International Journal of Phytomedicine 4 (2012) 272-276
http://www.arjournals.org/index.php/ijpm/index

Original Research Article

ISSN: 0975-0185

Antimicrobial activity of *Saraca indica* against clinical pathogens

Hema TA[1]*, Parvathy J[1],Shiny M[1], and S.Sujatha[1]

*Corresponding author:

Hema TA

[1]Department of Microbiology, Malankara Catholic College, Mariagiri, Kaliakkavilai, 629 153, Tamil Nadu.

Abstract

The antimicrobial activity of flowers of the medicinal plant *Saraca indica* collected from from the regions of Ambalathara, Kerala, South India was checked against the clinical pathogens by well diffusion method. *Saraca i1ndica* showed highest antimicrobial activity on methanolic extracts. The phytochemical evaluation showed the presence of alkaloids, tannins, proteins and reducing sugars. With the help of column chromatography the methanolic extract was purified and highest antimicrobial activity was observed in the concentration 2:8. By thin layer chromatography the compounds are separated and the R_f value obtained was 0.6. The compound responsible for the antimicrobial activity was partially identified as alkaloids.

Keywords: *Saraca indica*, antimicrobial activity, alkaloids, Rf value.

Introduction

Traditional medical practices have been known for centuries in many parts of the world for the treatment of various human ailments. The use of antibiotics has revolutionized the treatment of various bacterial infections. However, their indiscriminate use has lead to an alarming increase in antibiotic resistance among microorganisms, thus necessitating the need for development of novel antimicrobials. Medicinal plants are relied upon by 80% of world's population, and in India, the use of medicinal plants as therapeutic agents remains an important component of the traditional system [1].

According to World Health Organization, medicinal plants are the best source to obtain a variety of new herbal drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to understand their properties, safety and efficacy. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency. Many plants have been used because of their antimicrobial traits, which are chiefly due to synthesized during secondary metabolism of the plant [2].

Approximately 25 percent of all prescription drugs are derived from trees, shrubs or herbs. Nature has bestowed our country with an enormous wealth of medicinal plants therefore India has often been refered to as the medicinal garden of the world [3]. Plants are still widely used for ethno medicine around the world and phytomedicines derived from plants have shown great promise in the treatment of intractable infectious diseases including those caused by opportunistic pathogens. Microorganisms have been developing resistance to many antibiotics due to the indiscriminate use of antimicrobial drugs, increasing clinical problems in the treatment of infections [4].

Plants contain numerous biologically active compounds, many of which have been shown to have antimicrobial properties. 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 microbial diseases [5]. Medicinal plants are rich source of metabolites that are potential sources of drugs and essential oils. Some of them are semi domesticated and mostly grow as weeds. The nutritive values of these plants are important as they act as component for human consumption. Nearly all cultures from ancient times until today have used plants as source of medicine. In many developing countries traditional medicine is still the mainstay of health care and most of the drugs come from plants. The wide geographical and climatic diversity of Assam provides a repository of valuable indigenous system of medicine, as their extracts in various forms are being used in traditional system of medicine for the treatment of human ailments particularly those caused by *Escherichia coli* present in the gut of human beings [6].

Antibiotic resistance has become a global concern in recent years. This problem is of great significance especially in developing countries because infectious diseases are one of the major causes of mortality in these countries [7]. Various plant species have been serving as the best natural source of drugs and medicines since the beginning of civilization. Most of the plant constitutes, particularly the secondary metabolites possess potent antibacterial and antifungal activity. Among the different plant derived secondary metabolites, alkaloids proved to be the most important group of compounds that showed wide range of antimicrobial activity [8].

Saraca indica belongs to the family Fabaceae. It occurs almost throughout India up to an altitude of 750 m, in the central and the eastern Himalayas and in the Khasi, Garo and Lushai hills, a small evergreen tree. It is found in India, China, Ceylon and Malaysia. It has become quite scarce in several localities and is reported to be threatened in North Eastern Region of India. The leaves are paripinnate and the leaflets 6-12, oblong and rigidly sub-coriaceous. The flowers are orange or orange-yellow, eventually turning vermillion, very fragrant, in dense axillary corymbs; the pods, flat, leathery, the seeds, 4-8, ellipsoid-oblong and compressed. Flowers of this tree are used to treat cervical adenitis, biliousness, syphilis, hyperdipsia, burning sensation, hemorrhagic dysentery, piles, scabies in children and inflammation. Scant literature is available on the antimicrobial effect of their extracts.

