Journal of Applied Pharmaceutical Science Vol. 5 (09), pp. 124-126, September, 2015
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2015.50923
ISSN 2231-3354

In vitro evaluation of *Schima wallichii* (DC.)Korth.fruit for potential antibacterial activity

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ARTICLE INFO

Article history:
Received on: 02/07/2015
Revised on: 05/08/2015
Accepted on: 29/08/2015
Available online: 27/09/2015

Key words:
Schima wallichii (DC.) Korth. fruit, antibacterial activity, agar disc diffusion assay, minimum inhibitory concentration (MIC).

ABSTRACT

Schima wallichii (DC.) Korth. is a large evergreen and well known tree of Sikkim Himalayan region. The bark of this plant is traditionally used as antipyretic, antiseptic, anthelmintic, wound healing agent. In the present study an attempt has taken to investigate for potential antibacterial activity by taking different extracts of fruits of *Schima wallichii* (DC.) Korth. against Gram-positive bacteria (*Staphylococcus aureus* NCTC 8530 and *Bacillus liherfernis* 10341) and Gram-negative bacteria (*Escherichia coli* HD_{10}; *Salmonella paratyphi* A_2 and *Vibrio cholera* 64). Antibacterial activity of *Schima wallichii* (DC.) Korth. fruit extracts (benzene, acetone and aqueous) were assayed by the disc diffusion method. Among all the extracts, acetone extract was found most active against *Escherichia coli* HD_{10} and *Bacillus liherfernis* 10341 but this extract have no effect in case of *Vibrio cholera* 64. The MIC (Minimum Inhibitory Concentrations) of the extract was found 100 µg/ml for *Escherichia coli* HD_{10} and 150 µg/ml for *Bacillus liherfernis* 10341. The study promises an interesting future for designing a potentially active antibacterial agent from *Schima wallichii* (DC.) Korth. fruit.

INTRODUCTION

In the developing countries as well as in worldwide, the most human died due to infectious bacterial diseases. The bacterial organisms including Gram positive and Gram negative like different species of Staphylococcus, Bacillus and Salmonella are the main source to cause severe infections in humans. Because these organisms have the ability to survive in harsh condition due to their multiple environmental habitats (Bibi*et al*., 2011). There are several medicinal plants which are having antibacterial activity are used traditionally against bacteria (Khan *et al*., 2012). *Schima wallichii* (DC.) Korth. is a well-known plant of Sikkim Himalayan region, belonging to the family Theaceae. It is 40-100ft height and distributed 2000-5000ft. in Eastern Himalayan Region. It is used as anti-inflammatory and antidote agent by the local healers (Gurung, 2002). *Schima wallichii* barks having anthelmintic (Dewanjee *et al*., 2007), antibacterial (Dewanjee *et al*., 2008), antinociceptive (Dewanjee*et al*., 2009), anti-inflammatory (Dewanjee *et al*., 2011), antioxidant (Das *et al*., 2012), analgesic and antipyretic activity (Das and Ghosh, 2013). In this paper by considering the antibacterial activity of bark of *Schima wallichii* an attempt has taken to test the antibacterial activity of various extracts of *Schima wallichii* (DC.) Korth. fruits by using gram positive and gram negative bacteria.

MATERIALS AND METHODS

Plant material

Fruits of *Schima wallichii* (DC.) Korth. were collected from Majhitar, East Sikkim, India in the month of December-January, 2014 and identified by Dr. Dinesh Agarwala, Scientist C, Botanical Survey of India, Sikkim Himalayan Regional Center, Gangtok, India. A voucher specimen (Accession Number 0056 & 57) has been preserved at our laboratory for future reference.

Preparation of the extract

The fruits were dried under shade, pulverized into coarse powder with the help of mortar and pestle. The coarse powder was extracted with different solvent as given in protocols below:

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Organic solvent extraction

150 gm. of coarsely shade dried powder was extracted successively first with 500 ml of benzene and then with 500 ml of acetone by cold maceration process. The mixtures were placed at room temperature for 72 hrs. The solutions were filtered through muslin cloth and filtered again by passing through filter paper. The filtered solutions were concentrated separately by using rotary evaporator and finally transferred to petridishes at room temperature for complete evaporation. The solvents present in liquid extracts were collected separately from the rotary evaporator and kept into bottles at 4˚C for further use.

