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Research article

Comparative studies on the antimicrobial activity of *Terminalia arjuna* and *Aloe vera* against community associated drug resistant *Staphylococcus aureus* in pus from carbuncles of adults

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Key words: *Staphylococcus aureus*, Antibiotic susceptibility, *Terminalia arjuna, Aloe vera.*

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Abstract

The emergence of methicillin (oxacillin) resistant *Staphylococcus aureus* (MRSA) causes a serious health problem in developing as well as developed countries. Present study was conducted to isolate community associated *Staphylococcus aureus* in the pus from the carbuncles of adult persons including their antibiotic susceptibility and to study the antimicrobial effect of aqueous extract of bark of *Terminalia arjuna* and *Aloe vera* gel against drug resistant *Staphylococcus aureus*. Pus samples from carbuncles of adult persons of both sexes belonging to low socio-economic condition were collected and *Staphylococcus aureus* were identified through several biochemical tests. Antibiotic susceptibility and antimicrobial effect of aqueous extract of bark of *Terminalia arjuna* and *Aloe vera* gel were studied by agar well diffusion method. Moreover, present study revealed that 49 (65.3%) and 47 (62.67%) of *Staphylococcus aureus* isolates were resistant to Erythromycin and Clindamycin respectively while 39 (52.0%) *Staphylococcus aureus* were resistant for both Erythromycin and Clindamycin. Beside this 19 (48.72%) of them (resistant to both Erythromycin and Clindamycin) were also resistant to Methicillin and 11 (57.89%) of the Methicillin-resistant *Staphylococcus aureus* isolates were observed to be resistant to Vancomycin. Present study revealed that community associated Vancomycin resistant *Staphylococcus aureus* (which are also MRSA) were present in carbuncle. It was observed that aqueous extract of bark of *Terminalia arjuna* had good antimicrobial role against the Vancomycin resistant *Staphylococcus aureus* (which were also MRSA) whereas *Aloe vera* gel was found to be less important against them.

Introduction

Staphylococcus aureus is reported to cause pyogenic infection in man and is the common cause of boils, carbuncles, impetigo, infection of surgical or accidental wounds and burns and device associated infections [1-2]. Beside this, the organisms are able to colonize healthy individuals asymptomatically [3]. Now multidrug resistant human pathogenic bacteria which are commonly reported fall the developing as well as developed countries in an alarming condition due to indiscriminate use of antibiotics [4].

It is reported that community-acquired multidrug resistant *Staphylococcus aureus* are found to be increased leading to Staphylococcal scalded skin syndrome [5]. Boucher and Corey (2008) [6] reported that the frequency of methicillin resistant *Staphylococcus aureus* (MRSA) infections is found to be increased continuously in both hospital and more recently, in community settings and incidence of community acquired MRSA (CA-MRSA) is increased [7-8]. Additionally, vancomycin-resistant *Staphylococcus aureus* (VRSA) is found to be increased globally which causes increased morbidity and mortality [9-10]. Hence this adds urgency to the research to develop new infection fighting strategies. Additionally the antimicrobial properties of the medicinal plants are being increasingly reported recently from different parts of the world [11-13]. So, researchers are involved to turn their attention to medicinal plants for the development of better drugs against microbial infections [14].

Terminalia arjuna is a deciduous large sized fluted tree belonging to Combretaceae family which is found throughout the South Asian region. In India it is an exotic tree and one of the most versatile medicinal plants having a wide spectrum of biological activity. The bark of *Terminalia arjuna* is reported to act as anti-dysentric, antipyretic, astringent, cardiotonic, lithotriptic, anticoagulant, hypolipidemic, antimicrobial [15] and antiuremic [16] agent. On the other hand *Aloe vera* is a green color plant having thick, fleshy, tapered, spiny, marginated and dagger shaped leaves [17] which is grown from a short stalk near ground level. Beside this it is reported to be the most widely recognized herbal remedy which helps to stimulate the body's immune system [12].

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Depending on this background the present study was conducted to isolate community associated *Staphylococcus aureus* in the pus from the carbuncles of adult persons of both sexes belonging to low socio-economic condition from Gandhi colony, Berhampore, India including their antibiotic susceptibility and to study the antimicrobial effect of aqueous extract of bark of *Terminalia arjuna* and *Aloe vera* gel against drug resistant *Staphylococcus aureus*.

