International Journal of Natural and Engineering Sciences 3 (1): 25-28, 2009
ISSN: 1307-1149, www.nobel.gen.tr

Evaluation of Antimicrobial Activity of Saponin Isolated From Solanum Xanthocarpum and *Centella asiatica*

Krishnan KANNABIRAN* Thenmozhi MOHANKUMAR Vinitha GUNASEKER

Division of Biomolecules and Genetics School of Biotechnology, Chemical and Biomedical Engineering, VIT University, Vellore-632 014, Tamil Nadu, INDIA

*Corresponding Author
mail: kkb@vit.ac.in

Received: August 10, 2008
Accepted: November 23, 2008

Abstract

The aim of the present study was to evaluate the antimicrobial potential of aqueous and solvent (ethanol, methanol, acetone and ethyl acetate) extracts and saponin fraction isolated from the leaves of Solanum xanthocarpum and Centella asiatica against selected bacterial and fungal species. The antimicrobial activity was tested by agar disc diffusion and agar well diffusion method. The aqueous, ethanol, acetone and ethyl acetate extracts of *S. xanthocarpum* and *C. asiatica* were inhibited the growth of bacterial pathogens. The most susceptible Gram -negative bacterial pathogens were *Klepsella pneumoniae* (20 mm) and *Escherichia coli* (17 mm). The saponin fraction of *S. xanthocarpum* and *C. asiatica* inhibited the growth of Gram positive bacterium *S. aureus* (21 mm and 22 mm). The antimicrobial activity exerted by the saponin fraction was higher than the aqueous and organic solvent extracts against tested pathogenic bacteria and fungi and standard antibiotics. *Aspergillus fumigatus* was more susceptible fungal pathogen than *Aspergillus niger*. Preliminary phytochemical screening revealed the presence of saponins; phytosterols and carbohydrates in both the plants. Based on the results of this study, it can be concluded that the saponin fraction might be responsible for the antimicrobial potential of *S. xanthocarpum* and *C. asiatica*.

Key words: Medicinal plant, Solanum xanthocarpum, Centella asiatica antimicrobial activity, phytochemicals

INTRODUCTION

Medicinal plant based therapies are practiced traditionally in Ayurveda, Siddha and other indigenous systems of medicines including folklore medicines to treat numerous microbial infections.

Medicinal plants have been used for centuries as remedies for human diseases because they contain numerous phytochemicals with immense therapeutic value and more over considered to be natural and safe when compared to synthetic drugs [1]. Emergence of more and more multidrug –resistant pathogens was reported to be the leading cause for death world-wide [2]. Many infectious microorganisms are resistant to synthetic drugs; hence an alternative therapy is very much needed to control microbial pathogenesis. Medicinal plants have long been used as an alternative source for medicines and remedy for treating human diseases [3]. Presently 80 percent of the world population relies on plant derived medicines for maintaining good health and combating many diseases [4]. 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 [5].

Solanum xanthocarpum (Solanaceae), the Indian nightshade, commonly known as 'baigan kateli, 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], diabetics [8], rheumatism, catarrhal 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].

Centella asiatica (L), synonym Hydrocotyle asiatica, (Family Umbelliferae) is found almost all over the world. In Ayurveda, it is used for the management of disorders of central nervous system, skin and gastrointestinal system. The biological activity of *C. asiatica* includes anti-inflammatory, anti-cancer [10] anti-ulcer [11, 12] and wound healing activities [13]. *C. asiatica* has reported to be rich in antioxidants [14] due to the presence of phenolic compounds (3.23-11.7 g / 100 g dry sample) [15,16]. Triterpenoids such as asiatic acid, asiaticoside and madecasic acid were also reported to be the major biologically active constituents of *C. asiatica* [17]. Recently a bioactive polyacetylene compound has been isolated from *C. asiatica* [18].

The aim of the present study was to investigate the antimi crobial activity of aqueous and organic solvents extracts; and saponin fraction of *S. xanthocarpum* and *C. asiatica* against few selected bacterial and fungal pathogens.

MATERIALS AND METHODS

Collection of plants

Fresh plants *S. xanthocarpum* and *C. asiatica* were collected from the VIT University medicinal garden in the

month of December 2006 and were authenticated with the help of botanist.

Voucher specimens were prepared and deposited in the herbarium section of the VIT University, Vellore, Tamil Nadu, India. Leaves of *S. xanthocarpum* and *C. asiatica* were washed with distilled water, shade dried, powdered and stored in an air-tight container until further use.

