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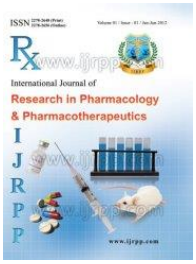

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Preliminary phytochemical screening and in vitro antioxidant activity of *Sida Acuta* Burm

Krishnaveni A*[1], Ezhilarasan B[1], Iyappan [1] A, Abdul Hasan Sathali A[2]

*[*1]Asst.Prof, [1] II M. Pharm , Department of Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai, Tamilnadu, India.*

[2]Principal, College of Pharmacy, Madurai Medical College, Madurai, Tamilnadu, India.

***Corresponding author: Krishnaveni A**

Email: akrishnaveni72@rediffmail.com

ABSTRACT

Sida acuta is shrub indigenous to pantropical areas, widely distributed in regions and found in pastures, waste lands, cultivated lands, roadsides, lawns and planted forests. *Sida acuta is* ethanomedically used as treatment of diuretic, asthma, fever, headache, cough, cold, ulcer, anthelmintic, snake bite, urinary disease, female disorders, sedative, eczema, kidney stone, elephantiasis, testicular swelling, poultice for dandruff, rheumatic affections, facial paralysis, pulmonary tuberculosis, gonorrheae. The phytochemical screening revealed the presence of tannin, saponin, flavonoid, terpenoids, cardio glycoside, vitamin composition was thiamine, niacin, ascorbic acid, tocopherol, riboflavin and mineral composition was calcium, magnesium, zinc, steroids, phenolic compounds, sesquiterpene, alkaloid cryptolepine, quindoline, quindolinone and fixed oil. The hydro alcohol extract was subjected to preliminary phytochemical analysis and the same was evaluated for its antioxidant studies by in vitro methods such as hydrogen peroxide scavenging activity, reducing power assay and total antioxidant capacity. The phytochemical analysis indicated the presence of alkaloids, carbohydrates, cardio glycoside, sterols, saponins, flavonoids,protein and aminoacids, terpenoids, resins, gum mucilage, quinone and coumarin. The antioxidant studies concentration (IC_{50}) of *Sida acuta* of hydrogen peroxide scavenging effect was found to be 1.83µg/mL in comparison with ascorbic acid (0.95µg/mL). Inhibitory concentration (IC_{50}) of *Sida acuta* for reducing power effect is found to be 142µ/mL in comparison with ascorbic acid 15µg/mL and Inhibitory concentration (IC_{50}) of *Sida acuta* for total antioxidant capacity was found to be 185µg/mL in comparison with ascorbic acid 159µg/mL. The present research draws the conclusion that this *Sida acuta* plant showed mild antioxidant effect, which may be due to the phytoconstituents.

Keywords: Antioxidant, Malvaceae , Phytochemical, *Sida acuta*

INTRODUCTION

Sida acuta is shrub indigenous to pantropical areas, weed is frequently found in pastures, waste lands, cultivated lands, roadsides, lawns and planted forests.

Sida acuta used in ayurvedic preparation as diuretic, sedative, abortifacient for the treatment of, asthma, fever, headache, cough, cold, ulcer, anthelmintic, snake bite, urinary disease, female disorders, [1], eczema, kidney stone, elephantiasis, testicular swelling, poultice for dandruff , rheumatic affections, azoospermia, oligospermia, spermatorrhea, leucorrhoea, wounds, sciatica, nervous and heart disease, facial paralysis, pulmonary tuberculosis, gonorrheae [2-5].

The literature survey of the plant revealed the presence of tannin, saponin, flavonoid, terpenoids, cardio glycoside, vitamin composition was thiamine, niacin, ascorbic acid, tocopherol, riboflavin and mineral composition was calcium, magnesium , zinc, steroids (ecdysterone, β-sistosterol, ampesterol), phenolic compounds (evofolin-A and B, scopoletin, loliolid and 4-ketopinoresinol, polyphenol, sesquiterpene[7], alkaloid cryptolepine, quindoline and quindolinone and fixed oil [6-9].

