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Research Article

ASSESSMENT OF *SEMECARPUS ANACARDIUM* (LINN.F.) LEAF METHANOLIC EXTRACT FOR THEIR ANTIBACTERIAL, ANTIFUNGAL AND ANTIOXIDANT ACTIVITY

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ABSTRACT

The antimicrobial activity of the methanolic leaf extract of *Semecarpus anacardium* L. was evaluated against medicinally important bacteria *Staphylococcus epidermidis* (ATCC 12228), *Micrococcus luteus* (ATCC10240), Methicillin-resistant *Staphylococcus aureus* (Hospital-isolate), *Propionibacterium acnes* (MTCC 1951) and yeast, *Malaassezia furfur* (MTCC 1374) using the MIC and MBC/MFC analysis. The lowest MIC and MBC values were obtained for *Staphylococcus epidermidis* which was 100 mg/mL. The methanolic leaf extracts were subjected to evaluation for antioxidant activity by DPPH free radical scavenging method and Nitric oxide radical scavenging method. Using DPPH method, IC_{50} values for methanolic leaf extract was 916.58 ± 2.14 µg/mL and for ascorbic acid was found to be less than 20 µg/ml respectively. IC_{50} values for methanolic leaf extract and ascorbic acid was found to be 51.33 ± 2.00 µg /ml and 36.81 ± 2.06 µg /ml respectively using Nitric oxide radical scavenging method. The antioxidant activity of these extracts can be attributed to the presence of tannins, saponins and flavonoids.

A high potential for scavenging of free radicals at relatively low concentrations and inhibiting the growth of the bacteria used in this study is an indication that the leaves of *Semecarpus anacardium* L. can be used as a source for the development and formulation of drugs, thus justifying the use of the leaves in herbal medicines to treat a variety of infectious conditions caused by test microorganisms.

Keywords: Antimicrobial, *Semecarpus anacardium* Linn.f., MIC, MBC, MFC, Antioxidant activity.

INTRODUCTION

Despite the huge number of antimicrobial agents for various purposes that already exist, the search for new drugs is a continuous task since the target microorganisms often develop new genetic variants which subsequently become resistant to available antimicrobial agents and the effective lifespan of any antibiotic is thus limited. The world's attention is now increasingly directed towards plant sources for developing antimicrobial drugs, since natural products are considered safer than synthetic ones. According to the World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy [1]. There are several published reports describing the antimicrobial activity of various crude plant extracts [2,3].

Semecarpus anacardium is a deciduous tree belonging to Anacardiaceae family and is growing in tropical and temperate regions of south east asian countries. Its seed, commonly known as 'marking nut' is largely used in Indian traditional medicine 'Ayurveda' for the treatment of rheumatoid arthritis, gout and other inflammatory diseases, tumours, asthma, epilepsy, psoriasis and leprosy [4]. A wide spectrum of physiological activities including anti-inflammatory [5], antiarthritic [6]and anticancer [7] properties have been attributed to the seed and its preparations. In our previous preliminary phytochemical studies, the leaves of *Semecarpus anacardium* showed the presence of alkaloids, saponins, tannins, flavonoids, steroids, glycosides, hexose sugars, diterpenes, mucilages and gums [8].

