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# ANTIMICROBIAL ACTIVITIES OF SOME MEDICINAL PLANTS OF WEST BENGAL

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## ABSTRACT

In rural and backward area of West Bengal, India several plants are commonly used as herbal medicine for the treatment of infectious diseases. Four such plants commonly used by the people of the area were screened for potential antibacterial activity. Antibacterial activity of both aqueous and methanol extracts of the plants parts were used for screening. The plants screened were *Psidium guajava*, *Andrographis paniculata*, *Terminalia arjuna* and *Adhatoda vasica*. Antibacterial activity was tested against six strains of both Gram positive and Gram negative bacteria. The susceptibility of the microorganisms to the extracts of these plants was compared with each other and with selected antibiotics. The result showed that, the methanol extracts of selected medicinal plants exhibited high activity against the tested organisms rather than aqueous extract of those plants. So, the minimum inhibitory concentration (MIC) of the methanol extract of selected plants was studied. Extract from *Terminalia arjuna* showed higher antimicrobial activity than the extract of *Psidium guajava*.

**KEY WORDS:** *Psidium guajava*, *Andrographis paniculata*, *Terminalia arjuna*, *Adhatoda vasica*, Pathogen, Antibacterial activity.

## INTRODUCTION

Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being. In our country we are using crude plants as medicine since Vedic period. A major part of the total population in developing countries still uses traditional folk medicine obtained from plant resources (Srivastava et al, 1996).

Nowadays, multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease (Service, 1995). In addition to this problem, antibiotics are sometimes associated with adverse effects on the host, including hypersensitivity, immune-suppression and allergic reactions (Ahmad et al, 1998). This situation forced scientists to search for new antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance (Monroe and Polk, 2000), there is a constant need for new and effective therapeutic agents (Bhavnani and Ballow, 2000). Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants (Cordell, 2000). Several screening studies have been carried out in different parts of the world. There are several reports on the antimicrobial activity of different herbal extracts in different regions of the world (De Boer et al, 2005).

World wide, infectious disease is the number one cause of death accounting for approximately one-half of all deaths in tropical countries. Perhaps it is not surprising to see these statistics in developing nations, but what may be remarkable is that infectious disease mortality rates are actually increasing in developed countries, such as the United States (Pinner et al, 1996).

Herbal drugs have become increasingly popular and their use is widespread. Clear-cut proof of their efficacy in microorganisms inducing pathogenesis is yet to be explored. Various medicinal plants have been used for years in daily life to treat disease all over the world. Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times (Farombi, 2003). Over 50% of all modern clinical drugs are of natural product origin (Stuffness and Douros, 1982) and natural products play an important role in drug development programs in the pharmaceutical industry (Baker et al, 1995). It has been suggested that aqueous and ethanolic extracts from plants used in allopathic medicine are potential sources of antiviral, antitumoral and antimicrobial agents (Vlietinck et al, 1995).

The use of plant extract for their antimicrobial action has been subjected of research in India by some workers. The plant extracts of *Aegle marmelos, Mormordica indica*, *Calotropis procera* (Pattnaik and Sharma, 2004), *Catharanthus roseus* (Bera and Saha, 1983) and *Azsadirachta indica. Tamarindus indica, Zingiber officinales, Curcuma longa, Allium sativum* (Rao and Satyanarayan, 1977) have shown profound antibacterial activities.

In less developed states of India and particularly in West Bengal, low income people such as farmers, people of small isolate villages and native communities use herbal medicine for the treatment of common infections. It is necessary to evaluate, in a scientific base, the potential use of herbal medicine for the treatment of infectious diseases produced by common pathogens.

In the present study we have chosen some plants used in herbal medicine to determine their antibacterial property. Evidently, there are not sufficient scientific studies that confirm the antimicrobial activity of these plants. This study

looks into the *in vitro* antimicrobial activity of these plants against some gram positive and gram negative pathogenic microorganisms that causes the most common cases of infectious diseases of the rural and backward communities of West Bengal, India.

