International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 21-07-2021, Accepted: 06-08-2021, Published: 21-08-2021
Volume 6, Issue 4, 2021, Page No. 848-853

An *In-vitro* study on antioxidant and antibacterial activity of chloroform extract from the leaves of *Sida Rhombifolia*

Jerin J P
Professor, Department of Organon of Medicine, Sri Sai Ram Homoeopathy Medical College and Research Centre, Sai Leo Nagar, West Tambaram, Chennai, Tamil Nadu, India

Abstract
The total phenolic and flavonoid contents, antioxidant and antibacterial properties of chloroform extract of *Sida rhombifolia* were investigated. Chloroform extract of *Sida rhombifolia* showed significant amounts of phenolic and flavonoid compounds and exhibited strong antioxidant activity. Also chloroform extract of *Sida rhombifolia* contained the highest phenolic and flavonoid contents (83.32 ± 2.34 mg GAE/g of extract and 63.21 ± 1.78 mg QE/g of extract, respectively). It also presented the highest $ABTS^{\bullet}$ scavenging activity with an EC_{50} of 58.32 µg mL, and the highest lipid peroxidation EC_{50} value of 64.32 µg mL. The extracts were found to exert moderate antibacterial activity against both Gram-negative and Gram-positive bacteria. These findings highlighted a scientific basis to the traditional usage of *Sida rhombifolia*, also showed its potential as a rich source of natural antioxidant and antibacterial compounds.

Keywords: *Sida rhombifolia*, antioxidant, antibacterial activity

Introduction

The term of medicinal plants include a various types of plants used in herbalism and some of these plants have a medicinal activities. Medicinal plants are the "backbone" of traditional medicine, which means more than 3.3 billion people in the less developed countries utilize medicinal plants on a regular basis (Davidson-Hunt, 2000) [3]. These medicinal plants consider as a rich resources of ingredients which can be used in drug development and synthesis. Besides that these plants play a critical role in the development of human cultures around the whole world. The Indian sub-continent has a very rich diversity of plant species in a wide range of ecosystems. There are about 17.000 species of higher plants, of which approximately 8.000 species, are considered medicinal and used by village communities, particularly tribal communities, or in traditional medicinal systems, such as the Siddha and Ayurveda.

Reactive oxygen species (ROS) including superoxide, hydroxyl radicals, singlet oxygen, and hydrogen peroxide are byproducts produced *via* biological reactions (Wang and Jiao, 2000) [15]. Increased levels of these ROS generate oxidative stress, which is considered to be associated with degenerative diseases such as diabetes mellitus, atherosclerosis, arthritis, and various cancers (Gackowski *et al.*, 2001) [4]. Many studies have shown that various plants extracts and a number of plant products including polyphenolic substances possess antioxidant properties and act against ROS (Lu and Yeap Foo, 2001) [7].

The use of natural antioxidants in food, cosmetic and therapeutic industry is a promising alternative for synthetic antioxidants due to its low cost, compatibility with dietary intake and no harmful effects in the human body. Many antioxidant compounds, naturally occurring in plant sources have been identified as free radical or active oxygen scavengers. Attempts have been made to study the antioxidant potential of a wide variety of vegetables like potato, spinach, tomatoes, legumes and fruits. Strong antioxidant activities have been found in berries, cherries, citrus, prunes and olives. Green and black teas have been extensively studied in the recent past for antioxidant properties since they contain up to 30% of the dry weight as phenolic compounds such as flavonols, flavandiols, flavonoids and phenolic acids. In addition to it, there are other phenolic acids (gallic acids) and characteristic amino acids (theanine) in tea (Aqil *et al*., 2006) [1].

It is widely accepted that a plant-based diet with a high intake of fruits, vegetables, and other nutrient-rich plant foods may reduce the risk of oxidative stress-related diseases. Most of the spices and herbs analysed have particularly high antioxidant contents. Although spices and herbs contribute little weight on the dinner plate, they may still be important contributors to our antioxidant intake, especially in dietary cultures where spices and herbs are used regularly. The antioxidant activity of spices and herb is due to the presence of antioxidants such as flavones, isoflavones, flavonoids, anthocyanin, coumarin lignans, catechins and isocatechins (Stoilova *et al*., 2007) [14].

