International Journal of Microbiological Research 6 (1): 27-33, 2015
ISSN 2079-2093

DOI: 10.5829/idosi.ijmr.2015.6.1.92214

In vitro Screening of Antibacterial Activities of Selected Ethiopian Medicinal Plants

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Abstract: *In vitro* antibacterial activity of hydro-alcoholic extract of four selected medicinal plants was screened using agar well diffusion and broth dilution techniques. The four medicinal plants were leaf of *Buddleja polystachya* and *Cordia Africana,* stem bark of *Albizia gummifera* and areal part of *Thymus schimperi.* Antibacterial activity was assessed against: *Pasteurella gallinarum, Salmonella gallinarum, Escherchia coli, Manhemia haemolytica, Staphylococcus aureus* and *Streptococcus dysagalactae.* The result of agar well diffusion showed that, hydro-alcoholic extract of *A. gummifera* was active against all tested bacteria, *B. polystachya* and *C. africana* did not inhibit growth of all test organisms. The highest zone of inhibition was exhibited by *A. gummifera* against *E.coli* (23.5mm) and *T. schimperi* against *S. dysagalactae* (23mm). The results of broth dilution method showed that plant extracts exhibited minimum inhibitory concentration (MIC) which varied from 10-10,000 µg/ml. The plant extract *B. polystachya* and *C. Africana* did not show MIC at 10,000 µg/ml against all test organisms. The good antibacterial activity of *A. gummifera* and *T. schimperi* observed in the current study suggests that there is a potential for discovery of novel broad spectrum antimicrobial agents from indigenous medicinal plants of Ethiopia.

Key words: Medicinal Plants · Bacteria · Antibacterial Activities · Hydro-Alcoholic Extracts · MIC

INTRODUCTION

Africa has a long and rich history of traditional plant use where Indigenous plants are still main source of medicine, craft materials and tools in Africa [1]. The branch of traditional medicine most amenable to scientific investigation is herbal medicine. Crude extractions of plants were the original therapeutic interventions used by man to control disease in human and livestock. Nearly all cultures from ancient times to the present day have used plants as a source of medicine. Development of herbal products depends on local botanical flora. As a result, different remedies tended to develop in different parts of the world. In some instances, related plants were used over wide geographical regions as a result of communication or importation of plant materials of high repute [2]. There is now resurgence in interest in natural products as sources of novel compounds to combat the ever present threat of disease. An antimicrobial resistance has become a global problem. Strategies to improve the current situation include research in finding new and innovative antimicrobials [3].

Governments of most countries in Africa have come to recognize the importance of recording traditional knowledge for further development, such as in Ethiopia [1], Tanzania [4] and South Africa [5]. Traditional medicinal plants are also a rich source of inexpensive and novel biologically active compounds [6], which are of great interest to both the developed and developing countries [7]. The evaluation of the efficacy, safety and dosage of traditional medicines is crucial, due to the reliance of the African population on plants as sources of medicines [8].

Ethiopia is a land of great topographical diversity, which is responsible for tropical, temperate and subtropical conditions. It is a land of multiplicity flora and fauna. This environmental mosaic accentuated the diversity of plant and animal life [9]. For centuries, most of

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the populations in Ethiopia, like other developing countries, have relied on a system of traditional medicine, which consists of both emperico-rational and magico-religious elements and at times a combination of both [10]. Tollosa [11] has identified and documented 37 herbal plant species from central high lands of Ethiopia used as remedy for various animal diseases. In developing countries like Ethiopia, cost of treatment is an important determinant of the use of veterinary drugs. Moreover, they cannot deliver complete coverage in preventive and curative health care practices because of inadequate labor, logistic problems and an erratic supply of drugs. Consequently, the majority of those raising stock in rural areas are far from site of veterinary stations so that applying veterinary herbal medicine is the only option they have [12]. Infectious diseases, which account for the significant proportion of the health problems, are most often catered for by this system of medicine [10]. *In view* of this, the search for new anti-microbial agents from medicinal plants is even more urgent in the context of countries like Ethiopia where infectious disease of bacterial and fungal are also developing an increasing resistance against many of the commonly used anti-microbial agents [9,13,14].

