

Evaluation of Antibacterial Activity and Phytochemical Constituents of Leaf Extract of *Lippia adoensis*

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ABSTRACT

There are quite large numbers of traditionally used medicinal plants that are used to treat skin disorder in the ethno medical system of Ethiopia. Medicinal plants namely *L. adoensis*, was screened for antibacterial activity against different strains of bacteria which are known to cause various types of skin infections and food poisoning

Anti bacterial effect of the plant species was evaluated against different bacterial strains. The leaves of plant species were extracted by maceration and soxhelt extraction technique for preparation of crude and fractional extract respectively. And anti bacterial screening of different concentration of both crude and fractional extract of the plant species were determined using agar well diffusion method. The test organisms were one gram positive (*S.aures*) and three gram negative (*Salmonella typhi*, *E.coli* and *P.aeruginosa*) standard organisms.

The results of the initial antibacterial screening test indicated the potential of these herbal drugs in treating bacterial infections of the skin and food poisoning. Among the different fractions (petroleum ether, chloroform, acetone and methanol) tested for antibacterial activity, the non-polar fractions were found to be more active than the polar fractions. The Phytochemical screening tests carried out on *L. adoensis* indicated the presence of tannins, flavonoids and saponins.

Different extracts *L.adoensis* were showed significant antibacterial activity against the *S.aures*, *P.aeruginosa*, *E.coli* and *S.typhi*. Hence further study is recommended to identify the specific active ingredient and potential formulation of effective antibiotic.

Key Words: Antibacterial activity, Phytochemical screening, plant species *Lippia adoensis*

INTRODUCTION

Even if there is progress of prevention and treatment of infectious disease, it is responsible for worsening the living condition of many million people around the world. Infectious disease is

the world's biggest killer of children and young adults. According to WHO report every year approximately 13 million people die of infectious diseases. Half of these deaths are from developing countries. Every hour around 1500 people die due to infectious diseases, majority of them are children. More over infectious disease is severe in countries which have unsanitary living conditions and malnutrition problem like Ethiopia.

Infectious/Communicable disease is caused by microorganism like bacteria, virus, fungus, parasite and other microbes. Bacterial infectious disease is most commonly caused by gram positive and gram negative bacteria. From gram positive bacteria *S.aureus* is known to cause different diseases like food poisoning, skin infection, wound infection and respiratory tract infection. Gram negative bacteria like *E.coli*, *Salmonella* and *Shigella* are commonly known to cause Gastro intestinal tract infection.

Previously with the advent of antimicrobials some Medical professional believed that the problem of infectious disease is over, but latter they understand microbes also arise with new survival strategies. Newly discovered and emerging infectious agents start to appear. Antimicrobial resistance is a worldwide problem. However the situation in developing countries is especially serious due to scarcity of modern medicine, poor primary health care and poverty (Inanir etal, 2002).

Infectious disease can be cured and controlled but appropriate drugs are critically important. In order to formulate potent anti-microbial drugs, plants used by herbalists are one of the possible options. It is believed that the increased use of plant medicine by different people of the world has potential for finding new and effective noble drugs.

According to literatures Many medicinal plants of Africa have been investigated for their chemical components. Accordingly some of the isolated compounds have been shown to possess interesting biological activities. for instance *Garicinia cola*, *Aframomum melegueta*, *Xylopiya aethiopica*, *Cryptolepis sanguinolent* and *Chasmanthera dependens* were found to possess different groups of compounds with wide ranging anti-inflammatory and antimicrobial activities (Taylor, 2000).

On the other hand Ethiopia is well known for its significant geographical diversity, which favored formation of different Habitat and vegetation zones. It is estimated that Ethiopia has a plant flora of about 700 specious. Moreover, about 12% of these plants are endogenous. The country Has high diversity of traditional knowledge and practice of the people (Evans, 2000). Even if traditional medicine has become an integral part of the cultures due to its long history, it experienced very little attention in modern research and development. For instance "Zingibil,Teji sar,Tenadam and *Lippia adonensis* were commonly used by different traditional medical practitioners (Evans, 2000).