Sida rhombifolia Linn. (Malvaceae) is known as arrowleaf sida by natives, and kurunthotti in Ayurvedic and Siddha medicine. It is a short-lived perennial shrub which can grow up to 1.5 m in height. The leaves of the plants are simple, narrowly ovate to lanceolate with entire leaf blade and without foliar nectarines. Flowers are solitary, axillary with cup-shaped calyx and yellow mericarps with awns, and glabrous with free petals. The plant has been widely used as traditional remedies for diarrhea, malarial, gastrointestinal dysentery, fevers, asthma and inflammation. *Sida* spp. have been proven scientifically to exhibit antibacterial, antioxidant, anti-anxiety, anti-obesity properties. In this study, antioxidant and antibacterial properties of chloroform extract of *Sida rhombifolia*.

Materials and Methods

Plant Materials

The *Sida rhombifolia* whole plant were collected from Government siddha medical college, herbal garden, Arumbakkam, Chennai, Tamilnadu, during January 2017 and it was taxonomically identified and authenticated as *Orthosiphon stamineus* by Dr. S. Sankaranarayanan, Head, Department of Medicinal Botany, Government Siddha Medical College, Arumbakkam, Chennai-600 106, Tamilnadu. A voucher specimen was deposited in the herbarium for future reference (Ref.No. MB/2021/GSMC).

Preparation of Extracts

The *Sida rhombifolia* whole plant was thoroughly cleaned, dried under the shade and coarsely powdered. The chloroform extract was prepared according to a previously reported method Kumarappan *et al*. (2012). The powdered plant material was mixed with chloroform and stored at room temperature for 5 days. After 5 days, it was filtered and the solvent was evaporated. The residue was dissolved in water, and the aqueous layer was washed with petroleum ether several times until a clear upper layer of petroleum ether was obtained. Extraction of the chloroform was performed for 36 h at room temperature, then concentrated. The residue was lyophilised. The extract obtained was dried and stored in an airtight container at 4◦C.

Phytochemical Screening

The aqueous extract of *Sida rhombifolia* were subjected to phytochemical screening to determine the presence of secondary metabolites such as alkaloids, flavonoids, terpenoids, tannins, glycosides, saponins and polyphenols using standard procedures (Harborne 1973) [5].

Thin Layer Chromatography

Thin layer chromatography of chloroform extract of *Sida rhombifolia* was performed using standard procedures (Harborne 1973) [5]. The ethyl acetate extract was placed carefully in precoated aluminum silica gel 60 F, Merck F 254 using a microcapillary tube. The spots were allowed to dry for few minutes and the TLC plate was placed in the solvent mixture, Toluene, acetone and Formic acid (6:6:1). After drying, the TLC plates were observed under UV at 240nm and 360 nm in UV TLC viewer.

ABTS (2, 2'-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid) radical scavenging assay

ABTS radical scavenging activity of chloroform extract of *Sida rhombifolia* was followed by Re *et al*. (1999) [13]. ABTS radical was newly prepared by addition 5 ml of 4.9 mM potassium persulfate solution to 5 ml of 14 mM ABTS solution and kept for 16 h in dark. This solution was diluted with distilled water to produce an absorbance of 0.70 at 734 nm and the same was used for the antioxidant activity. The final solution of standard group was made up to 1 ml with 950 µl of ABTS solution and 50 µl of Ascorbic acid. Correspondingly, in the experiment group, 1 ml reaction mixture encompassed 950 µl of ABTS solution and 50 µl of different concentration of each extracts. The reaction mixture was vortexed for 10 s and after 6 min, absorbance was recorded at 734 nm against distilled water by using a Deep Vision (1371) UV–Vis Spectrophotometer and compared with the control ABTS solution. Ascorbic acid was used as reference antioxidant compound. ABTS Scavenging Effect (%) = $[(A_0 - A_1/A_0) \times 100]$ Where A_0 is the absorbance of the control reaction and A_1 is the absorbance of extract.

