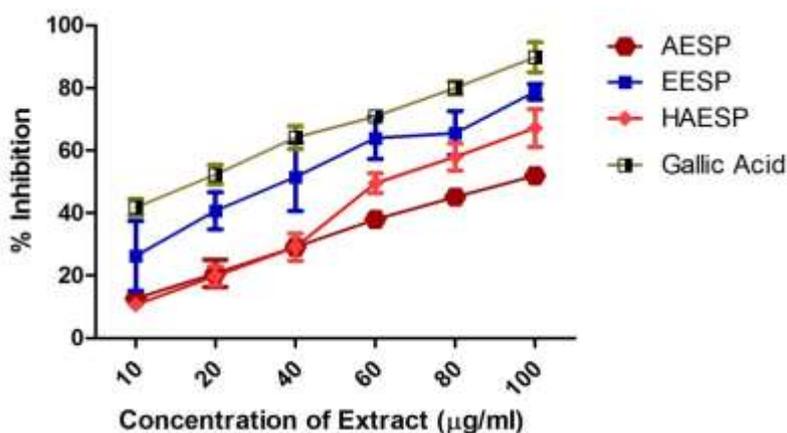


Figure 4: Percentage inhibition of AESP, EESP, HAESP and standard on ABTS radical scavenging assay.

Table 8: IC₅₀ Values for ABTS radical scavenging assay by AESP, EESP, HAESP and standard.

Test Drug / Standard	IC ₅₀ Value ABTS Assay \pm SD ($\mu\text{g/ml}$)
AESP	92 \pm 3.79
EESP	41.82 \pm 12.6
HAESP	69.11 \pm 6.38
GALLIC ACID	18.87 \pm 2.528

Data are given as Mean \pm SD (n=3)

DISCUSSION

Numerous physiological processes can produce oxygen-centered free radicals in the human body. With the accumulation of reactive oxygen species, some free radicals can cause oxidative damage to biomolecules (*e.g.*, lipids, proteins, DNA), triggering cancer, diabetes, atherosclerosis, aging, and other degenerative disorders.^[29,30,31] Hence, antioxidant activity is considered a fundamental property for human health and has attracted widespread research

interest. Many plant-derived substances, such as alkaloids, terpenoids, and flavonoids that exhibit potent antioxidant effects can scavenge free radicals. In DPPH radical assay method all three extracts showed potent antioxidant activity, with value ranges from 44.06 to 50.22 $\mu\text{g/mL}$. However, the standard ascorbic acid exhibited IC_{50} value of 17.96 $\mu\text{g/mL}$.

The biological activity of medicinal plants from all over the world has been studied by several groups of researchers. These studies are based on the popular uses of different species^[32], as well as on popular knowledge and scientific studies describing medical plant use, with a focus on how these plants could benefit the pharmaceutical industry.

Antioxidants are known to protect the humans against free radical mediated toxicities. Phyto components years together plays a significant role quenching such free radicals. In the present study in vitro antioxidant screening using nitric oxide methods reveals that among all tested extracts the lowest IC_{50} value of 54.46 $\mu\text{g/mL}$ was exhibited by EESP when compare to that of the standard gallic acid with IC_{50} 15.97 $\mu\text{g/mL}$. Where the results further clarifies that other two extracts also shown promising antioxidant activity in which AESP with IC_{50} 159.86 $\mu\text{g/mL}$ and HAESP with IC_{50} 110.9 $\mu\text{g/mL}$.

Approximately 50% of the drugs approved during 1981–2006 were directly or indirectly derived from natural products. In H_2O_2 radical assay method ethanol extract had shown potent activity with IC_{50} 58.48 $\mu\text{g/mL}$ when compare to aqueous (81.2 $\mu\text{g/mL}$) and hydro-alcoholic extract (80.13 $\mu\text{g/mL}$). In this IC_{50} of standard BHA was found to be 17.27 $\mu\text{g/mL}$.

Ethnobotany plays an increasingly important role in preserving disappearing traditional knowledge, especially traditional medicinal knowledge. Ethnobotanical surveys to document the traditional uses of various indigenous plants not only recognize this undocumented knowledge but also provide new avenues for pharmacological investigations to improve healthcare for a range of ailments.^[33,34] In addition, ethnobotanical information offers a viable alternative to high-throughput screening of bioactive substances.^[35] In ABTS assay method all three extracts exhibited a potent antioxidant activity but the highest activity was ranked by EESP with lowest IC_{50} value of 41.82 $\mu\text{g/mL}$ when compare to that of the standard gallic acid with IC_{50} 18.87 $\mu\text{g/mL}$. Followed by this the second best IC_{50} value of 69.11 $\mu\text{g/mL}$ possessed by HAESP and the least will be AESP with IC_{50} value of 92 $\mu\text{g/mL}$.

For drug discovery, phytochemical and pharmacological research based on modern ethnobotany is considered a validating approach in the search for novel chemical entities and frameworks with potential as drug leads.^[36] It is estimated that 122 drugs from 94 plant species have been discovered through ethnobotanical leads^[37,38], such as morphine, the main anesthetic alkaloid in opium, or vincristine, an antitumor compound.^[39]

CONCLUSION

Antioxidant and free radical scavenging potential of *Strychnos potatorum* was evaluated using *in vitro* methods like, DPPH, hydrogen peroxide, nitric oxide and ABTS radical scavenging assay method. The percentage inhibition of three different extract such as EESP, AESP and HAESP was compared with a standard ascorbic acid, gallic acid and BHA. Further from the results of the study it was concluded that the ethanol extract of *Strychnos potatorum* (EESP) had possess significantly higher level of free radical scavenging property than the other two extracts. Still future studies need to be carried out with respect to the components responsible for its antioxidant potential.

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