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IN VITRO ANTIOXIDANT SCREENING OF BIO-ACTIVE FRACTION OF SEEDS OF *STRYCHNOS POTATORUM* LINN

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ABSTRACT: *Strychnos potatorum* Linn. is a medium sized deciduous tree belonging to the family Loganiaceae. The collected seeds of *Strychnos potatorum* were dried and powdered. Methanolic extract of these seeds was prepared by cold maceration. The preliminary phytochemical screening of the crude extract revealed the presence of flavonoids and phenolic compounds. The crude extract was partitioned (gradient) with different solvents like chloroform, ethyl acetate, acetone and water. After conducting preliminary *in vitro* DPPH free radical scavenging assay on these fractions, the one with maximum antioxidant activity (bioactive fraction) was selected and screened with different *in vitro* antioxidant methods. The different *in vitro* antioxidant methods include DPPH free radical scavenging assay, nitric oxide radical scavenging assay, superoxide radical scavenging assay, hydroxyl radical scavenging assay and inhibition of peroxide formation method. The percentage inhibition of free radicals in each method was compared with standard drug. Ascorbic acid and α- tocopherol were used as standard drugs. The seeds of *Strychnos potatorum* have shown significant antioxidant activity.

INTRODUCTION: Free radicals are naturally occurring unstable molecules formed in the body as a result of chemical reactions during normal cellular processes[1]. They play a vital role in the pathogenesis of several human diseases and ageing. Antioxidants are compounds which help to inhibit the various oxidation reactions caused by these free radicals and prevent the damage of cells. So they are very important in providing good health[2].

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Antioxidants act in many ways like acting as the scavengers of free radicals, decreasing the oxygen concentration, preventing the formation of free radicals by chelation etc. Various plants and their parts are used as antioxidants. In this study the antioxidant potential of the seeds of *Strychnos potatorum* Linn.[3] (fam- Loganiaceae) has been conducted.

It is a moderate sized tree commonly found in southern and central part of India, Sri Lanka and Burma. This plant has been used for the treatment of various ailments such as jaundice, diabetes, chronic diarrhoea, dysentery, bronchitis etc. Traditionally seeds are used to clear muddy water [4, 5]. As per literature review it is clear that seeds are having a good hepatoprotective activity [6].

Preliminary phytochemical screening reveals seeds are rich in flavonoids and phenolic compounds. Free radical scavenging activity of bioactive fraction of seeds of *Strychnos potatorum* has been carried out using different *in vitro* antioxidants assay techniques.

MATERIALS AND METHODS:

Plant material:

The seeds of *Strychnos potatorum* were collected from the suburbs of Thrissur district, Kerala and the plant specimens were authenticated by Dr. Sujanapal, Scientist-B, KFRI, Kerala. The air dried seeds were powdered and defatted with petroleum ether. The methanolic extract was then prepared by cold maceration. Methanolic extract was partitioned with different solvents like, chloroform, ethyl acetate, acetone and water. Each solvent fraction analysed for their free radical scavenging activity by *in* vitro DPPH free radical scavenging assay. Since aqueous fraction of seeds of *Strychnos potatorum* showed maximum free radical scavenging activity, this was selected for further studies and was called bioactive fraction.

Phytochemical study:

Crude methanolic extract was analysed for the qualitative chemical composition using commonly used chemical reactions[7].

In vitro anti-oxidant screening:

The bioactive fraction (aqueous fraction) was tested for the anti-oxidant activity. Different *in vitro* methods such as DPPH free radical scavenging assay, nitric oxide radical scavenging assay, superoxide radical scavenging assay, hydroxyl radical scavenging assay and inhibition of peroxide formation method, was carried out. The percentage inhibition of free radicals produced was found by spectrophotometric method and the values are represented as mean±SD. The absorbance obtained for test and control are noted and the percentage inhibition of radical scavenging activity was calculated using the equation,

$$\text{Free radical scavenged (\%)} = \frac{\text{A Control} - \text{A Test}}{\text{A Control}} \times 100$$

Where,

A Control-Absorbance of control

A Test -Absorbance in the presence of the samples of extracts.

DPPH free radical scavenging assay: [8]

A stock solution of DPPH (1.3 mg/ml in methanol) was prepared such that 75µl of it in 3 ml methanol give an initial absorbance of 0.9. The decrease in the absorbance in the presence of sample extract and standard at different concentrations (10-100µg/ml) was noted at 517 nm after 30 minutes, using methanol as blank in UV-Visible Spectrophotometer. IC_{50} (Inhibitory concentration to scavenge 50% free radicals) value which denotes the concentration of sample required to scavenge 50% of the DPPH free radicals was also determined.