Although, many studies have been done on *Semecarpus anacardium*, none of them were performed on the methanolic extract of leaf part of the plant against *Staphylococcus epidermidis* (ATCC 12228), *Micrococcus luteus* (ATCC10240), Methicillin-resistant *Staphylococcus aureus* (Hospital isolate), *Propionibacterium acnes* (MTCC 1951) and *Malaassezia furfur* (MTCC 1374). *Staphylococcus epidermidis* is Gram-positive which is not usually pathogenic but patients with compromised immune systems are often at risk for developing an infection. These infections can be both nosocomial or community acquired, but they pose a greater threat to hospital patients [9]. *Micrococcus luteus* is Gram-positive. It may occasionally be opportunist pathogen which can cause septic shock, pnuemonia and urinary tract infections in an immune-deficient person [10]. Methicillin-resistant *Staphylococcus aureus* (Hospital isolate) is a Gram-positive bacterium responsible for several difficult-to-treat infections in humans. It is also called multidrug-resistant *Staphylococcus aureus.* MRSA is especially troublesome in hospitals and nursing homes, where patients with open wounds, invasive devices, and weakened immune systems are at greater risk of infection than the general public [11]. *Malassezia furfur* is the causative agent of Pityriasis versicolor, Pityriasis folliculitis and it has recently been implicated as a causative agent of seborrhoeic dermatitis and dandruff. It is a lipophilic yeast, therefore in vitro growth must be stimulated by natural oils or other fatty substances. The most common method used is to overlay Sabouraud's dextrose agar containing cycloheximide (actidione) with olive oil or alternatively to use a more specialized media like Dixon's agar which contains glycerol mono-oleate (a suitable substrate for growth) [12]. In the proposed work use of Sabouraud's agar with Corn oil and Tween 80 has been made for *Malassezia furfur*. *Propionibacterium acnes* is the relatively slow growing, typically aerotolerant anaerobic, Gram-positive bacterium (rod) linked to the skin condition acne, it can also cause chronic blepharitis and endophthalmitis, the latter particularly following intraocular surgery [13].

Antioxidants are important in preventing human diseases. Naturally occurring antioxidants in leafy vegetables and seeds such as ascorbic acid, vitamin E and phenolic compounds possess the ability to reduce oxidative damage associated with diseases like cancer, diabetes, arthritis, cardiovascular diseases, cataracts and ageing [14-16]. Antioxidant compounds may function as free radical scavengers, reducing agents and quenchers of singlet oxygen species [17]. Synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have restricted use in foods as they are carcinogenic. Therefore need of natural antioxidant has increased in recent years. The antioxidant activity in the leaf of *Semecarpus anacardium* was evaluated using DPPH and Nitric oxide radical scavenging activity.

MATERIALS AND METHODS

Plant collection and authentification

The leaves of *Semecarpus anacardium* were collected from an open field around Mumbai, Maharashtra. The identification of the plant was done at the Blatter Herbarium, St. Xavier's College, Mumbai. The

plant specimen matches with the Blatter Herbarium specimen no.T-472 of S. C. Tavakari. Leaves were shade dried and made into coarse powder with mechanical grinder and then passed through sieve, B.S.S Mesh No.60.

Instrument and chemicals

Jasco V-630 spectrophotometer was used for the measurement of absorbances of solution mixtures. Stardard used was Ascobic acid (S.D Fine Chemicals, Mumbai), 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) (Sigma Aldrich, Germany), Sodium nitroprusside (Fisher scientific, Mumbai), Sulphanilamide (S.D Fine Chemicals, Mumbai), N(1-Naphthyl) ethylene diamine dihydrochloride (S.D Fine Chemicals, Mumbai), DMSO (Himedia, India), Soyabean Casein Digest Agar medium (Himedia, India), Sabouraud's agar (Himedia, India), Tween 80 (Himedia, India), Tryptone soy broth (TSB) (Himedia, India), HiAnero Gas Pack (Himedia India), Equitron Anaerobic jar.

Preparation of the plant extract

The leaf powder of *Semecarpus anacardium* (20 gm) was extracted with 250 ml methanol by soxhlet extraction for 8 hrs. The extract was concentrated on water bath at 60ºC. The obtained dark brown thick liquid was stored in a glass vial in refrigerator.

Bacterial strains

Four bacterial strains, namely *Staphylococcus epidermidis* (ATCC 12228), *Micrococcus luteus* (ATCC10240),Methicillin-resistant *Staphylococcus aureus* (Hospital isolate), *Propionibacterium acnes* (MTCC 1951) and yeast, *Malaassezia furfur* (MTCC 1374) were used for antibacterial testing. The microbial isolates were procured from National Chemical Laboratories (NCL), Pune, Maharashtra, India and Microbial Type Culture Collection (MTCC) Chandigarh, India. The microorganisms were maintained at 4°C temperature.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal and Fungicidal Concentration (MBC / MFC)

