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EVALUATION OF *INVITRO* ANTIOXIDANT ACTIVITY AND ESTIMATION OF TOTAL PHENOL AND FLAVONOIDS CONTENT OF VARIOUS EXTRACTS OF *Stachytarpheta jamaicensis* (L)Vahl. LEAVES

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ABSTRACT

In the present investigation was to evaluate the antioxidant capacity of various extracts from leaf of *Stachytarpheta jamaicensis* using by *in-vitro* antioxidant methods were carried out for total antioxidant activity, DPPH, Superoxide radical scavenging activity, iron chelating activity, Nitric oxide radical scavenging activity, Hydroxyl radical scavenging activity, FRAP assay, total phenol content and flavonoids content. Methanolic extract of leaf of *Stachytarpheta jamaicensis* was showed more effective in total antioxidant activity, DPPH and FRAP. The results obtained in the present study indicate that the methanolic extracts of leaf are potential source of natural antioxidant.

KEY WORDS: *Stachytarpheta jamaicensis*, Total antioxidant activity, DPPH, FRAP assay, Total phenol content and flavonoids content.

INTRODUCTION

An antioxidant is “any substance when found at low concentration compared to that of an oxidizable substrate significantly delays or prevents oxidation of that substrate” [1]. Antioxidants are important as part of an organism’s defense mechanisms against free radical production and also in the prevention and repair of free radical generated molecular damage in a variety of situations. Cells have developed a comprehensive array of antioxidants that act co-operatively *in vivo* to combat the deleterious effects of toxic oxymetabolites [2]. Antioxidants found in biological system comprise a number of interconnecting and overlapping components, which include both enzymatic and non-enzymatic factors. Antioxidant enzymes primarily account for intracellular defense, while several non-enzyme molecules, small molecule weight antioxidants, protect various components against oxidation in plasma [3].

Oxidative stress play a crucial role in the development of aging process and some chronic diseases such as cancer, neurodegenerative and cardiovacsular diseases and diabetes in living organisms [4]. Dietary antioxidants protect the body against free radicals. There is an increasing interest in the antioxidant effects of compounds derived from plants, which could be relevant in relation to their nutritional incidence and their role in health and disease. Bioactive natural substances having the additive and synergistic effects in plant food are responsible for their potent antioxidant activities [5]. Plants have been an important source of medicine for thousands of years. Even today, the World Health Organization estimates that up to 80 per cent of people still rely mainly on traditional remedies such as herbs for their medicines [6]. Medicinal plants constitute one of the main sources of new pharmaceuticals and healthcare products. A whole range of plant-derived dietary supplements, phytochemicals and pro-vitamins that assist in maintaining good health and combating disease are now being described as functional ingredients and nutraceuticals. The role of medicinal plants in disease prevention or control has been attributed to antioxidant properties of their constituents, usually associated to a wide range of amphipathic molecules, broadly termed polyphenolic compounds [7]. The number of reports on the isolation of natural antioxidants, mainly of plant origin, has increased immensely during the last decade [8].

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Several methods have been developed to assay the antioxidant activity of herbal and plant extracts. The most common methods involve the determination of the ability to scavenge free radicals using DPPH assay, ferric reducing antioxidant power (FRAP) assay and ferrous-ion chelating assay [9-13]. The main objectives of this study were to determine Invitro antioxidant activity of various extracts of *S.jamaicensis* the total phenolic contents and total flavonoids contents.

MATERIALS AND METHODS

Preparation of various extracts of *Stachytarpheta jamaicensis* (L) Vahl.

The different parts of *S.jamaicensis* were dried in shade and powdered. The powdered plant materials were successively extracted with methanol (80°C), ethanol (45°C), petroleum ether (40-60°C), chloroform (60°C) and acetone (50°C) by hot percolation method in soxhlet apparatus (Harborne, 1984) for 24h. The solvent from the extracts was recovered under reduced pressure using rotary evaporate and subjected to freeze drying in a lyophilizer till dry powder was obtained.