Inhibition of Lipid Peroxidation Activity

Lipid peroxidation induced by Fe^{2+}ascarbate system in egg yolk was assessed as thiobarbituric acid reacting substances (TBARS) by the method of Ohkawa *et al*. (1979) [11]. The experimental mixture contained 0.1 ml of egg yolk (25% w/v) in Tris-HCl buffer (20 mM, pH 7.0); KCl (30 mM); $FeSO_4$ $(NH_4)_2SO_4.7H_2O$ (0.06 mM); and different concentrations of chloroform extract of *Sida rhombifolia* in a final volume of 0.5 ml. The experimental mixture was incubated at 37°C for 1 h. After the incubation period, 0.4 ml was collected and treated with 0.2 ml sodium dodecyl sulphate (SDS) (1.1%); 1.5 ml thiobarbituric acid (TBA) (0.8%); and 1.5 ml acetic acid (20%, pH 3.5). The final volume was made up to 4.0 ml with distilled water and then kept in a water bath at 95 to 100 °C for 1 hour. After cooling, 1.0 ml of distilled water and 5.0 ml of n-butanol and pyridine mixture (15:1 v/v) were added to the reaction mixture, shaken vigorously and centrifuged at 4000 rpm for 10 min. The absorbance of butanol-pyridine layer was recorded at 532 nm in Deep Vision (1371) UV–Vis Spectrophotometer) to quantify TBARS. Inhibition of lipid peroxidation was determined by comparing the optical density (OD) of test sample with control. Ascorbic acid was used as standard.

> Inhibition of lipid peroxidation (%) by the each extracts was calculated according to 1-(E/C) × 100

Where C is the absorbance value of the fully oxidized control and E is absorbance of the test sample.

Superoxide Radical Scavenging Assay

This assay was based on the capacity of the chloroform extract of *Sida rhombifolia* to inhibit the photochemical reduction of Nitroblue tetrazolium (NBT) in the presence of the riboflavin-light-NBT system (Tripathi and Pandey Ekta, 1999; Tripathi and Sharma, 1999). Each 3 ml reaction solution contained 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 2 µM riboflavin, 100 µM Ethylene diamine tetra acetic acid (EDTA), NBT (75 µM) and different concentration of extracts. It was kept visible in fluorescent light and absorbance was taken after 6 min at 560 nm by using a Deep Vision (1371) UV–Vis Spectrophotometer. Identical tubes with reaction mixture were kept in the dark served as blanks. The percentage inhibition of superoxide radical activity was measured by comparing the absorbance of the control with test sample solution:

> % Super oxide radical scavenging capacity= $[(A_0 - A_1)/A_0] \times 100$

Where A_0 was the absorbance of control and A_1 was the absorbance of both plant extracts fraction.

Nitric Oxide Radical Scavenging Activity

Nitric oxide scavenging ability of chloroform extract of *Sida rhombifolia* was measured according to the method described by Olabinri *et al*. (2010) [12]. 0.1 ml of sodium nitroprusside (10 mM) in phosphate buffer (0.2 M, pH 7.8) was mixed with different concentration of extracts and

incubated at room temperature for 150 min. After treated period, 0.2 ml of Griess reagent (1% Sulfanilamide, 2% Phosphoric acid and 0.1% N-(1-Naphthyl) ethylene diamine dihydrochloride) was added. The absorbance of the experimental sample was read at 546 nm against blank. All readings were taken in triplicate and ascorbic acid was used as standard. The percentage of inhibition was calculated by following equation:

$$\% \text{ Nitric oxide radical scavenging capacity} = [(A_0 - A_1)/A_0] \times 100$$

Where A_0 was the absorbance of control and A_1 was the absorbance of flavonoid rich fraction.

Culture Collection and Maintenance

The bacterial strains of *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa.* These standard strains were obtained from Microbial Type Culture Collection and gene bank (MTCC); Institute of Microbial Technology, Chandigarh, India. The stock culture was maintained on Mueller Hinton agar medium at 4 ◦C.

Antibacterial Activity

The antibacterial activities of the polyphenol rich fraction were assayed using the disc diffusion method (Drago *et al.*, 1999). Bacteria were grown overnight on Mueller Hinton agar plates, five colonies were suspended in 5 ml of sterile saline (0.9%) and the bacterial population in the suspension was adjusted to ~$3x10^8$ CFU/ml. A sterile cotton swab was dipped into the suspension and the swab rotated several times with firm pressure on the inside wall of the tube to remove the excess fluid. The swab was used to inoculate the dried surface of MH agar plate by streaking four times over the surface of the agar, rotating the plate approximately by 90^o to ensure an even distribution of the inoculums. The medium was allowed to dry for about 3 min before adding a sterile disc of 6 mm diameter. Each disc was placed firmly on to the agar to provide uniform contact with the bacteria. Bioactive compound (50 µg) was weighed and dissolved in 1 ml of 7% ethyl acetate. The different concentration of chloroform extract of *Sida rhombifolia* was introduced on to each disc and the control disc received only 7% ethanol. The plates were incubated at 37°C for 24 h and the inhibition zone was measured and calculated. The experiments were carried out in duplicate three times. The results (mean value, *n*=3) were recorded by measuring the zones of growth inhibition surrounding the discs.