## MATERIALS AND METHODS

**Selection of medicinal plants for this study:**
In the present work a few selected medicinal plants were screened for potential antibacterial activity. These are as follows:

***Psidium guajava***
**Family:** Myrtaceae
**Parts used:** Leaf
**Traditional uses**: Diarrhea, dysentery, gastroenteritis, anti cough, ulcers, bowels , cholera , hypoglycemic, anti-inflammatory, analgesic, antipyretic,

***Andrographis paniculata***
**Family:** Acanthecae
**Parts Used:** Leaf
**Traditional uses**: Digestive, Hepatoprotective, Vermicidal, Anti-acne, Analgesic, Anti-inflammatory Hypoglycemic and Immune Enhancemen.

***Terminalia arjuna***
**Family**: Combretace
**Parts used**: Bark
**Traditional uses:** Cardiovascular diseases, myocardial infarction, degenerative neurological diseases, cancer, amyloidosis, acute pancreatitis, arthritis, atherosclerosis, inflammatory bowel disease, diabetes, senile dementia, retinal degeneration and senile cataract.

***Adhatoda vasica***
**Family:** Acantheceae
**Parts Used:** Leaf
**Traditional uses**: Asthma, dermatitis, antispasmodic and chronic bronchitis.

**Identification and Preservation of Plant materials:**
Fresh plants parts were collected from the different rural and backward area of West Bengal, India. The taxonomic identities of this plant were determined by the expertise of the Department of Botany of our University. Each specimen were labeled, numbered and noted with date of collection, the locally and their medicinal uses and their approximate dosages of administration were recorded. Plant parts were washed with 70% alcohol and then rinsed with sterilized distilled water, air dried and stored in airtight bottles at 4$^{o}$C for further use.

**Preparation of extracts:**
Clean dry plant samples were collected in a cotton bags. The materials were grinded to fine power with the help of mixer grinder. Then these powered materials were used for the preparation aqueous and methanol extracts.

a) **Preparation of aqueous extract**
2 gm of powered materials were mixed with 20 ml of sterile distilled water and kept on a rotary shaker for 12 hours at 30$^{o}$C. Thereafter, it was filtered with the help of Whatman No. 1 filter paper. The filtrate was then centrifuged at 2000 rpm for 10 min. Then the supernatant was collected and stored at 4$^{o}$C for further use.

b) **Preparation of methanol extract**

10 gm. of powered materials were soaked in 30 ml of 70% methanol and were kept at 30$^{o}$C for 12 hours on a rotary shaker. After 12 hours the previous portion of added methanol was evaporated so to make the same volume methanol was added and then it was placed on a rotary shaker for another 12 hours at 30$^{o}$C. After that it was filtered through Whatman No. 1 filter paper. The filtrate was centrifuged at 2000 rpm for 10 min. The supernatant was collected

and the supernatant was allowed to evaporate until completely dry. The extracts were kept sterile bottles under refrigerated condition until use. Then 30 mg of dry extract was resuspened in 1 ml of 70 % methanol. The final concentration of the extract was 30 mg/ml.

**Microorganisms used:**
Two gram positive (*Staphylococcus aureus and Bacillus cereus)* and four gram negative (*Escherichia coli, Vibrio cholerae, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) pathogenic bacterial samples were collected from the Department of Microbiology, Vidyasagar University, Midnapore. The organisms were sub-cultured in nutrient broth and nutrient agar for use in experiment.

***In vitro* Antibacterial Study:**
Following methods were performed to determine the antimicrobial activity of plant extracts –

a) The modified agar-well diffusion method of Cappuccino and Sherman (1999) was employed to study the antibacterial activity of the plant extracts. 3.7% of Muller Hinton Agar was mixed with hot distilled water and autoclaved at 15 lb pressure for 15 minutes. After autoclaving, it was allowed to cool to 45$^{o}$C-50$^{o}$C. Then the medium was poured into sterilized Petri dishes with a uniform depth of approximately 4 mm. The agar medium was allowed to cool to room temperature. To standardize the inoculums density for sensitivity test, a $BaSO_4$ turbidity standard, equivalent to 0.5 Mac Farland standards were used. For the transformation of bacteria to Petridish a swab dipped in standard inoculums was used. After dipping, the swab was used to spread the bacteria on the media in a confluent lawn. Then the Petri dishes were left for 3 to 5 minutes. Using cork borer, 6 mm diameter wells were made in all the plates. Different extracts were added to the groove with one blank of each. Plates were incubated for 24 hours at 37$^{o}$C. After 24 hours the plates were examined. Results were recorded, as the presence or absence of inhibition zone. The inhibitory zone around the well indicated absence of bacterial growth and it was reported as positive and absence of zone is negative. The diameters of the zones were measured using diameter measurement scale. The effect of plant extract was compared with that of standard antibiotic tetracycline and levofloxacin.