Preparation of extracts

Solvent extracts were prepared by transferring 1g of the powder to sterile wide-mouthed screw-capped bottles containing the solvent. It was allowed to soak for 24 hours at room temperature then heated for an hour at 100°C. The mixture was then centrifuged at 2000 rpm for 10 minutes at 4°C. The supernatants were filtered through a sterile funnel containing sterile Whatmann filter paper no.1 and then filter sterilized using syringe filter containing 0.2µ cellulose acetate membrane (Sartorius).

Preparation of saponin fraction

The powdered sample was defatted by using petroleum ether 3 x1h at 40 °C. After filtering the petroleum ether, the sample was extracted with methanol 3x1 h with mild heating. The combined methanol extract was concentrated and the methanol extract was dissolved in methanol – acetone mixture (1:5 v/v) to precipitate the saponins [19]. The precipitate was dried under vacuum, turning to a whitish amorphous powder named as Crude Saponin Extract (CSE). The CSE was loaded on silica gel-60 (230-400 mesh, Merck) chromatography column and eluted with chloroform-methanol-water (70:30:10) [20]. The first fraction collected was evaporated under reduced temperature; the resultant residue was called Pure Saponin Fraction (PSF).

Bacterial and fungal species

Staphylococcus aureus (ATCC 700699), *Escherichia coli* (ATCC 10412), Pseudomonas aeruginosa (ATCC 27853) and Klebsiella pneumoniae (ATCC 2719) were used as test organisms.

Exactly 0.2ml of overnight cultures of each organism was inoculated into 20ml of sterile nutrient broth and incubated for 3-5 hours to standardize the culture to106 cfu mL-1. Mueller Hinton Agar solid media was used for culturing of bacteria and agar disc diffusion assay was used to assess the antibacterial activity. *Aspergillus fumigatus*, and *Aspergillus niger* species were clinical isolates obtained from Christian Medical College, Vellore,

Tamil Nadu, India. The fungal species were maintained in Sabouraud Dextrose Broth at 4˚C. Antifungal activity was tested by well diffusion method. Each fungal culture inoculum spores mL-1 2.5x103 was applied on plate and evenly spread on Sabouraud Dextrose Agar using a sterile swab. At the end of the 48 h period, the inhibition zones (including the well diameter 7mm), formed in the medium were measured.

Assay of antimicrobial activity

Agar diffusion assay was carried out to check the antimicrobial activity as described [21]. The plates were incubated at 37oC for 24 hours during which activity was evidenced by the presence of a zone of inhibition surrounding the well. Each test was repeated three times and the antimicrobial activity was expressed as the mean of diameter of the inhibition zones (mm), including the diameter of the well (7mm) produced by the plant extracts when compared to controls.

Standard antibiotic discs (7mm) were purchased from Himedia Chemicals, Mumbai, India. Antibiotic discs Penicillin, Streptomycin and Ampicillin (25 µg disc-1) were used to compare antibacterial activity with that of plant extracts. For fungal species Amphotericin-B was used to compare antifungal activity. The zones of inhibition observed were considered to be significant provided the value was greater than 7mm.

Phytochemical analysis

Standard procedures were followed to identify the phytochemical constituents in the aqueous extracts of the powdered leaves of *S. xanthocarpum* and *C. asiatica* [22].

RESULTS AND DISCUSSION

The antimicrobial activity of *S. xanthocarpum* and *C. asiatica* extracts was tested under in vitro conditions by agar disc diffusion and well diffusion method against four bacterial and two fungal pathogens. The zone of inhibition of microbial growth by aqueous, solvent (ethanol, methanol, acetone and ethyl acetate) extracts and saponin fraction is given in Table 1. Aqueous extracts of *C. asiatica* and S. xanthocapum leaves (50 mg/ml) inhibited the growth of Gram negative bacteria K. pneumoniae (20 mm and 11 mm) and E. coli (17 mm and 11 mm) respectively. Solvent extracts of *C. asiatica* and S. xanthocapum leaves (50 mg/ml) exhibited mild to moderate inhibition over the growth of tested bacterial pathogens. Ethanol extract of *C. asiatica* has no inhibition over tested bacterial strains.

The saponin fraction of S. xanthocapum and *C. asiatica* inhibited (21 and 22 mm respectively) the growth of Gram –positive bacteria S. aureus. The order of inhibition of the Gram-negative bacteria was K. pneumoniae followed by E. coli and *P. aeruginosa* by the saponin. The saponin fraction has shown comparatively higher inhibition on bacterial pathogens than the standard antibiotics (penicillin, streptomycin and ampicillin).