The most important characteristics of traditional herbal therapies is their crude preparations. A herb with different ingredients and properties is administered in its natural state in the form of water mixture, boiled or unboiled and is applied topically as pastes as well as other forms. This herbal heritage passes from generation to generation orally rather than in writings. With the aging and subsequent death of herbalists, it may become very difficult to get reliable information about the herbs in the future [15]. Therefore, the objectives of this study were: To evaluate the *In vitro* antibacterial property of *Albizia gummifera, Buddleja polystachya, Cordia africana* and *Thymus schimperi* against *P. gallinarum, S. gallinarum, E. coli, M. haemolytica, S. aureus and S. dysagalactae.*

MATERIALS AND METHODS

Study Area: The major component of the study, i.e. *In vitro* screening of antibacterial activity of selected medicinal plants was conducted at National Animal Health Diagnostic and Investigation Center (NAHDIC), Sebeta, Ethiopia. It is located 25 Kms west of Addis Ababa in Oromia region, Addis Ababa Zuria Leyu Zone. The mean annual temperature and rainfall ranges between 15°C-21°C and 800mm-1199mm respectively. The major types of soil in the area supporting the crops and the flora of the area are 60% black, 33% red, 5% brown and 2% grey soil. Agriculture is the main occupation of the population of the area. The agricultural activities are mainly mixed type with livestock and crop production under taken side by side [16].

Study Design: The type of study that used to evaluate antibacterial activity of the four medicinal plants against six bacterial species was Experimental design.

Description of Plants Used in the Current Study:
Albizia Gummifera (Mimosaceae): Locally called sessa (Amharic); is an umbrella like medium or large size tree to about 50m high commonly growing in upland and reverine forest at altitude range of 1700 -2400 m.a.s.l. It is also commonly found in eastern tropical Africa. In Ethiopia, it is widely distributed in the south west highland of the country [17].

Buddleja Polysatachya (Loganiaceae): Locally called Atquar (Amharic), is a much-branched shrub or small tree, 4-5m high; occasionally it can grow up to 12m. It has a red-brown or grey bark. The leaves are long and narrow to 15cm with a pointed tip and are light grey-green on top. The flowers are tubular, in small groups with a sharp and rather unpleasant smell. The fruits are small dry orange capsules that are open at the tip. It grows at altitude of 2200-3600 m.a.s.l. [18].

Cordial Africana (Boraginaceae): Locally called Wanza (Amharic), is a small to medium-sized evergreen tree, 4-15 m high, heavily branched with a spreading, umbrella-shaped or rounded crown. Bole typically curved or crooked. Bark grayish-brown to dark brown, smooth in young trees, but soon becoming rough and longitudinally fissured with age; young branchlets with sparse long hairs. Leaves are broad thinly dark green. It grows at Altitude of 550-2,600 masl and mean annual rainfall 700-2,000 mm large leafed cordia thrives in forest soil. Mature fruits have a sweet, mucilaginous, edible pulp [10].

Thymus Schimperi (Lomiaceae): Locally called Tossign (Amharic), is perennial herb or shrubs, woody at the base and it is 5-40cm long. Stems prostrate, sometimes erect in younger parts, quadrangular, green to purplish. Leaf blade concolorous blue green or pale green or discolorous, it mostly grows at altitude of 2250-4000 masl (afromantane and afro alpine vegetation zones) [19].

Fig. 1: Photos on leaves of *Albizia gummifera*, around Bedelle.

Fig. 3: Leaves and flowers of Cordia africana, along Jimma road

Fig. 2: Leaves and flowers of *Buddleja polystachya*, a road towards Suba forest.

Fig. 4: Leaves and branches of Thymus schimperi, from Debrebrhan in Gudoberet.

Table 1: Species, herbarium Voucher No, local name and plant parts used in current study

Species name (Family)	Herbarium Voucher no	Local Name	Area of collection	Parts used
Albizia gummifera (Mimosaceae)	Ag / 04 / 06	Sesa	Around Bedelle	Bark
Buddleja polystachya (Loganiacceae)	TE / 56 / 07	Atquar	A road towards Suba forest 14 KM from Sebeta	Leaf
Cordia africana (Boraginaceae)	TE / 57 / 07	Wanza	166Km along Jimma road near Welkite town	Leaf
Thymus schimperi (Lamiaceae)	TE / 54 / 07	Tossign	30KM away from Debrebrhan (Gudoberet PA)	Areal

Ag - Albizia gummifera, TE - Tadesse Eguale

Plant Material Collection and Pre Extraction Preparation: Fresh plants /plant parts was selected based on the literature survey on traditional uses of plants in Ethiopia and other part of the world. Those plants which claimed to have antimicrobial effect but not scientifically evaluated was selected and investigated. The plant materials were collected from their natural habitat. Parts of all plant species were collected and transported to National Animal Health Diagnostic and Investigation Center (NAHDIC) Sebeta. Voucher specimens for species identification of the plants were submitted to herbarium of the Addis Ababa University, biology department for identification by plant taxonomist. The garbled plants were dried in air at room temperature, pounded using pestle and mortar and kept in umber colored (air tight) bottle for further use.

