

Evaluation of Antibacterial Activities and Phytochemical Screening of the Crude Extracts of Roots of *Caylusea abyssinica* (Fresen.)

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Abstract: The main objective of this study was to evaluate *in vitro* antibacterial activities of crude extracts of the roots of *Caylusea abyssinica* extracted using different solvents namely methanol, acetone, chloroform and petroleum ether. The antibacterial activity tests were carried out against *Staphylococcus aureus* (DSMZ346), *Escherichia coli* (KL2DSM 498), *Pseudomonas aeruginosa* (DSMZ 1117) and *Salmonella thyphimurium* (ATCC 13311) using agar disk diffusion method. The methanol extract was found to be superior against all the tested bacterial strains followed by acetone, chloroform and petroleum extract regardless of the solvents (DEMSO or Tween 20) used to dissolve the crude extracts. *Staphylococcus aureus* (DSMZ346) was found to be more sensitive followed by *Salmonella thyphimurium* (ATCC 13311). Moreover, the methanol extract was found to be more active than the reference drug (Gentamycine). The qualitative phytochemical analyses were carried on methanolic crude extract of the root of *Caylusea abyssinica*. The results showed the presence of alkaloids, tannins, terpenes, saponins, flavonoids, polyphenols and steroids and absence of coumarins and anthraquinones. From the present study, it is possible to suggest that the extract could be potential source of new antimicrobial agents.

Key words: Antibacterial Activities · Crude Extract · Medicinal Plants · Phytochemical Screening · Jimma University

INTRODUCTION

Medicinal plants have been used for centuries to treat numerous human diseases [1]. Reports indicate that estimate of about 70-80% of world population; most of who are from developing countries still relies on medicinal plants for their primary health care systems [2, 3, 4, 5]. Moreover, several drugs currently in use in modern medicine are originated from medicinal plants [6, 7] and still there is a huge potential to discover more drugs in the future. Ethiopia is the home of many plant species that are commonly used in disease treatment by local healers. Similar to people of other developing countries, majority of the people in Ethiopia depend on traditional medicine that involve use of plant parts of medicinal plants [8, 9]. Some of the reasons in this regard are accessibility, affordability as compared to modern drugs, socio-cultural background and their effectiveness against a number of health problems [10].

Caylusea abyssinica (fresen) is a plant that belongs to the family of Resedaceae and it is distributed in Mediterranean region and Northern and Eastern Africa. Sudan, Ethiopia, Kenya, Uganda, Rwanda, Burundi, Tanzania and Malawi are some of the Eastern African countries where the plant is found in abundance [11]. There are reports that showed medicinal use of *Caylusea abyssinica* by people living in areas where it is growing. For instance, its leaves are used to expel intestinal worms and to treat stomachache [11], skin diseases [12], diabetes mellitus [13] and amoeba [14]. Similarly, its roots are used to treat abdominal pain [11], rabies [15], impotency and Scabies [16], diarrhea and expel intestinal parasites in humans [12, 17]. In Ethiopia, it is also used to treat internal diseases, fever, shivering and skin diseases of domestic animals [12, 18]. There are some experimental studies to explore the potentials of crude extracts of *Caylusea abyssinica* against different human diseases. In a recent report by [19], 80% methanolic extract of leaves

of this plant showed antidiabetic and oral glucose tolerance improving actions, particularly at the dose of 200 mg/kg in experimental animals. The report supported the traditional claims of use the leaves of *Caylusea abyssinica* for management of diabetes mellitus [13]. Although the plant is reported to be used traditionally for treating human health problems, there are no previous reports on antibacterial activity studies on the extract of the root of *Caylusea abyssinica*. Thus, as part of our search for new antibacterial agent from medicinal plants of Ethiopia, the roots of *Caylusea abyssinica* has been selected for the study. The antibacterial activity test and phytochemical screening results of crude extracts roots of *Caylusea abyssinica* are reported in this paper.

MATERIALS AND METHODS

Collection and Preparation of Plant Material: The root of *Caylusea abyssinica* was collected (in November 2012) from around Jiren and King Abba Jifar Palace, near Jimma city, Ethiopia. Botanical identification of the plant was made by a botanist and a specimen with voucher number AE 001 was deposited at the Herbarium of the Department of Biology, Jimma University. The collected plant material was chopped into small pieces and air-dried under shade without exposing it to direct sunlight and the dried plant material was grounded to 0.5 μ m sizes.