This is in agreement with previous reports that plant extracts are more active against Gram-positive bacteria than Gram-negative bacteria [23, 24]. The reason for the different sensitivity between Gram-positive and Gram–negative bacteria could be ascribed to the morphological differences between these micro-organisms, Gram-negative bacteria having an outer phospholipidic membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to lipophilic solutes, while porins constitute a selective barrier to the hydrophilic solutes with an exclusion limit of about 600 Da [25]. Hence, the Gram-positive bacteria should be more susceptible having only an outer peptidoglycan layer which is not an effective permeability barrier [26].

The antifungal activity of solvent (ethanol and metha nol) extracts and saponin fraction of *S. xanthocarpum* and *C. asiatica* was tested against two fungal pathogens and the results are given in Table 2. Methanolic extracts have greater inhibitorypotential than the ethanolic extracts against tested fungi. *A. fumigatus* was more susceptible (34 and 28 mm) to inhibition than A.

xanthocarpum and *C. asiatica* exhibited good inhibition against *A. fumigatus* (30 and 31 mm respectively). The methanolic extracts and saponin fractions were comparatively higher inhibition than the standard fungicide, amphotericin-B. Our results supports the earlier findings that the methanol extracts of *S. xanthocarpum* possess significant antifungal activity [27].

niger to the methanolic extracts. The saponin fraction of S.The results indicate that the saponins of *S. xanthocarpum* and *C. asiatica* are responsible for exerting antimicrobial activity against tested bacterial and fungal pathogens. Several new steroidal compounds have been isolated from the fruits of *S. xanthocarpum* [28]. Preliminary screening of phytochemicals revealed the presence of carbohydrates, saponins and

Table 1. Antibacterial activity of aqueous, ethanol, acetone and ethyl acetate extracts and saponin fractions of *S. xanthocarpum* and *C. asiatica* leaves

Plant parts/Extracts	Diameter of zone of inhibition (mm)*			
	S. aureus	***E. coli***	***P. aeruginosa***	***K. pneumoniae***
Penicillin disc (25 µg)	11	-[a]	-	-
Streptomycin disc (25 µg)	78			7
Ampicillin disc (25 µg)	10	10	-	-
***S. xantocarpum* leaves**				
Aqueous extract (50 mg/ml) 1	2	17 7	1	1
Ethanol extract (50 mg/ml)	-	13	14	5
Acetone extract (50 mg/ml)	11	41	2	10
Ethyl acetate extract (50 mgml)	41	2	-	4
Saponin fraction (10 mg/ml) 2	1	19 2	0	19
***C. asiatica* leaves**				
Aqueous extract (50 mg/ml) 1	0	11 1	2	20
Ethanol extract (50 mg/ml)	-	-	-	-
Acetone extract (50 mg/ml)	12	15	16	15
Ethyl acetate extract (50 mgml) 1	1 1	2	14 1	5
Saponin fraction(10 mg/ml) 2	2	19	18	21

Table 2. Antifungal activity of ethanol, methonal extracts and saponin fractions of *S. xanthocarpum* and *C. asiatica* leaves

Medicinal plants	Diameter of zone of inhibition (mm)*	
	A.niger	***A.fumigatus***
Amphotericin – B disc (25 µg)	22	14
S. xanthocarpum		
a. Ethanol extract (100 mg/ml)	22	26
b. Methanol extract (100 mg/ml)	32	34
C. asiatica		
a. Ethanol extract (100 mg/ml)	16	20
b. Methanol extract (100 mg/ml)	25	28
S. xanthocarpum		
Saponin fraction (10 mg / ml)	8 3	0
C. asiatica		
Saponin fraction (10 mg / ml)	8 3	1

Values are mean three experiments

* Inhibition zones are including the disc and the well diameter

Values greater than 7mm (size of the well) indicates significant activity.

phytosterols in both the plants whereas phenolic compounds, tannins and terpenoids are present only in *C. asiatica* (Table 3). It has been reported that the antibacterial activity depends on the total saponins and tannins content of the plant extract [29]. The triterpene asiaticoside of *C. asiatica* has been reported to be very effective against enteropathogens [30].Our results support the fact that the antimicrobial activity of plant extracts depends on the presence of phytochemicals.

