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Research Article

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Phytochemical Screening and *In-vitro* Antimicrobial Activity of *Acacia Brevispica* Harms Leave Extract Collected from East Hararge, Ethiopia

Teshome Gonfa Hordofa*, Fenan Tola Dida

Department of Chemistry, Haramaya University, Alem Maya, Ethiopia

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ABSTRACT

Acacia Brevispica Harms is one of the medicinal plants widely distributed in low land of Ethiopia. The leaves of this plant possess various medicinal properties such as of headache and Cancer related fever (Hyperpyrexia). Therefore, this study was aimed to evaluate the antimicrobial activity of the leaves and screen major phytochemical constituents present within the plants responsible for those activities. The plant leaves were extracted successively with petroleum ether, chloroform and ethanol. Accordingly, a crude yield of 0.38% from petroleum ether, 0.47% from chloroform and 8.5% (w/w) from ethanol were extracted. The phytochemical analysis showed the presence of steroids and anthraquinones in all ethanol, chloroform and petroleum ether crude extracts. The phytochemical analysis also indicated the presence of flavonoids and phenol in ethanol extract, alkaloid in chloroform, terpenoids in petroleum ether and carbohydrates in both chloroform and petroleum ether crude extracts. The antimicrobial activity of the crude extracts was determined by agar diffusion method. The ethanol extracts exhibited relatively higher zone of inhibition (6.42 ± 0.13 mm) against Escherchia coli and Salmonella typhus (6.37 ± 0.08 mm) 5.26 ± 0.08 mm) as compared to chloroform extract (5.33 ± 0.07 mm against Escherchia coli and 5.26 ± 0.08 mm Salmonella typhus). Chloroform extract also showed. The results indicated that Acacia brevispica could be used as a source of antimicrobial agents to treat various diseases.

Keywords: *Acacia brevispica*; Phytochemicals; Antimicrobial activity; Secondary metabolites; Medicinal plants

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INTRODUCTION

Now For thousands of years, medicinal plants and uses of traditional medicines have been closely linked and important roles through in the world in the treatment of human illnesses. Clinical, pharmacological, and chemical studies of these traditional medicines, which were derived predominantly from plants, were the basis of most early medicines. Nowadays, natural medicines not only provide the primary health care needs for the majority of the population in developing countries, but have attracted more and more attention even in developed countries [1]. Pharmacological studies of these traditional medicines, which were derived primarily from plants parts, were not only the basis of most early medicines but also important component of present day human healthcare and the sources of the present day drugs [2]. They are universally accepted because of the fact that medicinal plants continue to play important role in healthcare system of a large number of world's population [3]. According to estimation from the World Health Organization (WHO), more than 60% of the world's population and over 80% of the people in developing countries utilize mostly plant parts as traditional medicine for their main health care [4].

Medicinal plants have been receiving significant attention due to they are source of secondary metabolites which differ widely in terms of structure and therapeutic properties [5]. The important active biological substances such as alkaloids, glycosides, saponins, resins, oleoresins, terpenes/terpenoids, flavonoid, tannins, anthraquinones, steroids, volatile oils, resins and phenols are deposited different parts of plants. Since plants synthesize an extremely diverse chemical compounds, medicinal values of these plants are due to some bioactive compounds that produce definite physiologic action on the human body since [6]. These phytochemicals are derived from their whole plant parts such as leaves, barks, seed, seed coat, flowers, roots and pulps and thereby used as some of direct medicinal agents. These bioactive constituents of plants compounds have anticancer, anti-inflammatory, antimicrobial, antioxidant, antidiarrheal and anticarcinogenic properties [7]. They are also uses as a remedy for the treatment of gastropathy, hepatitis, nephritis, edema, chest pain, fever and cough of pneumonia, bronchitis, and arthritis.

In recent years, much attention has been paid to plants as a source of therapeutic agents due to their medicinal value, the reasonable cost, easy availability, and relatively lower incident of other adverse effects compared to synthetic pharmaceutical synthetic drugs are expensive and insufficient for the treatment of diseases, much attention has been paid to plants as a source of therapeutic agents. [8]. Therefore, there is still need to find and search new medicinal with their antimicrobial activity to treat infectious diseases.