Extraction of Plant Material: The medicinal plants were extracted using hydro-alcoholic extraction method according to Debella [20] at National Animal Health Diagnostic and Investigation Center (NAHDIC), Sebeta. A weighed amount of grounded powder of each plant parts (100g) was soaked separately in 500 ml of 80% methanol contained in a 1lit. capacity flask for 5 days and it was shacked occasionally. The samples were then strained using a tea strainer to remove solids. The resulting solution was again filtered using Whatman's filter paper No.1 to obtain a solution free of solids. The solution was then concentrated in a rotary evaporator to remove the solvent or methanol and stored at +4°C until used for the assay.

Preparation of Bacteria for Experiment: Different bacterial isolates was obtained from microbiology department of NAHDIC, Sebeta. The bacteria species used were*, P. gallinarum, S.gallinarum, E. coli M. hemolytica, S. aureus* and *S. dysagalactae*. They were isolated from animals during disease investigation and were deposited in microbiology laboratory in lyophilized form. These isolated bacterias' were homogenized in nutrient broth and cultured onto blood agar to check their viability. The suspected colonies were Gram stained and microscopically examined. Biochemical test was conducted to confirm and maintain purity of the

organisms for the experiment [21]. The bacterial cultures were standardized using the method of Baker and Thomsberg [22]. The test organisms were suspended in to sterile test tubes containing nutrient broth and normal saline was added gradually to it so as to compare the culture turbidity to that of McFarland standard, which corresponds to approximately $1.0x10^7$ cell ml^{-1}.

Determination of Antibacterial Activity: The antibacterial screening was conducted using agar well diffusion Lino and Deogracious [23] and Akoma and Olawepo [24] and broth dilution methods [25].

Data Analysis: Simple arithmetic mean was employed to present the mean zone of inhibition recorded during each observation in agar well diffusion tests.

RESULTS

Extraction Yield: The different species of plants has shown variation in percentage yield upon hydro-alcoholic extraction.

Zone of Inhibition and Minimum Inhibition Concentration (MIC): The highest antibacterial activity was seen for hydro-alcoholic extract of *Albizia gummifera* against *E.coli* at concentration of 1gm/ml. Of all plant tested *Albizia gummifera* was showed anti bacterial activity against all test organisms. The methanol extract of *Buddleja polystachya* and *Cordia africana* did not show anti bacterial activity against all test organisms.

Hydro-alcoholic extract of *Albizia gummifera* was found highly effective against *s. dysagalactae* (10µg/ml), *Salmonella gallinarum* was less sensitive (MIC= 10,000). *Albizia gummifera* showed a minimum bactericidal concentration (MBC) at MIC 1000µg/ml for *E.coli, M. haemolytica* and *S. aureus* and 100µg/ml for *P. gallinarom*. The extract of *Thymus schimperi* inhibit the growth of *M. hemolytica* at MIC= 1000µg/ml, but for other test organisms, it showed similar activity (MIC = 10,000µg/ml). *Albizia gummifera* showed higher activity against *S. dysagalactae* (MIC=10µg/ml) and *P. gallinarum* (100µg/ml), both of these organisms were representative of Gram positive and Gram negative bacteria respectively. The hydro-alcoholic extract of *Cordial Africana* and *Buddleja polysatachya* were not able to inhibit growth of bacteria at concentration of 10,000µg/ml for all type of test organisms.

DISCUSSION

The current experimental study showed that out of four claimed medicinal plant extracts screened, only two extracts namely *A. gummifera* and *T. schimperi* have showed remarkable anti-bacterial activity against most of the common microorganisms of veterinary importance investigated. This could justify their use in treatment of microbial infection in human and livestock. Previous study has also shown that aqueous extract of *A. gummifera* has shown magnificent antibacterial activity against several microorganisms that causes various diseases in human [10]. Another study also showed

Table 2: Percentage yield of different plants using hydro alcoholic extraction method

Plant species	Parts used	Extraction method	% yield (W/W)
Albizia gummifera	Bark	80% methanol (hydro alcoholic)	10.75
Buddleja polystachya	Leaf	80% methanol (hydro alcoholic)	19.5
Cordia africana	Leaf	80% methanol (hydro alcoholic)	8.96
Thymus schimperi	Areal	80% methanol (hydro alcoholic)	12

Table 3: Antibacterial activity of hydro-alcoholic extract of *A. gummifera, B. polystachya, C. Africana* and *T. schimperi* at concentration of 1gm/ml and gentamycine (13.13µg/ml).