Determination of the minimum inhibitory concentration (MIC) was carried out only on leaves using the Broth dilution method [18,19] with slight modification. The extracts were reconstituted in 10% v/v aqueous dimethyl sulfoxide (DMSO) at the concentration of 1600 mg/ml. A serial two fold dilution of reconstituted extract was performed to obtain 800, 400, 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.562, 0.781, 0.391, 0.195 mg/ml concentrations. Then 100 µl of an 18 hrs old culture of each of the bacteria earlier adjusted at10^6Colony Forming Unit per milliliter (CFU/ml) was added to all tubes and thoroughly vortexed. The tubes were incubated at 37°C for 48 hrs and observed for growth in form of turbidity. The test tube with the lowest dilution with no detectable growth by visual inspection was considered as MIC.

The MBC / MFC value was determined by spot innoculating of bacterial suspension from the MIC tubes that did not show any growth and subcultured onto Soyabein casein digest agar plates and incubated at 37°C for 48 hrs for *S. epidermidis*, *M. luteus*, MRSA. For *M. furfur* Sabouraud's agar with 0.2% corn oil and 0.5% tween 80 was used and the incubation period was 37°C for 72 hrs. For *P.acne* media used was Tryptone soy broth (TSB) and the incubation period was 7 days at 37°C under anaerobic conditions. After incubation, the concentration at which no visible growth was seen on the agar plate was recorded as MBC / MFC.

DPPH Scavenging Activity

The free radical scavenging activity of the methanolic leaf extract of *Semecarpus anacardium* was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH (2, 2-diphenyl-2-picryl hydrazyl). 0.1 mM of DPPH in methanol was prepared.

Different concentrations of sample and standard used were 20, 40, 60, 80, 100, 200, 500, 1000µg/ml. After 30 minutes the absorbance was measured at 517 nm. Ascorbic acid was used as the standard. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity [20,21]. Radical scavenging activity was expressed as inhibition percentage of free radical by the sample and was calculated using following formula:

$$\% \text{ DPPH Scavenged} = \frac{A_{Control} - A_{Test} \times 100}{A_{Control}}$$

Where, $A_{Control}$ was the absorbance of control (Methanol and DPPH). A graph is plotted using % Inhibition v/s Concentrations (Table 2 & Fig1 & 2).

Nitric oxide Scavenging Activity

The chemical source of Nitric oxide was sodium nitroprusside (10mM) in phosphate buffer (pH 7.4), which spontaneously generates nitric oxide. Nitric oxide interacts with oxygen to produce stable products, leading to the production of nitrites. 0.5 ml of 10mM sodium nitroprusside in phosphate buffer was mixed with 2 ml of different concentrations (20, 40, 60, 80, 100, 200, 500, 1000µg/ml) of the sample and standard and incubated at room temperature for 150 mins. After 150 mins the samples from the above were reacted with 1.2 ml Greiss reagent (1gm sulphanilamide in 5 ml ortho phosphoric acid and 104 mg N-(1-napthyl) ethylene diamine dihydrochloride in 100 ml of distilled water. The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with N-(1-napthyl) ethylenediamine was read at 546 nm. The reaction mixture without plant extracts but with methanol and sodium nitroprusside served as control. Ascorbic acid was used as positive control [22,23]. The results of anti-oxidant activity of methanolic extracts using Nitric oxide radical scavenging method were shown below (Table 3 and Fig 3 & 4).

The capability to scavenge the NO radical was calculated using the following equation:

$$\% \text{ NO Scavanged} = \frac{A_{Control} - A_{Test} \times 100}{A_{Control}}$$

Where, $A_{control}$ is the absorbance of the control (Methanol and Sodium nitroprusside). A_{test} is the absorbance in the presence of the extracts. The antioxidant activity of the extracts were expressed as IC_{50}.

RESULTS

The minimum inhibitory concentrations of the methanolic leaf extract of *Semecarpus anacardium* on the selected microorganisms are shown in Table 1. The MIC values were tested from 800 to 0.195 mg/mL. *S. epidermidis* showed MIC and MBC values at 100 mg/ml whereas for *M.Luteus* it was 100 and 400 mg/ml. MRSA showed a higher MIC at 200 mg/ml and MBC at 400 mg/ml. *P.acne* showed MIC at 800 mg/ml and MBC at 800 mg/ml. *M. furfur* showed MIC and MFC at 400 mg/ml.