Nitric Oxide Radical Scavenging Assay: [9]

The reaction mixture (3ml), containing 2.0ml of sodium nitroprusside, 0.5ml of phosphate buffered saline and 0.5ml of different concentrations (10µg-100µg/ml) of various extracts was incubated at 25°C for 5 hours. Control experiments without the test compounds, but with equivalent amounts of buffer were added conducted in an identical manner. After 5 hours, 0.5ml of Griess reagent was added. The absorbance of the chromophore formed during diazotization coupling with naphthylethylenediamine was measured at 546nm.

Superoxide Radical Scavenging Assay: [10]

Superoxide radical was generated from the photo reduction of riboflavin and was deducted by nitroblue tetrazolium dye (NBT) reduction method. The scavenging activity towards the superoxide radical was measured in terms of generation of O_2. The reaction mixture consisted of phosphate buffer (50mM, pH 7.6), riboflavin (2µm), EDTA (6µm), nitroblue tetrazolium (NBT) (50µm) and sodium cyanide (3g). Test compounds at various concentration of 10µg to 100µg / ml were added to make a final volume of 3ml. The absorbance was read at 530nm before and after illumination under UV lamp for 15 minutes against a control instead of sample. Ascorbic acid was used as the reference compound. All the tests were performed in triplicate and the results averaged. The percentage inhibition was calculated by comparing the results of control and test samples.

Hydroxyl Radical Scavenging Activity: [11]

The scavenging capacity for hydroxyl radical was measured according to the modified method

Rajeshwar Y *et al*. The assay is based on quantification of degradation product of 2-deoxy ribose by condensation with TBA. Hydroxyl radical was generated by the Fe3+- Ascorbate-EDTA-H_2O_2 system (Fenton reaction). The reaction mixture contained 0.1ml of deoxyribose, 0.1ml of EDTA, 0.1ml of H_2O_2, 0.1ml of ascorbic acid, 0.1ml of KH_2PO_4-KOH buffer and various concentrations of different extracts in a final volume of 1.0ml. The reaction mixture was incubated for an hour at 37^0C. At the end of the incubation period, 1.0ml of colour developed was measured at 535nm in a spectrophotometer. Deoxyribose degradation was measured as thiobarbituric acid reactive substance (TBARS) and the percentage inhibition was calculated. The percentage of inhibition was calculated from the control where no test extracts were added.

Lipid peroxide inhibition: [12]

Different concentrations (10-100µg/ml) of the plant extracts were incubated at 37^0C with 0.1ml of rat liver homogenate (25%) containing 30mM KCl, Tris-HCl buffer (0.04M; pH 7), ascorbic acid (0.06mM) and ferrous iron (0.16mM) in a total volume of 0.5ml for 1 hour. At the end of incubation time, TBARS produced was measured at 532 nm using UV-Visible spectrophotometer. The percentage of inhibition was calculated from the control where no test extract was added.

Statistical analysis

The experimental results were expressed as the mean ± SD. Data were assessed by student's t-test. $p< 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION:

Yield of the extract:

The percentage yield of the methanolic extract obtained by cold maceration was 11.29 % w/w and was in brown gummy in nature.

Phytochemical screening:

Seeds of *Strychnos potatorum* have shown the presence of carbohydrates, phenolic compounds, flavonoids, glycosides, saponins, alkaloids, terpenoids and steroids. The results are shown in **Table 1.** The presences of phenolic compounds and flavonoids have already proved to exhibit anti-oxidant property[13].

TABLE 1: RESULTS OF PRELIMINARY PHYTOCHEMICAL SCREENING OF METHANOLIC EXTRACT OF *STRYCHNOS POTATORUM* (SEEDS).

