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Phytochemical Screening and In-Vitro Antimicrobial Activity of Acacia Brevispica Leave Extract Collected around Haramaya Wereda

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Abstract
Medicinal plants play an important role in the discovery of novel drugs used in modern medicine. The Acacia Brevispica leaves as medicinal plant possesses the wide range of medicinal properties. This study was to determine leaves have any antimicrobial activity and also to check whether their phytochemical constituents responsible for those activities. The leaves of Acacia Brevispica were extracted successively with petroleum ether, chloroform and ethanol. Accordingly, a crude yield of 0.38% from petroleum ether, 0.437% 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 extract. flavonoids and phenol in ethanol extract, alkaloid in chloroform, terpenoids in petroleum ether and carbohydrates in ether extracts. The antimicrobial activity of the extracts was determined by agar diffusion method. The ethanol extract showed more significant activity against Escherchia (6.42±0.13) and salmonella typhus (5.33±0.07) coli as compared to chloroform extract (Escherchia coli, 6.37±0.08 and Salmonella typhus, 5.26±0.08). The results indicated that Acacia brevispica could be used as a source of antimicrobial agents to treat diseases.
Keywords: Acacia brevispica, phytochemicals, antimicrobial activity, secondary metabolities, medicinal plants
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1. INTRODUCTION

Medicinal and aromatic plants have always been intimately linked with human health and culture. For thousands of years medicine and natural products have been closely linked through the use of traditional medicines and natural poisons. Clinical, pharmacological, and chemical studies of these traditional medicines, which were derived predominantly from plants, were the basis of most early medicines. Plant derived medicines constitute a substantial component of present day human healthcare systems [Gómez et al 2007 and, Butler et al, 2004].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. In fact there are several medicinal plants all over the world which are being used traditionally in the prevention and treatment of several diseases [Vasanthi et al, 2014].These plants are used medicinally in different countries and are the source of potential and powerful drugs. According to World Health Organization (WHO), more than 60% of the world's population and over 80% of the people in developing countries depend upon traditional medicine for their primary health care needs [Sukirtha et al, 2012].

Diseases that have been managed traditionally using medicinal plants include malaria, epilepsy, infantile convulsion, diarrhea, dysentery, fungal and bacterial infections [Sofowora, 1996]. Medicinal herb is considered to be a chemical factory as it contains multitude of chemical compounds like alkaloids, glycosides, saponins, resins, oleoresins, sesquiterpene, lactones and oils (essential and fixed). Natural antimicrobials can be derived from plants, animal tissues and microorganisms. The shortcomings of the drugs available today propel the discovery of new pharmacotherapeutic agents from medicinal plant research [Amri, 2005]. The amount of phytochemical substances varies considerably from species to species and even from plant to plant, depending on the age and various ecological and climatic factors [Kumar et al, 2013].

Various parts of plant are known to be important source of secondary metabolites as alkaloids, glycosides, gums, terpenes (including essential oils, diterepenes, phytosterol and triterpene genins and saponin). It has been scientifically demonstrated that plants contain also other secondary metabolites such alkaloids, resins, oleosins, steroids, tannis and etc. to which biological properties are attributed, and from these properties, drugs have been developed to care diseases. Due to this, there is a constant need to find and develop new compounds with antimicrobial potential, and to continue the search of medicinal plants with new mechanisms of action to treat infectious diseases [Lewington, 1990].

Plants have limitless ability to synthesize aromatic substances, mostly phenols or their oxygen-substituted derivatives. Most of the natural products are secondary metabolites and about 12,000 of such products have been

isolated so far. These products serve as plant defense mechanisms against predation by microorganisms, insects and herbivores [Kumar et al, 2013]. Today there is growing interest in chemical composition of plant based medicines. Several bioactive constituents have been isolated and studied for pharmacological activity. During the last two decades, the pharmaceutical industry has made massive investment in pharmacological and chemical researches all over the world in an effort to discover much more potent drugs, rather, a few new drugs. Plants have successfully passed the tests of commercial screenings [Wink, 2010].