Using the DPPH scavenging activity, the IC_{50} value of methanolic leaf extract of *Semecarpus anacardium* was found to be 916.58 ± 2.14 µg/ml and for standard ascorbic acid it was less than 20 µg/ml respectively. By using Nitric oxide scavenging activity of methanolic leaf extract of *Semecarpus anacardium*, the IC_{50} value was found to be 51.33 ± 2.00 µg/ml and for standard ascorbic acid was 36.81 ± 2.06 µg/ml respectively.

Table 1: Minimum Inhibitory (MIC) and Minimum Bactericidal / Fungicidal Concentration (MBC / MFC) of *Semecarpus anacardium* L. leaf extract.

Microorganism	MIC (mg/mL)	MBC & MFC(mg/mL)
Staphylococcus epidermidis (ATCC 12228)	l00 mg/ml	l00 mg/ml
Micrococcus luteus(ATCC 10240)	100 mg/ml	400 mg/ml
Methicillin-resistant *Staphylococcus aureus* (Hospital isolate)	200 mg/ml	400 mg/ml
Propionibacterium acnes (MTCC 1951)	800 mg/ml	800 mg/ml
Malaassezia furfur (MTCC 1374)	400 mg/ml	400 mg/ml

Table 2: Data of DPPH scavenging activity of *Semecarpus anacardium* leaf extract.

Concentration Used (µg/ml)	Standard Ascorbic acid		Leaf Extract Sample	
	Ascorbic acid Absorbance Mean ± SD	Ascorbic acid % Inhibition Mean ± SD	Sample Absorbance Mean ± SD	Sample % Inhibition Mean ± SD
20	0.0166 ± 0.0004	93.3 ± 0.21	0.2244 ± 0.0005	9.58 ± 0.21
40	0.0115 ± 0.0007	95.3 ± 0.31	0.2190 ± 0.0004	11.7 ± 0.15
60	0.0105 ± 0.003	95.7 ± 0.10	0.2125 ± 0.0004	14.3 ± 0.15
80	0.0093 ± 0.0007	96.2 ± 0.29	0.2107 ± 0.0003	15.1 ± 0.12
100	0.0090 ± 0.0005	96.3 ± 0.17	0.2062 ± 0.0012	16.9 ± 0.51
200	0.0090 ± 0.0004	96.3 ± 0.10	0.1961 ± 0.0004	20.9 ± 0.15
500	0.0084 ± 0.0008	96.6 ± 0.29	0.1559 ± 0.0003	37.1 ± 0.12
1000	0.0083 ± 0.0006	96.6 ± 0.26	0.1191 ± 0.0004	52.0 ± 0.15