The plant exhibited various pharmacological activities such as antibacterial [10], antimicrobial [11], larvicidal and repellent [12], gastric anti-ulcer [13], insecticidal [14], hypoglycemic [15], anti-pyretic [16], anthelmintic [17], antioxidant and thrombolytic [18], electrolytes and organ function parameters [19], diuretic and anti-urolithiatic [20], invitro stability and aggregatory [21], anti – inflammatory [22], alpha amylase Inhibitory [23] , hepataprotective [24], calcium oxalate crystal growth inhibitory [25], corrosion inhibitory [26], antiplasmodial [27], analgesis [28], anti-venom [29], anti-malarial [30], anti-ulcer [31], wound healing [32], cytototoxicity [33], cardiovascular [34], antifungal [35], anticancer [36].

An the endeavor was taken to investigate the preliminary phytochemical screening and invitro antioxidant effect for this plant.

MATERIALS AND METHODS

Plant collection & authentication

Fresh leaf of *Sida acuta* Burm were collected from the komanampatty village Dindigul (Dist), (Tamil Nadu) during the month of August-2017 was authenticated by Dr. D.Stephen, M.Sc., Ph.D., Assistant Professor, Department of Botany, American College, Madurai-20.The herbarium of this specimen was kept in the department for further references

Preparation of hydro-alcoholic extract of *Sida acuta* Burm. (HAESA)

Procedure

The shade dried and coarsely powdered leaf of *Sida acuta Burm.* (Leaf) was defatted with petroleum ether (60-80˚c). The residue was dried and extracted with hydroalcohol (70%) by maceration until the complete extract of the material and filtered. The extract was concentrated under reduced pressure to obtain a solid residue (dark brown).

Phytochemical studies

The hydro-alcoholic extract was subjected to qualitative analysis to detect the presence of screening of secondary metabolites such as flavonoids, carbohydrates, alkaloids, glycosides, sterols, tannin, protein, amino acids, carotenoids, volatile oil, quinone, terpenoids, phenolic content and extract were determined as per (Harborne;1998)[37]. The results are Tabulated in **Table: 1**

INVITRO ANTIOXIDANT ACTIVITY

Hydro-alcoholic extract was subjected to in-vitro antioxidant studies. It includes hydrogen peroxide scavenging activity, reducing power activity and total antioxidant capacity.

Determination of hydrogen peroxide scavenging activity of HAESA

The hydrogen peroxide scavenging was determined for the hydro-alcoholic extract of *Sida acuta* Burm as per

(MG.Rana et al., 1996)38

Procedure

To 1 mL of different concentrations of HAESA and ascorbic acid was treated with 3.8 mL of 0.1 M phosphate buffer solution (pH 7.4) and then 0.2 mL of hydrogen peroxide solution were added. The absorbance of the reaction mixture was measured at 230 nm after 10 min. The results are tabulated in **Table: 2** and **fig: 1&2**

The percentage inhibition of hydrogen peroxide was calculated using the formula,

% inhibition = [(Control–Test) / Control] × 100

Determination of reducing power assay of HAESA

The reducing power assay was determined for the hydro-alcoholic extract of *Sida acuta* Burm as per **(navnath et al, 2010)** [39]

Procedure

The reducing power ability of plant extracts was screened by assessing the ability of the test extract to reduce $FeCl_3$ solution as mentioned by Oyaizu *et al.*, (1986). 0.1 to 0.5 mL of plant extract solution (1 mg/mL) was mixed with 0.75 mL of phosphate buffer and 0.75 mL of 1 % potassium ferricyanide [$K_3Fe(CN_6)$] and incubated at 50°C for 20min. About 0.75 mL of 10 % trichloro acetic acid was added to the mixture and allowed to stand for 10min. The whole mixture was then centrifuged at 3000 rpm for 10min. Finally 1.5 mL of the supernatant was removed and mixed with 1.5 mL of distilled water and 0.1mL of 0.1 % ferric chloride solution and the absorbance was measured at 700 nm in UV-Visible Spectrophotometer. Ascorbic acid was used as standard. and phosphate buffer was used as blank solution. The results were tabulated in **Table: 3** and **fig: 3&4**

