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PHYTOCHEMICAL SCREENING AND EVALUATION OF ANTIMICROBIAL ACTIVITY OF SARACA ASOCA FLOWER EXTRACT

Ravindran Jaganathan¹, Megala Parasuram², Rajeswari Ravindran³, Durga Devi Vijayaragavan^{4,*}

¹Pathology Unit, Lab Based Department, Universiti Kuala Lumpur Royal College of Medicine Perak (UniKL-RCMP), Ipoh, Perak, Malaysia.

²M.Sc. Biochemistry, Department of Biochemistry, Sacred Heart college (Autonomous), Tirupattur - 635 601, Tamil Nadu, India.

³Department of Medical Biochemistry, Dr. ALM PG Institute of Basic Medical Sciences, University of Madras, Taramani campus, Chennai-6000113, India.

^{4*} Assistant Professor, Department of Biochemistry, Sacred Heart college (Autonomous), Tirupattur - 635 601, Tamil Nadu, India.

Email: durgadevi@shcpt.edu

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Abstract:

Aim: The aim of the present study is to identify the presence of various phytochemicals that are present in the flower extract of *saraca asoca* and to evaluate its antimicrobial activity.

Methods: Qualitative and quantitative analysis of phytochemical constituents such as steroids, phenols, flavonoids, tannins, and saponins were carried out using standard protocols. Minerals, metals and trace elements of the sample were analyzed using Atomic Absorption Spectrophotometry. Antimicrobial activity of the extract was analyzed using clinical isolates of the microorganisms together with the standard strain.

Results: The results of the present study shows that phytochemicals such as tannins, flavonoids, saponins and coumarin were found to be present in methanolic extract. Among that coumarin was found to present in higher amount followed by flavonoids and Tannins. The methanolic flower extract was subjected to Atomic Absorption Spectroscopy to find the presence of minerals and trace elements. It was observed that minerals like zinc was found to be present in higher amount followed by Iron, and magnesium and the trace element like chromium was found to be absent. The methanolic extract was also found to possess potent antimicrobial activity against various pathogenic microorganisms.

Conclusion: The methanolic extract was found to have less toxic effects and it is shown to possess maximum inhibitory action against various infectious pathogens which might pave the way to treat infectious diseases caused by microorganisms.

Keywords: Methanolic extract, Microorganisms, Phytochemicals, *Saraca asoca*

1. Introduction

Phytochemicals are primary and secondary compounds that are occurring naturally in various medicinal plants, leaves, vegetables and they are found to exert defence mechanism to protect plants against various diseases [1]. Scientific evaluation of medicinal plants are important not only to the discovery of novel drugs but also it put forth to assess toxicity risks associated with the use of herbal preparations. Plant derived extracts contain numerous biologically active compounds, many of which have been shown to have antimicrobial properties [2]. Plant-derived medicines have been part of traditional healthcare in most parts of the world for thousands of years and there is increasing interest in plants as sources of agents to fight against microbial diseases [3].

Saraca asoca is an indigenous herbal drug belonging to the family *Caesalpinaceae* and it is distributed in evergreen forests of India up to an elevation of about 750 meters. It is a medium sized evergreen tree up to 9 m height with numerous branches [4]. It is cultivated in many gardens because of its decorative orange red flowers and evergreen beautiful foliage. *Saraca asoca* is reported to contain glycoside, flavonoids, tannins and saponins [5]. The *asoca* tree has many health benefits and has long been used in traditional Indian medicine as a key ingredient in various therapies and cures. It is used as protective drug for spasmogenic, oxytocic, uterotonic, anti-bacterial, anti-implantation, anti-tumour, antiprogestational, antiestrogenic activity against menorrhagia and anti-cancer. One of the uses of the *asoca* herb is in the treatment of menstrual disorders associated with excessive bleeding, congestion, pain, dysmenorrhoea, abdominal pain, uterine spasms and miscarriage [5, 6, 7]. It also has a nourishing effect on the circulatory system, thereby making it an effective remedy in arrhythmia and cardiac weakness [8]. The *asoca* herb also helps in encouraging urine flow and thus helps in treating conditions that cause painful urination. It also has specific analgesic properties and it is said to improve the complexion of skin [9]. Though various phytoconstituents have been reported earlier in leaves and bark of the plant but very few literature evidences are reported with the presence of phytoconstituents in flowers. The present study attempts to evaluate the phytoconstituents in flowers that can be useful for identification of the drug to treat certain diseases. However, the presence of gallic acid, an antioxidant molecule, has already been reported in *Saraca asoca* flower [10].