Calculation of percentage yield

The percentage yield was calculated for the extracts and major compounds with reference to the crude material taken using the formula given below:

$$\text{Percentage yield} = \frac{\text{Wt in grams of extracts}}{\text{Wt in grams of plant}} \times 100$$

EVALUATION OF *INVITRO* ANTIOXIDANT ACTIVITY OF VARIOUS EXTRACTS OF *S.jamaicensis*

Free Radical Scavenging Capacity on DPPH Radical

The free radical scavenging activity of all plants different solvent extracts was measured by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH). For DPPH assay, the method of (Blois MS 1958) was adopted. The capacity of all plants different solvent extract to scavenge the lipid-soluble DPPH radical was monitored at an absorbance of 517 nm. methanolic, ethanolic, chloroform, acetone & petroleum ether solvent extracts (1 ml) of plant *S.jamaicensis* at several concentrations ranging from 100-600 µg / ml was allowed to react with DPPH. Thirty minutes later, the absorbance was measured at 518 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging. The percentage inhibition of absorbance was calculated for each concentration relative to a blank absorbance using the spectrophotometer. The DPPH scavenging capacity of the extract is compared with that of BHT (Butylated hydroxytoluene). All determinations were carried out three times, and in five times. Percentage inhibition was calculated as DPPH radical scavenging activity [14].

$$\text{DPPH radical Scavenging effect (\%)} = \frac{(\text{Abs of control} - \text{Abs of activity sample}) \times 100}{(\text{Abs of control})}$$

Where, Abs control is the absorbance of initial conc. of DPPH radical

Abs sample is the absorbance of DPPH radical + sample Extract / standard

Superoxide radical scavenging activity

Superoxide radical (O_2) was generated from the photoreduction of riboflavin and was deducted by by nitroblue tetrazolium dye (NBT) reduction method. Measurement of superoxide anion scavenging activity was performed based on the method described by Winterbourne et al.,(1975). The assay mixture contained sample with 0.1 ml of nitroblue tetrazolium (1.5mM NBT) solution, 0.2 ml of EDTA (0.1 m EDTA), 0.05ml riboflavin (0.12mM riboflavin) and 2.55ml of phosphate buffer (0.067 M phosphate buffer). The control tubes were also set up in DMSO was added instead of sample. The reaction mixture was illuminated for 30 min and absorbance at 560 nm was measured against the control samples. Ascorbate was used as the reference compound. All the tests were performed in triplicate and results averaged. The percentage inhibition was calculated by comparing the results of control and test samples.

Iron chelating activity

The method of Benzie and Strain [15] was adopted for the assay. The principle is based on the formation of a-phenanthroline–Fe^{2+}complex and its disruption in the presence of chelating agents. The reaction mixture containing 1 mL of 0.05% a- phenanthroline in methanol, 2mL ferric chloride (200 µM) and 2mL of various concentrations ranging from 10 to 1000 µg was incubated at room temperature for 10 min and the absorbance of the same was measured at 510 nm. EDTA was used as a classical metal chelator. The experiment was performed in triplicates.

Nitric oxide radical scavenging activity

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which were measured by the method of Garrat(1964). The reaction mixture (3mL) containing 2ml of sodium nitroprusside (10mM), 0.5 mL of phosphate buffer saline (1M) were incubated at 25◦C for 150 min. After incubation, 0.5 mL of the reaction mixture containing nitrite was pipetted and mixed with 1ml of sulphanilic acid reagent (0.33%) and allowed to stand for 5min for completing diazotization. Then 1mL of naphthylenediamine dihydrochloride (1% neda) was added, mixed and allowed to stand for min. sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to

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produce nitrite ions which can be estimated by the use of Griess Illosvery reaction at 540 nm.