Minimum Inhibitory Concentrations (MICS)

The minimum inhibitory concentrations of the isolated compounds were determined by dilution method (Brantner and Grein, 1994). The strains were grown in Mueller Hinton broth to exponential phase with an *A*560 of 0.8, representing 3.2×10^8 CFU/ml. Different dilutions of the chloroform extract of *Sida rhombifolia* were prepared to give solutions of 25, 50, 75, and 100 µg/ml. 0.5 ml of each concentration was added into separate test tubes containing 4ml of MH broth inoculated with 0.5 ml bacterial suspension at a final concentration of 10^6 CFU/ml. Each MIC was determined from five independent experiments performed in duplicate. The tubes containing 4.5 ml of bacterial inoculates and 0.5 ml of 7% ethyl acetate used as bacterial control, 4.5 ml of uninoculated MH broth and 0.5 ml PBS served as a blank. The tubes were incubated at 37 ◦C for 18 h; inhibition of bacterial growth was determined by measuring the absorbance at 560 nm.

Statistical Analysis

The outcomes are shown as mean ± S.E.M. ($n = 6$). Statistical significance was determined by one-way analysis of variance with $p < 0.01$ and $p < 0.05$ considered significant followed by Dunnett Multiple Comparisons Test.

Results and Discussion

Phytochemical Screening

The phytochemical screening of aqueous extract of *Sida rhombifolia* studied presently showed the presence of alkaloids, flavonoids, polyphenol, terpenoids, and absence of glycosides and tannin (Table -1).

Table 1: Phytochemical screening of aqueous extract of *Sida rhombifolia*

S/No.	Constituents	Aqueous extract of *Sida rhombifolia*
1.	Alkaloids-Dragendroffs reagent	+
	Alkaloids- Mayers Test	
2.	Flavonoids- Alkali reagents	+
3.	Tannin- $Fecl_3$ test	+
4.	Saponins- Frothing test	-
5.	Terpenoids - Nollers test	+
6.	Glycosides- Keller-Killiani Test	-
7.	Polyphenols-Ferrozine	+
8.	Anthocynin- Ammonia Test	+

The Partial Characterization of Chloroform Extract of *Sida Rhombifolia* by TLC

The chloroform extract of *Sida rhombifolia* loaded on Pre-coated TLC plates (60F 254 Merck) and developed with a solvent system of hexane, ethyl acetate and acetic acid in the ratio of 10:5:0.5. The developed plate was viewed under UV 240nm and 360nm. The Rf value of compounds were shown in Fig-1.

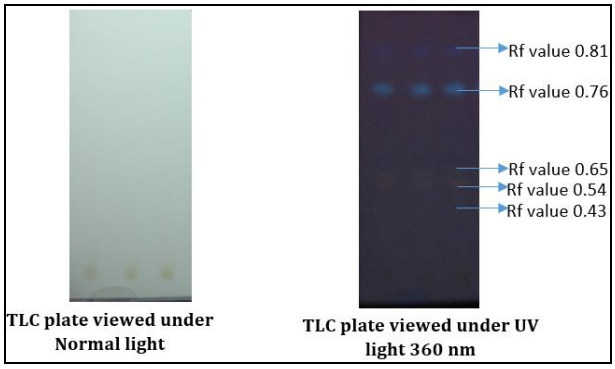

Fig 1: Partial characterization of chloroform extract of *Sida rhombifolia* by TLC

Free Radical-Scavenging Ability Using ABTS Assay

The radical scavenging ability was measured by ABTS assay as per given in table 3. The inhibition percentage of the ABTS radical activity was assessed on average and high free radical-scavenging values were found in chloroform extract of *Sida rhombifolia.* In ABTS assay, inhibition percentage was high in chloroform extract of *Sida rhombifolia* 79.32% with EC_{50} value 58.32 µl/ml. The pure ascorbic acid was lower activity (Table-2). Nevertheless, in present study, it is showed that these activities were mainly due to phenolics and flavonoids. It is known that vitamin C (ascorbic acid) and carotenoids are chief source of discrepancy of antioxidant/ antiradical activities in plant materials.

