Research Journal of
Phytochemistry

ISSN 1819-3471

Academic
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www.academicjournals.com

OPEN ACCESS Research Journal of Phytochemistry

ISSN 1819-3471
DOI: 10.3923/rjphyto.2021.30.40

Research Article
Safety Standards and Antimicrobial Activity of Root of *Salacia reticulata*

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Abstract

Background and Objective: The safety and efficacy of natural products mostly depend upon the standardization of medicinal plants. *Salacia reticulata* widely used traditionally, the hence present study was designed to develop a safety profile and antimicrobial activity of different extracts of *S. reticulate* root. **Materials and Methods:** Antimicrobial activity of different solvent extract of standardized *Salacia reticulata* evaluated. All extracts of *S. reticulata* were tested against gram-positive and gram-negative strains using zone of inhibition and minimum inhibitory concentrations. The *S. reticulata* was subjected for physiochemical standardization, phytochemical analysis, microbial analysis, heavy metal analysis and antibacterial activity. The antibacterial activity was tested against different types of microorganisms such as *Pseudomonas fluorescence*, *Proteus vulgaris*, *Staphylococcus epidermis*, *Vibrio cholerae*, *Pseudomonas aeruginosa*, *Salmonella aboni*, *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhi* and *Shigella sonnei*. **Results:** The quality control measures such as microbial load and heavy metal toxicity were performed and they were found in normal values compared to WHO Standards. The antibacterial activity was tested against pathogenic bacteria and found that four bacteria such as *P. vulgaris*, *S. epidermis*, *S. sonnei* and *B. subtilis* which produces a significant zone of inhibition for all the solvents (chloroform, methanol, petroleum ether and ethyl acetate). The maximum zone of inhibition obtained for *P. vulgaris* is 37, 16, 26 and 15 mm. **Conclusion:** In the present study, the developed standardization protocol for different extracts of *S. reticulata* and the standardized extracts of *S. reticulata* shows significant antibacterial activity.

Key words: *Salacia reticulata*, antimicrobial activity, safety profile, standardization, antibacterial activity

Citation: Uthirapathy, S., J. Ahamad and M.S.M. Ameen, 2021. Safety standards and antimicrobial activity of root of *Salacia reticulata*. Res. J. Phytochem., 15: 30-40.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Generally, food spoilage caused by microorganisms silent usually affects all types of food products and causes food waste and loss, even in developed countries. It has been estimated that the yearly losses of global food reach up to 40% due to various factors including decomposition by microorganisms[1]. Bacteria, yeast and moulds are the common types of microorganisms responsible for the spoilage of a food products[2]. Once these microorganisms reach food products, they grow by consuming the nutrients and produce metabolites that cause food wastage[3]. Foodborne disease is another unescapable food safety problem caused by the consumption of contaminated food products. Microorganisms are accessible naturally in the surrounding environment. Therefore, they can easily reach food during cultivation, collecting, slaughtering, processing and packaging[4]. These microorganisms can survive under adverse conditions used in food preservation such as low temperature, modified atmosphere packaging, vacuum packaging as well as resist conventional pasteurization[5]. Thus, there is a substantial concern among clients regarding the risk of using synthetic additives or preservatives for human health, which led to a decrease in the use of these chemicals in food preservation[6]. Therefore, new sustainable methodologies are essential to reduce the growth of pathogenic bacteria and prolong the shelf-life of natural products, without using any preservatives. Recently, many researchers explored the possible utilization of some herbal plant extracts as effective natural preservatives[7]. Traditionally, the plant crude extracts of different parts of medical plants, including leaves, stem, flower, roots, seeds and fruits were commonly used for treatments of some human diseases. Medicinal plants contain several bioactive constituents such as alkaloids, saponins, flavonoids, tannins and terpenoids, which possess antioxidant and antimicrobial properties[8]. The antimicrobial activities of some plant species have been widely investigated. For example, the crude extracts of cardamom, clove, cinnamon, ginger, mustard, garlic, basil, curry leaves, sage and other herbs reveal antimicrobial properties against a wide range of gram-positive and gram-negative bacteria[9].

Besides, Mau *et al.*[10] have been reported that the extracts from Chinese chives and cassia can effectively reduce the growth of *Escherichia coli* and other bacteria during the storage of meat, juices and milk. In a similar study, Doddanna *et al.*[11] examined the effect of some plant extracts on the growth of *Candida albicans*, the results indicated that the alcoholic extract of curry leaves effectively inhibit the growth of *C. albicans*. Moreover, Alwakeel[12] informed that thyme oil extract could decrease the growth of *C. albicans* and *Pseudomonas aeruginosa*.