***Saraca asoca* flower: (Figure 1)**

Kingdom : Plantae

Division : Magnoliophyta

Class : Mgnoliopsida
Order : Fabales
Family : *Caesalpinaceae*
Genus : *Saraca*
Species : *asoca*



Figure 1. *Saraca asoca* Flower.

The flowers are regarded as medicinally important plant part and used as therapeutic agent in treatment of diabetes, cancer and hemorrhagic dysentery, bleeding piles, uterine infections and bacillary dysentery. Dried flower buds are reported to have antibacterial activity [11]. Aqueous suspension of *Saraca asoca* flower has antiulcer activity in albino rats [12]. *Saraca asoca* bark and flowers exhibit antitumour activity against DLA, S-180 and Ehrlich ascites carcinoma tumour cell lines [13]. Larvicidal activity has also been recorded [14]. Chemopreventive activity of flavonoid fraction of *Saraca asoca* is reported in skin carcinogenesis [15]. Flower extract is bitter in taste and bark has a stimulatory effect on endometrium and ovarian tissue and used in uterine fibrosis, menorrhagia, bleeding hemorrhoids and also as astringent.

A preliminary study showed the presence of catechols, sterols, tannins, glycosides, terpenoids, flavonoids, and saponins in flower extract. Chloroform, ethanol, methanol and water extracts of dry *Saraca asoca* flower powder were used to determine the qualitative and quantitative presence of phytochemicals [16]. Ethanol and water extract of flower of *Saraca asoca* exhibited antibacterial activity against *staphylococcus aureus*, *aeromonas liquefaciens*, *trichophyton candida alicans*, *klesiella pneumoniae* and *E. faecolls*. The treatment of breast cancer and other type of cancer involves use of *Saraca asoca* which have the ability to provide accessible, cost effective and also a relatively safe treatment in comparison to the standard method [11, 17, 18]. Flowers of this tree are used to treat cervical

literature is available on the antimicrobial effect of their extracts.

The present study will be helpful in understanding the activity of *Saraca asoca* flower extracts in prevention and treatment of cancer as well as to evaluate any adverse effects associated with health benefits. The aim of the present study is to identify the phytochemicals like Alkaloids, Sterols, Tannins, Glycosides, Terpenoids, Flavonoids, Saponins, Coumarins, Oxalate, Quinones, Phlobatanins and Phenols, present in the extracts of flower and to evaluate its antimicrobial activity.

2. Materials and Methods

2.1 Plant Materials

The plant was collected from Pondicherry, Tamilnadu, India, in the month of May, and authenticated by Dr. Pannerselvam, Dept. of Botany, Annamalai University.

2.2 Chemicals

Merck, Hi-media, SRL, and Analytical grade chemicals/reagents were used in this study.

2.3 Instruments Used

Laminar airflow (Clean air systems), Deep freezer (Cryo Scientific), Micro pipettes (eppendorf), Shaker (Scientific engineering corporation), Water bath (Hitech), Lyophiliser (Lark), Centifuge (Eppendorf), Rotary evaporator (BUCHI Rotavapor R-200), UV-spectrophotometry, Atomic absorption spectrometry.

2.4 Sterilization

The glassware used in this study was sterilized by dry-heat sterilization (hot air oven) at 180 °C for 30 mins. The broth was sterilized by moist heat sterilization in an autoclave at 121 °C for 15 mins at 15 psi. Similar type of sterilization and cleaning methods were followed throughout the study.

2.5 Preparation of plant extract

The flowers of *Saraca asoca* were collected from Pondicherry during the month of May. Flower samples were washed with water and air dried at room temperature for 3 days, then oven dried at 40° C to remove the residual moisture. The dried flower were pulverized and stored in air tight container for future use. Two different solvents methanol and water were used for preliminary phytochemical screening.

About 5 g of dry powder was extracted with methanol at room temperature using soxhlet apparatus for 8 hours and the extraction was continued until the liquid was clear. The extracts were then filtered and concentrated to a dry mass

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under vacuum. The filtrate was treated with water to get aqueous extraction and the extracts were freeze-dried and lyophilized to powder and the percent extract yield (%) was calculated. The samples were stored in air-tight containers at 4° C for further use.