Determination of total antioxidant activity of HAESA

The total antioxidant effect was determined for the hydro-alcoholic extract of *Sida acuta* Burm as per **(prieto *et al.*, 1999)** [40]

Procedure

An aliquot of 0.3 mL of different concentrations of sample was treated with 2.7 mL of the reagent (H_2SO4, sodium phosphate and ammonium molyptate). In case of blank, 0.3 mL of methanol was used in place of sample. The tubes were incubated in a boiling water bath at 95°C for 90 min. The samples were cooled to room temperature; the absorbance of the aqueous solution of each concentration was measured at 695 nm against blank. The standard vitamin C was treated in a similar manner. The antioxidant activity was expressed as equivalents of Vitamin C (µg/mL). The results are tabulated in **Table: 4** and **fig: 5&6.**

RESULTS AND DISCUSSION

Phytochemical screening

The phytochemical screening of the hydro-alcoholic extract showed the following result and was displayed in table:1

TABLE: 1 PRELIMINARY PHYTOCHEMICAL SCREENING OF HYDRO – ALCOHOLIC EXTRACT OF *SIDA ACUTA* BURM. (LEAF)

S. No	Test	Hydro-alcoholic extract of *Sida acuta* (Leaf)
1	Alkaloids	Positive
2	Carbohydrates	Positive
3	Antharaquinone Glycoside	Negative
4	Cardiac glycoside	Positive
5	Cyanogenic glycoside	Negative
6	Sterols	Positive
7	Saponins	Positive
8	Flavonoids	Positive
9	Protein and Amino acids	Positive
10	Terpenoids	Positive
11	Resins	Positive
12	Gum and Mucilage	Positive
13	Quinone and Coumarine	Positive
14	Volatile oil	Negative
15	Colouring Pigments	Negative

The phytochemical screening of the hydro-alcoholic extract (70%) *Sida acuta* (Leaf) powder revealed the presence of alkaloids, carbohydrates, cardiac glycosides, coumarine glycosides, sterols, saponins, tannins, phenolic compounds, flavonoids, proteins, amino acids, terpenoids, fixed oils, gum, mucilage, quinone, coumarine, resins,. It shows the absence of anthraquinone glycosides, cyanogenetic glycosides, volatile oils, betacyanins, anthocyanins, lecothiocyanins, emodin, pholoptannins.

INVITRO ANTIOXIDANT STUDIES

Hydro-alcoholic extract was subjected to in-vitro antioxidant studies. It includes hydrogen peroxide scavenging activity, reducing power assay and total antioxidant capacity.

Determination of hydrogen peroxide scavenging activity of *Sida acuta* Burm.(leaves) (HAESA)