Hydroxyl radical scavenging activity

This was assayed as described by Elizabeth and Rao [15]. The assay is based on quantification of degradation product of 2-deoxyribose by condensation with TBA. Hydroxyl radical was generated by the Fe^{3+}-ascorbate –EDTA-H_2O_2 system (Fenon reaction). The reaction mixture contained 0.1ml deoxyribose (2.8mM), 0.1 mL EDTA (0.1mM), 0.1 ml H_2O_2 (1mm), 0.1mL ascorbate (0.1 mM), 0.1 mL KH_2PO_4-KOH buffer, pH 7.4 (20 mM) and various concentrations of plant extract in a final volume of 1mL. The reaction mixture was incubated for 1h at 37˚C. Deoxyribose degradation was measured as thiobarbituric acid reactive substances (TBARS) and the percentage inhibition was calculated.

Total antioxidant activity (phosphomolybdic acid method)

The antioxidant activity of the sample was evaluated by the transformation of Mo (VI) to Mo (V) from phosphomolybdenum complex (Prieto et al., 1999). An aliquot of 0.4 ml of sample solution was combined in a vial with 4ml of reagent solution (0.6M sulphuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate). The vials were capped and incubated in a water bath at 95˚C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The antioxidant activity was expresses relative to that of ascorbic acid.

FRAP assay

A modified method of Benzie and Strain (1996) was adapted for the FRAP assay. The stock solutions included 3000mM acetate buffer, pH 3.6, 10mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mM HCL and 20mM $FeCl_3$ $6H_2O$. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5mL TPTZ and 2.5mL $FeCl_36H_2O_2$. The temperature of the solution was raised to 37◦C before using. Plant extracts (0.15ml) were allowed to react with 2.85 ml of FRAP solution for 30 min in the dark condition. Reading of the colored product (ferrous tripyridyltriazine complex) were taken at 593nm. The standard curve was linear between 200 and 1000 µM $FeSO_4$. Results are expressed in µg/mL dry mass and compared with that of ascorbic acid.

Estimation of total phenol and flavonoids content in various extract of *S.jamaicensis* leaves [16]

The sample (0.5 g) was homogenized in 10X volume of 80% ethanol. The homogenate was centrifuged at 10,000rpm for 20min. the extraction was repeated with 80% ethanol. The supernatants were pooled and evaporated to dryness. The residue was then dissolved in a known volume of distilled water. Different aliquots were pipette out and the volume in each tube was made up to 3.0 ml with distilled water. Folin-Ciocalteau reagent (0.5 ml) was added and the tubes were placed in a boiling water bath for exactly one minute. The tubes were cooled and the absorbance was read at 650nm in a spectrophotometer against a reagent blank. Standard catechol solutions (0.2-1mL) corresponding to 2.0-10 µg concentrations were also treated as above. The concentration of phenols was expressed as mg/g extract.

ii) Estimation of flavonoids

A known volume of the extract was pipetted out and evaporated to dryness. Vanillin reagent (4.0 mL) was added and the tubes were heated in a boiling water bath for 15 min. varying concentrations of the standard were also treated in the same manner. The optical density was read in a spectrophotometer at 340nm a standard curve was constructed and the concentration of flavonoids in each sample was calculated. The values of flavonoids were expressed as mg/g extract.

Statistical analysis

The data are expressed as mean ±SD. Statistical comparisons were performed by one-way analysis of variance (ANOVA), followed by Tukey's multiple range test. The results were considered statistically significant at '*p*' values less than 0.05.

RESULTS

Percentage of extractive values of *S.jamaicensis* leaf

S.jamaicensis leaf extracts were stored in screw cap vials further use. The percentage of the leaf are shown table 1: 2, 2-diphenyl-1-picryl hydrazyl (DPPH) activity of different extracts of *S.jamaicensis* leaf

The percentage of DPPH scavenging activities of leaf of S. jamaicensis extracts were summarized in table 1. At all concentrations tested, leaf of S. jamaicensis exhibited a dose- dependent DPPH radical scavenging activity. The order of the scavenging activity was found to be IC50 values of Ethanol(164 µg/mL) > Methanol(210 µg/mL) > Petroleum ether(598 µg/mL) > Chloroform(635 µg/mL) > Acetone(720 µg/mL). The scavenging effect was comparable to that of the standard BHT with IC50 value 284 µg/mL. The results suggested that the medicinal property exhibited by the plant might be due to the radical scavenging activity.