Experimental

Materials and methods

Collection of samples

Pus samples were collected from carbuncles of 27 adult subjects of both sexes (age group of 21-50 years) belonging to low socio-economic condition from Gandhi colony, Berhampore, Murshidabad, India during the period of March 2017 to June 2017. This study was conducted as per the human ethical guidelines and pus samples were taken with informed consent from the subjects and institutional authority.

Bacterial strains and culture method

Pus samples which were collected aseptically, spread on the LB agar media and incubated overnight at 37ºC. The bacterial colonies obtained from the pus samples were cultured on sheep blood agar and incubated overnight at 37ºC.

Identification

Bacterial isolates which were obtained from pus samples were undergone several tests i.e., Gram stain, colony morphology, catalase, coagulase, heat-stable nuclease production on thermonuclease test agar, mannitol salt agar fermentation to identify as *Staphylococcus aureus* [18-20].

Antibiotic susceptibility

Antibiotic susceptibility test was performed against the *Staphylococcus aureus* isolates by disc diffusion method. *Staphylococcus aureus* isolates were spread on tryptic soy agar and antibiotic discs for Erythromycin (15 µg), Clindamycin (2 µg), and Vancomycin (30 µg) (HiMedia Laboratories Pvt. Ltd., India) were used according to standard recommendation [21]. Isolates were considered as susceptible and resistant according to interpretative criteria given by the Clinical and Laboratory Standards Institute (CLSI) (2005) [22].

Methicillin-resistant *Staphylococcus aureus* (MRSA) was identified according to the method described by the CLSI, using Tryptic soy agar plate supplemented with NaCl (0.68 mol/L) and Oxacillin (6 mg/ml, w/v) (HiMedia Laboratories Pvt. Ltd., India) [23].

Collection of plant material

Terminalia arjuna and *Aloe vera* plants were selected for the evaluation of antimicrobial activity against *Staphylococcus aureus* isolates. Bark of *Terminalia arjuna* was collected from the square field, Berhampore, Murshidabad, India. Leaves of *Aloe vera* were collected from the department of Botany, Krishnath College, Berhampore, Murshidabad, India. Both types of plant specimens were authenticated by the department of Botany, Krishnath College, Berhampore, Murshidabad, India. These specimens have been preserved by the department for future references. Authentication No. for Aloe vera is KNC/BOT 2 and Arjun is KNC/BOT 20.

Preparation of extract

10 g of bark of *Terminalia arjuna* which was collected was washed thoroughly in running tap water, rinsed in sterile distilled water and shade dried in open air and ground to powder. Then the powder was extracted by maceration in 100 ml of hot sterile double distilled water. Then the preparation was filtered through a Whatman No. 1 filter paper and finally the plant extracts obtained were allowed to concentrate under vacuum at 40°C and stored at 4°C in labeled sterile bottles until further use. The extract was dissolved in DMSO treated with sterile double distilled water during the antimicrobial assay [24]. In case of Aloe vera, mature, healthy and fresh leaves were washed in the running tap water for 5 min. After rinsing with sterile distilled water, leaves were dissected longitudinally and the colourless aloe gel was scraped out using a sterile knife. The mortar and pestle were used to ground aloe gel with DMSO [25]. The extract was centrifuged at 5000 rpm for 5 min. The supernatant was collected and stored in refrigerator at 4°C.

Antimicrobial assay

The antimicrobial activities of the plant extracts are determined using Agar well diffusion method [26] with further modifications in our laboratory.0.1 ml of diluted inoculum (105 CFU/ml) of each pathogenic *Staphylococcus aureus* isolate (Erythromycin, Clindamycin, Methicillin and Vancomycin resistant) was spread on the Nutrient agar plates and also MSA plates. Wells of 2 mm diameter were made in the agar plates and also MSA plates. 40 µl of the plant extracts were added to the wells made in the plate and all plates were kept aerobically at 37°C for 24-48 h in incubator. Antimicrobial activity was evaluated by measuring the zone of inhibition (mm) against the VRSA (which are also resistant to Erythromycin, Clindamycin and Methicillin) strains. All data on antimicrobial activity were average of triplicate.

Phytochemical screening

Phytochemical analysis of secondary metabolites was carried out qualitatively according to the standard methods [27-28].