Table 3. Preliminary screening of phytochemicals of aqueous extracts of *S. xanthocarpum* and C. aisatica leaves

Phytochemicals	***S.xantocarpum***	***C. asiatica***
Carbohydrates	+	+
Saponins	+	+
Phytosterols	+	+
Phenolic compounds	-	+
Tannins	-	+
Flavanoids	-	-
Terpenoids	-	+

+ : Present

- : Absent

S. xanthocarpum is non toxic and has been reported to be safe for human use [7] and *C. asiatica* was already in use, it is clinically safe to consume and it was long been used as a component of herbal remedies. 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]. Allergic Bronchio Pulmonary Aspergillosis (ABPA), an immunologic lung disease has been reported to be mediated by the antigens of the genus Aspergillus [31]. Since the saponins of *S. xanthocarpum* and *C. asiatica* are found to be very effective against A. fumigatous it could be used as a potential agent in controlling ABPA. The petroleum ether extract of *S. xanthocarpum* was found to be very effective against *Culex quinquefasciatus* (Say) larvae [32]. Its larvicidal properties have also been reported against vectors of malaria and dengue/DHF [33].

The present study further confirms the traditional use of *S. xanthocarpum* as popular complementary medicine to relieve throat congestion, cough, cold, bronchitis and bronchial asthma. The results of this study further reaffirm the existence of strong correlation between plant phytochemicals and antimicrobial activity.

Thus the use of *S. xanthocarpum* and *C. asiatica* as lead candidate medicinal plants with immense therapeutic potential can be further substanstiated with respect to its phytochemical and its efficacy to control bacterial and fungal infections with special reference to ABPA to make it as an effective therapeutic agent. However, further studies are needed to establish its chemical structure and to confirm its broad spectrum of antimicrobial activity against pathogenic microorganisms.

Acknowledgements

The authors are grateful to the management of VIT University for providing necessary facilities to carry out this study. Financial support in the form of L-RAMP Student Project Scheme to Dr.K.Kannabiran by VIT-TBI (under Lemelson foundation support) is gratefully acknowledged.

REFERENCES

[1] Szelenyi I, Brune K. 2002. Herbal remedies for asthma treatment: Between myth and reality. Drugs Today. 38: 265.

[2] Bandow JE, Brotz H, Leichert LIO, Labischinski H, Hecker

M. 2003. Proteomic approach to understanding antibiotic action.

Antimicrobial Agents for Chemotherapy. 47: 948-955.

[3] Nostro A, Germano MP, D'Angelo V, Marino A, Cannatelli MA. 2000. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Letters in Applied Microbiology. 30: 379 - 384.

[4] Maridass M, De Britto AJ. 2008. Origins of plant derived medicines. Ethnobotanical Leaflets. 12: 369-383.

[5] Inamdar N, Edalat S, Kotwal VB, Pawar S. 2008. Herbal drugs in milieu of modern drugs. International Journal of Green Pharmacy. 2: 2-8.

[6] Govindan S, Viswanathan S, Vijayasekaran V, Alagappan R. 1999. A pilot study on the clinical efficacy of Solanum xanthocarpum and *Solanum trilobatum* in bronchial asthma. Journal of Ethnopharmacology. 66: 205-210.

[7] Govindan S, Viswanathan S, Vijayasekaran V, Alagappan R. 2004. Further studies on the clinical efficacy of *Solanum trilobatum* in bronchial asthma. Phytotherapy Research. 18: 805-809.

[8] Kar DM, Maharana L, Pattnaik S, Dash GK. 2006. Studies on hypoglycaemic activity of Solanum xanthocarpum Fruit extract in rats. Journal of Ethnopharmacology. 108: 251-256.

[9] Mirza M, Yaqeen Z, Zakir-ur-Rehman, Ismail S. 2003. Hypotensive activity of Solanum xanthocarpum in dog. Hamdard Medicus. 46: 110-112.

[10] Babu TD, Kuttan G, Padikkala J. 1995. Cytotoxic and antitumor properties of certain texa of Umbelliferae with specific reference to Centella asiatica (L.) urban. Journal of Ethnopharmacology. 48: 3–57.

[11] Sairam K, Rao VC, Goel RK. 2001. Effect of Centella asiatica Linn on physical and chemical factors induced gastric ulceration and secretion in rats. Indian Journal of Experimental Biology. 39: 137–142.

[12] Cheng CL, Guo JS, Luk J, Koo MWL. 2004. The healing effect of Centella extract and asiaticoside on acetic acid induced gastric ulcers in rats. Life Sciences. 74: 2237–2249.

[13] Suguna L, Sivakumar P, Chandrakasan G. 1996. Effects of Centella asiatica extract on dermal wound healing in rats. Indian Journal of Experimental Biology. 34:208–211.

[14] Jayashree G, Kurup MG, Sudarslal VS, Jacob VB. 2003. Antioxidant activity of Centella asiatica on lymphoma-bearing mice. Fitoterapia. 74: 431–434.