Botanical back ground of Acacia brevispica

The genus Acacia belongs to the family *Leguminosae.* It is a cosmopolitan genus containing more than 1350 species, distributed throughout tropical and warm temperate areas of the world. [Beentje, 1994 and Singh et al, 2016].

Acacia brevispica a species belongs to the family Fabaceae. It is also known as *Senegalia brevispica*. It was reclassified after molecular phylogenetic studies resulted in the transfer of many *Acacia* species into the genera *Vachellia*, *Senegalia*, *Mariosousa* and *Acaciella*, with only Australian species remaining the *Acacia* genus [Kyalangalilwa et al, 2013].

Acacia brevispica is native to Africa and common in widespread dry as well as semi-humid parts of Africa, being found in Ethiopia, Sudan, Somalia, Kenya, Zaire, Angola, Natal and Cape Province (Skerman, 1982 and Bekele, 2007). In Ethiopia, it can be found between 400 m and 2000m in the southern range lands of the Borana Plateau, Harerge, Bale, Welo, Sidamo, Gamo Gofa, Kefa and Shoa regions [Skerman, 1982 and Coppock, 1994]. *It* is mainly used as a forage tree whose foliage, pods and seeds are readily eaten by goats. It is used as firewood and live fence. The roots are used in ethnomedicine [Bekele, 2007]

Ethiopia has a long history with traditional medicine. From the different forms of specialized areas, herbal therapy appears to play a prominent role in Ethiopian traditional medicine for treatment of various diseases. Ethiopia is the most diverse plant species in Africa that serve sources of many traditional medicinal plants. In Ethiopia, medicinal plants contribute, to about 80% of the traditional medicines used in the country. Most of these plants are obtained from local sources in the wild by knowledgeable traditional practitioners [Worku, 2016].

Healing in Ethiopian traditional medicine is not only concerned with curing of diseases but also with the protection and promotion of human physical, spiritual, social, mental and material wellbeing. The current Ethiopian health care system is primary health care focused. However, traditional healers continue to practice outside of the health services although they are the main health care providers for many Ethiopians [WHO/EDM/TRM, 2000].

Ethiopian plants have remarkably effective medicinal values for many ailments that affect people and livestock. *Acacia brevispica* is one of plant eaten by goats and camels. Camel and goats milk contains enriched with minerals and organic nutrients. Therefore, human being indirectly gets such minerals and organic nutrients from these species. By considering the importance of *Acacia brevispica* leaves as a medicinal plant, this study aimed to explore the preliminary phytochemical screening and anti microbial activity for their pharmacological properties.

MATERIALS AND METHODS

Plant Material

The leaves of Acacia brevispica were collected from the local area of east Hararge distinct, Babileand shade-dried at room for temperature three weeks. The dried leaves were then crushed to fine powder using an electric grinder.

Extraction of Acacia brevispica leaves

The air dried and finely powdered leaves (100 g) were soaked and extracted successively with 300 mL each petroleum ether, chloroform, and ethanol for 3 days. Each extract was filtered out using Whatman No.1 filter paperand the solvent was removed by rotary evaporator under reduced temperatureand pressure. The crude extracts werecollected in vials andstored in the refrigerator for further analysis.

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

Material and chemicals

The apparatus used for extraction and testing were round bottom flask, test tubes, cylinder, filtiration paper, conical flasks. Heidolph rotary evaporator was used for removal of solvent under reduced pressure

Petroleum ether, Chloroform and ethanol, were used for extraction while conc. HCl,2%NaOH, Lead acetate, hagers reagents(picric acid) phytochemical test while mullerhintonagar, Dimethyl sulphoxide, $BaCl_2$ used for

antimicriobial activity.