Lippia adoensis is included under Verbenaceae that is a large family with about 70 to 80 genera and over 3,000 species; distributed throughout the world mainly in the tropics and temperate regions. In Ethiopia, the family is represented by 9 genera and 30 species. *Lippia* is a genus with 200species in tropical Africa and America. Five species have been described in the country. *L.adoensis* (locally known as "Kesse") is a shrub having a height of 1 to 3 meters. Two varieties are recognized in Ethiopia, the wild variety (var. *adoensis*) and the cultivated variety (var.*koseret*) (Toshihiro etal, 1992).

In Ethiopia, *Lippia adoensis* extracts were used medicinally by a variety of indigenous people for treatment of skin infection. However little is known about the chemical constituents present in the plant. Information on the biological activities of this plant including its anti microbial property are sketchy. The essential oil has been investigated but only a small fraction were identified .Chemical characterization of the oils imperative to determine the commercial value

and potential application. A rapid, simple method through a chemical marker, which can be used to evaluate the anti microbial activity and Phytochemical screening of *Lippia adoensis* is important. Hence this study was done to assess the anti bacterial activity and phytochemical constituents of leaf extract of *Lippia adoensis*.

METHODOLOGY

Descriptive study design was conducted in laboratories of department of chemistry, biochemistry and Medical microbiology at Jimma University. The leaves of *Lippia adoensis* were collected, characterized in Jimma university herbarium and deposited there. All parts of the plant materials were dried in an open air protected from direct exposure to sunlight. The dried plant materials were separately powdered to suitable size and made ready for extraction.

PREPARATION OF THE FRACTIONAL EXTRACTS

Fifty gm of leaf powder was extracted with 80% methanol by maceration. This was continued for 48 hours with frequent agitation and the resulting liquid was filtered by using filter paper. Extraction was repeated five times and the filtrates of all portions were combined in one vessel. The organic solvent was removed by evaporation using rota vapor at not more than 40°C. The aqueous residue was then placed in an oven at 40°C for about 48 hours to remove the water. The resulting dried mass was converted to powder then, packed into a glass vial and stored in desiccators over silica gel until use. Likewise hundred gm of *L. adoensis* was extracted with petroleum ether, chloroform, acetone and methanol by the use of soxhelt extraction technique. Polarity gradient extraction (PGE) was operated by injecting four solvents into parts of extraction column. Each solvent was removed by evaporation using a rota vapour and the fractions were then placed in an oven at not more than 40°C for about 24 hours to remove any residual solvent. The resulting semisolid mass of each fractional extracts was stored in desiccators until use in the same way as the crude extract.

ANTIBACTERIAL SCREENING OF THE CRUDE EXTRACTS AND FRACTIONS

The antibacterial activities of the hydro-alcoholic extracts of the plant species was determined using agar well diffusion method. Eighty percent Methanol, methanol and chloroform were used as negative controls during the whole test on bacteria.

Standard bacteria strains of human pathogens were used for screening of anti microbial activity. These are one gram positive (*s.aures* (ATCC25925)) and three gram negative [*E.coli* (ATCC25922), *salmonellatyphi* (ATCC83859) and *P.aeruginosa* (ATCC27853)]. All slandered organisms were first grow on 5% sheep red blood agar plates. Few colonies (4 to 5) of similar morphology of the respective bacteria were transferred with a sterile inoculating loop to a liquid medium (peptone water) and incubated until adequate growth of turbidity equivalent to McFarland 0.5 turbidity standard was obtained. The inoculums of the respective bacteria were streaked on to the Mueller Hinton agar plates using a sterile swab in such a way as to ensure thorough coverage of the plates and a uniform thick lawn of growth following incubation. Wells of 10 mm in diameter were formed on to Mueller Hinton plates using a sterile cork borer. The wells were filled with the test agents (100µl) each and the plates were allowed to stay for 1 to 2 hours at room temperature. Finally, the plates were then incubated at 37°C for 18 to 24 hours. The resulting diameters of zones of inhibition were measured and recorded in mm. Gentamycin was used as a positive control at a concentration of 0.1 mg/ml. Moreover tube dilution method was performed to determine the minimum inhibitory concentration of the crude extracts.