Superoxide radical scavenging activity of different extracts of *S. jamaicensis* leaf

The present study indicated that the inhibition seen in a dose dependent manner (table 2). Extracts found to possess the activity in the order of their scavenging effective IC50 value of methanol (390 µg/mL) > ethanol (445 µg/mL) > petroleum ether (560 µg/mL) > chloroform (640 µg/mL) > acetone (710 µg/mL). The scavenging effect was comparable to that of the standard Quercetine (355 µg/mL).

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Iron chelating activity of different extracts of *S.jamaicensis* leaf

The iron chelating activity of leaf of S.jamaicensis was shown in table 3. The extracts found to possess the activity in the order of order of their chelating effect methanol (IC50 value 460 µg/ml) > Ethanol (IC50 value 560 µg/mL) > Petroleum ether (IC50 value 630 µg/mL) > Chloroform (IC50 value 675 µg/mL) > Acetone (IC50 value 820 µg/mL). the chelating effect was comparable to that of the standard IC50 value of 260 µg/mL.

Nitric oxide radical scavenging activity in different extracts of *S.jamaicensis* leaf

The nitric oxide radical scavenging activity of various extracts of leaf of *S.jamaicensis* and EDTA were presented in table 4.the IC50 values of various extracts methanol (IC50 value 395 µg/ml) > Ethanol (IC50 value 495 µg/mL) > Chloroform (IC50 value 580 µg/mL) > Petroleum ether (IC50 value 580 µg/mL) > Acetone (IC50 value 980 µg/mL). The nitric oxide radical scavenging effect was comparable to that of the standard IC50 value of 579 µg/mL. The methanolic extract of leaf of *S.jamaicensis* was more effective in scavenging nitric oxide radical than that of ethanol, chloroform and petroleum ether extracts.

Hydroxyl radical scavenging activity in different extracts of *S. jamaicensis* leaf

The percentage of hydroxyl radical scavenging activity of various extracts of leaf of *S. jamaicensis* was presented in table 5. The IC 50 values of methanol (IC50 value 580 µg/ml) > Ethanol (IC50 value 610 µg/mL) > Petroleum ether (IC50 value 670 µg/mL) > Chloroform (IC50 value 780 µg/mL) > Acetone (IC50 value 1322 µg/mL). The Hydroxyl radical scavenging effect was comparable to that of the standard IC50 value of 710 µg/mL. the methanolic extract was significantly increase the radical scavenging activity in comparison with standard.

Total antioxidant activity in different extracts of *S.jamaicensis* leaf

The total antioxidant activity of various extracts of leaf of *S. jamaicensis* was presented in table 6. The IC 50 values of methanol (IC50 value 195 µg/ml) > Ethanol (IC50 value 299 µg/mL) > Petroleum ether (IC50 value 560 µg/mL) > Chloroform (IC50 value 590 µg/mL) > Acetone (IC50 value 620 µg/mL). The Hydroxyl radical scavenging effect was comparable to that of the standard IC50 value of 270 µg/mL. The methanolic extract was significantly increase the total antioxidant activity in comparison with standard.

Ferric Reducing Antioxidant Power Assay in different extracts of *S.jamaicensis* leaf

Table 7 illustrated the FRAP values of various extracts of leaf of *S. jamaicensis* and ascorbate at various concentrations. The IC 50 values of methanol (IC50 value 280 µg/ml) > Ethanol (IC50 value 495 µg/mL) > Petroleum ether (IC50 value 580 µg/mL) > Chloroform (IC50 value 620 µg/mL) > Acetone (IC50 value 745 µg/mL). The Hydroxyl radical scavenging effect was comparable to that of the standard IC50 value of 275 µg/mL. The methanolic extract was significantly decrease the FRAP activity in comparison with standard.