Extraction: The dried and powdered plant material (100 g) was sequentially extracted with petroleum ether, chloroform, acetone and methanol by employing maceration technique with continuous shaking (at 25°C for 72 hrs) using a shaker (GSL 400). The extracted matter from each solvent was filtered first using a cotton plug followed by Whatman No 1 filter paper. The filtrates were concentrated using rotary evaporator (Laborota 4000) at a temperature not more than 40°C. The resulting crude extracts were weighed and stored in refrigerator below 4°C [20] until further use for antibacterial activity tests and phytochemical screening.

Screening for Antibacterial Activities of the Crude Extracts

Bacterial strains: Antibacterial activity of gradient extracts of the roots of *Caylusea abyssinica* was investigated against bacterial strains namely *Staphylococcus aureus* (DSMZ346), *Escherichia coli* (KL2DSM 498), *Pseudomonas aeruginosa* (DSMZ 1117) and *Salmonella thyphimurium* (ATCC 13311) based

on disc diffusion method using standard procedures [21]. The bacterial strains were obtained from the Department of Biology, Jimma University.

Preparation of Media and Antibacterial Activity Test:

The antibacterial activity tests were carried out using a standard procedure reported in literature [21] as described briefly below. All bacterial cultures were first grown on 5% sheep red blood agar plates at 37 °C for 24 hrs prior to inoculation onto the nutrient agar. Few colonies (4 to 5) of similar morphology of the respective bacteria was transferred with a sterile inoculating loop to a liquid medium and this liquid culture was incubated until adequate growth of turbidity equivalent to McFarland 0.5 turbidity standard was obtained. The turbidity of the actively growing broth culture was adjusted with sterile saline solution to obtain turbidity optically comparable to that of the 0.5 McFarland standards that was resulted in a suspension containing approximately $1-2 \times 10^8$ CFU/ml for different strains. The inoculate of the respective bacteria was streaked on to the Hinton-Muller 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. Then 6 mm diameter sterile discs (Whatmann No. 3 paper) were placed on the surface of the inoculated Agar in Petri dishes and 50 mg/ml concentration of the test solutions (that were prepared by dissolving isolated compounds in DMSO) were also applied onto the discs using micropipette. After addition of test solutions on the discs, they were allowed to diffuse for 5 minutes and the plates were then kept in an incubator at 37 °C for 24 hrs. The antibacterial activity was evaluated, after 24 hrs, by measuring the diameter of zone of growth inhibitions surrounding the discs (in mm) using transparent ruler. In this experiment, Gentamycin (10 μ g) was used as positive control whereas dimethyl sulfoxide (DMSO) and Tween 20 were used as negative control in the experiment.

Phytochemical Screening: The methanol extract of *Caylusea abyssinica* was subjected to standard phytochemical screening to test the presence of phytoconstituents such as alkaloids, terpenoids, flavonoids, tannins, resins, coumarins, saponins, anthraquinones, polyphenols and steroids using standard procedures reported for each class of compound as briefly described below. The tests were based on the visual observation of color change and in some cases formation of a precipitate after addition of a particular reagent.

Test for Alkaloids: 0.2 g of the crude methanolic extract was dissolved in 10 ml of 1% HCl solution. The solution was placed in a water bath for few minutes and then 1 ml of the solution was placed into two test tubes. One of the tubes was then treated with 2-4 drops of Dragendorff's reagent and the other with 2-4 drops of Mayer's reagent. The presence of alkaloids is indicated by the appearance of an orange reddish precipitation and white-precipitate for Mayer's reagent, respectively [22, 23].

Test for Terpenoids: A small quantity of the methanolic extract was dissolved in 2 ml of chloroform. Then 3 ml of conc. sulfuric acid (H_2SO_4) was carefully added to form a layer. Formation of a reddish-brown colored interface can be used to prove the presence of terpenoid [24].

Test for Flavonoids: A small quantity of the crude extract was dissolved in 5 ml of ethanol. In another test tube, a mixture of 5 ml of ethanol and 5 ml of 50% KOH solution was prepared. Then the two solutions were mixed together. Formation a yellow colored product was used to confirm presence or absence flavonoids [25].