PHYTOCHEMICAL SCREENING

Extracts of the plant species namely *L. adoensis*, was subjected to Phytochemical screening using standard screening procedures. For alkaloids; 0.5 g crude extract was stirred with 5 ml of 1% HCl on a steam bath. 1 ml of the filtrate was treated with a few drops of Mayer's reagent and another ml was similarly treated with Dragendorff's reagent. Turbidity or precipitation with both reagents was taken as preliminary evidence for the presence of alkaloids. For saponins; 0.5 g of crude plant extract was shaken with water in a test tube. Frothing which persists on warming was taken as preliminary evidence for the presence of saponins. by using Chloroform - glacial acetic acid - methanol - water (64:32:12:8) as a mobile phase and vanillin-sulphuric acid as a spraying reagent for detection. Formation of a blue, blue violet, red or yellow brown zone is considered as positive test for saponins [18-20]. For tannins; 0.5 g crude extract was stirred with 10 ml of distilled water and filter. The addition of FeCl₃ reagent to the filtrate resulting in blue, blue-black, green or blue-green coloration or precipitation was taken as evidence for the presence of tannins. For anthraquinones; A sample (5 g) of *L.adoensis* extract was shaken with 10 ml of benzene and filter. A 10% ammonium hydroxide solution (5 ml) was added to the filtrate and the mixture was shaken. The presences of a pink, red or violet color in the ammoniacal phase was taken as an indication of the presence of Anthraquinones. For poly phenols; for 2 ml of the aqueous solution of the crude extract, 3 drops of a mixture of 1 ml 1% FeCl₃ and 1 ml 1% K₃Fe(CN)₆ were added. Formation of green blue color was taken as an indication of the presence of Polyphenols. For flavonoids, for 2 ml of the alcoholic solution of the crude extract 4 drops of 2% lead acetate solution were added. The development of yellow or orange color was taken as an indication of the presence of flavonoids.

RESULTS

The study showed that on the antibacterial activities of the leaf extract of *L. adoensis* and its phytochemical constituents. The fresh leaves of this plant have very high water content and shrink extremely to a light weight dried mass with a partial loss of its green color. In general, the yields obtained from the plant are quite adequate. The largest yield was recovered from methanol fractional extract. The solvents used for extraction and reconstitution of the extracts were water, ethanol petroleum, acetone and ether. None of this chemical showed significant activity against tested bacteria. The results of the antimicrobial screening assay of the crude extracts of *L.adoensis* on the selected bacterial strains indicated that all the extracts of *L. adoensis* shows significant activity against tested bacteria with varying zone of inhibition (Table 2). The petroleum ether extract was the most active of the four extracts. It showed activity against all the organisms tested. The results illustrated that the non-polar fractions (i.e. petroleum ether and chloroform) were stronger in their activity compared to the relatively polar fractions (Table 2). The MIC values indicated that 2.5mg/ml extracts of *L.adoensis* inhibited the growth of *Pseudomonas aeruginosa*, *Salmonella* and *Staphylococcus aureus* (Table 3). The leaves of *L. adoensis* were screened for the presence of different Phytochemical compounds of therapeutic interest using chemical (Table 4).