	Mean, Zone of inhibition (mm)					
Test plants	*P.gallinarum*	*S.gallinarum*	*E.coli*	*M.haemolytica*	*S.aureus*	*S.dysagalactae*
A. gummifera	23	20	23.5	19.5	19.5	22
B. polystachya	-	-	-	-	-	-
C. africana	-	-	-	-	-	-
T. schimperi	15	-	-	15.5	23	19.5
*Gentamycin	23.5	17.8	12.5	16.5	18.5	20
**Distilled water	-	-	-	-	-	-

**Positive control, Gentamycin (13.13µg/ml), **Negative control, sterile distilled water, (-) = no zone of inhibition*

that extract of *A. gummifera* was effective against *S. pneumonia* and *S. pyogens* [26]. Previous studies have shown that different secondary metabolites such as alkaloids, polyphenols, triterpens, saponins and glycosides are found in hydro-alcoholic extract of *A. gummifera* [10]. Some or all of these chemicals could be responsible for the observed antibacterial activity.

The other two plants extracts, *C. Africana and B. polystachya* did not induce any antibacterial activity at concentrations tested in spite of their traditional use for treatment of different ailments [10,27]. Methanol extract of *B. polystachya* and aqueous extract of *C. africana* inhibited growth of *Neisseria gonorrhea* at concentration of 250µg/ml [10]. The probable reason for the absence of antibacterial activity could be due to the fact that the hydro-alcoholic extraction could not be able to extract active principles responsible for antimicrobial activity or the bacterial species used in the current experiment may not be sensitive to active chemicals in these plants.

The results of the current study showed that extracts of *A. gummifera* and *T. schimperi* have antibacterial activity against both Gram positive and Gram negative organisms. *A. gummifera* was active against all test organisms evaluated including *E. coli* in contrast to the finding of Lino and Deogracious [23] and Yitbarek[28] who reported absence of activity of plant extracts against this species. In the present study *E. coli* was found highly sensitive to the methanol extract of *A. gummifera.* In line with the finding of Unasho *et al*. [26], the hydro alcoholic extract of *Thymus schimperi* were highly active against *S. aureus* and *S. dysagalactae*. These plants could be effective and suitable for general use against Gram positive and Gram negative pathogens of zoonotic importance.

The lack of susceptibility of all tested bacteria to the plant extracts against *Cordia Africana* and *B. polystachya* could be attributed to the fact that, unlike conventional pharmaceutical products which are usually prepared from synthetic materials by means of reproducible manufacturing techniques and procedures, herbal medicinal products are prepared from materials of plant origin which may be subjected to contamination and deterioration [29]. Other researchers also reported that it could be due to the storage of extracts which may require special condition of humidity or temperature or protection from light or due to the plant extracts which might contain little of the active ingredients [23]. There is also always the possibility that a given plant extract which were in active *In vitro* may exhibit properties of pro-drugs which are administered in an inactive form, but their metabolites could be active *in vivo* [30].

Hydro-alcoholic extract of *A. gummifera* showed MIC of 100µg/ml for *P. gallinarum*, 1000µg/ml for *M. haemolytica* and *S. aureus* and 10µg/ml for *S. dysagalactae*. This finding is similar with the finding of Yitbarek [28]. In his experiment, the water and hydro alcoholic extract of *Marsine africana* showed similar MIC for the bacteria mentioned above. Devilleries [31] and Yitbarek [28] reported that *E.coli* was not sensitive by water and methanol extract of test plants. But, in the current study 80% methanol extract of *A. gummifera* inhibits its growth at MIC of 1000µg/ml. *T. schimperi* showed MIC of 10000µg/ml for *P. gallinarum, S. aureus* and *S. dysagalactae*. A similar result was reported by Unasho *et al*. [26] for *A. gummifera* against *S. payogens* and *S. pneumonia*.

CONCLUSION

From the finding of this study, it is possible to conclude that *A. gummifera* and *T. schimperi* have shown good antibacterial activities. The demonstration of antibacterial activity in the extracts would justify their use in treatment of microbial infection or to use in traditional medicine. The finding of this study further suggests that there is a potential in discovery novel antimicrobial agents from indigenous medicinal plants in Ethiopia.

ACKNOWLEDGEMENTS

Great gratitude goes to NAHDIC in providing all materials needed to conduct the research work and for allowing to participate in different trainings and also to W/o Letebrhan and Ato Melaku and Ato Tibebu for their technical support and advise.

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