Procedure of Phytochemical Tests
The different qualitative chemical tests were performed for establishing the profile of given extracts to detect various phyto-constituents present in them. The phytochemical were analyzed according to standard screening tests using conventional procedures.

Test for flavonoids
Alkaline reagent test; - Crude extract was mixed with 2ml of 2% solution of NaOH. An intense yellow color was formed which turned colorless on addition of few drops of diluted acid which indicated the presence of flavonoids. Lead acetate Test:-Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Test for alkaloids
Hager's Test: -5 ml of 2% HCl was poured in a test tube having plant extract. The test tube having the mixture was heated, filtered it and poured few drops of picric acid in a mixture. Formation of yellow color precipitate indicates the presence of alkaloids.

Test for saponins
Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Test for Tannins
Gelatin Test;-To the extract, 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 f concentrated H_2SO_4 was added to $1cm^3$ of eachcrude extract. A reddish brown colour indicates the presence of steroids.

Test for terpenoids
Salkowski test; - 5 ml of each solvent extract 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 colouration was formed at the interface thus indicating a positive result for the presence of terpenoids.

Detection of phenols
Ferric Chloride Test: Extracts 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)
0.5g of the powdered sample was taking in separate test tube and 10mls of chloroform was added then shake for 5 minutes again. Then the extracts were filtered and shake again. A bright pink colour in the upper aqueous layer indicates the presence of free anthraquinones.

Test for carbohydrates:
Molisch test: Treat extract with few drops of alcoholic alpha-naphthol. Add 0.2 ml concsulphuric acid slowly along the sides of test tube, purple to violet colour ring appears at junction

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 ^{0}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_2SO4 (1% V/V) with constant stirring to make 0.5 McFarland standards (Andrews and Wise, 2002).

Antimicrobial ActivityTest

Previously prepared paper discs containing different extracts were placed individually on the surface of the petriplates, 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 DISCUSSIONS

Percentage yield

From successive soaking extraction of *Acacia brevispica* leaves, crude yield of 0.38% w/w from petroleum ether, from 0.437%w/w from chloroform and 8.5%w/w from ethanol were obtained. From the percentage extract yield of the leaves plant extracts, the ethanolic extracts were the one that extract more percentageyield which indicated presence of more polar compounds in the plant.

Phytochemical Tests

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

No.	Phytochemical test	ethanol	chloroform	Petroleum ether
1	flavonoids	+	-	-
2	alkaloids	-	+	-
3	saponins	-	-	-
4	tannins	-	-	-
5	steroids	+	+	+
6	terpenoids	-	-	+
7	phenols	++	-	-
8	Quinones	-	-	-
9	anthraquinones	+	+	+
10	carbohydrates	-	+	+

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

The result of the phytochemical analysis of the leaf extracts in petroleum ether, chloroform and ethanol solvents has shown the presence of active phytochemical compounds in *Acacia brevispica* leaves. From the Table 1, it could be seen that, flavonoids, alkaloids, steroids, terpenoids, phenols, antraquinones and carbohydrateswere present in all the plants.

The petroleum ether and chloroform extracts of the plants were containing steroids, antraquinones and carbohydrates while terpenoids and alkaloids were found only in petroleum ether and chloroform extracts respectively. The leaf extracts of *Acacia brevispica*also showed presence of maximumphenolic, anthraquinonesand flavonoids in aqueous ethanolic extracts. Saponins,quinones and tannins are completely absent in all solvent extracts. In contrast to all anthraquanones and steroids are maximum present in all the solvent extract including petroleum ether the least non polar one. This result indicated that steroids and anthraquinones were isolated with non-polar solvents.

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 favorably with that of the standard antibiotics streptomycin and have appeared to be broad spectrum as its activities were independent on gram reaction. 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±0.08mm). 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 analysis 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 results revealed the presence of medicinally important constituents in this plant.

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. Therefore, extracts from these plants could be seen as a good source for useful drugs.

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