Classes of Compounds	Chemical Tests Performed	Observations
Carbohydrates	Molisch's test	SP
	Fehling's test	+
		+
Phenolic compounds	Phosphomolybdic acid test	++
	Ferric chloride test	++
	Lead acetate test	++
Flavonoids	Shinoda test	++
	Lead acetate test	++
Alkaloids	Wagner's	++
	Mayer's	++
	Dragendorff's test	
Glycosides	Wagner's	+++
	Mayer's	+++
	Dragendorff's test	+++
	Hager's test	+++
Glycosides	Legal's test	++
	Borntrager's test	++
Saponins	Foam test	+
	Haemolysis test	+
Aminoacids	Ninhydrin test	-
Terpenoids and Steroids	Liebermann-Burchard test	+

SP - *Strychnos potatorum*, NB: indicate not present, + in traces, ++ present in moderate amount, +++ more amount is present

Bioactive fraction of *Strychnos potatorum:*

The dried seeds were powdered and defatted with petroleum ether and partitioned with chloroform, ethyl acetate, acetone, and water. Each fraction was screened for their anti-oxidant potential by DPPH free radical scavenging assay method. Aqueous fraction of *Strychnos potatorum* (ASP) showed maximum free radical scavenging activity. So this fraction (bioactive fraction) was used for further studies.

In vitro anti-oxidant screening:

The bioactive fraction was screened for its *in vitro* anti-oxidant activity by DPPH free radical scavenging assay, nitric oxide radical scavenging assay, superoxide radical scavenging assay, hydroxyl radical scavenging assay and inhibition of peroxide formation method. The percentage inhibition in each method was compared with standard drug. The IC_{50} values were also calculated. The results are tabulated in **Table 2** to **Table 6.**

TABLE 2: RESULTS SHOWING DPPH RADICAL SCAVENGING ACTIVITY OF AQUEOUS EXTRACT OF *STRYCHNOS POTATORUM* AND ASCORBIC ACID.

Concentration of the extract (µg/ml)	Percentage Inhibition by *Strychnos potatorum* extract	Concentration of the standard (µg/ml)	Percentage Inhibition by Ascorbic acid (Standard)
Control	0	Control	0
20	6.19±1.12	20	25.46±0.65
40	21.71±0.63	40	42.80±1.19
60	48.48±0.84	60	57.92±1.51
80	64.31±1.71	80	68.87±0.99
100	75.26±1.29	100	75.77±0.88

IC_{50} for *Strychnos potatorum* = 61 µg/ml
IC_{50} for Ascorbic acid = 48 µg/ml
Values are Mean ± SD (n=3)

TABLE 3: RESULTS SHOWING NITRIC OXIDE RADICAL SCAVENGING ACTIVITY OF AQUEOUS EXTRACT OF *STRYCHNOS POTATORUM* AND ASCORBIC ACID

Concentration of the extract (µg/ml)	Percentage Inhibition by *Strychnos potatorum* extract	Concentration of the standard (µg/ml)	Percentage Inhibition by Ascorbic acid (Standard)
Control	0	Control	0
20	22.18±1.46	20	24.78±1.35
40	30.77±1.54	40	39.95±1.41
60	47.76±0.85	60	57.52±0.13
80	61.21±1.64	80	71.18±0.26
100	71.29±0.51	100	77.69±1.29

IC_{50} for *Strychnos potatorum* = 63µg/ml
IC_{50} for Ascorbic acid = 51 µg/ml
Values are Mean ± SD (n=3)

TABLE 4: RESULTS SHOWING SUPER OXIDE RADICAL SCAVENGING ACTIVITY OF AQUEOUS EXTRACT OF *STRYCHNOS POTATORUM* AND ASCORBIC ACID

Concentration of the extract (µg/ml)	Percentage Inhibition by *Strychnos potatorum* extract	Concentration of the standard (µg/ml)	Percentage Inhibition by Ascorbic acid (Standard)
Control	0	Control	0
20	17.46±1.28	20	18.79±1.33
40	35.22±1.54	40	35.93±1.03
60	54.91±1.08	60	45.93±0.93
80	63.89±1.55	80	51.74±0.90
100	73.18±1.54	100	56.13±0.80

IC_{50} for *Strychnos potatorum* = 54µg/ml
IC_{50} for Ascorbic acid = 73µg/ml
Values are Mean ± SD (n=3)

TABLE 5: RESULTS SHOWING HYDROXYL RADICAL SCAVENGING ACTIVITY OF AQUEOUS EXTRACT OF *STRYCHNOS POTATORUM* AND ASCORBIC ACID

Concentration of the extract (µg/ml)	Percentage Inhibition by *Strychnos potatorum* extract	Concentration of the standard (µg/ml)	Percentage Inhibition by Ascorbic acid (Standard)
Control	0	Control	0
20	10.25±0.83	20	24.23±0.68

40	23.18±1.39	40	44.35±1.31
60	39.85±0.86	60	58.05±0.88
80	64.94±1.63	80	71.27±0.70
100	68.87±1.78	100	78.55±1.17

