



Original Research Article

Antibacterial activity of leaf extract of *Tarennia asiatica* (L.) Kuntze ex K.Schum.

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ABSTRACT

Keywords

Resistance monitoring tools, Insecticide, Similarity, Malaria vectors, Benin

The Knowledge regarding the similitude between both WHO and CDC methods, was useful in the assessment of insecticide susceptibility tests in malaria vectors. The current study aimed at an investigation on the similitude between both WHO and CDC methods useful for the determination of the insecticide susceptibility in malaria vectors. Larvae and pupae of *Anopheles gambiae s.l.* mosquitoes were collected from breeding sites in Littoral and Alibori departments. WHO susceptibility tests were conducted on unfed females mosquitoes aged 2–5 days old with impregnated-papers with fenitrothion (1%) and bendiocarb (0.1%) whereas CDC susceptibility tests were conducted with stock solutions of bendiocarb (12.5µg per bottle) and fenitrothion (50µg per bottle). Both *An. gambiae* Suru-lere and Kandi populations were susceptible to fenitrothion and bendiocarb according to both methods. The current study clearly shows that both WHO susceptibility test and CDC bottle bioassay are two important tools for the monitoring of insecticide resistance, a necessary element of any medium-scale or large-scale deployment of an insecticidal intervention. There is similarity between both methods.

Introduction

The medicinal plants fetch greater significance in the human life because of the widespread belief that green medicines are healthier and safer than the synthetic products. The medicinal plants possess secondary metabolites to inhibit various disease causing microorganisms. The antimicrobial activity is the capacity of the substance to kill or inhibit micro organism. There are several reports (Srinivasan *et al.*, 2001; de Boer *et al.*, 2005; Soneja

et al., 2009) that plants are acting against microorganisms. Hence, in the present study the aqueous and ethanol leaf extracts of *Tarennia asiatica* belongs to Rubiaceae are used against five bacterial organisms.

Materials and Methods

Fresh leaves were washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

For aqueous extraction, 10g of air-dried powder was added to distilled water and boiled on slow heat for 2 hours. It was then filtered through 8 layers of muslin cloth and centrifuged at 5000 rpm for 10min. The supernatant was collected. This procedure was repeated twice. After 6hours, the supernatant collected at an interval of every 2 hours, was pooled together and concentrated to make the final volume one-fourth of the original volume (Parekh *et al.*, 2005). It was then autoclaved at 121° C temperature and at 15 lbs pressure and stored at 4°C. For solvent extraction, 10g of air-dried powder was taken in 100ml of ethanol in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 190-220 rpm for 24h. After 24 hours the supernatant was collected and the solvent was evaporated to make the final volume one-fourth of the original volume and stored at 4°C in airtight bottles.

Bacterial culture

Pure culture of the human pathogenic bacteria such as two gram positive strain, *Bacillus subtilis*, *Staphylococcus aureus* and three gram negative strain *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* obtained from MTCC Chandigarh were used for the present *in vitro* antimicrobial assay.

Determination of Antibacterial assay

Antibacterial activity of the aqueous and ethanol extracts of the leaf sample was performed by the agar well diffusion method (Perez *et al.*, 1990). The bacterial cultures were adjusted to 0.5 McFarland turbidity standards and inoculated on to Muller-Hinton Agar (MHA) plates. A well was prepared in the plates with the help of cork borer (0.85cm). 10µl of the test compounds was introduced in to the well. A well was

made in each of the culture plates and filled with 20 µl of 10 mg/ml of gentamycin as positive control and sterile water and ethanol served as a negative control. Antimicrobial activity was determined by measuring the zone of inhibition around each well. For each extract three replicate trials were conducted against each organism and the mean values were statistically analysed and recorded.

Results and Discussion

The results of the antimicrobial testing using agar well diffusion method are presented in Table 1. The results showed that the ethanol and aqueous leaf extracts of *Tarenna asiatica* possessed antimicrobial activity.