b) The Minimum inhibitory concentration (MIC) was evaluated by dilution method (Prescott et al, 2005) on plant extracts to observe the antimicrobial activity. Anti-bacterial agents were incorporate in different concentration with liquid media. These media were inoculated with the test bacteria and incubated. The lowest dilution at which there is no growth of organisms is considered significant. The turbidity of the test sample is measured by spectrophotometer with respect to blank.

**Statistical Analysis:**
Since the readings of control (distilled water) in the *in vitro* antibacterial studies of medicinal plant were zero, the data was analyzed by simple arithmetic means of the different extracts and standard error was compared to the control. No other statistical test was applied to show significance since the extracts were either positive or negative for the antibacterial studies.

## RESULTS

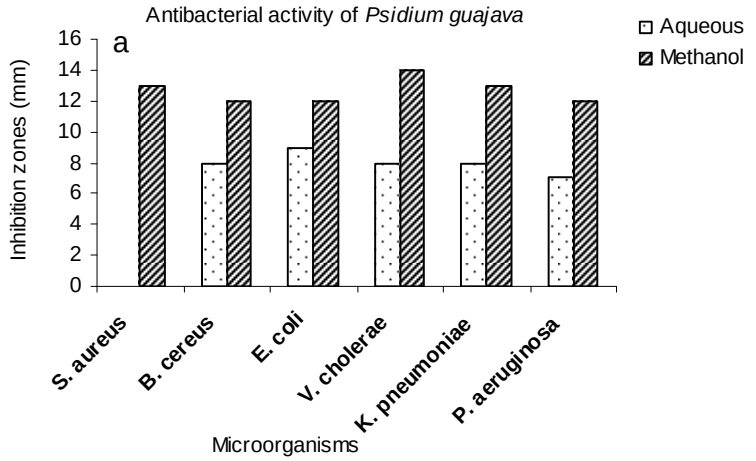
The aqueous extract of *Andrographis paniculata* and *Adhatoda vasica* were not found to be active against all organisms tested (Fig. 1b &1d). But the aqueous extract of *Psidium guajava* and *Terminalia arjuna* showed low activity against most of the tested organisms. Aqueous extract of *Psidium guajava* (Fig. 1a &1c) is not active against *Staphylococcus aureus*.

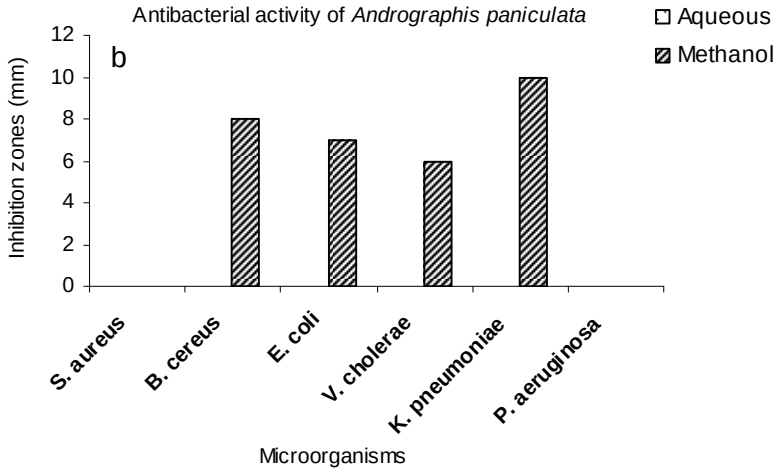
The methanol extract showed different levels of antimicrobial activity toward test organisms. The methanol extract of *Psidium guajava* showed highest antimicrobial activity against all the