Absorbance of Control: 0.248 ± 0.001

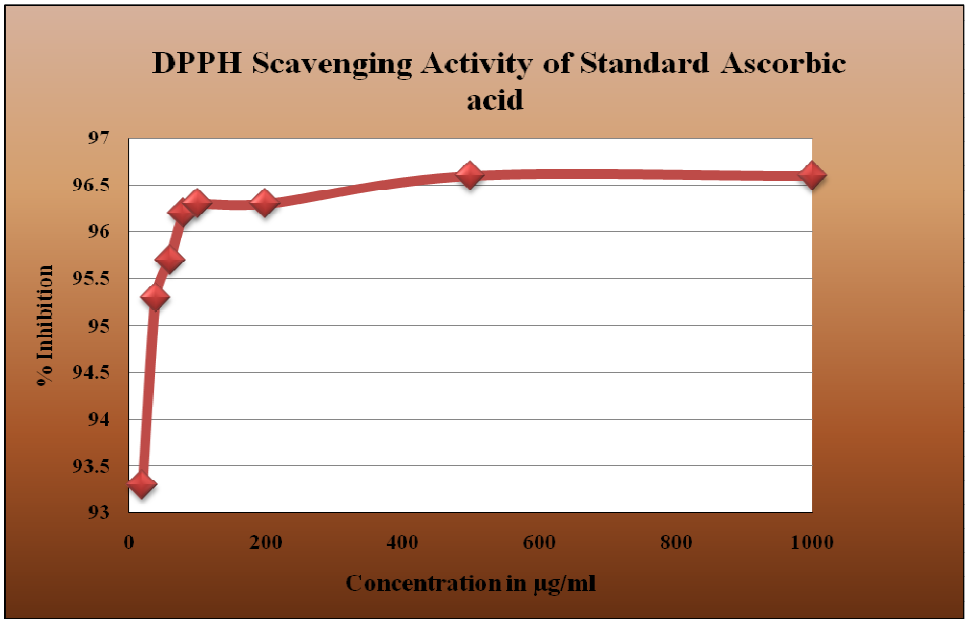

Fig. 1: DPPH scavenging activity of Standard Ascorbic acid.

IC_{50} value of Standard Ascorbic acid less than 20 µg/ml.

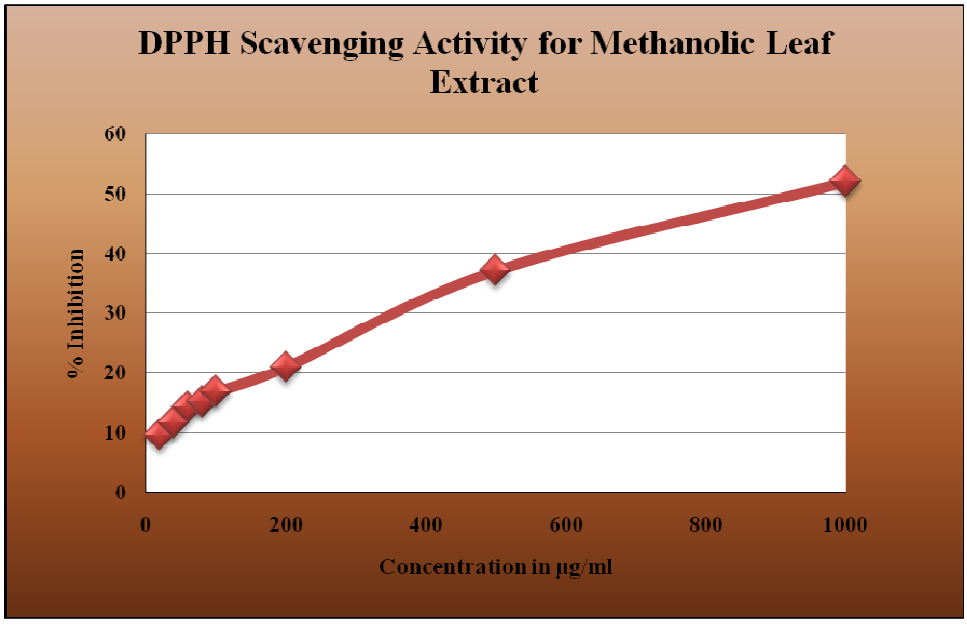

Fig. 2: DPPH scavenging activity of *Semecarpus anacardium* methanolic leaf extract.

IC_{50} Value of methanolic leaf extract of *Semecarpus anacardium*: 916.58 ± 2.14 µg/ml.

Table 3: Data of Nitric oxide scavenging activity of *Semecarpus anacardium* L. leaf extract.

Concentration Used (µg/ml)	Standard Ascorbic acid		Leaf Extract Sample	
	Ascorbic acid Absorbance Mean ± SD	Ascorbic acid % Inhibition Mean ± SD	Sample Absorbance Mean ± SD	Sample % Inhibition Mean ± SD
20	0.1114 ± 0.0001	35.0 ± 0.06	0.1124 ± 0.0002	34.4 ± 0.10
40	0.0850 ± 0.001	50.4 ± 0.06	0.0934 ± 0.003	45.5 ± 0.20
60	0.0846 ± 0.0001	50.7 ± 0.06	0.0843 ± 0.0002	50.8 ± 0.06
80	0.0772 ± 0.0001	55.0 ±0.06	0.0773 ± 0.0002	54.9 ± 0.06
100	0.0720 ± 0.0002	58.0 ±0.06	0.0717 ± 0.0002	58.1 ± 0.12
200	0.0690 ± 0.0001	59.7 ±0.06	0.0687 ± 0.0002	59.9 ± 0.15
500	0.0680 ±0.0002	60.3 ± 0.10	0.0679 ± 0.0002	60.4 ± 0.10
1000	0.0501 ± 0.0002	70.7 ± 0.12	0.0673 ± 0.0002	60.7 ± 0.06