Salacia reticulata Wight (Celastraceae) is a medicinal plant with a restricted distribution in Sri Lanka and India. It is commonly called 'Ponkoranti' due to its characteristic golden yellow coloured roots. In traditional Indian medicine, its aqueous infusion is used in the treatment of diabetes[13-15]. The major phytoconstituents of *S. reticulata* are anthocyanidins, catechins, phenolic acids, quinones and related triterpenoids[16,17]. Herbal plants are rich sources of bioactive molecules that can be used in drug synthesis and development. Mangiferin, salacinol and kotalanol have been reported to be potent α-glucosidase inhibitors that have been shown to inhibit increases in serum glucose levels. Mangiferin also inhibits aldose reductase activity, thereby delaying the onset or progression of diabetic complications[18,19]. The *S. reticulata* having several biological remedies including hepatoprotective[20], antidiabetic[20,21], hypolipidemic[21,22], gonorrhea, rheumatism, itching asthma, inflammation[23,24] and antimicrobial[24-26]. The understanding of the mechanism of antimicrobial action of herbal plants extracts is the first stage in the optimal utilization of these extracts as natural antimicrobial agents to prolong the shelf-life and preserve the food quality. With this objective, antimicrobial activity of different extracts of *S. reticulate* against ten common food pathogens and spoilage microorganism was examined and to understand the mechanism of action of *Salacia reticulate* root extract concerning the potential disruption in the membrane of microorganism and changes in cytoplasmic pH. In additionally the extract of *S. reticulate* was tested for quality control analysis such as microbial load, heavy metal toxicity and physiochemical analysis.

MATERIALS AND METHODS

Study area: The roots of the *Salacia reticulate* Wight (Family: Celastraceae) was collected in July, 2019 from Kollimalai, Tamil Nadu and was identified and authenticated by Dr. V. Chelladurai, M.Sc., PhD, (Retired Scientist), Research Officer-Botany, Central Council for Research in Ayurveda and Siddha, Govt. of India. Tirunelveli, Tamil Nadu, (the Voucher specimen No. PGP/Ph.Cog/118) has been deposited in the Department of Pharmacognosy, Center for advanced research in Indian system of medicine (CARISM), Sastra University, Thanjavur, Tamil Nadu, India.

Plant material and chemicals: Dried plant roots were air-dried for 2 weeks and ground into fine powdered form, by using a grinder, kept in plastic bags and subjected later to extraction. All the reagents such as HNO_3 and H_2O purchased from MERCK (Analytical Grade). De-ionized water will be used for all analytical work and all the glassware, polyethene bottles, pipette tips and others will be washed with1% HCl, rinsed with de-ionized water before preparing standards, reagents and samples.

Extraction of plant materials: Ten grams of air-dried powder was placed in 100 mL of each organic solvent (acetone, methanol, hexane, ethyl acetate and chloroform) in a conical flask, plugged with cotton and then kept on a rotary shaker at 190-220 rpm for 24 hrs. After 24 hrs, it was filtered through 8 layers of muslin cloth and centrifuged at 5000 × g for 15 min. The supernatant was collected and the solvent was evaporated to make the final volume one-fourth of the original volume, giving a concentration of 40 mg/0.1 mL. It was stored at 4°C in airtight bottles for further studies.

Standardization of *S. reticulate*: The standardization of *S. reticulata* root samples were performed as a method described in WHO guidelines[27]. The following parameters of standardization are performed as organoleptic characters, chemical constituents, loss on drying at 105°C, total ash, acid -insoluble ash and water-insoluble ash. Then extraction of acetone, chloroform, ethyl acetate, hexane, methanol and water, etc. was done. The individual standardization methods described below:

Identification of phytoconstituents: Phytochemical screening of different solvent extracts *S. reticulata* root samples were performed as per the method described by Farnsworth[28] and Ali *et al.*[29].

Loss on drying: The percentage of active chemical constituents in the crude drug is mentioned on an air-dried basis. Hence the moisture content of a drug should be determined and also be controlled. The moisture content of a drug should be minimized to prevent the decomposition of crude drugs either due to chemical changes or microbial contaminations.

About 1 g of the drug (without preliminary drying) is accurately weighed and placed in a tared evaporating dish. The moisture content was determined by heating a drug at 105°C in an oven to constant weighed.