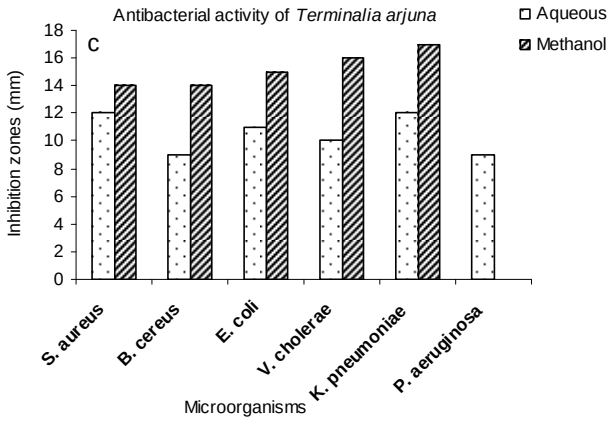
tested organisms. The methanol extract of *Andrographis paniculata* exhibited low antimicrobial activity against *Bacillus cereus*, *Escherichia coli*, *Vibrio cholerae* and *Klebsiella pneumoniae* and is inactive against rest of the test organisms (Fig. 1a & 1b).

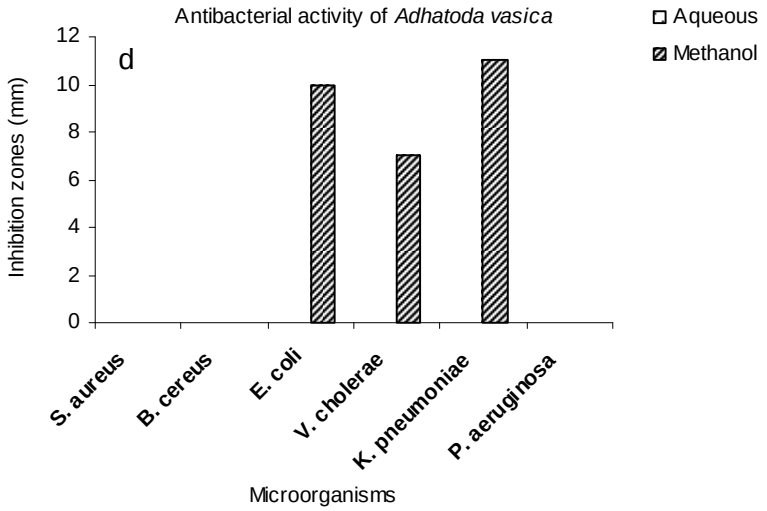
Methanol extract of *Terminalia arjuna* was active against all the test organisms except *Pseudomonas aeruginosa* (Fig. 1c). On the other hand, it was found that the methanol extract of *Adhatoda vasica* exhibited low activity against *Escherichia coli*, *Vibrio cholerae* and *Klebsiella pneumoniae* and was inactive against *Staphylococcus aureus*, *Bacillus cereus* and *Pseudomonas aeruginosa* (Fig. 1d)

To screen the antibacterial activity against tested organisms, tetracycline and levofloxacin were used as a standard. It was found that levafloxacin (5µg/ml) standard showed higher activity than tetracyclin (30µg/ml) standard against tested microorganisms (Fig. 1e).




Antibacterial activity of *Terminalia arjuna*
c
Aqueous
Methanol
Inhibition zones (mm)
0
2
4
6
8
10
12
14
16
18
S. aureus
B. cereus
E. coli
V. cholerae
K. pneumoniae
P. aeruginosa
Microorganisms


Antibacterial activity of *Adhatoda vasica*
d
Aqueous
Methanol
Inhibition zones (mm)
0
2
4
6
8
10
12
S. aureus
B. cereus
E. coli
V. cholerae
K. pneumoniae
P. aeruginosa
Microorganisms