Estimation of total phenol content in different extracts of *S.jamaicensis*

The total phenolic content of various extract of leaf of *S.jamaicensis* was presented in table 8. The methanolic extract of leaf of *S.jamaicensis* was noticed higher amount of phenolic components than that of other extracts.

Estimation of total flavonoids content in different extracts of *S.jamaicensis*

The total flavonoids content of various extract of leaf of *S.jamaicensis* was presented in table 8. The methanolic extract of leaf of *S.jamaicensis* was noticed higher amount of flavonoids components than that of other extracts.

Table 1. Extractive value of *Stachytarpheta jamaicensis* (leaf)

S.no	Petroleum ether	acetone	Distilled water	chloroform	ethanol	Methanol
1	7.32	3.78	1.28	11.62	12.28	14.62
2	7.38	3.68	1.32	11.76	12.34	14.71
3	7.42	3.79	1.33	11.88	12.25	14.56
4	7.38	3.79	1.46	11.67	12.32	14.53
5	7.40	3.67	1.57	11.70	12.27	14.77
Mean±SD	7.38±0.03	3.74±0.06	1.39±0.1	11.72±0.09	12.29±0.03	14.63±0.1

DISCUSSION

In recent years much attention has been devoted to natural antioxidant and their association with health benefits. Plants are potential sources of natural antioxidants and produce various antioxidative compounds that have therapeutic potentials. Antioxidant-based drug formulations are used for the prevention and treatment of many complex diseases. Naik et al examined *Momardica charantia* Linn, *Glycyrrhiza glabra*, *Acacia catechu*, and *Terminalia chebula* as antioxidants. The methanol extract of

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Helichrysum plicatum has been reported to have antioxidant activity using two in vitro methods, namely DPPH and β-carotene linoleic acid assays Tepe et al., 2005**.** Antioxidant activity of *Cyperus rotundus* Rhizomes Extract (CRRE) was evaluated in a series of *in vitro* assay involving free radicals and reactive oxygen species and IC50 values were determined.

Table 2. 2, 2-diphenyl-1-picryl hydrazyl (DPPH) activity of different extracts of *S.jamaicensis* leaf

S.No	Plant extracts/solvents	Concentration μg/ml						IC50 Value(μg/ml)
		10	50	100	200	400	600	
1	Petroleum ether	9.65 ± 0.36	18.52 ± 007	24.49 ± 0.05	34.19 ± 0.04	48.74 ± 0.05	50.89 ± 0.10	598
2	Chloroform	5.39 ± 0.09	9.22 ± 0.18	18.20 ± 0.07	22.61 ± 0.15	35.51 ± 0.09	43.05 ± 0.03	635
3	Acetone	3.02 ± 0.01	7.04 ± 0.02	10.14 ± 0.04	18.81 ± 0.08	22.38 ± 0.22	30.45 ± 0.30	720
4	Ethanol	34.66 ± 0.09	38.91 ± 0.08	48.18 ± 0.06	64.86 ± 0.04	69.34 ± 0.03	79.94 ±0.03**	164
5	Methanol	37.05 ± 0.02	48.71 ± 0.07	58.63 ± 0.12	68.30 ± 0.06	76.89 ± 0.06	88.14 ±0.04**	210
6	BHT	35.37 ± 0.03	46.26 ± 0.04	52.22±0.05	64.41 ± 0.03	70.47±0.05	82.19 ± 0.03	284

