JOURNAL OF ADVANCED
BOTANY AND ZOOLOGY

Journal homepage: http://scienceq.org/Journals/JABZ.php

Research Article Open Access

Phytochemical Screening and Antibacterial Activity of Leaves Extract of *Bersama abyssinica*

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Received: June 12, 2015, Accepted: July 23, 2015, Published: July 23, 2015.

ABSTRACT

The present work investigate the phytochemical screening and antibacterial activities of leaves extract of *B. abyssinica* by disc diffusion method .The leaves of *B. abyssinica* were extracted with increasing gradient of organic solvents (petroleum ether, chloroform, ethyl acetate and methanol). Phytochemical screening results confirmed the presence of alkaloids, glycosodes, flavonoids, steroids, phenols, tannins, triterpenene, anthraquinones, polysterols, coumarins and absence of saponins. Evaluation of antibacterial activity of crude leaves extracts of *B. abyssinica* via disc diffusion method against *Escherichia coli*, *Staphylococcus aureus Klebsiella pneumonia* and *Salmonella typhi* showed dose dependant activities in both microorganisms. *B. abyssinica* methanol and chloroform leaves extracts exhibited much higher antibacterial activity against the tested pathogenic bacteria. Results have proved that *B. abyssinica* leaves possess antibacterial activity.

Key words: *B. abyssinica,* phytochemical screening, anti-bacterial activity.

INTRODUCTION

Bersama abyssinica (Melianthaceae) belongs to the genus *Bersama* which comprises four species [1]. It grows in lowland bush savanna, gallery forests and montane forests, from sea-level up to 2700 m altitude. In East Africa, there are two subspecies of *B. abyssinica* namely; *B. abyssinica* Fresen. subssp. *abyssinica and B. abyssinica subsp. paullinioides*. It is distributed in Democratic Republic of Congo, Tanzania, Mozambique, Zambia, Zimbabwe, Angola, Nigeria, Ethiopia, Kenya, Sudan and Uganda [2], is used by local communities for the treatment of microbial infections including mycobacterial infections [3].

B. abyssinica grows in Ethiopia is recognized as subspecies *B. abyssinica* where it is known locally as Azamer (Amharic) and Lolchissa (Oromeffa) [4, 5]. In Ethiopian traditional medical practices, the leaves extracts of *B. abyssinica* are administered orally for treating dysentery, tumour [6], stomach disorders such as abdominal pain, colic, diarrhoea, cholera, intestinal worms, amoebiasis, rabies, syphilis, gonorrhoea and malaria are also treated with these decoctions [5].

In continuation of the ongoing project to study the chemical constituents and biological activity of medicinal plants of Ethiopian flora, we hereby report the phytochemical screening of various bioactive constituents and evaluate antimicrobial activity of leaves crude extract of *B. abyssinica.*

MATERIALS AND METHODS

Plant material collection: The leaves of *B. abyssinica* was collected in January, 2015 from Amahara region, Awi Zone near Enjibara Town from Jibaber, located 475 km from Addis Ababa, and capital of Ethiopia. The plant material was identified by Mr. Mark botanist in SIM forestry project study, where voucher specimens were deposited.

Extraction: The collected plant material was dried and powdered with mechanical grinder. 250g dried leaves powder of *B. abyssinica* was extracted by cold percolation with 1.5L (petroleum ether, chloroform, ethyl acetate and methanol) separately two times for 48 h while shaking at speed of 220r/min at room temperature. The solution was filtrated by using Whatmann No.1 filter paper by suction filtration and the filtrate was concentrated by using a rotary evaporator to yield the crude extract. The amount of the crude extract and color of the extract in each solvent indicated in (Table 1).