2.6 Phytochemical analysis

The presence of important phytochemical constituents such as steroids, phenols, flavonoids, tannins, and saponins was assessed using standard techniques.

2.6.1 Test for Steroids

2.6.1.1 Salkowski Test

To two ml of methanolic extract two ml of chloroform and two ml of H₂SO₄ is added. A red colour layer is appeared. This shows the presence of steroids.

2.6.2 Test for Terpenoids

Take two ml of methanolic extract add two ml of chloroform and allowed to stand until it become dry. After that water and two ml of Conc. HCl is added. Grey colour is appeared .This shows the presence of terpenoids.

2.6.3 Test for Saponins

2.6.3.1 Foam Test

To two ml of plant extract add five ml of water and shake vigorously and the formation of foam indicates the presence of saponins.

2.6.4 Test for Tannins

Take two ml of plant sample and add two ml of ferric chloride solution a blue green (or) black colour is developed. This shows the presence of tannins.

2.6.5 Test for Flavonoids

2.6.5.1 Shinoda Test

To two ml of sample, add few magnesium turnings followed by two ml of Conc. HCl dropwise and pink colour is developed. This shows the presence of flavonoids.

2.6.5.2 Alkaline Reagent Test

(i) Wagner's test:

To a few ml of filtrate, few drops of Wagner's reagent were added by the side of the test tube. A reddish-brown precipitate confirmed the test as positive.

Wagner's reagent: Iodine (1.27 g) and potassium iodide (2 g) were dissolved in 5 ml of water and made up to 100 ml with distilled water.

(ii) Hager's test:

To few ml of the filtrate, 1 or 2 ml of Hager's reagent (saturated aqueous solution of picric acid) was added. A prominent yellow precipitate indicated the test as positive.

2.6.6 Test for Glycosides

2.6.6.1 Keller Killani Test

Two ml of flower extract is treated with two ml of acetic acid and two ml of 2% ferric chloride and mixed thoroughly and this mixture was poured into another tube containing two ml of sulphuric acid. A brown colour is appeared. This shows the presence of Glycosides.

2.6.7 Test for Coumarins

Two ml of sample is treated with two ml of ethanol. To this add two ml of 2% alkaline NaOH. A dark yellow colour is obtained. This indicates the presence of coumarins.

2.6.8 Test for Quinones

To 1 ml of the extract, 1 ml of concentrated sulphuric acid was added. Formation of red colour shows the presence of Quinones.

2.6.9 Test for Oxalates

Two ml of flower sample is added with five ml of ethanolic Hcl (1:1). A greenish black colour is formed. This shows the presence of oxalates.

2.6.10 Test for Phlobatannins

Take two ml of sample and one ml of Hcl are added and boiled. A red colour precipitate is not developed. This shows the absence of phlobatannins.

2.6.11 Test for Phenols

Take two ml of flower extract and add five ml of water and two ml of ferric chloride solution. Presence of dark green colour indicates the presence of phenol.

2.7 Quantification of Phytochemicals

2.7.1 Estimation of Tannin content: Weigh 5 gm of sample, dissolved in 50 ml of distilled water. From this 1 ml of sample is pipetted out and used for the further test. To 1 ml of sample, add 1 ml of folin reagent, 1ml of sodium

carbonate and 8ml of distilled water and stabilized for 30 minutes at room temperature. A blue colour is developed and read at 760 nm using UV visible spectrometer.

2.7.2 Estimation of Flavonoids

Weigh 5 gm of plant sample dissolved in 50 ml of distilled water and 1 ml of sample is pipetted out for further test. To 1 ml of sample add 1ml of acetic acid, 2 ml of pyridine solution and 1ml of albumin chloride and 6 ml of 80% methanol (8 ml of methanol + 2 ml water) and finally incubated at room temperature for 30 minutes. The colour developed is read at 420 nm using UV visible spectrometer.

2.7.3 Estimation of Coumarin

Weigh 5 gm of plant sample dissolved in 50 ml of distilled water. From this 1ml of sample is pipetted out for the further test.

2.7.4 Estimation of Elements

The Minerals, metals and trace elements of the sample were analyzed using Atomic Absorption Spectrophotometry (Perkin Elmer 2380). Quantification of elements such as magnesium, iron, zinc, and chromium was carried out.

2.7.4.1 Test for Iron: Five mg of sample was weighed and transferred into 100 ml of conical flask. Then add 5 ml of Conc. Nitric acid and digested and makeup to 100 ml using deionized water. Then the sample was mixed well and filtered. The filtrate was used for further test.