Aqueous extraction

2.5 ml of chloroform was mixed with distilled water to get a final volume of 1 litre and treated as same as the organic solvents to get dry aqueous extract.

Phytochemical Screening

Organic (Benzene & Acetone) and aqueous extracts of *Schima wallichii* (DC.) Korth. fruits were containing cardiac glycosides, tannins, flavonoids, steroids and saponins by using standard procedures (Jeyaseelan and Jashothan, 2012).

Bacterial strains

The bacterial strains selected for present study were collected from Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India. A total of five bacteria namely *Escherichia coli* HD_{10}; *Salmonella paratyphi* A_2; *Vibrio cholera* 64; *Staphylococcus aureus* NCTC 8530 and *Bacillus liherfernis* 10341 were screened for present investigation. The first three were Gram-negative and remaining were Gram-positive bacteria.

These bacterial cultures were maintained in nutrient agar slants at 37°C. Microorganisms of each type were reactivated by sub-culturing in nutrient broth media, incubated at 37°C over-night.

Antibacterial activity

In-vitro antibacterial susceptibility test was carried out by disc diffusion method against 2 Gram-positive (*Staphylococcus aureus* NCTC 8530 and *Bacillus liherfernis* 10341) and 3 Gram-negative bacteria (*Escherichia coli* HD_{10}; *Salmonella paratyphi* A_2 and *Vibrio cholera* 64). The turbidity of bacterial suspension in Mueller Hinton Broth (Himedia, India) was adjusted to McFarland standard number 0.5. With a sterile cotton swab bacterial culture was streaked on previously prepared Mueller Hinton agar plate (Himedia, India).The dried and sterilized filter paper discs were treated separately with desired concentration of previously prepared organic and aqueous solution of the root extract using a micropipette. Then it was dried in air under aseptic condition and placed at equidistance on a circle of the seeded plate. The concentrations of fruit extract used were 100µg/disc, 150µg/disc, 200µg/disc, 250 µg/disc, 300 µg/disc. These plates were kept for 4-6 hours at low temperature to diffuse the test materials from disc to the surrounding medium and then incubated at 37°C for 24 hours.

The diameter of the zone of inhibition produced by the fruit extract was then compared with standard antibiotic Streptomycin (100µg/disc). Each sample was used in triplicate for the determination of antibacterial sensitivity test. Distilled water and DMSO soaked discs were used as negative control (Fakruddin *et al*., 2012).

Statistical analysis

The values of antibacterial sensitivity test of the different extracts of *Schima wallichii* (DC.) Korth. fruits were expressed as mean ± standard deviation (n=3) for each sample (Rao *et al*., 2010).

RESULTS

Predictions of antibacterial activity in herbal compounds extracted from plant parts depend largely upon the type of solvent used for extraction. This fruit extracts were containing cardiac glycoside, tannin, flavonoid, steroid and saponin which is identified by the Preliminary phytochemical screening.The benzene, acetone and aqueous extract of *Schima wallichii* (DC.) Korth. fruit exhibited the antibacterial activity against 5 bacterial strains (table 1) and the result were expressed as mean ± standard deviation (n=3). Among the various extract acetone extract showed maximum antibacterial activity. The antibacterial activity of acetone extract was highest against *Escherichia coli* HD_{10} (14.9±0.200), moderate against *Bacillus liherfernis* 10341(14.8±0.264) and lowest in case of *Staphylococcus aureus* NCTC 8530 (13.43±0.351) as compared with streptomycin as positive control and negative Control as distilled water and DMSO (Dimethyl sulfoxide).