TLC profile: For TLC analysis plate with silica gel 60 F_{254} TLC (Merck, Germany) was used. Plate markings were made with soft pencil. Glass capillaries were used to spot the sample. TLC applied sample by using capillary at distance of 1 cm at 4 tracks with petroleum ether: ethyl acetate (9:1) and petroleum ether: Ethyl acetate (7:3) solvent system. After pre-saturation with mobile phase, freshly prepared iodine spray reagents were

used to detect the bands on the TLC plates. The movement of the analyte was expressed by its retention factor (R_f), values

Preliminary phytochemical screening

Phytochemical screening tests were done to determine the class of compounds present in the crude leaves extract *B. abyssinica* following the standard protocols [7, 8]. The results were reported as (+Ve) for presence and (-Ve) for absence in (Table 2).

Alkaloids (Wagner's test): Extracts were dissolved individually in dilute Hydrochloric acid and filtered. Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Glycosides (Modified Borntrager's test): Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides. Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

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.

Phytosterols (Libermann Burchard's test): Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

Triterpenes (Salkowski's test): Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

Phenols (Ferric Chloride test): Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

Tannins (Gelatin Test):To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

Anthraquinones: About 0.5 g of the extract was boiled with 10% HCl for few minutes in water bath and filtered. The filtrate was allowed to cool and equal volume of $CHCl_3$ was added to the filtrate. Few drops of 10% ammonia was added to the mixture and heated. The formation of rose-pink colour was taken as an indication for the presence of anthraquinones.

Coumarins: 3 ml of 10% NaOH was added to 2 ml of extract formation of yellow colour indicates the presence of coumarins.

Flavonoids (Alkaline Reagent Test): Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

Steroids (Salkowski test):Treat about 0.2 g of the extract was mixed with 2 mL of chloroform and 3 mL of conc. Sulphuric acid, red color at lower layer indicates presence of steroids and formation of yellow colored lower layer indicates presence of triterpenoids.

Microorganism Strain: The antibacterial activity of extracts of *B. abyssinia* were evaluated by using four bacterial strains: One Gram-positive bacteria *Staphylococcus aureus* and three Gram- negative *Escherichia coli*, *Klebsiella pneumoniae* and *Salmonella typhi*. These microorganisms were cultured in microbiology laboratory of Department of biology, Bahri Dari University, Ethiopia.

Evaluation of Antibacterial Activity: The antibacterial activity of leaves extract of *B. abyssinia* was evaluated by the disc diffusion method and performed in the accordance with the guidelines of National Committee for Clinical Laboratory Standards [9], with minor modification. A 24 hour old culture of selected bacteria was mixed with sterile physiological saline (0.85%) and the turbidity was adjusted to the standard inoculum of MacFarland scale 0.5 [~106 colony forming units (CFU) per milliliter]. Petri dishes containing 20µL of Mueller-Hinton agar were used to inoculate bacterial suspension. Filter paper discs (Whatman no. 1, diameter = 6 mm) impregnated with the extract solutions prepared in DMSO (100mg/ml, 75mg/ml, 50mg/ml and 25mg/ml) were placed on the inoculated plates and petri dishes were incubated for 24 h at 37°C. A paper disc impregnated with Ciprofroxine (50mg/ml) was used as positive control. The inhibition zone diameters were measured in millimeters as indicated (Table 3).

RESULTS

Percentage of yield extract

The yield of sequential extracts (gm) is shown in (Table 1). The amount obtained from Petroleum ether, chloroform, ethyl acetate and methanol extracts are presented respectively (Table1).

Table 1:- extractive values of different extract of B. abyssinica leaves

S.No	Solvent	Colour of extract	Yield of the extract (in gm)	Percentage yield(%w/w)
1	Petroleum ether	Brownish	14	5.6%
2	Cholroform	Brownish	16	6.4%
3	Ethyle acetate	Brownish	19	7.6%
4	Methanol	Brownish	21	8.4%

TLC profile

TLC of petroleum ether extract of *B. abyssinica* revealed the presence of four spots having R_f values of 0.15, 0.27, 0.45 and 0.74 respectively with Petroleum ether: ethyl acetate (9:1) as eluent. Similarly, two spots were observed with R_f of 0.69 and 0.84 using Petroleum ether: Ethyl acetate (7:3) as eluent.