[a]-mean of three assays; ± - standard deviation
** significant at $p < 0.05$

Table 3. Iron chelating activity of different extracts of *S.jamaicensis* leaf

S.No	Plant extracts/solvents	Concentration μg/ml						IC50 Value(μg/ml)
		100	200	300	400	500	600	
1	Petroleum ether	10.53±0.01	13.46 ± 0.07	17.19 ± 0.06	19.46 ± 0.02	22.23 ± 0.02	44.16 ± 0.02	630
2	Chloroform	8.23 ± 0.03	9.39 ± 0.08	12.61 ± 0.09	14.55 ± 0.06	18.13 ± 0.02	35.35 ± 0.03	675
3	Acetone	3.23 ± 0.03	6.26 ± 0.04	9.38 ± 0.04	11.14 ± 0.03	16.22 ± 0.02	30.33 ± 0.06	820
4	Ethanol	12.73 ± 0.03	16.13 ± 0.04	19.71 ± 0.15	22.75 ± 0.09	42.45 ± 0.03	58.96 ± 0.02**	560
5	Methanol	15.17 ± 0.03	21.70 ± 0.04	33.46 ± 0.03	38.20 ± 0.02	58.16 ± 0.01	66.64 ± 0.01**	460
6	EDTA	28.3 1± 0.06	42.80 ± 0.03	58.05 ± 0.04	63.20 ± 0.03	68.81 ± 0.02	87.13 ± 0.05	260

[a]-mean of three assays; ± - standard deviation
** significant at $p < 0.05$

Table 4. Nitric oxide radical scavenging activity in different extracts of *S.jamaicensis* leaf

S.No	Plant extracts/solvents	Concentration μg/ml						IC50 Value(μg/ml)
		100	200	300	400	500	600	
1	Petroleum ether	20.83 ± 0.05	22.15 ± 0.04	28.54 ± 0.01	31.31 ± 0.01	45.26 ± 0.06	51.78 ± 0.03	595
2	Chloroform	17.96 ± 0.07	19.37 ± 0.06	22.57 ± 0.02	28.43 ± 0.09	31.58 ± 0.06	46.87 ± 0.08	580
3	Acetone	14.42 ± 0.07	17.58 ± 0.04	19.29 ± 0.09	21.45 ± 0.09	26.22 ± 0.01	32.36 ± 0.21	980
4	Ethanol	24.73 ± 0.08	28.19 ± 0.07	31.21 ± 0.07	33.53 ± 0.08	51.33 ± 0.02	58.40 ± 0.05**	495
5	Methanol	26.50 ± 0.08	31.52 ± 0.07	33.76 ± 0.09	51.24 ± 0.05	60.03 ± 0.03	66.41 ± 0.03**	395
6	EDTA	23.47 ± 0.08	28.83 ± 0.04	32.04 ± 0.01	41.95 ± 0.04	48.05 ± 0.02	54.71 ± 0.05	579

[a]-mean of three assays; ± - standard deviation
** significant at $p < 0.05$

Table 5. Hydroxyl radical scavenging activity in different extracts of *S. jamaicensis* leaf

S.No	Plant extracts/solvents	Concentration μg/ml						IC50 Value(μg/ml)
		100	200	300	400	500	600	
1	Petroleum ether	7.16 ± 0.01	9.73 ± 0.03	11.21 ± 0.51	19.43 ± 0.06	22.42 ± 0.1	42.31 ± 0.12	670
2	Chloroform	5.28 ± 0.05	7.23 ± 0.07	9.63 ± 0.06	15.42 ± 0.03	19.32 ± 0.07	36.59 ± 0.22	780
3	Acetone	2.92 ± 0.01	5.01 ± 0.03	7.38 ± 0.08	11.30 ± 0.07	15.80 ± 0.11	22.78 ± 0.06	1322
4	Ethanol	8.39 ± 0.06	10.20 ± 0.03	14.48 ± 0.22	21.35 ± 0.09	26.28 ± 0.7	48.33± 0.03**	610
5	Methanol	10.60 ± 0.08	12.29 ± 0.10	19.62 ± 0.09	26.69 ± 0.08	35.77 ± 0.06	59.30± 0.03**	580
6	Thiobarbituric acid	9.05 ± 0.01	11.14 ± 0.02	11.27 ± 0.02	13.94 ± 0.02	23.42 ± 0.02	44.42 ± 0.02	710

[a]-mean of three assays; ± - standard deviation
** significant at $p < 0.05$

Table 6. Total antioxidant activity in different extracts of *S.jamaicensis* leaf

