A Comparative study of antibacterial potential and phytochemical analysis between field grown and tissue cultured plant - Solanum xanthocarpum Schrad. and Wendl.

K. Poongothai SK, P. Ponmurugan SK, K. Syed Zameer Ahmed SK

1. Research and Development Centre, Bharathiar University, Coimbatore - 646 041, Tamil Nadu, India.
2. Department of Biotechnology, K.S.Rangasamy College of Technology, K.S.R. Kalvina gar, Thiruchengode - 637 215, Tamil Nadu, India.

ABSTRACT

A study was conducted to screen the efficacy of various solvents covering polar, non polar and neutral such as ethyl acetate, methanol, ethanol, acetone and aqueous extracts of in vitro raised leaves was compared with field grown leaves of S. xanthocarpum for antibacterial activity against Gram-positive and Gram-negative bacteria using agar well diffusion technique. The diameter of zone of inhibition was taken as an indicator of antibacterial effect. The result showed that except aqueous leaf extract, extracts prepared from various organic solvents showed effective antibacterial activity against the test organisms. A strong inhibition zone was recorded in ethyl acetate and methanol extract of in vitro and field grown leaves against the test organism. Preliminary phytochemical analysis of field grown and tissue cultured plants aqueous leaf extract of S. xanthocarpum showed the presence of alkaloids, saponins, steroids, amino acids, and reducing sugars. The investigation indicated that S. xanthocarpum is one of the potential medicinal plant for therapeutic purpose. Among the two extracts tested for phytochemical analysis as well as antibacterial activity, extracts prepared from tissue cultured plants was found to be superior than field grown plants.

Key words: Antibacterial activity, Agar well diffusion method, inhibition zone, tissue cultured plant, Phytochemical.

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an enormous number of modern drugs have been isolated from natural sources especially from plants; many of these isolations were based on the uses of the agents in traditional medicine. This plant based, traditional medicine system continues to play an essential role in health care, with about 80% of the world’s inhabitants relying mainly on traditional medicines for their primary health care (1).

The increase in number of antibiotic resistant bacteria is no longer matched by expansion in the arsenal of agents available to treat infections. Literature reports and ethno botanical records suggest that plants are the sleeping giants of pharmaceutical industry (2,3). They may provide natural source of antimicrobial drugs that will provide novel or lead compounds that may be employed in controlling some infections globally. Antibiotic resistance has become a global concern and is being threatened by emergence of multidrug resistance-pathogens. Therefore, increase in failure due to chemotherapeutics and antibiotic resistance leads to screening of several medicinal plants for their antimicrobial effect. The World Health Organization (WHO) has also recommended the evaluation of plants for effectiveness against human diseases and for the development of safe modern drugs (4).

Solanum xanthocarpum is a prickly diffuse bright green perennial herb and distributed throughout India, found mostly in dry places as a weed on road side and waste lands (5). It is one of the members of the dasamula (ten root) of the Ayurveda, which is considered to be a noxious weed. Numerous reports are available on the medicinal use of S. xanthocarpum, especially in Ayurvedic medicine for asthma (6,7), diabetes (8), rheumatism, catarhal fever, cough, chest pain, stone in the bladder, flatulence, toothache, bronchospasm, constipation and gonorrhea. The fruits are used as anthelmintic agent, and also used to get relief from sore throat and indigestion. Pharmacological studies on this herb have shown that the aqueous and alcoholic extracts possess a good hypotensive effect (9). The aim of the present study was to investigate the antimicrobial activity of the extracts from in vitro raised leaves and Field grown leaves of S. xanthocarpum using aqueous and organic solvents against selected pathogenic microorganisms.

MATERIALS AND METHOD

Plant material

The field grown leaves was collected from the habitats near to the bank of Cauvery river, Tamil Nadu, India, during the month of August to November (2009). The plant was identified and authenticated from standard resources. The plant was brought to the laboratory and thoroughly washed in running tap water to remove debris and dust particles and then rinsed in distilled water, shade dried, coarsely powdered and stored in an air tight container for further use. Similarly in vitro raised leaves were collected from our laboratory and the above said procedure was followed.