IC_{50} for *Strychnos potatorum* = 68µg/ml
IC_{50} for Ascorbic acid = 44µg/ml
Values are Mean ± SD (n=3)

TABLE 6: RESULTS SHOWING INHIBITION OF LIPID PEROXIDE FORMATION OF AQUEOUS EXTRACT OF *STRYCHNOS POTATORUM* AND α-TOCOPHEROL

Concentration of the extract (µg/ml)	Percentage Inhibition by *Strychnos potatorum* extract	Concentration of the standard (µg/ml)	Percentage Inhibition by α-Tocopherol (Standard)
Control	0	Control	0
20	21.35±0.51	20	17.3±0.53
40	32.89±0.51	40	27.49±0.81
60	50.32±1.75	60	34.36±1.07
80	55.22±1.37	80	42.7±0.98
100	63.2±1.13	100	51.28±0.87

IC_{50} for *Strychnos potatorum* = 60µg/ml
IC_{50} for α-Tocopherol = 88µg/ml
Values are Mean ± SD (n=3)

From the results, it is observed that *Strychnos potatorum* seeds are having good anti-oxidant property. A graphical representation has also been made for each method to compare the activity with standard drug (**Fig. 1 to Fig. 5**).

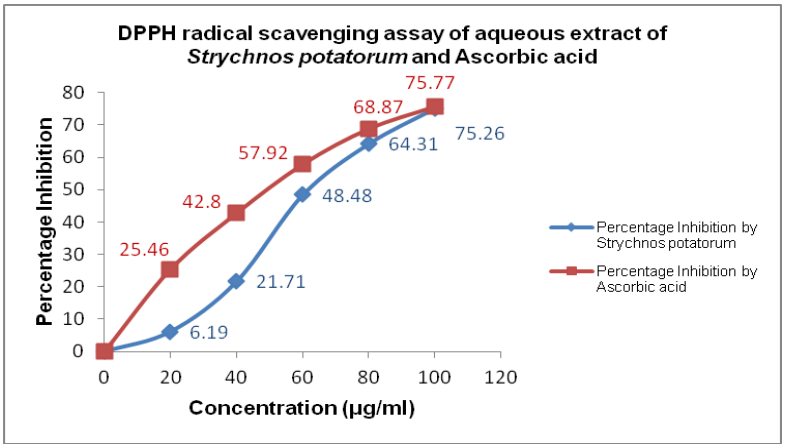

FIG.1: RESULTS SHOWING DPPH RADICAL SCAVENGING ACTIVITY OF AQUEOUS EXTRACT OF *STRYCHNOS POTATORUM* AND ASCORBIC ACID.

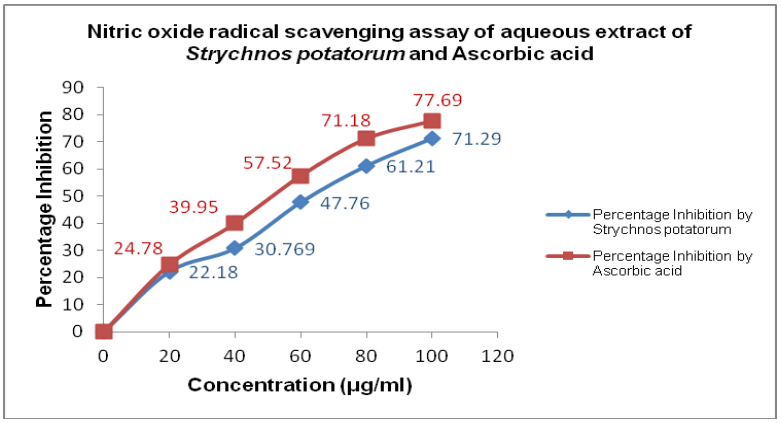

FIG.2: RESULTS SHOWING NITRIC OXIDE RADICAL SCAVENGING ACTIVITY OF AQUEOUS EXTRACT OF *STRYCHNOS POTATORUM* AND ASCORBIC ACID.