Atomic Absorption Spectroscopy Condition:

Mode : absorption
Primary wavelength : 248.3 nm
Fuel flow rate : 0.8 to 1.0 L/min
Gas : air/acetylene

Finally scan the standard solution and sample solution in Atomic Absorption Spectroscopy (AAS).

2.7.4.2 Test for Magnesium

Five gm of plant sample was weighed and transferred into 100ml of volumetric flask. Add 5ml of nitric acid and digested. The solution was made up with deionized water. Finally filter the solution through 0.45 micron membrane filter. From this pipetted out 1ml and dissolved in 100ml of deionized water.

Atomic Absorption Spectroscopy Condition:

Mode : absorption
Primary wavelength : 285.2nm

Fuel flow rate : 0.9 to 1.2 L/min

Gas : air/acetylene

2.7.4.3 Test for Zinc

Five mg of plant sample was weighed and transferred into 100 ml volumetric flask. Add 5 ml of Conc. Nitric acid and digest the sample. Then the solution was makeup with deionized water. The sample was filtered using 0.45 micron Membrane filter.

Atomic Absorption Spectroscopy Condition:

Mode : absorption

Primary wavelength : 213.9

Fuel flow rate : 0.9 to 1.2 L/min

Gas : air/acetylene

Finally, the standard solution and sample solution was scanned in atomic absorption spectroscopy (AAS).

2.7.4.4 Test for Chromium

Five gram of plant sample was weighed and transferred into 100ml of standard flask. Add 5 ml of Conc. Nitric acid was added and digest for 20-30 minutes and makeup with deionized water. Finally filter the solution through 0.45 micron membrane filter.

Atomic Absorption Spectroscopy Condition:

Mode : absorption

Primary wavelength : 357.9 nm

Flow : 4.0 to 4.4 L/min

Gas : nitrous oxide/acetylene gas

Finally scan the standard solution and sample solution using atomic absorption spectroscopy (AAS).

2.8 Determination of *In vitro* Antimicrobial activity

2.8.1 Test Organisms

Clinical isolates of the microorganisms were used along with the standard strain. Six pathogenic strains like *Aeromonas liquefaciens* MTCC2645 (B4), *Bacillus subtilis* NCIM 2920(B2), *Enterococcus faecalis* MTCC439 (B1), *Klebsiella pneumonia* NCIM2883 (B3), *Candida albicans* MTCC1637 (F1), *Trichophyton rubrum* MTCC3272 (F2) were obtained from Microbial Type Culture Collection (MTCC) Chandigarh and National Collection of Industrial

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Microorganisms (NCIM), Pune, India were maintained on nutrient and Potato Dextrose Agar slants at refrigerated condition and used in the present study.

2.8.2 Drug preparation

The methanolic extract of the flower part of the plant *Saraca asoca* was used in the present study. The solvent free extract of the sample was prepared at the concentration of 0.1 g/ml in 10% DMSO and stored in a sterile vial for further investigation.

2.8.3 Mc Farland Method

2.8.3.1 Preparation of Mc Farland Standard

Sulphuric acid	-	995 ml
Barium chloride	-	5 ml

Mc Farland, standard was prepared before plating the culture on Muller Hinton Agar medium. The test organism was adjusted with Mc Farland standard to check the anti microbial activity of the plant extracts. When the density of the organisms in MHB is high, saline was added to reduce the density of the micro organisms. After comparison, the broth was used to test the anti microbial activity.

2.8.3.2 Muller Hinton Broth (MHB)

Meat infusion	-	0.2 g
Casein hydrolysate	-	1.75 g
Starch	-	0.15 g
Distilled water	-	100 ml
pH	-	7.4

Five ml of the above broth was transferred to each test tube and sterilized by autoclaving at 121° C for 15 mts.

After sterilization the pathogenic strains like *Aeromonas liquefaciens* MTCC2645 (B4), *Bacillus subtilis* NCIM 2920 (B2), *Enterococcus faecalis* MTCC439 (B1), *Klebsiella pneumonia* NCIM2883 (B3), *Candida albicans* MTCC1637 (F1) *Trichophyton rubrum* MTCC3272 (F2) were inoculated into Muller-Hinton Broth (MHB) and Potato Dextrose Broth (PDB) under aseptic condition and kept in a shaker for 18 h. Before inoculation this broth were compared with Mc Farland standard and adjusted using saline.