S.No	Plant extracts/solvents	Concentration µg/ml						IC50 Value(µg/ml)
		100	200	300	400	500	600	
1	Petroleum ether	22.39 ± 0.07	26.40 ± 0.13	31.35 ± 0.07	40.18 ± 0.03	46.33 ± 0.07	56.83 ± 0.08	560
2	Chloroform	18.26 ± 0.08	24.57 ± 0.05	28.20 ± 0.09	37.78 ± 0.03	41.26 ± 0.02	52.60 ± 0.07	590
3	Acetone	7.02 ± 0.01	11.05 ± 0.03	15.66 ± 0.08	19.62 ± 0.07	24.68 ± 0.12	46.46 ± 0.04	620
4	Ethanol	38.31 ± 0.08	41.40 ± 0.08	50.62 ± 0.02	57.35 ± 0.03	62.77 ± 0.07	82.42±0.05**	299
5	Methanol	40.49 ± 0.05	51.73 ± 0.06	58.90 ± 0.01	78.85 ± 0.08	84.28 ± 0.08	92.98±0.44**	195
6	Ascorbic acid	33.93±0.03	42.07 ± 0.01	56.86 ± 0.03	61.49 ± 0.06	66.92 ± 0.02	71.23 ± 0.02	270

[a]-mean of three assays; ± - standard deviation
** significant at $p < 0.05$

Table 7. Ferric Reducing Antioxidant Power Assay in different extracts of *S.jamaicensis* leaf

S.No	Plant extracts/solvents	Concentration µg/ml						IC50 Value(µg/ml)
		100	200	300	400	500	600	
1	Petroleum ether	17.85±0.04	21.38±0.12	24.71±0.16	28.46±0.07	32.75±0.09	54.17±0.03	580
2	Chloroform	11.04±0.02	14.91±0.05	16.37±0.08	18.04±0.02	23.44±0.01	46.72±0.09	620
3	Acetone	6.83 ± 0.11	9.72 ± 0.13	10.92±0.07	12.18±0.05	18.84±0.08	38.57±0.09	745
4	Ethanol	22.48±0.31	30.58±0.09	33.52±0.07	36.72±0.06	52.19±0.04	62.82±0.07**	495
5	Methanol	25.24±0.08	38.57±0.19	51.44±0.09	60.86±0.05	67.81±0.13	75.82±0.04**	280
6	Ascorbic acid	38.93±0.03	44.72±0.12	52.95±0.03	61.62±0.06	69.94±0.03	78.56 ±0.07	275

[a]-mean of three assays; ± - standard deviation, ** significant at $p < 0.05$ 0.44 ± 0.12

Table 8. Estimation of total phenol content in different extracts of *S.jamaicensis*

S.No	Plant extracts/solvents	Concentration µg/ml	
		Phenol	Flavonoids
1	Petroleum ether	0.44 ± 0.12	4.93 ± 0.13
2	Chloroform	0.25 ± 0.08	3.00 ± 0.08
3	Acetone	0.19±0.06	1.16 ± 0.39
4	Ethanol	1.51±0.28	6.14 ± 0.05
5	Methanol	2.45±0.02**	6.98 ± 0.04**
6	1.Ascorbic acid 2.Rutin	1) 2.51±0.01	2) 9.89±0.09

[a]-mean of three assays; ± - standard deviation, ** significant at $p < 0.05$

CRRE exhibited its scavenging effect in concentration dependent manner on superoxide anion radicals, hydroxyl radicals, nitric oxide radical, hydrogen peroxide, and property of metal chelating and reducing power. The results obtained in the present study indicate that *C. rotundus* rhizomes extract can be a potential source of natural antioxidant Nagulenran. In this study the Methanol extract rendered a better antioxidant potential than other extract by exhibiting a high scavenging activity.

CONCLUTION

Searching plant sources may bring new natural products into pharmaceutical, cosmetic and food production. In the present work, the high antioxidant capacity observed for methanolic extract of leaf of *Stachytarpheta jamaicensis*, suggested that it may play a role of preventing human diseases. These *invitro* assays indicate that this leaf extracts is a significant source of natural antioxidant. which might be helpful in preventing the progress of various oxidative stresses.

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