In Ethiopia, plants have been used as a source of medicine for a long time to treat various human and livestock ailments. About 80% of Ethiopian people still depend on traditional medicine for their primary health care and more than 95% of traditional medicinal preparations are plant origin [9]. One of the medicinal plants used in low land of Ethiopia is *Acacia brevispica* which belongs to the family *Fabaceae*. *Acacia brevispica* is an indigenous plant to Africa and widespread in dry as well as semi-humid parts of Africa such as Ethiopia, Sudan, Somalia, Kenya, Zaire, Angola, Natal and Cape Province [10]. In Ethiopia, it is distributed widely in southern range lands of the Borana Plateau, Harerge, Bale, Welo, Sidamo, Gamo Gofa, Kefa and Shoa regions [11]. It is mainly used as a forage tree whose foliage, pods and seeds are readily browsed by goats and camels. Traditionally, it used for the treatment of headache [4] and Cancer related fever (Hyperpyrexia). It is known that, camels and goats milk are enriched with minerals and organic nutrients [12]. Therefore, human beings indirectly obtained such minerals and organic nutrients from these plant species. But no researchers have been carried out on the leaves *Acacia brevispica* regarding to the phytochemical studies and antimicrobial activities. By considering the importance of *Acacia brevispica* leaves as a medicinal plant, this study aimed to investigate the preliminary phytochemical screening and evaluate its antimicrobial activity.

MATERIALS AND METHODS

Plant Material

The leaves of *Acacia brevispica* were collected from the local area of east Hararge distinct, Babile. The scientific name of each plant was confirmed at the herbarium of Haramaya University and voucher specimen was deposited. The plant leaves were shade-dried at room temperature and the dried leaves were then crushed to fine powder using an electrical grinder [13].

Preparation of Extracts

The air dried and finely powdered leaves (100 g) were soaked and extracted successively with 300 mL petroleum ether, chloroform, and ethanol for 3 days. Each extract was filtered out using Whatman No.1 filter paper and the filtrate concentrated by rotary evaporator under reduced temperature and pressure. The crude extracts were collected

in vials and stored in the refrigerator for further analysis. Each crude extract was were collected in vials and stored in the refrigerator at -4ºC until used.

Bacterial isolates: Different two clinical microbial isolates gram negative were isolated and identified by using conventional biochemical tests and cultivated in pure culture, at microbiological laboratory/college of agriculture in Haramaya University.

Materials and Chemicals

The apparatus used for extraction and testing were round bottom flask, test tubes, cylinder, filtration paper, conical flasks. Heidolph rotary evaporator was used for removal of solvent under reduced pressure. Petroleum ether, chloroform and ethanol, were used for extraction, conc. HCl, NaOH, Lead acetate, hagers reagents (picric acid) for phytochemical test while muller hinto nagar, Dimethyl sulphoxide, $BaCl_2$ used for antimicriobial activity [14].

Procedure of Phytochemical Tests

The qualitative chemical tests were conducted for detecting the profile of various phytochemical constituents present in each crude extract of *Acacia brevispica* leaves. The phytochemical were analyzed according to standard screening tests using conventional procedures [15].

Test for flavonoids: The Alkaline reagent test: Each crude extract (5 mL) was mixed with 2 ml of 2% solution of NaOH. Then five drops of diluted HCl was added. An intense yellow color which turned colorless on addition drops of diluted acid indicates the presence of flavonoids. This test also confirmed by lead acetate test (Each crude extract 5 mL) was treated with five drops of lead acetate solution. Formation of yellow colour precipitate shows the presence of flavonoids.