2.8.3.3 Muller Hinton Agar (MHA)

Meat infusion	-	0.2 g
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Casein hydrolysate	-	1.75 g
Starch	-	0.15 g
Agar	-	2 g
Distilled water	-	100 ml
pH	-	7.3

Microbial strains were tested for their sensitivity by disc diffusion method. The test sample was prepared in DMSO. This method was used to evaluate in vitro antibacterial and antifungal activity of the test sample against certain human pathogenic microorganisms on Muller Hinton Agar (MHA). A sterile cotton swab was used to incubate the microbial suspension on the surface of agar plate rotating the plate at every 600 C to ensure homogeneous growth. 15 µL & 25 µL of the flower extract were loaded in each disc separately. The test sample loaded discs (6 mm diameter) were loaded in each disc by a sterilized forceps. One separate dish was used for control study by taking sterile triple distilled water (without test sample). The plates were incubated and the zone of inhibition was measured with rule/HiAntibioticZoneScale-C. The assays were performed in triplicate and the average values are presented. Methicillin – 10 mcg (for bacteria) was used as positive control. The assessment of antimicrobial activity was based on measurement of inhibition zones formed around the disc. The plates were incubated for 24 h at 37 °C and the diameter of the inhibition zones was recorded. Three independent trials were conducted for each extract.

3. Results and Discussion

3.1 Preliminary phytochemical analysis of the flowers of *Saraca asoca*

Saraca asoca was known to possess many medicinal values. This study was carried out to evaluate the properties exhibited by the flowers of *Saraca asoca*. The flower was subjected to extraction with methanol and water and the yield was tabulated (Table 1). It was observed that aqueous extract residue was found to be present in higher amount when compared to methanolic residue. It was known that most of the plant constituents are extracted in methanolic extract and used for further study.

Table 1. Amount of Residues Obtained.

S. No	Type of Extract	Residue/5 g of Sample (in gm)
1	Methanol	1
2	Water	1.8

Primarily the phytochemical screening of methanolic extract of the flower of *Saraca asoca* was investigated and the results were tabulated (Table 2). It was observed that most of the phytochemicals like tannins, flavonoids, saponins, and coumarins were found to be present.

Among those coumarins was found to be present in higher amount followed by flavonoids and tannins. This report was supported by [10, 20, 21] who reported that *Saraca asoca* contain glycoside, flavonoids, tannins, and saponins.

Table 2. Qualitative Analysis of Phytochemicals in methanolic extract.

S. No	Phytochemicals	Result
1	Steroids	-
2	Terpenoids	-
3	Saponins	+
4	Tannins	+
5	Flavonoids	
	a. Shinode test	-
	b. Alkaline reagent	+
6	Glycosides Keller killani test	-
7	Coumarins	+
8	Quinones	-
9	Oxalate	-
10	Phenols	-

(-) – “Absence”

(+) – “Presence”

The flower extract was subjected to Atomic Absorption Spectroscopy to find the presence of minerals and trace elements. It was observed that minerals like zinc was found to be present in higher amount followed by iron, and magnesium and the trace element like chromium was found to be absent (Table 3). From this it is evident that the flower extract does not contain any toxic substance and can be consumed orally. Since, this is the first report on mineral analysis there is no evidence to support the result.

Table 3. Quantitative Analysis of Phytochemicals in methanolic extract.

S. No	Phytochemical Present	in %
1	Coumarin	74
2	Flavonoid	70
3	Tannins	34

3.2 Antimicrobial activity

The antibacterial activity of the methanolic extract of the flower of *Saraca asoca* was examined against various pathogenic microorganisms like *Aeromonas liquefaciens* MTCC2645 (B4), *Bacillus subtilis* NCIM 2920 (B2), *Enterococcus faecalis* MTCC439 (B1), *Klebsiella pneumonia* NCIM2883 (B3), *Candida albicans* MTCC1637 (F1) *Trichophyton rubrum* MTCC3272 (F2) by disc diffusion method. It was observed that *B. subtilis* and *A. liquefaciens* has exhibited maximum zone of inhibition followed by *K. pneumoniae* and *E. faecalis*. Among the two concentrations (15 μ L and 25 μ L/disc) tested the higher concentration of the sample got greater sensitivity than the lower concentration (15 μ L of flower extract) against all the tested pathogenic bacteria (Figure 2 and Table 4). From this it is evident that *Saraca asoca* was found to be most active against certain Gram positive and Gram negative bacteria. The inhibition zone obtained is compared with standard Methicillin – 10 mcg which is used as positive control. The flower extracts of *Saraca asoca* has exhibited antibacterial activity against the above mentioned pathogenic organisms [9, 19, 20].