Absorbance of Control: 0.1713 ± 0.002

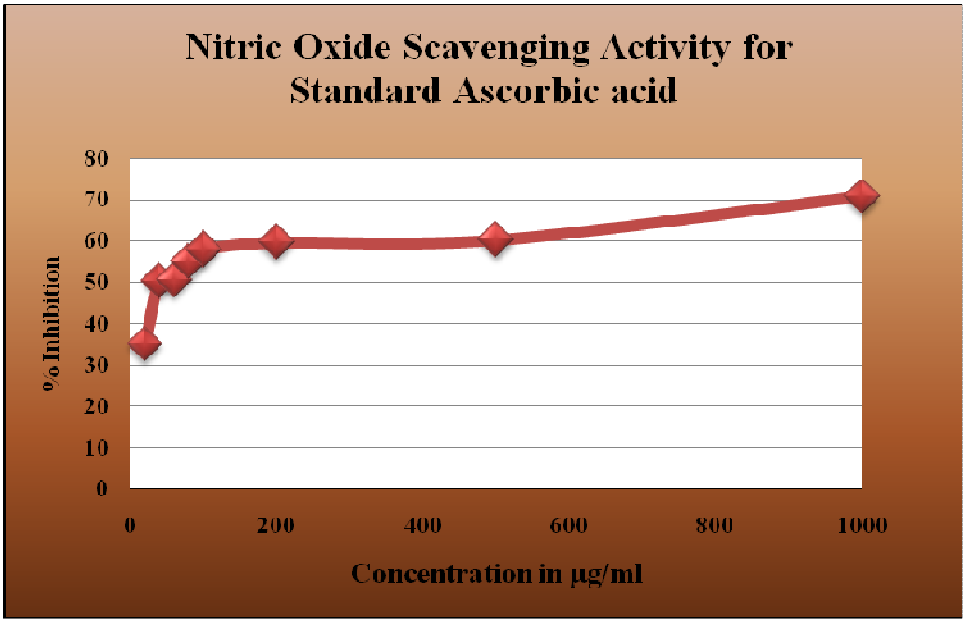

Fig. 3: Nitric oxide scavenging activity of Standard Ascorbic acid

IC_{50} Value of Standard Ascorbic acid : 36.81 ± 2.06 µg/ml.