The growth of *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis* was controlled by the leaf extract of *Tarenna asiatica*. Ethanol as well as aqueous extracts presented notable antibacterial activity against test organisms. But the extract in organic solvent showed more growth inhibitory activity on the bacteria than the aqueous extract. *Bacillus subtilis* and *Staphylococcus aureus* were most sensitive to both the extracts, while *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis* were slightly sensitive only to ethanol extract.

It is interesting to note that the values of the positive control Gentamycin obtained in this study were more or less equal to the values of *Bacillus subtilis* and *Staphylococcus aureus* in the ethanol extract. Both the ethanol and water (negative control) recorded no inhibition zone.

The demonstration of antibacterial activity against both gram positive and gram negative bacteria is an indication that the

plant is a potential source for production of drugs with a broad spectrum activity. The test organisms used in this study are associated with various forms of human infections. From a clinical point of view, *Klebsiella pneumoniae* is the most important member of the *Klebsiella* genus of Enterobacteriaceae and it is emerging as an important cause of neonatal nosocomial infection. *Escherichia coli* causes septicemias and can infect the gall bladders, meninges, surgical wounds, skin lesions and the lungs, especially in debilitate and immuno deficient patients (Black, 1996). *Proteus mirabilis* causes wound infections and urinary tract infections in the elderly and young males often following catheterization and cystoscopy and it is a secondary invader of ulcers and pressure sores (Cheesebrough, 2000).

Hence the usage of leaf extract of *Tarenna asiatica* against the human pathogenic bacteria may claim the therapeutic value of the plant. The results of the experiment thus justify the use of *Tarenna asiatica* in the traditional system of medicine for the treatment of various infectious skin diseases caused by human pathogenic bacteria. The results reveal that both ethanol and aqueous extracts were more active against *Bacillus subtilis* and *Staphylococcus aureus* than *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis*. Among the extracts, ethanol leaf extract had exhibited higher inhibitory activity than the aqueous extract. The results of this study showed that the ethanol extract was more effective than aqueous extract. This may be due to the better solubility of the active components in organic solvents as reported by de Boer *et al.* (2005).

This observation can be rationalized in terms of the polarity of the compounds being extracted by the solvent in addition to their intrinsic bioactivity, by their ability to dissolve or diffuse in the different media used in the assay. The growth media also seem to play an important role in the determination of the antibacterial activity. Lin *et al.* (1999) reported that Muller-Hinton agar appears to be the best medium to explicate the antibacterial activity and the same was used in the present study.

Basavaraja *et al.* (2011) reported that *Mussaenda frondosa* bark extract exhibited almost equipotent antibacterial activity as compared with that of standard drug. This is in line with the present study that the positive control Gentamycin showed more or less equipotent zone of inhibition with the ethanol extract only. The inhibitory activity may be due to the inhibitory effect of compounds on cell wall synthesis and nucleic acid production as reported by Hammer (1999). The above said antibiotic is reported to have inhibitory effect on cell wall synthesis and nucleic acid production. Generally glycosides, terpenoids and flavonoids are implicated in general resistance as anti pathogen principles. Such principles may be present in the *Tarenna asiatica*.

In the present work, it was confirmed that the leaves of *Tarenna asiatica* possess antibacterial property. Subsequently, pure chemical compounds responsible for this activity have to be identified; isolated and finally using these compounds as prototypes, synthetic chemical entities will be developed that will possess even greater bacterial activity.

Table.1 Antibacterial activity of ethanol and aqueous leaf extracts of *Tarenna asiatica*

S.No	Name of organism	Zone of inhibition (mm)				
		Ethanol extract	Aqueous extract	Gentamycin	Negative control	
				control	Ethanol	Water
	Bacteria					
1	<i>Bacillus subtilis</i>	17±0.8	13±0.11	18±0.3	-	-
2	<i>Staphylococcus aureus</i>	18±0.4	14±0.12	19±0.1	-	-
3	<i>Escherichia coli</i>	13±0.21	10±0.11	18±0.21	-	-
4	<i>Klebsiella pneumoniae</i>	12±0.2	10±0.14	17±0.11	-	-
5	<i>Proteus mirabilis</i>	10±0.11	9±0.02	18±0.12	-	-

Values are Mean ± SE (n=3)

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