Test for alkaloids: Hager's Test: 2% HCl was poured in a test tube having 5 mL crude plant extracts. The test tube having the mixture was heated then five drops of picric acid was added to the mixture. Formation of yellow color precipitate indicates the presence of alkaloids

Test for saponins: Froth Test: 10 mL of the extracts were diluted with distilled 20 ml water. Each diluted crude extracts was shaken in a 25 mL graduated cylinder for 15 minutes separately. Formation of layer of foam indicates the presence of saponins.

Test for tannins: Gelatin Test: To 5 mL each extracts, 1% gelatin (Gelatin dissolves in warm water immediately) solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

Test for steroid: 5 drops of concentrated H_2SO_4 was added to 1 cm^3 of each crude extract. A reddish brown colour was the preliminary evidence for the presence of steroids [16].

Test for terpenoids: Salkowski test: 5 mL of each solvent extracts was mixed in 2 ml of chloroform followed by the careful addition of 3 ml concentrated (H_2SO_4). A layer of the reddish brown colour at the interface indicates positive result for the presence of terpenoids.

Detection of phenols: Ferric Chloride Test: 5 mL xtracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Test for Quinones: One ml of each of the various extracts was treated separately with alcoholic potassium hydroxide solution. Quinines give coloration ranging from red to blue.

Test for free Anthraquinones (Bumtrager's): 0.25 g of each powdered sample was taking in separate test tube and 5 mls of chloroform was added then shake for 5 minutes again. Then the extracts were filtered and shake again. Then the extracts were filtered and shake again. A bright pink colour in the upper aqueous layer indicates the presence of free anthraquinones [17].

Test for carbohydrates: Molisch test: To 5 mL of each extract, 3 drops drops of alcoholic alpha-naphthol. 2 mL of conc. sulphuric acid added slowly along the sides of test tube. Purple colour ring appears at junction of the layers indicates the presence of carbohydrates.

Experimental Procedure for *in-vitro* Antimicrobial Activity Test

Preparation of culture media: Pure Muller Hinton Agar (MHA) was prepared by dissolving about 38 g of MHA in 1000 mL of distilled water and adjusted to pH 7.4 ± 0.2, sterilized by autoclaving at 121°C for 15 minutes at 15 psi pressure and used for sensitivity tests in the pathology laboratory, Haramaya University.
Preparation of discs: From the plant extracts, 100 mg of each crude extracts was dissolved in 1 mL of 4% Dimethyl sulphoxide (DMSO) and 0.2 mL of the prepared extracts were loaded on to the filterPaper discs (Sterilized Whatman No. 1 filter paper discs of 6 mm diameter) to get 20 mg/disc Concentration and allowed to dry at room temperature. Preparation of 0.5 McFarland standards: 0.5 mL of 0.048 M $BaCl_2$ (1.175% w/v, $BaCl_2.H_2O$) was added to 99.5 mL of 0.18 M H_2SO_4 (1% V/V) with constant stirring to make 0.5 McFarland standards (Andrews and Wise, 2002) [18].

Antimicrobial activity test: Previously prepared paper discs containing different extracts were placed individually on the surface of the petri plates containing 20 mL of respective media seeded with 0.1 mL of previously prepared microbial suspensions individually (10 CFU/mL). Standard antibiotic Streptomycin (20 µg/disc) obtained from Hi-media was used as positive controls. The discs containing n-hexane, dichloromethane, chloroform and methanol solvents were served as negative controls. The assessment of antimicrobial activity will be based on measurement of inhibition zones formed around the discs. The plates were incubated for 24 hrs at 37°C and the diameter of the inhibition zones was recorded.

RESULTS AND DISCUSSION

From soaking extraction of *Acacia brevispica* leaves, crude yield of 0.38% w/w, 0.47%w/w and 8.5%w/w were obtained from petroleum ether, chloroform and ethanol respectively, The highest yield of ethanol crude extracts indicated, the plant leaves were enriched with highly polar phytochemical constituents such as flavonoids and phenols. The preliminary phytochemical screening of the leaves of *Acacia brevispica* showed the presence of various secondary metabolites as indicated in Table 1 below. The result of the phytochemical analysis of the leaves extracts in various solvents has shown a remarkable presence of various phytochemical compounds in the plant species. The obtained result of the phytochemical screening from petroleum ether, chloroform and ethanol crude extracts indicated the presence of active phytochemical compounds in *Acacia brevispica* leaves [19].