Test for Tannins: 10 ml of the ethanolic solution of the crude methanol extract was taken in a test tube, few drops of 1% ferric chloride reagent was added. Appearance of bluish colored mixture could be used as indicator of the presence of tannins [23].

Test for Coumarins: 5 ml of previous filtered extracts were putted in a test tube and covered by a filter paper saturated in NaOH. Then, the test tube was placed in a water bath to heat it for 10 minutes. Finally, a filter paper was taken and exposed to UV light. In this test observation of a green bright yellow color is used to confirm the presence or absence of coumarins [26].

Test for Saponins: A small quantity of the crude extract was boiled with 5ml of distilled water in a water bath for 10 minutes. The mixture was filtered while hot and allowed to cool. The following test was then carried out. 2.5 ml of filtrate was diluted to 10 ml with distilled water and shaken vigorously for 2 minutes (Froth formation was used as an indicator for the presence of saponins in the filtrate) [27].

Test for Polyphenols: Five ml of previous filtered solution of the crude extract was taken and then 1 ml of 1% $FeCl_3$ and 1 ml of 1% $K_3(Fe(CN)_6)$ solutions were added. The appearance of fresh radish blue color indicated the presence of polyphenols [28].

Test for Anthraquinones: About 0.5 g of the crude extract was placed into a dry test tube and then 5 ml of chloroform was added and shaken for 5 minutes. The solution was filtered and the filtrate shaken with an equal volume of 100% ammonia solution. The formation of pink violet or red color indicates the presence of anthraquinones [29].

Test for Steroids: Two ml of acetic anhydride was added to a test tube containing 0.5 g of extract in 2 ml of H_2SO_4 . The color changed from violet to blue or green is used to confirm the absence or presence of steroids [30].

RESULTS AND DISCUSSION

Reports indicate that *Caylusea abyssinica* is known for its wide application as traditional medicine to treat different ailments [11, 31]. Thus, the classes of compounds need to be analyzed in its different morphological parts of the plant. In this section of our report, *in vitro* antibacterial activity tests of crude extracts of roots of are *Caylusea abyssinica* and also phytochemical screening of the methanol extract are presented.

Antibacterial Activities of Crude Extracts from Root of Caylusea Abyssinica: Air-dried root of *Caylusea abyssinica* (100g) were extracted with gradient solvent systems and maceration methods gave 0.292, 0.695, 1.438 and 2.360 g crude extracts for petroleum ether, chloroform, acetone and methanol, respectively. The crude extracts were then subjected to antibacterial activity tests using the method discussed in the methods and material section and the four bacterial species namely *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella thyphimurium*.

The results of antibacterial activity tests (for test solutions dissolved in DMSO) showed that the majority of the crude extracts are active in inhibiting the growth of the bacterial strains as demonstrated by the observed inhibition zone values. When the zone of inhibition values of the crude extracts are compared to each other, the activities of crude extracts of methanol, chloroform and methanol were found to be generally higher than that of the reference compound (Gentamycine) whereas that of petroleum extract were lower than that of Gentamycine (Table 1). Among all crude extracts tested, methanol extract was found to be superior in activity against all the bacterial species. The data revealed that the zone of

Table 1: Inhibition zones (in mm) of the crude extracts dissolved DMSO.

S. No	Extracts	Bacterial species			
		<i>S. aureus</i>	<i>S. thyphi</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
1	Methanol extract	31	29	24	17
2	Acetone extract	25	23	18	11
3	Chloroform extract	17	16	17	13
4	Petroleum ether extract	13	14	16	-
	DMSO (solvent)	-	-	-	-
	Gentamycin (10 μ g) (Ref.)	19	14	22	18

Table 2: Inhibition zones (in mm) of the crude extracts dissolved in Tween 20.

S. No	Extracts/Solvent/Drug	Bacterial species			
		<i>S. aureus</i>	<i>S. thyphi</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
1	Methanol Extract	30	28	19	13
2	Acetone extract	27	19	11	10
3	Chloroform extract	18	12	14	11
4	Petroleum ether extract	11	13	11	-
	Tween 20 (Solvent)	-	-	8	-
	Gentamycin (10 μ g) (Ref)	19	14	22	18

Table 3: Phytochemical screening results of methanolic extract of the roots of *Caylusea abyssinica*.