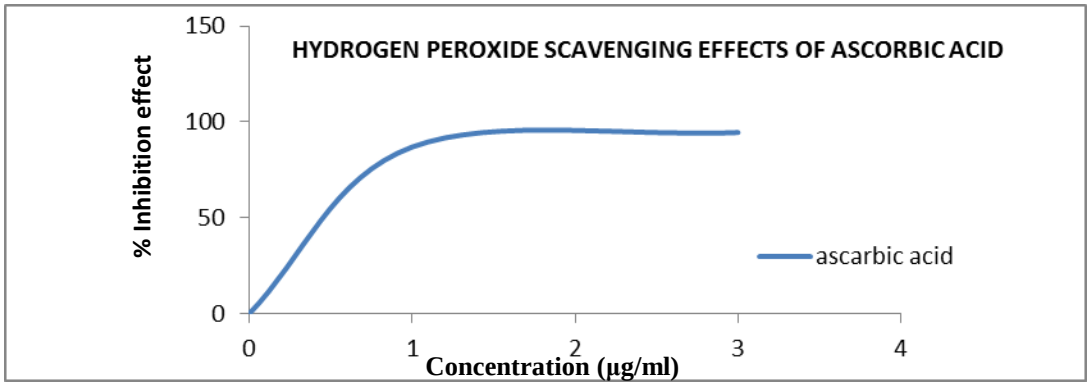

FIG: 1 Hydrogen peroxide scavenging effect of Ascorbic acid

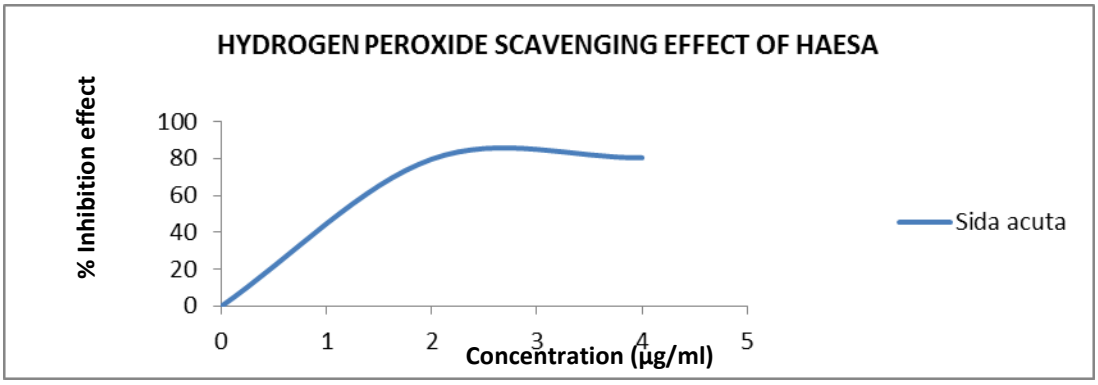

FIG: 2 Hydrogen peroxide scavenging effect of HAESA

TABLE: 2 Determination of hydrogen peroxide scavenging activity of *Sida acuta* Burm (leaf) (HAESA)

S. No.	Concentration of ascorbic acid (µg/ml)	Percentege inhibition of Ascorbic acid	Concentration of *Sidaacuta* (µg/ml)	Percentege inhibition of *Sidaacuta*
1	1	87±1.05	2	79.65
2	3	94±1.50	4	80.35
	IC_{50}	0.95µg/ml		1.83µg/ml

*****mean ± SEM,

It infers that the extract concentration was to be two fold then ascorbic acid with to inhibit the free radicals. It showed mild antioxidant effect.

Determination of reducing power assay of *Sida acuta* Burm Leaves (HAESA)