Active extracts thus obtained (fruits extracts prepared in organic and aqueous solvents) were further subjected for determination of minimum inhibitory concentration (MIC) by two-fold micro broth dilution method against susceptible bacterial species viz., Gram-positive (*Staphylococcus aureus* NCTC 8530 and *Bacillus liherfernis* 10341) bacteria and Gram-negative (*Escherichia coli* HD_{10}; *Salmonella paratyphi* A_2 and *Vibrio cholera* 64) bacteria. Table 2 indicated that acetone extract was found significant inhibitor than other extracts. MIC of this extract were 100 µg/ml , 150 µg/ml against gram negative *Escherichia coli* HD_{10} and *Salmonella paratyphi* A_2 respectively, while it was 100 µg/ml and 150 µg/ml against other two Gram-positive bacteria *Staphylococcus aureus* NCTC 8530 and *Bacillus liherfernis*10341 respectively.

The concentration of acetone extract tested was not enough to inhibit *Vibrio cholera* 64. The other extracts like benzene and aqueous extracts with MIC ranging between 200 µg/ml and 300 µg/ml showed inhibitory activity against both Gram-positive and Gram negative bacteria.

Table 1: *In vitro* antibacterial activity of organic and aqueous extracts of *Schima wallichii*(DC.)Korth.fruit

Test Sample		Zone of Inhibition* (in mm diameter)				
		E.coli	S. paratyphi	V. cholera	S. aureus	B. liherfernis
Benzene		10.8±0.655	10.9±0.200	9.46±0.404	10.56±0.776	11.93±0.152
Acetone		14.9±0.200	13.93±0.152	-	13.43±0.351	14.8±0.264
Aqueous		11.9±0.264	11.76±0.057	9.93±0.230	11.93±0.230	11.76±0.305
Positive Control	Streptomycin	20.46±0.702	18.66±0.057	17.13±0.251	21.7±0.400	20.93±0.585
Negative Control	Distilled water	-	-	-	-	-
	DMSO	-	-	-	-	-

*Zone of inhibition (in mm diameter) including the diameter of well (6 mm) in agar disc diffusion assay. The values are expressed as mean ± standard deviation (n=3) for each sample.

Microorganisms: E.coli-*Escherichia coli* HD_{10}; S.paratyphi-*Salmonella paratyphi* A_2; V.cholera-*Vibrio cholera* 64; S.aureus-*Staphylococcus aureus* NCTC 8530; B.liherfernis-*Bacillus liherfernis* 10341.

Table. 2: Minimum inhibitory concentration (MIC) of *Schima wallichii*(DC.)Korth.fruit extracts

Extract Type	Minimum Inhibition Concentration(µg/ml)				
	E.coli	S. paratyphi	V. cholera	S. aureus	B. liherfernis
Benzene	300	250	200	250	200
Acetone	100	150	ND	100	150
Aqueous	200	250	300	200	250

ND-Not Detected

Microorganisms: E.coli-*Escherichia coli* HD_{10}; S.paratyphi-*Salmonella paratyphi* A_2; V.cholera-*Vibrio cholera* 64; S.aureus-*Staphylococcus aureus* NCTC 8530; B.liherfernis-*Bacillus liherfernis* 10341.

DISCUSSION

In present study a variety of Gram positive and Gram negative bacteria strains were selected for the screening of antibacterial effect of benzene, acetone and aqueous extract of *Schima wallichii* (DC.) Korth. fruit to perceive the antibacterial spectrum. The results of this study showed that the fruit extracts of *Schima wallichii* (DC.) Korth. is active against both Gram positive and Gram negative bacterial strains, which may be indicative of the presence of broad spectrum chemical compounds in this extracts. So in future modern genera may be benefited by using this plant fruits.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENT

The authors are very grateful to Department of Pharmaceutical Technology, Jadavpur University and Department of Pharmacognosy, Himalayan Pharmacy Institute for providing necessary facilities and helps for this research work.

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How to cite this article:

Barma AD, Mohanty JP, Pal P, Bhuyan NR. *In vitro* evaluation of *Schima wallichii* (DC.)Korth.fruit for potential antibacterial activity. J App Pharm Sci, 2015; 5 (09): 124-126.