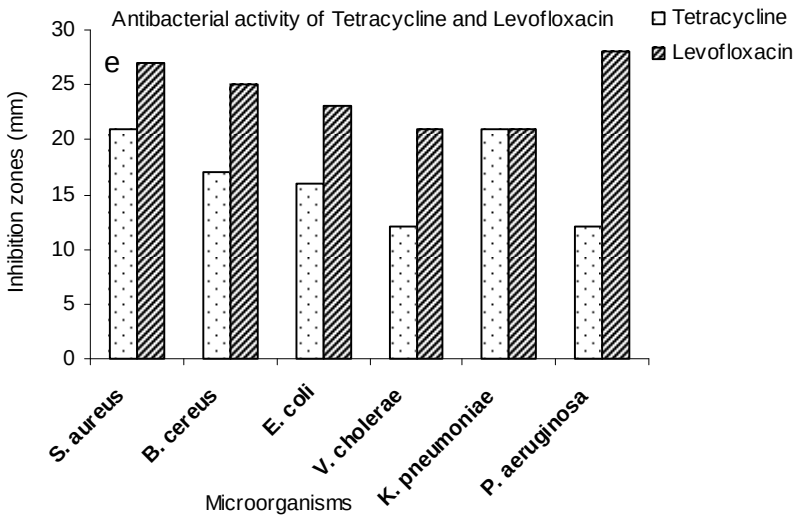

Antibacterial activity of Tetracycline and Levofloxacin
e
Tetracycline
Levofloxacin
Inhibition zones (mm)
0
5
10
15
20
25
30
S. aureus
B. cereus
E. coli
V. cholerae
K. pneumoniae
P. aeruginosa
Microorganisms

***Figure-1: Antibacterial activity of Psidium guajava (a), Andrographis paniculata (b), Terminalia arjuna (c) and Adhatoda vasica (d) in aqueous (2gm/20ml) and methanol (30mg/ml) extracts and also Tetracyclin (30µg/ml) and Levafloxacin (5µg/ml) standards (e) against both gram (+ve) and gram (-ve) microorganisms.***

Table 1 showed the MIC values of methanol extracts of selected plants and the standard levafloxacin. The result indicates that standard antibiotic levafloxacin has much higher antimicrobial activity than the two selected methanol plant extracts. Among the plant samples, extract from *Terminalia arjuna* showed better antimicrobial activity than *Psidium guajava*.

**Table – 1**

The MIC of the *Psidium guajava* and *Terminalia arjuna* methanol extracts and levofloxacin against the microorganisms.

| Microorganisms | MIC (mg/ml) | | |
|---|---|---|---|
| | ***Psidium guajava*** | ***Terminalia arjuna*** | ***Levofloxacin*** |
| *Staphylococcus aureus* | 0.835 ± 0.083 | 0.466 ± 0.014 | 0.039 ± 0.005 |
| *Bacillus cereus* | 0.713 ± 0.062 | 0.528 ± 0.008 | 0.067 ± 0.009 |
| *Escherichia coli* | 1.290 ± 0.042 | 0.625 ± 0.016 | 0.059 ± 0.007 |
| *Vibrio cholerae* | 0.874 ± 0.054 | 0.394 ± 0.015 | 0.031 ± 0.003 |
| *Klebsiella pneumoniae* | 0.792 ± 0.025 | 0.383 ± 0.012 | 0.096 ± 0.008 |
| *Pseudomonas aeruginosa* | 1.261 ± 0.098 | - | 0.062 ± 0.009 |

*Data represents Mean ±Standard Error of Mean*

## DISCUSSION

Successful prediction of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Traditional medicinal plants are use primarily water as the solvent but in our studies we found that plant extracts in organic solvent (methanol) provided more consistent antimicrobial activity compared to those extracted in water. These observations can be rationalized in terms of the polarity of the compounds being extracted by each solvent and in addition to their intrinsic bioactivity. The results of screening are presented in Figure 1. The aqueous and methanol extracts of *Psidium guajava*, *Andrographis paniculata*, *Terminalia arjuna* and *Adhatoda vasica* were subjected to a preliminary screening for antimicrobial activity against six standard bacteria: two gram positive (*Staphylococcus aureus and Bacillus cereus)* and four gram negative (*Escherichia coli, Vibrio cholerae, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). It was clear that the methanol extract of selected medicinal plants exhibited high activity against the tested organisms rather than aqueous extract of those plants. Methanolic extracts of plants generally posses terpines and phenolics, which are reported by different workers as antimicrobial compounds (Dwivedi, 2007 ; Sanches NR et al,

2005 ; Begum et al, 2004 ; Manach et al, 2001 ;). Methanol extract of *Psidium guajava* and *Terminalia arjuna* showed pronounced activity against all the tested gram positive and gram negative microorganisms except *Pseudomonas aeruginosa*. It was surprising that there is difference in the antibacterial activities of the extracts of the different plants. This could be due to the phytochemical differences between them.