E.faecalis (B1)



B.Subtilis (B2)



Klebsiella pneumoniae (B3)



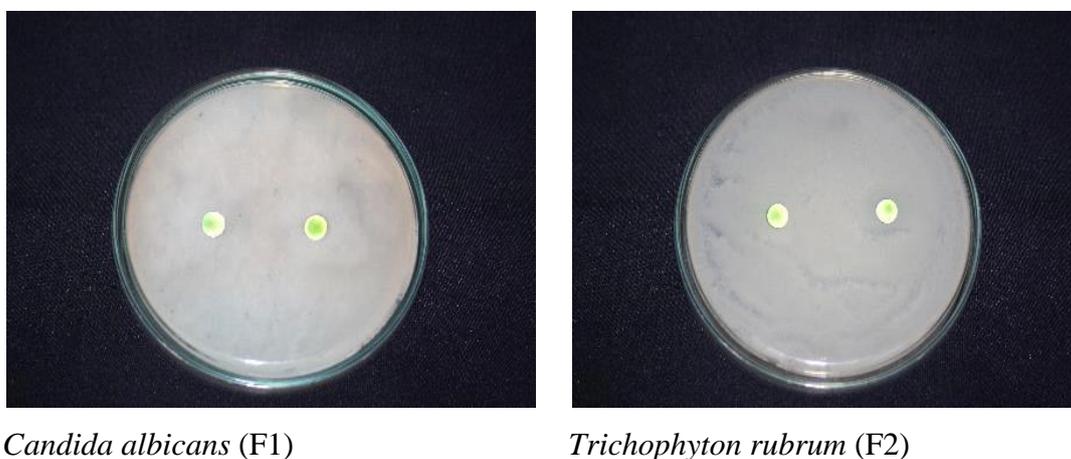
Aeromonas liquefaciens (B4)

Figure 2. Antibacterial activity of flower extract of *Saraca asoca*.

Table 4. Antibacterial activity of methanolic flower extract of *Saraca asoca*.

S. No	Test Organisms	Zone of inhibition (in mm)	
		15 μ L	25 μ L
1	<i>A. liquefaciens</i>	11	14
2	<i>E. faecalis B1</i>	10	12
3	<i>K. pneumoniae B3</i>	11	13
4	<i>B. subtilis B2</i>	14	14
5	<i>Candida albicans F1</i>	0	0
6	<i>Trichophyton rubrum F2</i>	0	0

In case of antifungal activity, the flower extract does not exhibit any potential effect against *Candida albicans* MTCC1637 (F1) and *Trichophyton rubrum* MTCC3272 (F2) tested. It has been reported that bark extract of *Saraca asoca* is inactive against fungal pathogens [20] (Figure 3).

**Figure 3. Antifungal activity of flower extract of *Saraca asoca*.**

The present study confirms the antimicrobial activity of flower extract of *Saraca asoca*. The extracts were found effective against certain Gram positive and Gram negative bacteria. Unfortunately, the fungal strain did not show the activity while bacterial strains showed good activity against test sample in both concentrations. The concentration dependent manner plays a very important role in the antimicrobial activity. Further work on extracts of the same plant is necessary to establish its exact antimicrobial activity.

4. Conclusion

Saraca asoca is highly accepted as universal solution in the ayurvedic medicine. It is age old and consistent source of medicine. It is used in many pharmacological activities like anti cancer, antimenorrhagic, anti, oxytoxic, antimicrobial activity and have extend uses in ayurveda, unani and homeopathy. This study provides important data on the acute and sub-acute oral toxicity profile of *Saraca asoca*. It can be concluded that methanolic plant extracts give the maximum inhibition against several pathogenic bacterial and fungal species, and in parallel the methanolic

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extracts was reported to have antibacterial activity. Thus, it can be said that common plants have great potential as antimicrobial compounds. They can therefore be used for the treatment of several harmful infectious diseases caused by resistant microorganisms. Hence, plant based compounds could be useful in meeting the demand of lesser side effects of synthetic drugs during drug development.

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Corresponding Author:

Durga Devi Vijayaragavan*,

Email: durgadevi@shcpt.edu