Determination of total ash: About 2-4 g of the ground air-dried material, accurately weighed, in a previously ignited and tarred crucible. Spread the material in an even layer and ignite it by gradually increasing the heat to 500-600°C until it is white, indicating the absence of carbon. Cooled in a desiccator and weighed. If carbon-free ash cannot be obtained in this manner, cool the crucible and moisten the residue with about 2 mL of water or a saturated solution of ammonium nitrate. Dry on a water bath, then on a hot plate and ignite to constant weight. Allow the residue to cool in a suitable desiccator for 30 min and then weighed without delay. Calculate the content of total ash in mg g^{-1} of air-dried material.

Determination of acid-insoluble ash: To the crucible containing the total ash, add 25 mL of hydrochloric acid, cover with a watch glass and boil gently for 5 min. Rinse the watch glass with 5 mL of hot water and add this liquid to the crucible. Collect the insoluble matter on an ashless filter paper and wash it with hot water until the filtrate is neutral. Transfer the filter paper containing the insoluble matter to the original crucible, dry on a hotplate and ignite to constant weighed. Allow the residue to cool in a suitable desiccator for 30 min and then weighed without delay. Calculate the content of acid-insoluble ash in mg g^{-1} of air-dried material.

Determination of water-soluble ash: The total ash obtained was boiled for 5 min with 25 mL of distilled water and filtered through an ashless filter paper. The filter paper was ignited in a silica crucible, cooled and percentage of water-soluble ash was calculated.

Determination of sulfated ash: The silica crucible is heated to redness for 10 min and then allowed to cool in a desiccator and then weighed. The 1 g of substance is weighed into the crucible, ignited gently until the substance is thoroughly charred. Then the residue is moistened with 1 mL of sulphuric acid and then heated in an oven. It is allowed to cool and then weighed.

pH in 1% solution: About 1 g of the sample was taken in a 200 mL beaker. Then make up to 100 mL with water and kept in a boiling water bath for 15 min. It is cooled and measured by a pH meter.

Determination of microbial load: One gram of plant sample weighed and added to 99 mL of sterile distilled water for preparing the serial dilution. The samples in the flask were

kept in a mechanical shaker for a few minutes to obtain a uniform suspension of microorganisms. The dilution is 1:100 or 10^{-2}. From that 1 mL of the 10^{-2} dilution was transferred to 9 mL of sterilized distilled water. This is 1:1000. This procedure was repeated up to 10^{-6} dilution, 0.1 mL of serially diluted sample was inoculated to the sterile plate containing Nutrient agar, SS agar and Potato Dextrose Agar (PDA) medium by spread plate method. Nutrient agar and SS agar plates were incubated at 37°C for 24 hrs and PDA plates were incubated at room temperature for 3-5 days. Bacterial and fungal colonies were counted using the colony counter.

Determination of heavy metals

Sample collection: The samples cleaned and dried under shade. Then the samples dried in an oven at 40-50°C to obtain a constant weight. The dried samples then ground and powdered in an agate pestle and mortar. Samples labelled and stored in pre-cleaned polyethylene bottles for further analysis.

Digestion of samples (sample preparation): A Multiwave 3000 micro oven system (Perkin Elmer, USA) with 16 position Teflon vessels with capping are being used here. The digestion vessels are provided with controlled pressure, temperature and release valve. Before use, all Teflon vessels are soaked with 10% HNO_3. The system is initially programmed by giving a gradual rise of 20, 40 and 50% power for 5, 15 and 20 min respectively for the due warming up. The powder samples are being used without any further treatment for sample preparation. 0.5 g of sample is weighed into the Teflon vessels followed by a digestion mixture of, HNO_3 and H_2O_2 in the ratio of 3:1, according to the nature of the samples is being applied.

The resulting solution after microwave digestion is filtered through Whatman # 40 filter paper (if necessary) and diluted to 50 mL with de-ionized water. A sample blank containing only an acid mixture is prepared at the same time. The method of standard addition is generally adapted to calibrate the instrument before going for the observation of the samples.

Heavy metals analysis by Flame AAS/graphite furnace (Fe, Mn, Zn, Ni, Co, Pb, Hg and As): After calibrating the instrument with prepared working standard, the digests liquid sample's solution is subjected to analysis of Fe, Mn, Zn, Ni, Co, Pb, Hg and As by AAS flame/Graphite furnace with specific instrumental conditions as given by instruments manufacturer. Introduce the solution into the flame, record the reading, using the mean of the three reading and quantified the concentration of the metals in the given samples against the standard calibration curve obtained from Concentration vs. Absorbance of the prepared known concentration on the day of the analysis[29,30].