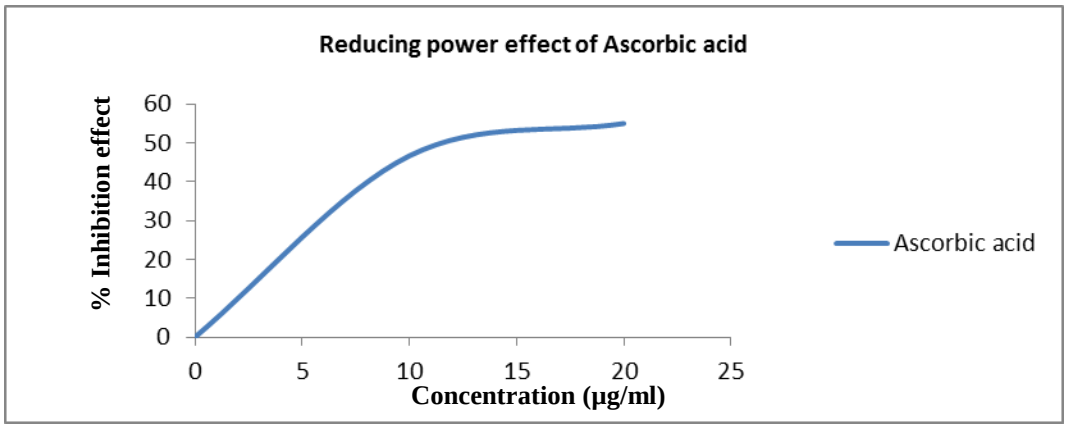

FIG: 3 Reducing power assay of Ascorbic acid

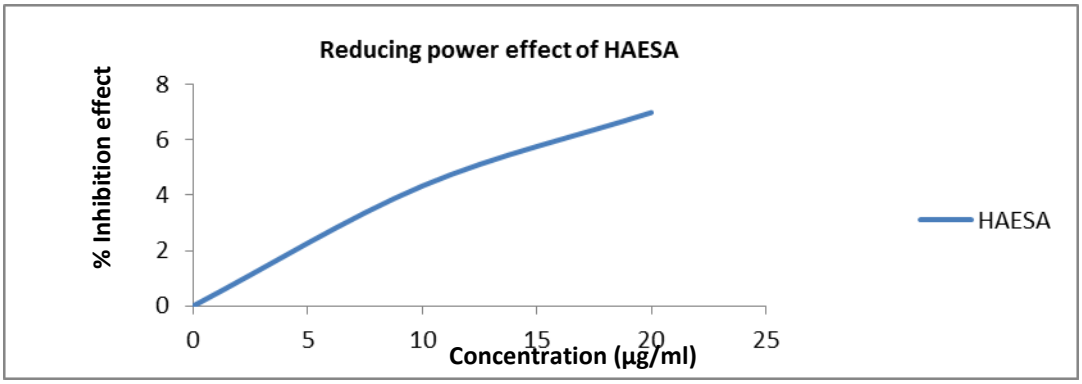

FIG: 4 Reducing power assay of HAESA

The inhibitory concentration (IC_{50}) of *Sida acuta* (leaf) against **reducing power assay** determined in comparison with ascorbic acid used as a standard. The inhibitory concentration (IC_{50}) of *Sida acuta* (leaf) in reducing power assay is found to be 142µg/ml in comparison with ascorbic acid 15µg/ml

Table: 3 Determination of reducing power assay of *Sida acuta* Burm. (Leaf) (HAESA)

S. No	Concentration of Ascorbic acid/ (HAESA) (µg/ml)	Percentage reduction of ascorbic acid	Percentage reduction of(HAESA)
1	10	46.6 ± 2.02	4.3 ± 0.100
2	20	55.0 ± 4.0	7.0 ± 0.02
	IC_{50}	15µg/ml	142µg/ml

*mean ± SEM

It is concluded that the HAESA required a nine times the concentration of ascorbic acid with to reduce the free radicals. It showed mild antioxidant effect

Determination of total antioxidant assay of *Sida acuta* Burm Leaf (HAESA)