Table 2: Free radical-scavenging ability using ABTS assay of chloroform extract of *Sida rhombifolia*

Different concentration of extract	Percentage of ABTS radical activity	
	Chloroform extract of *Sida rhombifolia*	Standard Vitamin-C
25 µl/ml	22.36±0.78	19.32±1.78
50 µl/ml	38.64±2.87	35.64±2.34
75 µl/ml	57.32±1.56	54.32±0.89
100 µl/ml	79.32±2.31	73.65±2.45
EC_{50} value	58.32	61.23

[a] Results are expressed as percentage inhibit of ABTS ability with respect to control. Each value represents the mean+SD of three experiments

Inhibition of Lipid Peroxidation Activity

Chloroform extract of *Sida rhombifolia* also inhibited the lipid peroxidation induced by ferrous sulfate in egg yolk homogenates. Maximum inhibition was recorded in Chloroform extract of *Sida rhombifolia* 72.34% with EC_{50} value 64.32µl/ml and lowest inhibition percentage ascorbic acid 67.32% (Table-3). As it is identified that lipid peroxidation is the net result of any free radical attack on membrane and other lipid components present in the system, the lipid peroxidation may be enzymatic (Fe/NADPH) or non-enzymatic (Fe/ascorbic acid). In the present study, egg yolk was used as substrate for free radical mediated lipid peroxidation, which is a non-enzymatic method. Normally, the mechanism of phenolic compounds for antioxidant activity includes neutralizing lipid free radicals and preventing decomposition of hydroperoxides into free radicals.

Table 3: Inhibition of lipid peroxidation activity of chloroform extract of *Sida rhombifolia*

Different concentration of extract	Lipid peroxidation inhibition percentage	
	Chloroform extract of *Sida rhombifolia*	Standard Vitamin-C
25 µl/ml	21.36±0.78	19.34±1.78
50 µl/ml	37.64±2.36	34.65±0.36
75 µl/ml	51.34±1.56	47.32±0.78
100 µl/ml	72.34±0.23	67.32±1.34
EC_{50} value	64.32	71.37

[a] Results are expressed as percentage inhibit of lipid peroxidation with respect to control. Each value represents the mean+SD of three experiments.

Superoxide Scavenging Assay Activity

Chloroform extract of *Sida rhombifolia* exhibited powerful scavenging activity for superoxide radicals in a concentration dependent process than positive control. Chloroform extract of *Sida rhombifolia* showed highest radical activity in the percentage of 67.32% with EC_{50} value 73.64 µl/ml when compared to positive control 65.32% with EC_{50} Value 78.34 µl/ml (Table-4). One of the standard method to produce Superoxide radicals is through photochemical reduction of nitro blue tetrazolium (NBT) in the presence of a riboflavin-light-NBT system. These superoxide radicals are extremely toxic and may be produced either through xanthine activity or through mitochondrial reaction. Superoxide radicals are reasonably a weak oxidant may decompose to form stronger reactive oxidative species, such as singlet oxygen and hydroxyl radicals (Jordan *et al*., 2009) [6].

Table 4: Superoxide scavenging assay activity of chloroform extract of *Sida rhombifolia*

Different concentration of extract	Percentage of Superoxide scavenging activity	
	Chloroform extract of *Sida rhombifolia*	Standard Vitamin-C
25 µl/ml	15.64±0.36	14.36±2.64
50 µl/ml	28.32±1.79	25.64±1.96
75 µl/ml	48.32±0.78	45.67±0.78
100 µl/ml	67.32±1.48	65.32±2.49
EC_{50} value	73.64	78.34

[a] Results are expressed as percentage of Superoxide scavenging activity with respect to control. Each value represents the mean+SD of three experiments.