Antimicrobial activity by Kirby-Bauer method

Test microorganisms: The microbial strains are identified strains and were obtained from the Centre for Advanced Research in Indian System of Medicine (CARISM), Sastra University, Thanjavur, Tamilnadu, India. The bacterial strains used in the entire studies are *Pseudomonas aeruginosa*, *Pseudomonas fluorescence*, *Staphylococcus epidermis*, *Bacillus subtilis*, *Proteus vulgaris*, *Salmonella typhi*, *Salmonella aboni*, *Shigella soni*, *Vibrio cholerae* and *Escherichia coli*.

Antibacterial assay: A loop full of the strain was inoculated in 30 mL of nutrient broth in a conical flask and incubated on a rotary shaker for 24 hrs to activate the strain. Mueller Hinton Agar No. 2 was prepared for the study. The assay was performed using agar disc diffusion for aqueous extract and solvent extract. The media and the test bacterial cultures were poured into Petri dishes (Hi-Media). The test strain (0.2 mL) was inoculated by swabbing into the media (inoculum size 10^8 cells mL^{-1}) when the temperature reached 40-4°C. Care was taken to ensure proper homogenization. The experiment was performed under strict aseptic conditions. For the Agar disk diffusion method, the test extract (0.1 mL) was introduced onto the disk (0.7 cm) (Whatman No. 2) and then allowed to dry. Thus the disk was completely saturated with the test extract. Then the disk was introduced onto the upper layer of the medium with the bacteria. The plates were incubated overnight at 37°C. Microbial growth was determined by measuring the diameter of the zone of inhibition. Chloramphenicol and Tetracycline were used as standard drug. Similarly, Methanol and distilled water were used as control. The control and Standard activity were deducted from the test and the result obtained was plotted.

RESULTS AND DISCUSSION

The organoleptic characters of root extract and average physicochemical parameters of the roots of raw material of *S. reticulata* coarse powder are tabulated in Table 1. This will aid in the physical and solubility identification of the root extract. Dried powder of *S. reticulata* root was continuously refluxed with different solvents such as methanol, chloroform, acetone, ethyl acetate, hexane and water separately at 40-800°C for 3 hrs using soxhlet apparatus. The solvent extract was then stored in air-tight

Table 1: Organoleptic characters of *S. reticulata*

Character	Observation
Colour	Straw yellow colour powder
Odour	Characteristic odour
Taste	Pleasant aromatic taste

Table 2: Extractive values of *S. reticulata* in different solvents

Solvent used	Yield (%)	Colour of the residue
Acetone	1.64	Light yellow
Chloroform	1.64	Yellow
Ethyl acetate	1.50	Yellow
Hexane	6.18	Light yellow
Methanol	3.53	Brownish-yellow
Water	14.57	Brown

containers at 40°C for further use. The different extractive values including yield and colour of the residue are presented in Table 2. The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in the estimation of specific constituents soluble in a particular solvent. The alcohol soluble extractive value of *S. reticulata* roots is 3.53%, compared with Bruce *et al.*[31], which reported the alcohol-soluble extractive value of 4.40%. The water-soluble extractive value of *S. reticulata* root is 14.57% as compared with Bruce *et al.*[31], which reported the water-soluble extractive value of (3.40%), This suggests that the use of alcohol as an extractive solvent is a better choice for the polar metabolites present in the root extracts. The qualitative phytochemical analysis of *S. reticulata* root extract reveals the presence of tannins, glycosides, carbohydrates, alkaloids, phenols and flavonoids. When compared with Bruce *et al.*[31], reported that the phytochemical analysis contains high amounts of alkaloids, tannins and flavonoids and moderate amounts of carbohydrates, glycosides and terpenoids with low concentrations. Estimation of different extractive values determines the amount of the active constituents in a given amount of plant material when extracted with a particular solvent. The extractions of any crude drug with a particular solvent yield a solution containing different phytoconstituents. The compositions of these phytoconstituents depend upon the nature of the drug and the solvent used. It also indicates whether the crude drug is exhausted or not[31].