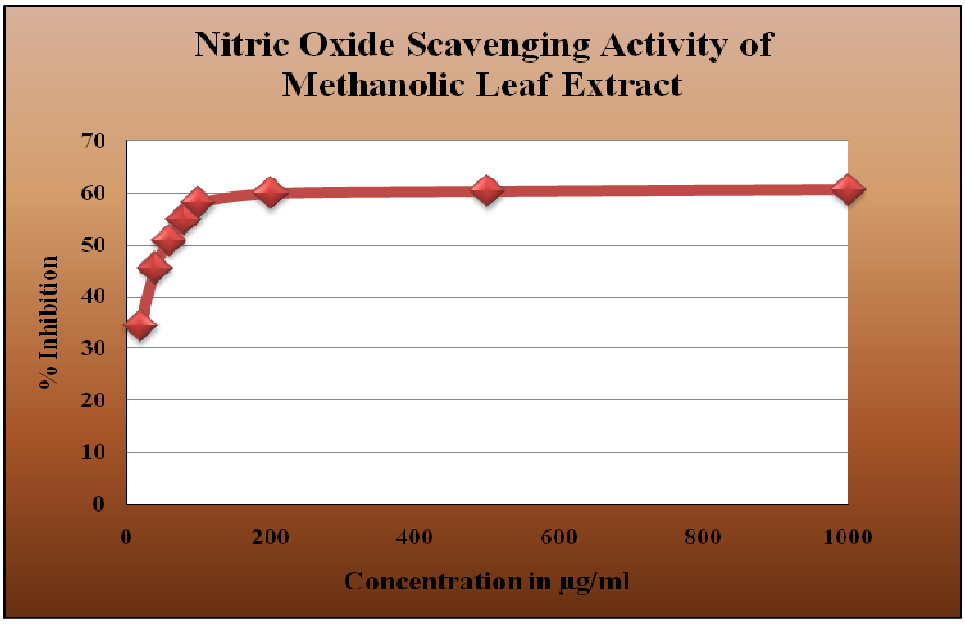

Fig. 4: Nitric oxide scavenging activity of *Semecarpus anacardium* methanolic leaf extract.

IC_{50} Value of methanolic leaf extract of *Semecarpus anacardium*: 51.33 ± 2.00 µg/ml.

DISCUSSION

Preliminary phytochemical studies revealed the presence of alkaloids, glycosides, tannins, flavanoids, steroids, phenols, proteins, hexose sugars, diterpenes, nonreducing polysaccharides, mucilages and gums and in the methanolic leaf extract of Semecarpus anacardium [8].

Earlier studies reported that the MBC values can either be the same or higher than the corresponding MIC values [24]which was proven in this study for *S. epidermidis,* the MIC was same as the MBC value (100 mg/ml). Higher MIC and MBC values were seen for *P.*acne at 800 mg/ml under anaerobic conditions. For *M. luteus,* MIC was observed at 100 mg/ml with MBC at 4 times the MIC value (400 mg/ml) while for MRSA, MIC was at 200 mg/ml with MBC value 2 times the MIC value (400 mg/ml). The yeast *M. furfur,* showed MIC and MFC both at 400 mg/ml. The MBC / MFC values which are obtained after plating various dilutions of the extracts are more reliable than the MIC values, obtained using turbidity as a measure of growth. Lower MIC and MBC values indicate higher efficacy [25]. Thus, the low MIC and MBC values exhibited by the leaf extract against *Staphylococcus epidermidis* is of potential importance in the health care delivery system, since it could be used as an alternative to orthodox antibiotics in the treatment of infectious diseases caused by these microbes, especially as they frequently develop resistance to known antibiotics [26].

The values of DPPH scavenging activity of methanolic extract are given in Table 2. From the analysis we can interpret that the effect of extract DPPH radicals increased with concentrations.

The measurement of the scavenging activity of DPPH radical allows one to determine exclusively the intrinsic ability of the antioxidant compound to donate hydrogen atoms or electrons to this reactive species in a homogenous system. The DPPH radical contains an odd electron which is responsible for the absorbance at 517 nm and also for the visible deep color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance. Higher reduction of DPPH is related to the high scavenging activity performed by particular sample.

Nitric oxide is a potent pleiotropic mediator of different physiological process and plays a vital role in smooth muscle relaxant, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical, important as an effectors molecule in different biological systems including neuronal messenger, vasodilatation and antimicrobial and anti-tumor activities. But over production of the radical is responsible for pathogenesis of different inflammatory diseases [27].

CONCLUSION

Bacterial infections can be treated with the *Semecarpus anacardium* leaf extract, since it exhibited favorable antibacterial and antifungal activity. The leaf of *Semecarpus anacardium* has never been evaluated for antibacterial activity against *Staphylococcus epidermidis*, *Micrococcus luteus,* MRSA, *Propionibacterium acnes* and *Malaassezia furfur* before. Also it is for the first time a detailed study has been undertaken to demonstrate that the methanolic *Semecarpus anacardium* leaf extract can effectively scavenge various reactive oxygen species or free radicals under in vitro conditions. This may be due to a number of stabilized oxidation products that it can form after oxidation or radical scavenging. Hence, this plant can be used to discover new bioactive natural products that may serve as lead in the development of new pharmaceuticals.

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