Nitric Oxide Radical Scavenging Assay

Nitric oxide radical quenching activity of chloroform extract of *Sida rhombifolia* were identified and compared with the standard ascorbic acid. The chloroform extract of *Sida rhombifolia* displayed the maximum inhibition of 84.13% at a concentration of 100 µg/ml, in a concentration-dependent process when compared to ascorbic acid with inhibition percentage 77.36% (Table-5). In the current study, nitrite was produced by incubation of sodium nitroprusside in standard phosphate saline buffer at 25°C was reduced by chloroform extract of *Sida rhombifolia*. Significant scavenging activity may be due to the antioxidant property of flavonoid, which compete with oxygen to react with nitric oxide, leading to less production of nitric oxide.

Table 5: Nitric oxide radical scavenging assay of the chloroform extract of *Sida rhombifolia*

Different concentration of extract	Percentage of Nitric oxide radical scavenging activity	
	Chloroform extract of *Sida rhombifolia*	Standard Vitamin-C
5 µl/ml	22.34±1.79	18.32±0.73
10 µl/ml	47.34±2.37	45.32±0.89
15 µl/ml	61.34±1.46	57.32±2.34
20 µl/ml	77.36±2.89	74.32±1.89
EC_{50} value	58.31	60.32

[a] Results are expressed as percentage of Nitric oxide radical activity with respect to control. Each value represents the mean+SD of three experiments.

Anrtibacterial Activity by Disc Diffusion Method

Antibacterial activity of chloroform extract of *Sida rhombifolia* tested against *Enterococcus faecalis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* were assessed as inhibition zones in the agar plates (Table-6). In this experimental all the bacteria were found to be sensitive to the polyphenol rich fraction. Additionally, the zone of inhibition reconsideration that the polyphenol rich fraction influenced antibacterial activity in proportion to concentration gradient ranges 25-100 µl/ml against the tested bacteria. Amongst the bacteria considered, *Staphylococcus aureus* and *Escherichia coli* was identified to be highly susceptible followed by *Pseudomonas aeruginosa* and *Enterococcus faecalis*. This may confirm the antibacterial property of chloroform extract of *Sida rhombifolia*. Polyphenols metabolites such as phenolic and flavonoids, are significant antibacterial activity (Machado *et al.*, 2002) [8].

The antimicrobial activity of phytochemicals are due to their capability to composite with extracellular and soluble protein and to complex with bacterial cell wall although polyphenol may be related to their ability to deactivate microbial adhesions, enzymes and cell envelop proteins (Cowan, 1999) [2].

Table 6: The antibacterial activity of the chloroform extract of *Sida rhombifolia* by disc diffusion method

Pathogenic organism	Different concentrations Crude extract (µl/ml)			
	25 µl/ml	50 µl/ml	75 µl/ml	100 µl/ ml
Staphylococcus aureus	10.2±0.3	12.6±1.2	15.6±0.4	17.3±1.3
Pseudomonas aeruginosa	8.2±2.5	10.8±1.5	13.2±1.3	15.6±0.8
Escherichia coli	9.3±1.3	11.5±0.8	14.6±2.1	16.7±2.1
Enterococcus faecalis	8.6±0.9	10.2±1.7	12.6±3.5	14.8±1.5

*The inhibitory Zone size measured included the 6.0 mm size of the well by means of caliper. All the assays were duplicated, and the mean values were recorded.

Minimum Inhibitory Concentration

In the complete sequences, the MIC of chloroform extract of *Sida rhombifolia* ranged between 25 to 100 µg/ml against gram positive bacteria and gram negative bacteria, (*Staphylococcus aureus, Enterococcus faecalis, Escherichia coli* and *Pseudomonas aeruginosa*) respectively. The Minimum inhibitory absorption value of chloroform extract of *Sida rhombifolia* increases with increase in concentration. *S. aureus* exhibited maximum inhibition when compared to the other pathogenic bacteria at 100 µl/ml concentration. *Enterococcus faecalis, Escherichia coli* appearances reasonable range of inhibition activity. *P. aeruginosa* display slighter activity. In comparison with gram positive bacteria and gram negative bacteria, the MIC of chloroform extract of *Sida rhombifolia* displayed highest inhibition in gram negative bacteria and among the gram positive bactertia *S. aureus* showed maximum inhibition (Graph-1).