Test	Test result
Alkaloids	+
Terpenes	+
Flavonoids	+
Tannins	+
Resins	+
Cumarins	-
Saponnins	+
Polyphenols	+
Anthraquinones	-
Steroids	+

Note: + = present; - = absent

inhibition values are 31 mm, 29 mm, 24 mm and 17 mm against *Staphylococcus aureus*, *Salmonella thyphimurium*, *Escherichia coli* and *Pseudomonas aeruginosa*, respectively. The corresponding inhibition zone values of Gentamycine were found to be 19 mm, 14 mm, 22 mm and 18 mm against *Staphylococcus aureus*, *Salmonella thyphimurium*, *Escherichia coli* and *Pseudomonas aeruginosa*, respectively (Table 1). The antibacterial activities of methanol were followed by that of acetone and chloroform that were found to be active against all test strains used in the experiment (See the data in Table 1). On the other hand, the petroleum

ether extract was found to be not active against *Pseudomonas aeruginosa* and lower antibacterial activities against *Staphylococcus aureus* (13 mm), *Escherichia coli* (16 mm) and *Salmonella thyphimurium* (14 mm) as compared to Gentamycine (Table 1). No antibacterial activities were observed for the DMSO that was used as negative control.

In our study, similar procedures were repeated for crude extracts dissolved in Tween 20 in order to investigate changes in antibacterial activity (if there are any). The results showed a similar trend as that of the crude extracts dissolved in DMSO (Table 1 vs. Table 2). The methanol extract was again found to be superior in activity against all the bacterial species used in the experiment as it was evidenced by observed high zone of inhibition values. The observed inhibition zone values of methanol extract dissolved in Tween 20 were comparable to the values of the methanol extract dissolved in DMSO against the bacterial species used in the experiment (Table 2). However, its inhibition zone values against *Escherichia coli* and *Pseudomonas aeruginosa* were found to be lower than the observed activity of Gentamycine against these two bacterial species (Table 2). Similar to DMSO, no inhibition was observed for Tween 20 against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella thyphimurium* and but zone inhibition value of 8 mm was observed against *Escherichia coli* (Table 2). Therefore, based on the

observed data, the methanol extract was subjected to phytochemical screening to investigate the phytochemicals that could be responsible for its superior antibacterial activity.

Phytochemical Screening Test: The phytochemical screening test of the methanol extract of *Caylusea abyssinica* showed the presence of some phytochemical constituents such as alkaloids, terpenes, flavonoids, resins, polyphenols, saponins, steroids and tannins. No anthraquinones and coumarins were detected from the phytochemical screening test (Table 3). This observation is consistent with previous report by [19]. The authors reported that phytochemical screening of 80% methanolic extract of leaf of *Caylusea abyssinica* showed positive test for secondary metabolites such as reducing sugars, alkaloids, steroidal compounds, phenolic compounds, tannins, saponins, flavonoids, cardiac glycosides and negative for the presence of anthraquinones and phlobatannins [19]. The observed superior antibacterial activity of the crude methanol extract as compared to other extracts and the reference drug (Gentamycin) could be due to the combined or individual effects of the secondary metabolites that are detected [32]. Therefore, further studies are recommended to confirm specific phytochemical(s) that can be attributed for the observed antibacterial activity of the methanol crude extract.

CONCLUSIONS

The *in vitro* antibacterial activity tests that were carried out using Agar Disc Diffusion method showed that antibacterial activity of the crude extracts of the root *Caylusea abyssinica* to be promising source of bioactive compounds that could be used as leads in finding new clinically effective antibacterial compounds. The majority of the extracts, especially those of polar solvents extracts namely methanol, acetone and chloroform extracts showed higher and/or comparable antibacterial activities as compared to the reference drug Gentamycin. Phytochemical studies revealed the presence of alkaloids, terpenes, flavonoids, polyphenols, saponins, steroids and tannins and absence of anthraquinones and coumarins in the methanolic extract that showed superior antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella thyphimurium* and *Escherichia coli*. Future studies or isolation of specific compounds are suggested in order to find out whether the observed high antibacterial activities of the crude extracts (e.g., methanol extract) is due to the combined or individual effects of the phytochemicals found in it or not.

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