Phytochemical screening: The present study showed that the roots of *S. reticulate* have pharmacologically important phytoconstituents such as alkaloids, carbohydrates, glycosides, tannins, steroids, flavonoids and phenolic compounds. The preliminary phytochemical screening for various functions group is tabulated in Table 3. The qualitative phytochemical test in *S. reticulata* their antioxidant and anti-inflammatory properties[35,36]. The phenolic unit can be found dimerized or further polymerized, creating a new class of polyphenol, for example, ellagic acid roots extracts revealed that flavonoids, phenolic and tannins are the much appreciable constituents in methanol extract. Tannins are much high level in water extract, to reduce the risk of coronary heart diseaseand have anticancer activities[32]. Flavonoids and phenols are excellent sources of natural antioxidants. Alkaloids are a group of naturally occurring chemical compounds that contain mostly basic nitrogen atoms. Roots *S. reticulata* contains alkaloids, which showed potential antimicrobial properties. Alkaloids have also pharmacological effects and are used as local anaesthetic and stimulants. Cocaine, caffeine, nicotine, analgesic morphine, the anti-bacterial berberine and antimalarial drug quinine are all Alkaloids[32,33]. Flavonoids are strong antioxidants and are effective antibacterial substances *in vitro* against a large number of microorganisms by inhibition of the membrane-bound enzymes[34]. They also showed substantial anticarcinogenic and antimutagenic activities due to is a dimer of gallic acid and forms the class of ellagitannins or catechin and a gallocatechin can combine to form the red compound of flavonoids. Tannins are a group of polymeric phenolic compounds and cause local tumours[37]. They can inactivate and kill microorganisms. They used in the treatment of varicose ulcers, hemorrhoids, minor burns, frostbite as well as inflammation of gums, in recent years, these compounds have demonstrated their antiviral diseases including AIDS[38]. The root of *S. reticulate* including alkaloids, flavonoids, tannin, steroids and phenolic groups are found in the sample. The results of the phytochemical analyses showed that flavonoids were more in quantity than the other phytochemicals tested. Flavonoids, according to the research by may modify allergens, viruses and carcinogens thereby acting as a biological response modifier and acting on bacteria by inhibiting its protein synthesis. Some *in vitro* studies exhibited that flavonoids could also possess anti-microbial[39], anti-allergic and anti-inflammatory properties[40]. Phytochemicals such as alkaloids, glycosides and carbohydrates were found to be moderate in concentration. It stimulates lean body mass and also plays vital roles in the prevention of bone loss in elderly men[22]. Phytochemicals such as tannins, flavonoids and tannins were found to be relatively high in concentration. Tannins could be an effective ameliorative agent of the kidney[41]. Tannins and flavonoids have also shown to be potential anti-oxidant, anti-bacterial and anti-viral agents[41]. Estimation of extractive values determines the amount of the active constituents in a given amount of plant material when extracted with a

Table 3: Results of phytochemical screening of *S. reticulata* roots

	Chemical constituents					
Solvent used	Alkaloid	Carbohydrate	Flavonoids	Glycosides	Phenol	Tannins
Acetone	-	-	++	-	+++	+
Chloroform	-	-	-	-	-	-
Ethyl acetate	-	-	-	+	-	-
Hexane	-	-	-	-	-	-
Methanol	+	+	+++	-	++	+++
Water	-	+	+++	-	++	++

+++: Appreciable amount, ++: Average amount, +: Trace amount, -: Absent

Table 4: Physico-chemical characters of *S. reticulata*

Parameter	Values in (%)
Loss on drying at 105°C	5.60
Total ash	1.80
Acid insoluble ash	0.92
Water-insoluble ash	1.80
Sulphated ash	2.06
Moisture content	0.034

particular solvent. The extractions of any crude drug with a particular solvent yield a solution containing different phytoconstituents. The compositions of these phytoconstituents depend upon the nature of the drug and the solvent used. It also indicates whether the crude drug is exhausted or not[42].

Physicochemical standards: The physicochemical analysis of *S. reticulata* powdered roots reveals the parameters such as moisture content, total ash values, acid insoluble ash values, water-soluble ash values, alcohol soluble extractive value and water-soluble extractive values presented in Table 4. Total ash value is (1.80%) as compared with Bruce *et al.*[31], which reveals the total ash value (3.85%), which can also be used to detect foreign organic matter and adulteration of sand or earth. Acid insoluble ash value is (0.92%) as compared with Bruce *et al.*[31] which reported the acid-insoluble value of (1.0%) and also compared to that of *Atropa belladonna* L. leaves which are not more than 4%, water-soluble ash value is (1.8%) as compared with Bruce *et al.*[31], reveals the water-soluble ash value of (0.50%). The water-soluble ash is used to estimate the amount of inorganic compound present in drugs. In the present study roots of *S. reticulata* was thoroughly investigated for their physicochemical characters to analyze their quality, purity and standardization for their safe use. The generated information of the present study provide data that is helpful in the correct identification and authentication of this *S. reticulata.* WHO encourages and supports countries to identify and provide safe and effective remedies for use in the public and private health service.