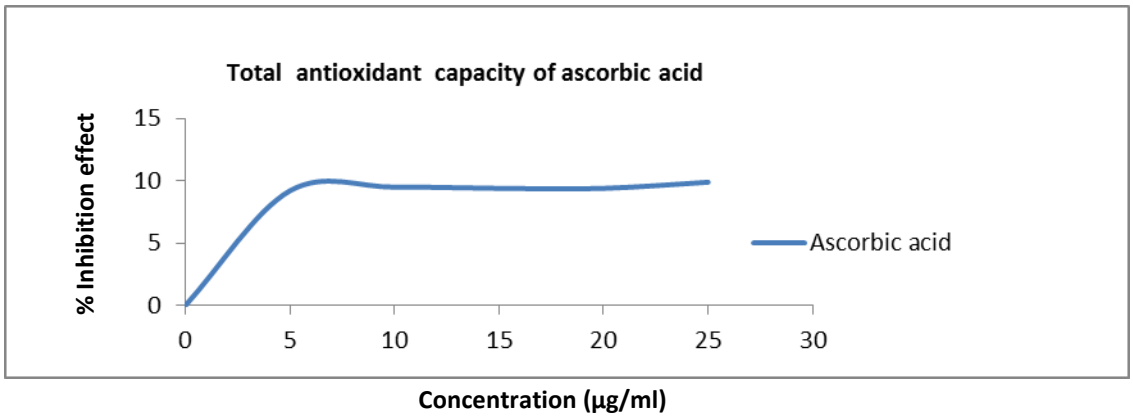

FIG: 5 Total antioxidant capacity of Ascorbic acid

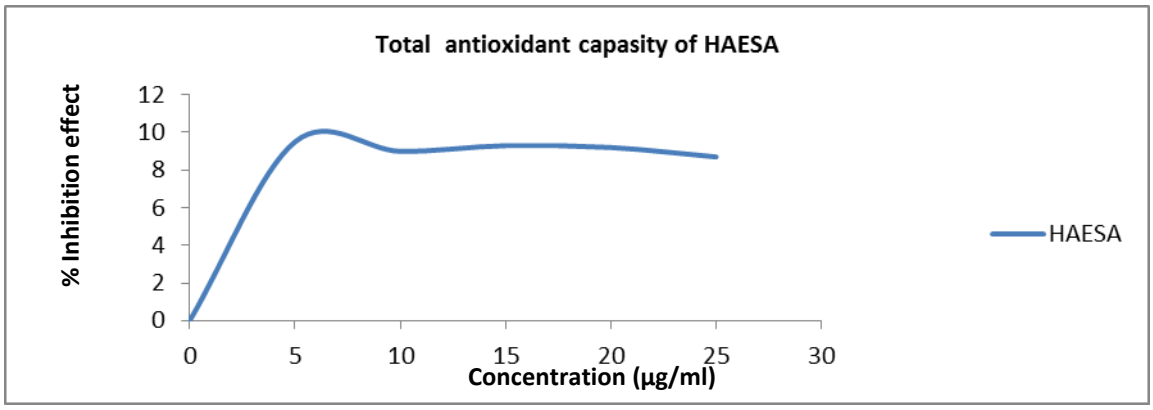

FIG: 6 Total antioxidant capacity of HAESA

The inhibitory concentration (IC_{50}) of *Sida acuta* (leaf) against **total antioxidant capacity** was determined in comparison with ascorbic acid used as a standard. The total antioxidant capacity is found to be 185.4µg/ml in comparison with ascorbic acid 159.8µg/ml.

S.no	Con of Ascorbic acid/HAESA (µg/ml)	Percentage inhibition of Ascorbic acid *	Percentage inhibition of HAESA*
1	5	9.2±0.2	8.7±0.05
2	10	9.5±0.5	9.0±0.3
3	15	9.4±0.3	9.2±0.2
4	20	9.4±0.3	9.3±0.20
5	25	9.9±0.4	9.5±0.3
	IC_{50}	159.8µg/ml	185.4µg/ml

*Mean±SEM

CONCLUSION

The preliminary phytochemical screening of hydro-alcoholic extract of *Sida acuta* showed presence of alkaloids, carbohydrates, cardiac glycosides, coumarine glycosides, sterols, saponins, tannins, phenolic compounds, flavonoids, proteins, amino acids, terpenoids, gum, mucilage, quinone, coumarin and resins. Hence, preparations may be formulated with aqueous extract. It concluded that the HAESA possess antioxidant effect when compared with ascorbic acid to inhibit the free radicals. It showed mild antioxidant effect, Hence preparations may be formulated with aqueous extract.

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