Results and discussion

Plant extracts were suggested to be the potential sources of antimicrobial compounds especially against bacterial pathogens [29]. Besides suggestion was there that a medicinal herb can be viewed as a synthetic laboratory as it produces and contains a number of chemical compounds and can be used against *Staphylococcus aureus* due to their availability, fewer side effects and reduced toxicity [30-31]. In the present study, out of the 105 bacterial isolates in pus collected from carbuncles of adult subjects of both sexes (age group of 21-50 years) belonging to low socio-economic condition 75 (71.42%) bacterial isolates were identified as *Staphylococcus aureus* through Gram staining, Catalase test, coagulase test, heat-stable nuclease production on thermonuclease test agar, Mannitol salt fermentation.

Moreover, present study revealed that 49 (65.3%) and 47 (62.67%) of *Staphylococcus aureus* isolates were resistant to Erythromycin and Clindamycin (Table 1) respectively while 39 (52.0%) *Staphylococcus aureus* were found to be resistant to both Erythromycin and Clindamycin (Table 1) which were usually used for the treatment of skin infection [32-33]. This may be due to different geographic location [34]. Moreover, it was found that 19 (48.72%) of these *Staphylococcus aureus* isolates (resistant to both Erythromycin and Clindamycin) were also resistant to Methicillin (Table 1) on agar plate containing Oxacillin (Table 1). Additionally in the present study 11 (57.89%) of the Methicillin-resistant *Staphylococcus aureus* isolates were observed to be resistant to Vancomycin (Table 1 and Figure 1).



Figure 1. "**Vancomycin-resistant "*Staphylococcus aureus* isolates on agar plate.**

All these findings demonstrated that multidrug resistant *Staphylococcus aureus* were present in the pus from carbuncles. It was reported that emergence of multidrug resistant *Staphylococcus aureus* in a community is due to acquiring mobile genetic element by a locally prevalent antibiotic susceptible strain [35]. Suggestion was there that indiscriminate use of antibiotics has become the major factor for the emergence and dissemination of multidrug resistant strains of several groups of microorganisms [36]. Several reports also had shown that incidence of community acquired MRSA (CA-MRSA) was increased [6-8]. According to several reports low level and intermediate Vancomycin-resistant *Staphylococcus aureus* (VRSA) have been found to be emerged in north India [37-38].

Whether aqueous extract of bark of *Terminalia arjuna* and *Aloe vera* gel had any role against the Vancomycin-resistant *Staphylococcus aureus* (VRSA) (which are also resistant to Erythromycin, Clindamycin and Methicillin) antimicrobial activities of them were studied. It was surprisingly found that *Terminalia arjuna* had good antimicrobial activity against all VRSA (Table 2 and Figure 2) and maximum zone of inhibition was found against the SR1 strain (Table 2). In the present study phytochemical screening revealed that phytosterol, lactones, flavonoids, phenolic compounds, tannins and glycosides were present in the aqueous extract of bark of *Terminalia arjuna*. These findings indicate that antimicrobial activity of aqueous extract of bark of *Terminalia arjuna* against the Vancomycin-resistant *Staphylococcus aureus* may be due to presence of flavonoid. Mandal*et al.,* (2013) [39] and Jaiswal *et al.,* 2015 [40] also reported that flavonoid extract of bark of *Terminalia arjuna* shows antimicrobial activity. Aneja *et al.,* (2012) [41] also said that aqueous extract of *Terminalia arjuna* shows the antimicrobial activity against *Staphylococcus aureus*. Nema *et al.,* (2012) also reported that many useful phytoconstituents have been isolated from *Terminalia arjuna* which include triterpenoids for cardiovascular properties, tannins and flavonoids for its anticancer, antimicrobial properties [42].