TLC of chloroform extract of *B. abyssinica* revealed the presence of six spots having R_f values of 0.25, 0.30, 0.35,0.42, 0.60 and 0.70 respectively using petroleum ether: ethyl acetate (9:1) as eluent. Likewise, four spots were observed with R_f of 0.15, 0.34, 0.78 and 0.90 using solvent system Petroleum ether: Ethyl acetate (7:3) as eluent. .

TLC of Ethyl acetate extract of *B. abyssinica* revealed the presence of six spots having R_f values of 0.26, 0.33, 0.40, 0.53, 0.83 and 0.88 respectively using petroleum ether: ethyl acetate (9:1). Similarly, six spots were obtained having R_f 0.16, 0.20, 0.40, 0.54, 0.81 and 0.92 with Petroleum ether: Ethyl acetate (7:3) as eluent.

TLC of Methanol extract of *B. abyssinica* revealed the presence of three spots having R_f values of 0.33, 0.71, and 0.91 (petroleum ether: ethyl acetate (9:1) as eluent) and five spots having R_f of 0.18, 0.33 0.40, 0.66 and 0.93 (Petroleum ether: Ethyl acetate (7:3) as eluent)

Phythochemical screening test

The leaves of *B. abyssinia* were used to investigate preliminarily phytochemical studies. The selected part of plant was analyzed for phytochemical screening for the extracts obtained from cold extraction successfully using gradient solvent system, petroleum ether, chloroform, ethyl acetate and methanol respectively. The extracts were subjected to various qualitative tests for phyto-constituents such as alkaloids, flavonoids, triterpenoids, tannis, steroids, Phytosterols, Coumerins, Sterols, Phenols Saponins and anthraquinones.

Alkaloids: The leaves extracts of *B. abyssinia* reacted positively with Wagner's reagent, showed that a reddish precipitate indicating the presence of alkaloids in all solvent system.

Flavonoids: The leaves extracts of *B. abyssinia* have shown positive response to alkaline reagent by the disappearing of the yellow color indicates the presence flavonoids in all solvent system.

Glycosides: extracts also reacted positively Borntrager's reagents in which observed that the formation of rose-pink colour in the ammonical layer indicated that the presence glycosides in all solvent system.

The results confirmed that alkaloids, flavonoids and glycosides are major secondary metabolites in leaves of *B. abyssinia.*

Phenols, Tannins, Coumarin and Anthraquinones: have shown positive response to the respective reagent tests of ethyl acetate and methanol leaves extracts of *B. abyssinia.* The results confirmed that phenols, tannins, coumarin and anthraquinones are also second major secondary metabolites in leaves of *B.abyssinia.*

Triterpenoids Phytosterols and Steroids: The extracts were shown that positive test with respective reagents; only in methanol extract of *B. abyssinia* but absence in other solvents.

Saponins: the extracts was checked for presence of saponins, it showed that the negative result for Froth reagent test in all solvent system.

Table 2:- Phytochemical screening of leaf Bersama abyssinica

Chemical components	Reagents	Solvent system			
		P. ether	chloroform	Ethyl acetate	methanol
Alkaloids	Wagner reagent	+	+	+	+
Flavonoids	Alkalline reagent	+	+	+	+
Glycosides	Borntrager's reagents	+	+	+	+
Phenols	$FeCl_3$	-	-	+	+
Tannis	gelatin reagent				
Coumerins	Alkalline reagents	-	-	+	+
Anthraquinones	10%NH_3 solution	-	-	+	+
steroids	DiluteNH_3 solution	-	-	-	+
polysterols	Libermann Burchard's test	-	-	-	+
Triterpenenes	Salkowski's test	-	-	-	+
Saponins	Froth test	-	-	-	-