Table 1: Phytochemical profile of crude extracts of *Acacia brevispica* leaves

No.	Phytochemical test	Ethanol	Chloroform	Pet. ether	Results
1	Flavonoids	+	-	-	Yellow precipitate
2	Alkaloids	-	+	-	Yellow precipitate

3	Saponins	-	-	-	-
4	Tannins	-	-	-	-
5	Steroids	+	+	+	Reddish brown
6	Terpenoids	-	-	+	Reddish brown
7	Phenols	++	-	-	Deep blue
8	Quinones	-	-	-	-
9	Anthraquinones	+	+	+	Pink solution
10	Carbohydrates	-	+	+	Purple ring

Note ++=strong presence, +=moderate presence, -=absence

From Table 1, it could be seen that, flavonoids, alkaloids, steroids, terpenoids, phenols, antraquinones and carbohydrates were present in crude extracts of plant. The petroleum ether and chloroform crude extracts were containing steroids, antraquinones and carbohydrates while terpenoids and alkaloids were found only in petroleum ether and chloroform extracts respectively. The strong presence of phenolic, anthraquinones and flavonoids in ethanolic extracts showed the leaf of *Acacia brevispica*a was enriched with these constituents. Saponins, quinones and tannins are completely absent in all solvent extracts. In contrast to all, anthraquanones and steroids were present in all the solvent extract [20].

Antibacterial Activity

Table 2: Actimicrobialactivity of *Acacia brevispica* leaves

No	**Types of bacteria**	**Zone of inhibition(mean ± stardarddevaion)**		
		Ethanol extract	Chloroform extract	Streptomycin
1	*Escherchia coli*	6.42 ± 0.13	6.37 ± 0.08	9.23 ± 0.03
2	*Salmonella typhus*	5.33 ± 0.07	5.26 ± 0.08	9.57 ± 0.01

The two extracts (ethanol and chloroform) of plant tested showed varying degree of antibacterial activities against the test bacterial species (Table 2). The antibacterial activities of the ethanol and chloroform extracts compared favourably with that of the standard antibiotics streptomycin and have appeared to be broad spectrum as its activities were independent on gram reaction [21]. Among the two extracts of *Acacia brevispica* leaves, ethanol extract showed maximum inhibitory activity against *Escherchia coli* (6.42 ± 0.13 mm) than Chloroform extract (6.37 ± 0.08 mm). Similarly, Extract of ethanol showed maximum activity against *Salmonella typhus* (5.33 ± 0.07) than Chloroform extract (5.26 ± 0.08). All the two extracts effectively inhibit both bacterial.

CONCLUSION

From successive soaking extraction of *Acacia brevispica* leaves, crude yield of 0.38% 0.437% and 8.5%w/w were extracted from petroleum ether, chloroform and from ethanol respectively. The more yield of ethanol crude extract showed the plant was enriched with flavonoids and other compounds containing phenol groups. The phytochemical screening revealed the presence of steroids and anthraquinones in all ethanol, chloroform and petroleum ether extract. The phytochemical analysis also indicated the presence of flavonoids and phenol in ethanol extract, alkaloid in chloroform, terpenoids in petroleum ether and carbohydrates in both petroleum ether and chloroform extracts. The presence of such phytochemical constituents could be responsible for the observed antibacterial property. The

antimicrobial activity of the extracts was determined by agar diffusion method. The ethanol extract showed more significant activity against *Salmonella typhus* and *Escherchia coli* as compared to chloroform extract. The *Escherchia coli* bacteria more affected by acacia leave extract than *Salmonella typhus* under the same solvent extracts. Both of them are more inhibited by ethanol leave extracts than chloroform extracts. Finally, we recommended that further investigation could be conducted on the isolation and characterization of bioactive components in the plant.

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