The present study focuses on to evaluate the antimicrobial activity and phytochemical analysis of the flowers of medicinal plant *Saraca indica* collected from the regions of Ambalathara, Kerala, South India used for the treatment of clinical infections caused by pathogenic microorganisms.

Methodology

Collection of plant materials

The medicinal plant *Saraca indica* free from diseases were collected from the regions of Ambalathara, Kerala, South India and identified. A voucher specimen was deposited in the St. Xavier College, Centre for Biodiversity and Biotechnology, Palayamkottai, Tamilnadu, India. The collected plant parts were removed, washed thoroughly with running tap water and again washed with sterile distilled water to remove dirt prior to drying process. The flowers of *Saraca indica* were shade dried at room temperature for a week to remove the moisture content and powdered using mixer grinder.

Preparation of plant extract

The air dried finely ground plant parts (1 gm) were taken separately in air tight bottles and 10 ml of different solvents (ethanol, methanol, acetone, chloroform and distilled water) were added and kept under dark. After two days, the contents were stirred and filtered using Whatmann no: 1 filter paper. The filtrate was collected and stored in sterile glass beakers for further study.

Collection of test organisms

Seven clinical microbial cultures namely *Staphylococcus aureus, Klebsiella* sp., *Enterococci* sp., *Escherichia coli, Pseudomonas* sp., *Serratia* sp., and *Proteus* sp., were used in this study was collected from Travancore Medical College, Kollam. All the strains were confirmed by cultural and biochemical studies. These organisms were cultured on nutrient agar at 37 ºC for 24 h and maintained in nutrient agar slants at 4ºC for further use.

Antimicrobial activity assay

The antimicrobial activity of selected medicinal plants against clinical pathogens was determined by using agar well diffusion method [9]. The Mueller Hinton Agar Medium was prepared and poured onto 100mm petriplates (25-30 ml/plate) still molten. After solidification, 24 hrs nutrient broth grown pathogenic cultures were swabbed on the molten medium using sterile cotton swabs. Wells of 6 mm diameter were punched over the agar plates using a sterile gel puncher. 50 µl of each extract were poured into the wells and the plates were incubated at 37ºC for 24 hrs. After incubation, the antimicrobial activity was assayed by measuring the diameter of the inhibition zone formed around the well. All the solvents under study were used as negative control and the antibiotic tetracycline, penicillin and chloramphenicol as used as positive control. All the experiments were performed in triplicates and mean of the triplicate values was calculated.

Phytochemical analysis

Phytochemical analysis for major phytoconstituents of the plant extract was performed as follows [10]

Detection of alkaloids (dragendroff's test)

Filtrates were treated with Dragendroff's reagent (Solution of Potassium Bismath Iodide). Formation of an orange precipitate indicates the presence of alkaloids.

Detection of glycosides

Extracts were treated with 1ml of glacial acetic acid and a few drops of ferric chloride. To this few drops of conc. sulphuric acid was carefully added. Formation of reddish brown colour at the junction of two layers and bluish green colour in the upper layer indicates the presence of glycosides.

Detection of saponins (foam test)

Extracts were shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

Detection of phenols (ferric chloride test)

Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Detection of tannins

To 0.5ml of plant extracts, 1ml of distilled water and 1-2 drops of ferric chloride solution was added. Blue colour was observed for gallic tannins and green black for catecholic tannins.

Detection of flavonoids (alkaline reagent test)

Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Detection of proteins and amino acids (xanthoproteic test)

The extracts were treated with few drops of conc. nitric acid. Formation of yellow colour indicates the presence of proteins.

Detection of resins

Extracts were treated with acetone. Small amount of water were added and shaken. Appearance of turbidity indicates the presence of resins.

Detection of reducing sugar

To 0.5 ml of plant extracts, 1 ml of distilled water and 5-8 drops of Fehling's solution was added and heated over water bath. Formation of brick red precipitate indicates the presence of reducing sugar.

Detection of phytosterols (salkowski`s test)

Extract were treated with chloroform and filtered. The filtrates were treated with few drops of concentrated sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

Biochemical analysis

Column chromatography

Column chromatography was performed by packing a glass column with silica gel (1:2). A column may be packed wet by pouring solvent adsorbent slurry into the tube or dried by filling it with dry adsorbent. One ml of plant extract is then dissolved with appropriate solvent and added carefully at the top of the column, so as not to disturb the packing. Fractions are developed by adding different concentration of the solvent (methanol: water) to the column packed with silica gel and collecting the fractions of eluent in separate eppendorf tubes, which was subjected to antimicrobial activity.