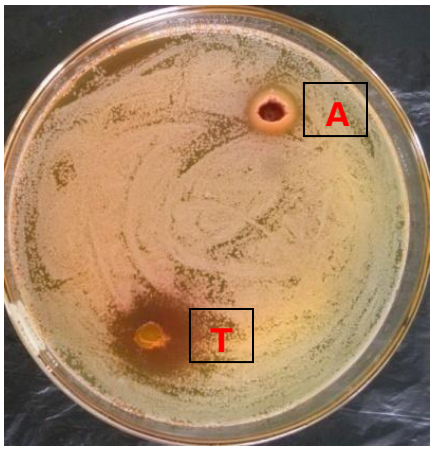


Figure 2. "**Erythro^R Clin^R Meth^R Vanc^R "*Staphylococcus aureus* isolate on MSA plate and aqueous extract of Bark of *Terminalia arjuna* (T) and *Aloe vera* (A) gel.**

Table 1. *Staphylococcus aureus* isolates and antibiotic susceptibility of *Staphylococcus aureus*

Staphylococcus aureus isolates (%)	Antibiotic susceptibility				
	Erythro^R strain	Clind^R strain	Both Erythro^R Clin^R strain	Meth^R among Erythro^R Clin^R strains	Vanc^R strain among Meth^R Erythro^R Clin^R strains
71.42% (75)	49 (65.3%)	47 (62.67%)	39 (52.0%)	19 (48.72%)	11 (57.89%)

N.B: R means resistant; Erythro=Erythromycin; Clin=Clindamycin; Meth=Methicillin; Vanc=Vancomycin

Table 2. Antimicrobial activity of aqueous extract of Bark of *Terminalia arjuna* and *Aloe vera* gel against "Erythro^R Clin^R Meth^R Vanc^R "*Staphylococcus aureus* isolates

Strains	Aqueous extract of Bark of *Terminalia arjuna* (Zone of inhibition in mm)	Aqueous extract of *Aloe vera* gel (Zone of inhibition in mm)
SR1	15.0 mm	No zone
SR2	14.0 mm	10.0 mm
SR3	11.0 mm	No zone
SR4	14.5 mm	No zone
SR5	12.5 mm	No zone
SR6	13.5 mm	No zone
SR7	14.0 mm	No zone
SR8	10 mm	No zone
SR9	10.5 mm	No zone
SR10	10 mm	No zone
SR11	12.5 mm	No zone

On the other hand it was observed that aqueous extract of *Aloe vera* gel had antimicrobial activity against only one (10%) (SR2 strain) of these drug resistant *Staphylococcus aureus* strains mentioned earlier (VRSA) (Table 2 and Figure 2). Moreover present study had shown that aqueous extract of *Aloe vera* gel possessed secondary metabolites such as triterpenoid, flavonoid, phenolic compounds, tannin, alkaloid, saponin (Table 3). These findings demonstrated that *Aloe vera* gel extract had antimicrobial activity against the antibiotic resistant strain (SR2 strain) in the study which may be due to presence of secondary metabolites such as steroid, triterpenoid, flavonoid, phenol, tannin, alkaloid, saponin (Table 3). It was also described earlier by Roopashree*et al.,* (2008) [43]. *Aloe vera* gel extract was proved to possess compounds with antimicrobial properties which could be used as an antimicrobial agent [44-45]. But it was also found that aqueous extract of *Aloe vera* gel was not effective for the rest of VRSA strains. On the other hand this present study revealed that inhibitory zone was larger in case of aqueous extract of bark of *Terminalia arjuna* than the *Aloe vera* gel (Table 2) against the VRSA (SR2) (which are also resistant to Erythromycin, Clindamycin and Methicillin). All these findings demonstrated that herbal products containing *Terminalia arjuna* can be used to treat carbuncle infected with VRSA and also MRSA as a better option than the *Aloe vera* gel against the VRSA in the present study.

Table 3. Secondary metabolites in aqueous extract of Bark of *Terminalia arjuna* and *Aloe vera* gel

Secondary metabolites	Aqueous extract of Bark of *Terminalia arjuna*)	Aqueous extract of *Aloe vera* gel
Steroid	-	+
Triterpenoid	+	+
Flavonoid	+	+
Phenolic Compounds	+	+
Alkaloid	+	+
Saponin	+	-
Phytosterol	-	+
Lactones	-	+
Tannins	+	+
Glycosides	-	+

Conclusion

Present study revealed that community associated Vancomycin resistant *Staphylococcus aureus* (which are also MRSA) were present in carbuncle. It was observed that aqueous extract of bark of *Terminalia arjuna* was more potent as an antimicrobial active against the Vancomycin resistant *Staphylococcus aureus* (which are also MRSA) isolates than the aqueous extract of *Aloe vera* gel.

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