Microbial load: Herbal medications are likely to be contaminated with a wide variety of others potentially pathogenic bacteria. In a study whose was evaluated the bacterial contamination of powdered herbal medicinal preparations sourced from identified herbal retail outlets in different parts of India, China and Nigeria, the results showed that several herbal remedies were contaminated with *Salmonella typhi* and *Shigella* spp., besides *E. coli* and *S. aureus*[1]. The main microbial contamination of plant materials, in general, are attributed to total aerobic mesophilic, enterobacterial, yeast and mould[4]. The values obtained for the total number of pathogenic bacteria, yeasts and moulds were following the limits set by the WHO recommended procedures for plant products to which hot water is added before use, in the case of specific pathogenic bacteria such as *E. coli*, *Salmonella* sp., *Shigella* sp., *Enterobacter* sp., total heterotrophic bacteria, yeast and mould. The results were displayed in Table 5. The most widely accepted and used technique is that recommended by WHO for the total count of microorganisms in plant materials. The value of *E. coli* was 5, within the limit but *Salmonella* sp., *Shigella* sp. was absent and *Enterobacter* sp., total heterotrophic bacteria, yeast and mould values were within specified limits. Those values were representing the final product of extract for oral use safely without microbial load contaminations.

Heavy metals: This study, therefore, sought to establish the presence, quantity and prevalence of eight heavy metals (Fe, Mn, Zn, Ni, Co, Pb, Hg and As) in *S. reticulate* root extract commonly used for the treatment, prevention and management of some diseases in India. Lead (Pb) was present in *S. reticulata* extract examined, not exceed the maximum safe limit for lead. Mercury was also detected in this extract (0.0053 ppm) at the same time as the majority of elements were below the detection limit. Nickel of the *S. reticulata root* extract has a very less ppm-level. Copper, zinc, manganese and arsenic in this root extract were the very low-level limit. These heavy metals results were presented in Table 6. The

Table 5: Microbial load of *S. reticulata*

Organisms	WHO standard	Microbial contamination	Suggestion
E. coli	10^2	5	Within limits
Salmonella sp.	Absence	Nil	Within limits
Shigella sp.	Absence	Nil	Within limits
Enterobacter sp.	10^4	Nil	Within limits
Total heterotrophic bacteria	10^7	22×10^3	Within limits
Yeast and mould	10^4	5×10^2	Within limits

Table 6: Heavy metal analysis of *S. reticulate*

Metal	RDA standard (ppm)	*S. reticulate* (ppm)	Limits
Fe	25	12.26	Within limit
Mn	5	1.90	Within limit
Zn	20	0.47	Within limit
Ni	100	0.06	Within limit
Co	1	0.02	Within limit
Pb	10	0.31	Within limit
Hg	0.5	0.0053	Within limit
As	10	0.0080	Within limit

findings of heavy metal toxicity suggest that the use of this *Salacia* species for the management of diseases did not cause heavy metal toxicity and may be beneficial to the users in cases of micronutrient deficiency as these metals were found to be present in readily bioavailable form. The uses of atomic absorption spectrophotometry for the determination of the amounts of heavy metals in both raw materials and extract of *S. reticulate* samples have been analyzed and well reported[43]. The macro and micronutrients can be good, toxic or lethal depending on the dose, the study also evaluated the health implications of the heavy metals quantified, based on the recommended daily intake of medicinal plant decoctions[44].

Heavy metals as micronutrients are important for the proper functioning of vital organs in the body. For example, iron is a component of haemoglobin and other compounds used in respiration. Heavy metals are widespread in the soil as a result of geo-climatic conditions and environmental pollution. Therefore, their assimilation and accumulation in plants are obvious. Together with other pollutants, heavy metals are discharged into the environment through industrial activity, automobile exhaust, heavy-duty electric power generators, municipal wastes, refuse burning and pesticides used in agriculture[45]. Human beings, animals and plants take up these metals from the environment through air and water. Heavy metals tend to accumulate in both plants and human organs. Since plants and animals are essential sources of micronutrients for man, it becomes necessary to monitor the levels in biological materials that are required by man for both dietary and medicinal purposes. This is because deficiency or excess of micronutrients can be factors of disease generation. Even though a lot of phytochemical and bioactivity studies have been carried out on several medicinal plants[46] not much has been reported on the heavy metal contents of many herbal plants.