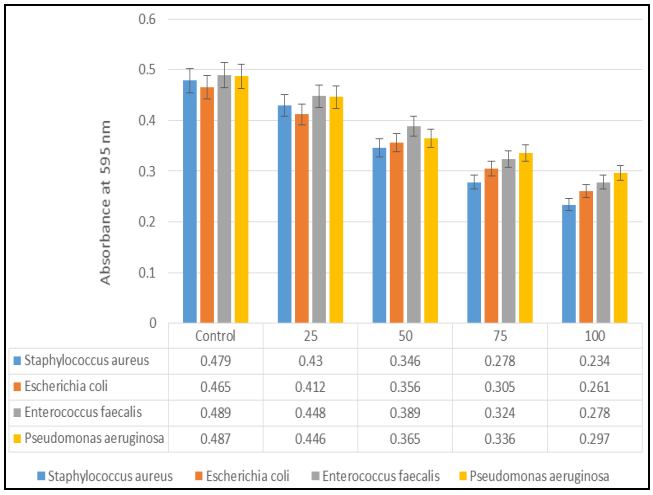

Fig 2: MIC of chloroform extract of *Sida rhombifolia*

Conclusion

Phytochemicals are valuable plant constituents for the scavenging of free radicals because of their phenolic hydroxyl groups. This, together with the obtained results, suggests that as the amount of phytochemical compounds increases, the antioxidant activity also increases. In conclusion, the present study demonstrates that the chloroform extract of *Sida rhombifolia* can protect the body from oxidative stress from ROS. These may be used in nutraceuticals and the food industry.

Acknowledgement

We thank Mr. SaiPrakash Leomuthu, CEO Sairam Institutions, Mr. SathishKumar CBO Sairam Institutions. Dr. Cheramanan M. D. (H), Principal Sri Sairam Homoeopathy Medical College West Tambaram for Support and Encouragement to carry out the study.

Reference

1. Aqil F, Ahmed I, Mehmood Z. Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. Turk J Biol,2006:30:177-183.
2. Cowan MM. Plants products as antimicrobial agents. Clin Microbiol Rev,1999:12:564-582.
3. Davidson-Hunt I. Ecological ethno botany: stumbling toward new practices and paradigms. MASA J,2000:16:1-13.
4. Gackowski D, Kruszewski M, Jawien A, Ciecierski M, Olinski R. Further protective role of antioxidants in wine and plant foods. Food Technol,2001:47:85-9.
5. Harborne JB. A guide to modern techniques of plant analysis. 3rd ed. London, New York: Chapman and Hall. Phytochemical methods, 1998.
6. Jordan MJ, Martınez RM, Martınez C, Monino I, Sotomayor JA. Polyphenolic extract and essential oil quality of *Thymus zygis* ssp. gracilis shrubs cultivated under different watering levels. Ind Crop Prod,2009:29:145-153.
7. Lu Y, Yeap Foo Y. Antioxidant activities of polyphenols from sage (*Salvia officinalis*). Food Chem,2001:75(2):197-202.
8. Machado TD, Leal ICR, Amaral ACF, dos Santos KRN, da Siva MG, Kuster RM. Antimicrobial ellagitannin of Punica granutumfruits. J Braz Chem Soc,2002:13:606-610.
9. Marin PD, Grayer RJ, Kite GC, Matevski V. External leaf flavonoids of *Thymus* species from Macedonia. Biochem Syst Ecol,2003:31:1291-1307.
10. Meda A, Lamien CE, Romito M, Millogo J, Nacoulma OG. Determination of the total phenolic, flavonoid and praline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chem,2005:91:571-577.
11. Ohkowa H, Ohisi N, Yagi K. Assay for lipid peroxides in animals tissue by thiobarbituric acid reaction. Analytical Biochemistry,1979:95:351-358.
12. Olabinri BM, Odedire OO, Olaleye MT, Adekunle AS, Ehigie LO, Olabinri PF. *In vitro* evaluation of hydroxyl and nitric oxide radical scavenging activities of artemether. Research Journal of Biological Science,2010:5(1):102-105.
13. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay," Free Radical Biology and Medicine,1999:26(9-10):1231-1237.
14. Stoilova I, Krastanov A, Stoyanova A, Denev P, Gargova S. Antioxidant activity of a ginger extract (*Zingiber officinale*). Food Chem,2007:102(3):764-770.
15. Wang SY, Lin HS. Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage, J. Agric. Food Chem,2000:48:140-146.