Table 1. Percentage Yields of Different Fractions of *L. adoensis* leaf extract. Jimma zone Oromia region June 2010

S.N.	Extraction technique	Wt.in gram	% of yield w/w
1	Methanol fractional extract	0.124	12.4
2	Crude extract	5.02	10.02
3	Petroleum ether fractional extract	0.0221	2.21
4	Chloroform fractional extract	0.0150	1.50
5	Acetone fractional extract	0.0690	6.90

Table 2. Antibacterial Activities of Different Fractions of *L. adoensis* against Selected Strains of Bacteria Jimma zone Oromia region June 2010

Fraction of extract	Concentration mg/ml	Bacterial strain Zone of inhibition (mm)			
		Sa (ATCC 25925)	Ec (ATCC 25922)	Pa (ATCC 27853)	Sal (ATCC 83859)
Petroleum ether	75	37	24	18	20
	50	33	22	18	18
	25	29	18	17	16
Chloroform	75	33	19	20	23
	50	27	18	19	20
	25	34	16	17	19
Acetone	75	31	19	21	20
	50	29	16	19	19
	25	24	14	16	15
Methanol	75	30	20	21	21
	50	26	19	17	19
	25	22	16	15	18
crude	75	32	19	23	24
	50	25	18	21	20
	25	21	16	19	17
Gentamycine	0.1	29	22	21	22

Sa = *S. aureus*, Ec = *E. coli*, Pa = *P. aeruginosa*, sal = *salmonella thyphi*

Table 3. Minimum Inhibitory Concentration (MIC) Values of the crude (80% Methanol) Extracts of *L.adoensis* on the Tested Strains Jimma zone Oromia region June 2010.

Plant species	Concentration mg/ml	Bacterial strain			
		Sa (ATCC 25925)	Ec (ATCC 25922)	Pa (ATCC 27853)	Sal (ATCC 83859)
<i>Lippia adoensis</i>	20	-	-	-	-
	10	-	-	-	-
	5	-	+	-	-
	2.5	-	+	-	-
	1.25	+	+	+	+
	0.625	+	+	+	+

(+) = presence of growth, (-) = absence of growth, Sa = *S. aureus*, Ec = *E. coli*, Pa = *P.aeruginosa*, Sal=salmonella

Table 4. Phytochemical screening of crude (80% methanol) extract of the Leaves of *L. adoensis* using chemical test methods Jimma zone Oromia region June 2010.

Metabolites tested for	crude (80% methanol) extract of <i>L. adoensis</i>
Alkaloids	+
Saponins	+
Tannins	++
Anthraquinones	-
Polyphenols	+
flavonoids	+

- = negative, ++ = strongly positive, + = positive

DISCUSSION

In our study it was observed that the percentage yields obtained from successive extraction of these plants indicated that increasing polarity of the extracting solvent increases the yield except chloroform which afforded quite low yield compared to petroleum ether. As a result, methanol, which is the most polar of all solvents used for fractionation, afforded the maximum yield. These fractions were then tested against the selected bacterial strains on which the crude extract showed activity.

The extracts of the *Lippia adoensis* species were screened for biological activity against different strains of bacteria. The antibacterial activity-screening tests were carried out on organisms that are known to be among the most common causative agents of both primary and secondary infectious skin disorders. *L. adoensis* was also found to be active on at least one of the selected bacterial strains. When the antibacterial activities of this herbal drug were compared to that of the positive controls, many of them (*L. adoensis* at a concentration of 75 mg/ml) were found to have almost comparable activity to the standard gentamycin against bacteria. *L. adoensis* at a concentration of 100 mg/ml was found to have greater activity against bacteria as compared with standard gentamycin. In some cases, antibacterial activities observed were even greater than the positive controls activities for example, *L. adoensis* against *S. aureus*. In comparison with similar study done in Addis Ababa University, Ethiopia, The finding of this study is consistent, the antibacterial activity of the tested plant showed that the zone of inhibitions increased with an increase in concentration. (Tadeg et al, 2004).