Earlier Perumalsamy et al, (1998) showed significant antibacterial activity of aqueous extracts of *Terminalia arjuna* bark against *Escherichia coli, Klebsiella aerogenes, Proteus vulgaris,* and *Pseudomonas aerogenes*. Recently, Parekh et al, (2005) reported that aqueous extract of *Euphorbia hirta* was inactive against both the *B. subtilis* and *S. epidermidis* and also suggested that the aqueous extracts of *Cynodon dactylon*, *Ficus benghalensis* and *Tecomella undulate* were inactive against *Bacillus subtilis, Staphylococcus epidermidis, Pseudomonas pseudoalcaligenes, Proteus vulgaris and Salmonella typhimurium*. Nair et al, (2005) reported that the antibacterial activity of *S. emarginatus* leaf extract of both solvents (aqueous and methanolic) against *P. testosteroni, K. pneumoniae, M. flavus, P. morganii, B. subtilis and S. epidermidis* . The methanolic extract showed considerably more activity than the aqueous extract. Singh et al, (2008) reported the presence of antibacterial principles in the bark of *Terminalia arjuna* with arjunetin particularly exhibiting selectively higher activity against *S. epidermidis*. Devi et al. (2007), evaluated the effect of methanolic extract of *T. arjuna* on diclofenac sodium induced gastric ulcer in experimental rats and concluded that extracts of *Terminalia arjuna* act as gastroprotective agent due to its free radical scavenging activity and cytoprotective nature. Studies have shown that bark of *Terminalia arjuna* contains glycosides, flavonoids, tannins and minerals (Ramya et al, 2008). Guava leaf extract have been shown to be effective against many bacterial species known to cause diarrhea, including *S. aureus, E. coli* and other common entero-pathogenic cultures (Jairaj et al, 1999 ; Coutino-Rodriguez et al, 2001). The methanolic extract of *P. guajava* (leaves) was the only agent showing significant inhibitory (and antidirrhoeal) activities against the growths of *Salmonella* and *Shigella* species and two isolates of enteropathogenic *E. coli* (Lin et al, 2002).

As we have found better result with methalonic extract of *Psidium guajava* and *Terminalia arjuna* against most of the tested pathogens. So, the MIC values of methanol extracts of those plants such as *Psidium guajava* and *Terminalia arjuna* were performed (Table-1). The results showed that the MIC of *Psidium guajava* extract against all the tested organisms varied between 0.713 ± 0.062 and 1.290 ± 0.042 while that *Terminalia arjuna* extract against all the tested microorganisms except *Pseudomonas aeruginosa* ranged between 0.383 ± 0.012 and 0.625 ± 0.016. The standard antibiotic levafloxacin had MIC values varying between 0.031 ± 0.003 and 0.096 ± 0.008. The results indicate that standard antibiotic has stronger activity than the two plants extracts (Table-1). Sanches et al, (2005) reported the different results of MIC values of ethanol:water extracts of *P. guajava* leaves, stem, bark and roots were active against the gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*. The ethanol:water extracts showed stronger antimicrobial activity as compared to aqueous extracts. All extracts were virtually inactive against the gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*. Recently, Shariff et al, (2006) suggested that the MIC of 2.0 and 4.0 mg/ml was found against all the tested bacteria like *Bacillus subtilis, Escherichia coli, Pseudomonas solanacearum, Xanthomonas axonopodis* pv*. malvacearum, Xanthomonas vesicatoria* when absolute alcohol and chloroform extracts of *P. minima* leaf were used.

Guava is rich in tannins, phenols, triterpins, flavonoids, essential oils, saponins, carotenoids,

lectins and all those compounds together showing antimicrobial activities (Kamath et al., 2008). Arjunic acid, arjungenin, arjunetin are some of the compounds of *Terminali arjuna* are reported as antimicrobial compounds (Singh et al., 2008). These findings support the traditional knowledge of local users about their selection of plant samples as antimicrobial agents and it is a preliminary scientific validation for the use of these plants for antibacterial activity. To promote proper conservation and sustainable use of such plant resources, awareness of local communities should be enhanced incorporating the traditional knowledge with scientific findings. The results of the present study also support the medicinal usage of the studied plants and suggest that some of the plant extracts possess compounds with antibacterial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens. The most active extracts can be subjected to isolation of the therapeutic antimicrobials and undergo further pharmacological evaluation.

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