Thin layer chromatography

To determine the purity of collected samples by column chromatography, the active fractions were again subjected to TLC on silica gel plates (silica gel G 60; Merck). The Rf value of each macromolecule was noted using the formula:

$$Rf = \frac{\text{Distance travelled by the substance}}{\text{Distance travelled by the solvent}}$$

Results

Antimicrobial activity assay

Antimicrobial activities of five solvent extracts (ethanol, methanol, acetone, chloroform and distilled water) were tested against seven clinical pathogens such as *Staphylococcus aureus* (Pus), *Klebsiella* sp. (Sputum), *Escherichia coli* (Urine), *Pseudomonas* sp. (Pus), *Enterococci* sp. (Urine), *Serratia* sp. (Sputum) and *Proteus* sp. (Sputum). Among the five solvents tested, methanolic extracts of *Saraca indica* showed higher significant activity against the pathogenic organisms such as *Klebsiella* sp. (Sputum), *Escherichia coli* (Urine) and *Proteus* sp. (Sputum), followed by *Serratia* sp. (Sputum), *Entericocci* sp. (Urine), *Staphylococcus aureus* (Pus) and *Pseudomonas* sp. (Pus) (Table 1).

Phytochemical evaluation

Methanolic extracts of *Saraca indica* which showed highest antimicrobial activity exhibited for phytochemical evaluation. Phytochemical analysis of methanolic extracts revealed the presence of secondary metabolites such as alkaloids, tannins, proteins and reducing sugar.

Column chromatography

Methanolic extracts of *Saraca indica* was purified using column chromatography. Column chromatography was done with the help of stationary phase and mobile phase. The mobile phase was prepared in different concentration and the fractions (1ml) were collected. The eluents were subjected to antimicrobial activity and highest activity was obtained in 2:8 fractions against the organism *Klebsiella* sp., *Escherichia coli and Proteus* sp.

Thin layer chromatography

The fraction which showed highest activity in column chromatography was subjected to TLC. The R_f value of the separated compound was 0.6. The compound may be identified as alkaloids.

Discussion

Saraca indica is highly regarded as a universal panacea in the ayurvedic medicine. It is one of the universal plant having medicinal activities. This versatile plant is the source of various types of compounds. In the present scenario many plants are used to treat many diseases. *Saraca indica* is used in many pharmacological activities like anti cancer, anti menorrhagic; anti oxytoxic, antimicrobial and have extend uses in Ayurveda, unani and homeopathy. It has many uses like to treat infections, Central nervous system function, genitor-urinary functions. As the global scenario is changing towards the use of non toxic plant product having traditional medicinal use, development of modern drug from

Table 1 Antimicrobial activity of *Saraca indica* against clinical pathogens

S. no	Tested organism	Zone of inhibition (mm)				
		Metha-nol	Ac-etone	Chlor-oform	Eth-anol	Distilled water
1	*Klebsiella* sp	18	15	12	10	8
2	*Escherichia* coli	15	12	11	11	-
3	*Proteus* sp	14	13	-	-	-
4	*Serratia* sp	13	-	12	-	-
5	*Enterococci* sp	14	-	-	10	-
6	*Staphylococcus aureus*	12	11	8	-	-
7	*Pseudomonas* sp	13	10	8	6	-

Saraca indica should be emphasized for the control of various diseases [3].

In the present study, an attempt was made to find out suitable phytoremedy, which can effectively inhibit the growth of clinical pathogens. The plant extracts with effective antibacterial properties were also subjected to phytochemical analysis. Sainath et al. [5] reported that the *Saraca indica* is used in traditional medicine against different ailments like, gynaecological disorders, uterine fibroids, burns, diarrhoea. The water extracts of *Saraca indica* were found to be most active against bacteria as well as fungal pathogens. The methanolic extract of stem bark of *Saraca indica* exhibited significant inhibitory activity against bacteria used [11]. The antibacterial activity by disc diffusion method showed that the methanolic and water extracts of stem bark of *Saraca indica* were very effective against most of the bacteria tested and especially against *Bacillus* species and *Pseudomonas aeruginosa* [5]. Water soluble fractions of the flowers and buds of *Saraca indica* were reported to have significant inhibitory effect against *Shigella boydis* [12].

In this study the flowers of *Saraca indica* is used for determining the antimicrobial activity against the clinical pathogens and showed highest antimicrobial activity. The methanolic extracts of *Saraca indica* exhibit significant antimicrobial activity against *Klebsiella* sp, *Escherichia coli* and *Proteus* sp followed by *Serratia* sp, *Enterococci* sp, *Staphylococcus aureus* and *Pseudomonas* sp. Phytochemical screening of *Saraca indica* indicates the presence of alkaloids, tannins, proteins and reducing sugar. These results indicate the presence of various phytochemical in this plant.