Antibacterial activity: The antimicrobial activities of the root of *S. reticulata* at different solvent extracts were screened by the disc diffusion method and the mean value of the zone of inhibition was assessed in millimetre diameter. The results are given in Table 7. The activity of the extracts was compared with the known antibacterial drugs, chloramphenicol and tetracycline. Disc diffusion method revealed good antibacterial activity of the chloroform, methanolic extracts and ethyl acetate compared to the petroleum ether extract. The methanolic extract was found to be most active against *P. vulgaris*, *S. aboni* and *Escherichia coli*. The zone of inhibition of methanolic extract against *P. vulgaris* and *Escherichia coli* at 50 mg concentration was 16.0 and 10.0 mm, respectively. Chloroform extract also exhibited good antibacterial activity against *P. vulgaris* and *B. subtilis*. The zone of inhibition of chloroform extract against *P. vulgaris* and *B. subtilis* bacterial strains was 37.0 and 15.0 mm, respectively. The zone of inhibition of the standard drugs chloramphenicol and against *P. vulgaris* and *B. subtilis* were 17 and 35 mm, respectively. Tetracycline showed the zone of inhibition to be 28.0 and 30.0 against *P. vulgaris* and *B. subtilis*, respectively. The petroleum ether extract and ethyl acetate exhibited good activity against *P. fluorescence*, *P. vulgaris* and *S. epidermis* of the three tested bacterial strains. All extracts of *S. reticulata* exhibited good activity against *P. vulgaris* and *S. aboni*. All the extracts of *S. reticulata* have no activity against the *V. cholerae*, *P. aeruginosa, S. typhi* and *S. sonnei* bacterial strains. The Minimum Inhibitory Concentration (MIC) was determined by the broth dilution method and the results are given in Fig. 1-3, respectively. It is worthy of note that most of the major compounds detected in the root of *S. reticulata*, in this work are biologically active molecules. Many of these compounds could be considered as part of plants defence systems. Antimicrobial activity of both polar as well as non-polar solvents for the extraction of active components from the roots of *S. reticulata*, was tested against gram-positive and gram-negative strains using zone of inhibition and minimum inhibitory concentrations. The microorganism *E. coli*, which is already known to be multi-resistant to drugs was also resistant to the plant extracts tested[46]. The results were observed that successive Soxhlet extracts were studied with different solvent (methanol, chloroform, petroleum ether and ethyl acetate) extracts have an inhibitory

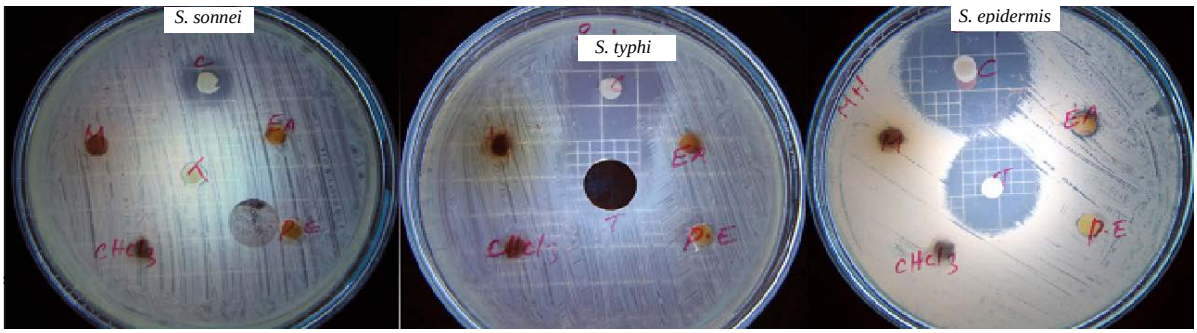

Fig. 1: Antibacterial activity of *S. reticulata* against *S. sonnei*, *S. typhi* and *S. epidermis*

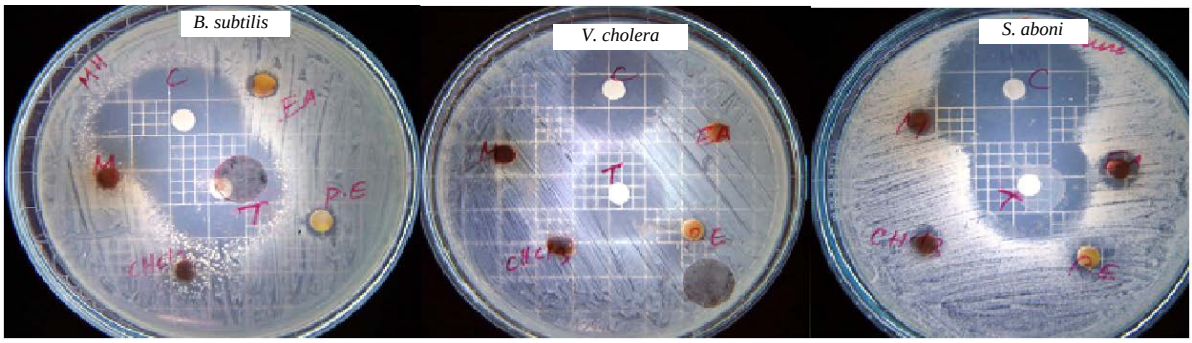

Fig. 2: Antibacterial activity of *S. reticulata* against *B. subtilis*, *V. cholera* and *S. aboni*