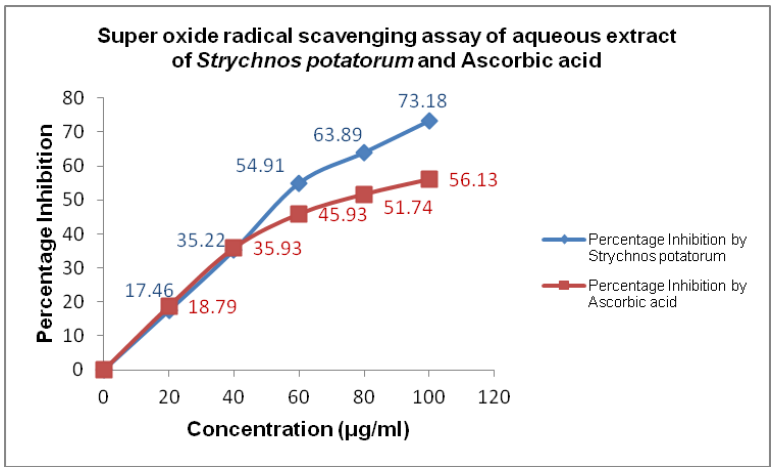

FIG.3: RESULTS SHOWING SUPER OXIDE RADICAL SCAVENGING ACTIVITY OF AQUEOUS EXTRACT OF *STRYCHNOS POTATORUM* AND ASCORBIC ACID.

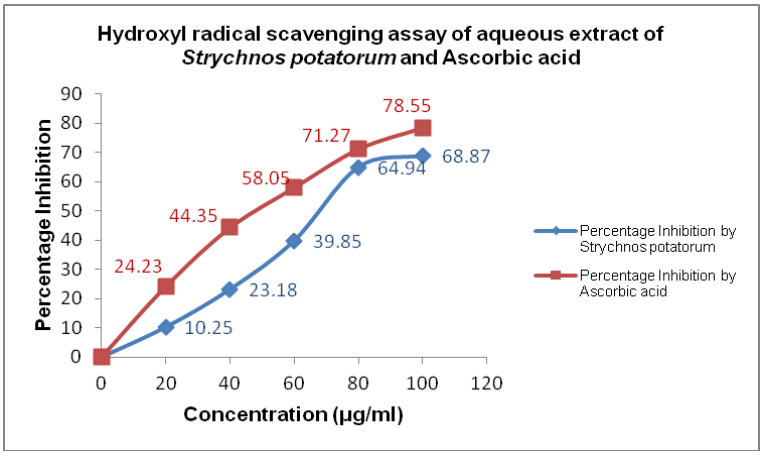

FIG. 4: RESULTS SHOWING HYDROXYL RADICAL SCAVENGING ACTIVITY OF AQUEOUS EXTRACT OF *STRYCHNOS POTATORUM* AND ASCORBIC ACID.

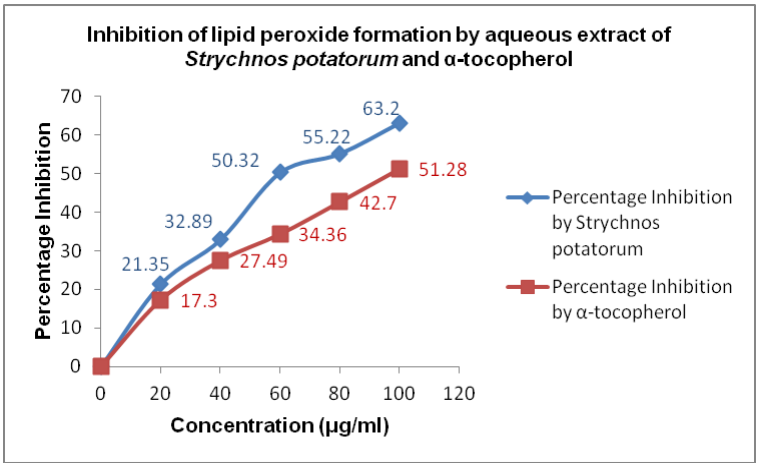

FIG.5: RESULTS SHOWING INHIBITION OF LIPID PEROXIDE FORMATION OF AQUEOUS EXTRACT OF *STRYCHNOS POTATORUM* AND A-TOCOPHEROL

CONCLUSION: Anti-oxidant property of seeds of *Strychnos potatorum* was carried out after preparing the bioactive fraction of *Strychnos potatorum*. Free radical scavenging activity was screened by different *in vitro* methods like, DPPH free radical scavenging assay, nitric oxide radical scavenging assay, superoxide radical scavenging assay, hydroxyl radical scavenging assay and inhibition of peroxide formation method. The activity was compared with a standard drug also. Ascorbic acid was used as standard for all methods except lipid peroxide inhibition method. α-

tocopherol was the standard used in lipid peroxide inhibition method. The percentage inhibition of free radicals in each method was compared with standard drug. The results revealed the high anti-oxidant potential of seeds of *Strychnos potatorum*.

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