According to Cibin et al.[13] reported that preliminary phytochemical screening revealed the presence of tannins, proteins, steroids, glycosides, carbohydrates, saponins, flavonoids in different extracts of the flower of *Saraca indica*. It has been observed that most active components present in the flowers are flavonoid, steroids, tannins and glycosides. These phytoconstituents may be responsible for various pharmacological actions of this plant part, like antibacterial, antiulcer, anticancer, larvicidal and chemo protective activities.

In the present study, TLC was performed against highly active methanolic extract from flowers of *Saraca indica*. The Rf value was obtained as 0.6 so that the results revealed the presence of alkaloids. Phytochemical analysis of 40 active extracts demonstrated the presence of common phytoconstituents like phenols (79.5%), epigallotannins or condensed salt tannins (77%), glycosides (49%), saponins (38%), flavonoids (28%) and alkaloids (25%). The presence of these compounds was also detected by thin layer chromatography (TLC). TLC analysis also differentiated between monomeric and dimeric forms of flavonoids. Their phytochemical analyses are in agreement with the reports of other workers [14].

In conclusion, methanolic extract showed higher antibacterial activity towards both Gram-positive and Gram-negative organisms, which supports the traditional use of this plant and paves a way for the development of novel drugs. Researches on the pharmacological properties of the plant extracts have several limits, due to unknown composition of all the components of the plant source investigated. All studies on this area need for other confirmations and require additional research. Thus, the present study reflects a hope for the development of novel drugs.

Acknowledgement

The authors are thankful to the Management of Malankara Catholic College, for providing all the needs throughout the study.

References

[1]. Arora DS, Kaur JK. Antibacterial activity of some Indian, medicinal plants. Journal of natural medicines. 2007; 61:313-319.Prusti A, Mishra SR, Sahoo S, Mishra SK. Antibacterial activity of some Indian medicinal plants. Ethnobotanical Leaflets. 2008; 12:227-230.

[2]. Pradhan P, Joseph L, Gupta V, Chulet R, Arya H, Verma R, Bajpai A. Journal of chemical and pharmaceutical research. 2009; 1(1): 62-71.

[3]. Duraipandiyan V, Muniappan A, Savarimuthu I. Antibacterial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. Complementary and alternative medicine. 2006; 10:6-35.

[4]. Sainath R, Prathiba J Malathi R. Antimicrobial properties of the stem bark of *Saraca indica* (Caesalpiniaceae). European Review for Medical and Pharmacological Sciences. 2009; 13: 371-374.

[5]. Unni BG, Borah A, Singh HR. Phytochemical and antibacterial study of North East India on Escherichia coli. Asian J. Exp. Sci. 2009; 23(1):103-108.

[6]. Solanki,R. Some medicinal plants with antibacterial activity. International journal of comprehensive pharmacy. 2010; 4(10):1-4.

[7]. Raman S, Sultana N, Anwar MN. Invitro Antimicrobial activity of Holarrifine-24ol isolated from the stem bark of Holarrhena antidysenterica. International journal of agriculture and biology. 2004; 4:698-700.

[8]. Bauer AW, Kirby WMM, Sherris JC and Truck M. Antibiotic susceptibility testing by standard single disk diffusion method. Am. J. Clin. Path. 1966;45:493-496.

[9]. Kepm W. Qualitative Organic Analysis: Spectrochemical Techniques. 2nd Edn., McGraw- Hill, London and New York, ISBN 0070841586. 1986;pp: 197.

[10]. Dabur R, Gupta A, Mandal TK, Singh DD, Bajpai V, Gurav AM, Lavekar GS. Antimicrobial activity of some Indian medicinal plants.Afr. J. Trad. Comp. Alt. Med. 2007; 4: 313-318.

[11]. Narang GD, Nayar S, Mcndiratta DK. Antibacterial activity of some indigenous drugs, J. Vet. Animal husb. Res. 1962; 6(1): 22-25.

[12]. Cibin TR, Gayathri DD, Abraham A. Chemoprevention of skin cancer by the flavonoid fraction of Saraca asoka. Phytotherapy Research . 2009; 24:666–672.

[13]. Cai L, Wu CD. Compounds of Syzygium aromaticum possessing growth inhibitory activity against oral pathogens. J of Nat. Prod. 1996; 59(9): 987–990.