Preparation of extract

100gms of both the field grown and in vitro raised leaves powder were taken separately and exhaustively extracted by soxhlet extraction method using each of the following solvents: ethyl acetate, methanol, ethanol and acetone. For the preparation of aqueous extracts, 10g of plant material was extracted with 100ml of sterile double distilled water. The mixture was heated slowly at 40°C for 16h in an oven and filtered through several layers of muslin cloth. The filtrate was again filtered by using Whatman no.1 filter paper and concentrated to 1/5 of the original volume by evaporation in shaded conditions and stored at 4°C until used.

Test microorganisms

Microorganisms used were Staphylococcus aureus (MTCC 96), Streptococcus pyogenes (MTCC 442), Streptococcus mutans (MTCC 97), Bacillus...
sphaericus (MTCC 511), Salmonella paratyphi (MTCC 735), E. coli (ATCC 10412), Pseudomonas aeruginosa (MTCC 424), Proteus vulgaris (ATCC 6308), Klebsiella pneumoniae (ATCC 70063) and Serratia marcescens (MTCC 97).

Screening for antibacterial activity

Antibacterial activities of leaf extracts of field grown and tissue cultured plants were tested by well-in-agar method. The culture plates were prepared by pouring 20 ml of Mueller-Hinton agar medium into sterile Petri plates. The inoculum suspension was spread uniformly over the agar medium using sterile cotton swabs to get uniform distribution of the organisms. Using flamed cork borer, well of 5 mm diameter was made in the media at a distance of 1-2 cm from the periphery of the plates. These plates were labeled and 0.2 ml of leaf extracts at different concentrations (75, 50, 25, and 10 ) was added aseptically into the well. The effect of the leaf extracts against the test organisms was recorded by measuring the diameter of inhibition zone. The experiment was repeated thrice and the mean values were recorded.

Phytochemical analysis of the extract

Aqueous leaves extract field grown and tissue cultured plants of S. xanthocarpum was subjected to preliminary phytochemical screening for the presence or absence of phytoconstituents [10, 11].

RESULT

The antimicrobial activity of S. xanthocarpum leaf extract of field grown and tissue cultured plants was tested under in vitro condition by agar well diffusion method against Gram-positive and Gram-negative bacterial pathogens. The results indicated that the zone of inhibition of microbial growth by aqueous, solvent (ethyl acetate, acetone, methanol and ethanol) extracts is given in Table 1 and Table 2. All the extracts exhibited different degrees of antimicrobial activity against tested organisms. The results indicated that all the organisms were found to be more susceptible to the higher concentrations of extracts. It is clear from the Table 1 and Table 2 that most of the extracts were effective against E. coli, S. aureus and S. pyogenes. Among the two extracts tested, extracts prepared from tissue cultured plants were found to be superior when compared to field grown plants.

Aqueous extracts of S. xanthocarpum leaf extract of field grown and tissue cultured plants inhibited the growth of Gram-negative bacteria E. coli (20mm, 18mm), K. pneumoniae (2mm, 2mm) and Gram-positive bacteria S. aureus (7mm, 7mm) respectively. Solvent extracts of S. xanthocarpum leaves at various concentrations exhibited mild to moderate inhibition over the growth of tested bacterial pathogens. In terms of specific inhibition, ethyl acetate leaf extract of S. xanthocarpum was found to be more effective against the entire tested microorganism with the zone ranging from 26 to 16mm. It was found that ethyl acetate extract exhibited highest degree of activity against S. pyogenes (26mm, 26mm), B. sphaericus (27mm, 24mm), P. vulgaris (18mm, 18mm), S. marcescens (17mm, 16mm), S. mutans (16mm, 14mm) etc. The extracts prepared from acetone had broad spectrum activity against the entire tested pathogen. Similarly ethanol leaf extracts, too exhibited mild to moderate activity against tested organism like S. marcescens, S. pyogenes, S. paratyphi, E. coli, and P. vulgaris. P. vulgaris, K. pneumoniae, and P. aeruginosa exhibit mild to moderate activity against all the extracts. The results of the present investigation were compared with standard antimicrobial drugs, it was observed that the antimicrobial effect of ethyl acetate, acetone, and methanol extracts were acceptable with respect to standard antibiotics. Hence all the leaf extracts have antibacterial activity against at least one microorganisms tested. Preliminary screening of phytochemicals revealed the presence of alkaloids, saponins, steroids, reducing sugars and amino acids in both field grown and tissue cultured (aqueous) leaf extracts of S. xanthocarpum given in Table 3.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Extract type</th>
<th>Zone of inhibition (mm)</th>
<th>Control (10µg/ml)</th>
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<td></td>
<td></td>
<td>Extract concentration (mg/ml)</td>
<td>Control</td>
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<td>75  50  25  10  G  C</td>
<td>75  50  25  10  G  C</td>
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Table 1. Antimicrobial sensitivity of field grown and tissue cultured plant extracts of S. xanthocarpum against pathogenic microorganisms:

All values are mean of three replicates.
"**" No activity. TC (Tissue cultured plant), F (Field grown), EA (Ethyl acetate), A (Acetone), M (Methanol), E (Ethanol), Aq (aqueous), G (Gentamycin), C (Choremphanicol).

Table 2. Antimicrobial sensitivity of field grown and tissue cultured plant leaves extracts of S. xanthocarpum tested against pathogenic microorganisms:

All values are mean of three replicates.
"**" No activity. TC (Tissue cultured plant), F (Field grown), EA (Ethyl acetate), A (Acetone), M (Methanol), E (Ethanol), Aq (aqueous), G (Gentamycin), C (Choremphanicol).
that the antimicrobial activity of plant extracts depends on the presence of higher concentration of the extract. The results of this study support the fact administration of 200 mg of deriphylline dry powder thrice a day for 3 days found to be very effective to controlling mild it was reported that oral administration of ity of saponins against bacterial and fungal pathogens have immune boosting, anti-inflammatory properties, antiviral and antibacte-
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DISCUSSION

The present study was carried out to evaluate the efficacy of S. xanthocarpum (Field grown plants and tissue cultured plants) leaf extracts against pathogenic microorganisms. Our results revealed that ethyl acetate and methanolic leaf extract of (in vitro and field grown) S. xanthocarpum possess remarkable growth inhibitory activity of tested microorganisms. The presence of antibacterial substances in the higher plants is well established (12). Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. Phytomedicine can be used for the treatment of diseases as is done in case of Unani and Ayurvedic system of medicines or it can be the base for the development of a medicine, a natural blueprint for the development of a drug (13). Various workers have already shown that Gram positive bacteria are more susceptible towards plants extracts as compared to Gram negative bacteria (14). (15) These differences may be attributed to fact that the cell wall in Gram positive bacteria is of a single layer, whereas the Gram negative cell wall is multilayered structure (16).

S. xanthocarpum has antimicrobial activity which was associated with alkaloids and saponins present in it. For instance, plants rich in saponins have immune boosting, anti-inflammatory properties, antiviral and antibacterial activities (20). Several reports are available in support of antimicrobial activity of saponins against bacterial and fungal pathogens (21,22). In a clinical study, it was reported that oral administration of S. xanthocarpum at a dose of 300 mg dry powder thrice a day for 3 days found to be very effective to controlling mild to moderate bronchial asthma and the bioactivity is equivalent to that of administration of 200 mg of deriphylline (6,7). The result of present study showed that the plants and their extracts had broad spectrum and magnitude of activity in higher concentration of the extract. The results of this study support the fact that the antimicrobial activity of plant extracts depends on the presence of phytochemicals.

CONCLUSION

This present study justifies the claimed uses of S. xanthocarpum Schard. and Wendl. in the traditional system of medicine to treat various infectious diseases caused by microbes. To conclude the present study, there is a strong correlation between phytoconstituents of the plant and antimicrobial activity between field grown and tissue cultured plants.

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REFERENCES