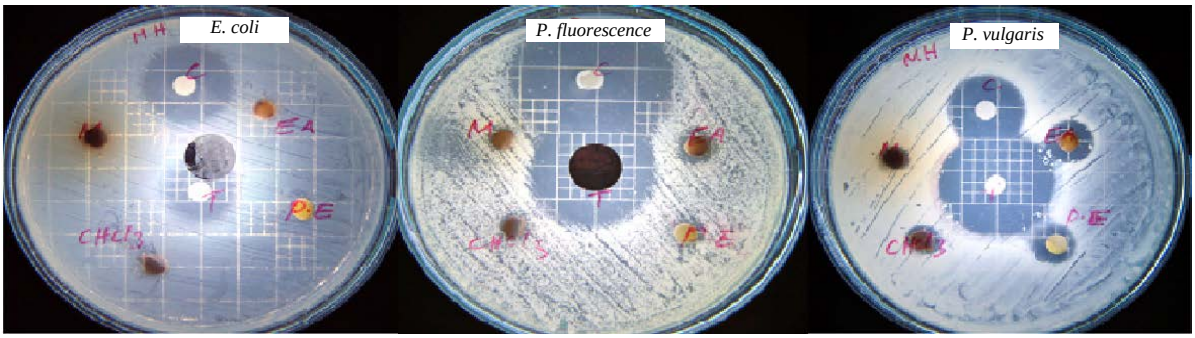

Fig. 3: Antibacterial activity of *S. reticulata* against *E. coli*, *P. fluorescence* and *P. vulgaris*
MH: Methanol, EA: Ethyl acetate, $CHCl_3$: Chloroform, PE: Petroleum ether, T: Tetracycline, C: Chloramphenicol

Table 7: Antibacterial activity of *S. reticulata*

	Zone of inhibition (mm)					
Organisms	Chloramphenicol	Tetracycline	Methanol	$CHCl_3$	Petroleum ether	Ethyl acetate
Pseudomonas fluorescence	38	26	Nil	Nil	9	10
Proteus vulgaris	17	28	16	37	26	15
Staphylococcus epidermis	37	27	Nil	Nil	22	10
Vibrio cholerae	28	22	Nil	Nil	Nil	Nil
Pseudomonas aeruginosa	Nil	Nil	Nil	Nil	Nil	Nil
Salmonella aboni	38	28	10	13	10	11
Escherichia coli	24	17	10	Nil	Nil	Nil
Bacillus subtilis	35	30	10	15	10	10
Salmonella typhi	28	24	Nil	Nil	Nil	Nil
Salmonella sonnei	12	Nil	Nil	Nil	Nil	Nil

effect towards all microorganisms used in the test such as *B. subtilis*, *P. vulgaris* and *S. aboni*. Chloroform extract was showed prominent activity than methanolic extract for *P. vulgaris bacteria.* But petroleum ether and ethyl acetate extract have an inhibitory effect towards only *P. fluorescence*. Methanolic extract of *S. reticulata* has an inhibitory effect on *E. coli*. These findings suggest that different solvent extract of *S. reticulata* has anti-bacterial potential and therefore should be investigated for biomolecular analysis.

CONCLUSION

The present investigation is focused on quality control, antibacterial and phytochemical investigation. The quality control measures such as microbial load and heavy metal toxicity were performed and they were found in normal values compared to WHO Standards. The antibacterial activity was tested against pathogenic bacteria and found that for bacteria such as *Proteus vulgaris*, *Staphylococcus epidermis*, *Shigella sonnei* and *Bacillus subtilis* which produces a significant zone of inhibition for all the solvents (Chloroform, Methanol, Petroleum ether and Ethyl acetate). The maximum zone of inhibition obtained was *Proteus vulgaris* 37, 16, 26 and 15 mm. The present research accomplishes that quality control standards were important for medicinal plants and standardization of quality control is the key factor.

SIGNIFICANCE STATEMENT

The *S. reticulata* extracts have great potential as antimicrobial compounds against microorganisms. Thus, they can be used in the treatment of infectious diseases caused by resistant bacteria. The synergistic effect from the association of antibiotics with plant extracts against resistant bacteria leads to new choices for the treatment of infectious diseases. This effect enables the use of the respective antibiotic when it is no longer effective by itself during treatment. Furthermore, they investigated the synergistic effects of extracts of *S. reticulata* with antimicrobial activity in connection with antibiotics against drugs resistant bacteria.

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