In our study the antibacterial activity profile of the plant species against the tested strains indicated that *S. aureus* was the most susceptible bacterium of all the bacterial test strains. On the other hand, *E. coli* was found to be the most insensitive strain of all bacteria. In fact, gram-negative bacteria are frequently reported to have developed multi drug resistance to many of the antibiotics currently available in the market of which *E. coli* is the most prominent. Therefore, it is not surprising to learn that *E. coli* is the least responding bacterial strain to the tested plant extracts (Salman et al, 2008).

The antibacterial activity was more pronounced on the gram-positive bacteria (*S. aureus*) than the gram-negative bacteria (*E. coli*, *salmonella typhi* and *P. aeruginosa*). The reason for the difference in sensitivity between gram-positive and gram-negative bacteria might be ascribed to the differences in morphological constitutions between these microorganisms, gram-negative bacteria having an outer lipopolysaccharide membrane. This makes the cell wall impermeable to antibacterial chemical substances. The gram-positive bacteria on the other hand are more susceptible having only an outer peptidoglycan layer which is not an effective permeability barrier. Therefore, the cell walls of gram negative organisms are more

complex in lay out than the gram positive ones acting as a diffusion barrier and making them less susceptible to the antibacterial agents than are gram positive bacteria (Alonso et al, 2000).

In spite of this permeability differences, however, some of the extracts have still exerted some degree of inhibition against gram-negative organisms as well. Several reports have indicated that infectious skin disorders are very common in Ethiopia. Among the pathogens most commonly known to cause infectious disorders of the skin is *S. aureus* (Hodges, 2002). Thus, *Lippia adoensis* showed activity against *S. aureus* might justify the extensive use of these agents for the treatment of skin disorders. In all species of plants tested for antibacterial activity, the zone of inhibitions increased with an increase in concentration i.e. stronger activity was observed at 100 mg/ml than lower concentrations. This study illustrated that the non-polar fractions (i.e. petroleum ether and chloroform) were stronger in their activity compared to the relatively polar fractions (i.e. acetone and methanol). Antibacterial activities were found to decrease with increasing polarity indicating that the active compounds responsible for antibacterial activities of the extract reside in the non-polar fractions in relatively higher concentrations. The MIC values indicated that extracts of *L. adoensis* were more potent against bacteria. *P. aeruginosa* was more sensitive to the antibacterial agents from among gram-negative bacteria being inhibited at 2.5 mg/ml by *L. adoensis* crude extracts. The MIC values of the extracts on *S. aureus* was found to be 2.5 mg/ml (*L. adoensis*). The overall antibacterial activity screening results is still indicative of the potential of these herbal drugs as effective medicaments in the treatment of infectious skin disorders.

The leaves of *L. adoensis* were screened for the presence of different Phytochemical compounds of therapeutic interest. *L.adoensis* showed positive test for the presence of alkaloids, saponins, polyphenols, flavonoids and tannins. Numerous studies conducted on the antimicrobial activities of the class of compound listed above reported the potential of each class of compound in inhibiting the growth of wide ranges of microorganisms. Phenolics and polyphenols are compounds having such potential. Reaction with sulfahydryl groups or more non-specific reactions with proteins is thought to be the possible mechanism for phenolic toxicity to microorganisms (Scalbert, 1991). Flavonoids and flavonoid-derived plant natural products have long been known to function as antimicrobial defense compounds (Kazmi et al, 1994). Different *in vitro* studies have shown that they are effective antimicrobial substances against a wide spectrum of microorganisms.

CONCLUSIONS AND RECOMMENDATION

Antibacterial activity profile of *L. adoensis* against the tested strains indicated that *S. aureus* was the most susceptible and *E. coli* was found to be the most insensitive strain of all bacteria. *Lippia adoensis* showed activity against *S. aureus* might justify the extensive use of these agents for the treatment of skin disorder. Phytochemical screening study on *L. adoensis* showed positive test for the presence of alkaloids, saponins, Polyphenols, flavonoids and tannins. The result from this study has shown that indicating their potential in treating infectious diseases of the skin. The possible synergistic effect between different combinations of these extracts must be taken into account. This speculation needs further study.

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