(+) indicates Present (-) indicates absent

Antibacterial activities evaluated by disc diffusion method. The antibacterial activity in terms of zone of inhibition (in mm diameter) of petroleum ether, chloroform, ethyl acetate and methanol extracts of *B. abyssica* leaves at the different concentrations of 25, 50,75 and 100mg/ml against four pathogenic organisms, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonae*, and *Salmonellae typhi* was presented in (table3.3). The activity of extracts has also been compared with the broad spectrum commercially available antibiotic (Ciprofloxacin). Ciprofloxacin showed the inhibition zone for *E. coli* (19 mm), *S. aureus* (20 mm), *K. pneumoniae* (18 mm) and *S. typhi* (18mm). The results indicated that the concentrations 25, 50, 75 and 100mg/ml of the all four types of *B. abyssica* extracts were active against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonae*, and *Salmonellae typhi.* The results also showed that the ethyl acetate and methanol was the best solvents for extracting antimicrobial substances from this plant compared to petroleum ether and chloroform. It can be suggested that *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonae*, and *Salmonellae typhi* were the most resistant organisms to the concentrations of 25 and 50mg/ml of the petroleum ether extract. Maximum activity of chloroform extract was seen against *Escherichia coli* at concentration of 25mg/ml (Table3). The plant extracts compared favourably with the standard antibiotic Ciprofloxacin (Table 3).

Table 3:- Antimicrobial activity of leaf extract of Bersama abyssinica.

Diameter of zone of inhibition (mm) *																	
Conc.	100mg/ml				75mg/ml				50mg/ml				25mg/ml				
Solvent	P. ether	chloroform	ethyl acetate	methanol	P. ether	chloroform	ethyl acetate	methanol	P. ether	chloroform	ethyl acetate	methanol	P. ether	chloroform	ethyl acetate	methanol	Cipro. (50mg/ml)
E. coli	*10*	*14*	*13*	*14*	*9*	*14*	*12*	*13*	*8*	*13*	*11*	*12*	*6*	*15*	*10*	*11*	19
S.aureus	*12*	*16*	*14*	*16*	*10*	*15*	*13*	*15*	*9*	*12*	*13*	*14*	*4*	*10*	*11*	*13*	20
K.pneumoniae	*11*	*13*	*14*	*13*	*9*	*12*	*12*	*12*	*8*	*10*	*12*	*11*	*5*	*9*	*10*	*10*	18
S. typhi	*9*	*12*	*15*	*15*	*6*	*11*	*14*	*14*	*5*	*10*	*13*	*13*	*4*	*7*	*12*	*12*	18

Conc.: concentration; *: mean of three replicates; Cipro: Ciprofloxacin

DISCUSSIONS

TLC profiling of all four extracts gives an impressive result that directing towards the presence of number of phytochemicals. Various phytochemicals gives different R_f values in different solvent system. This variation in R_f values of the phytochemicals provides a very important clue in understanding of their polarity and also helps in selection of appropriate solvent system for separation of pure compounds by column chromatography.

Mixture of solvents with variable polarity in different ratio can be used for separation of pure compound from plant extract. The selection of appropriate solvent system for a particular plant extracts can only be achieved by analysing the R_f values of compounds in different solvent system. Different R_f values of the compound also reflect an idea about their polarity. This information will help in selection of appropriate solvent system for further separation of compound from these plant extracts.

The plant *B. abyssinica* belonging to the family Melianthaceae was taken up for the study by here to screen and give a report on the possible preliminary phytochemical screening and exhaustive extraction of the plant material was done with gradient solvent system, petroleum ether, chloroform, ethyl acetate and methanol respectively, and the extracts were screened for the presence of various medicinally active phyto-constituents.

The leaves extracts of the plant of *B. abyssinica* was subjected to phytochemical screening which reveals the presence of various pharmacological active components. The traditional use of the plant may be attributed to its high contents of alkaloids, flavonoids, triterpenoids, tannis, steroids, phytosterols, coumerins, sterols, terpenoids, phenols and anthraquinones constituents. *B. abyssinica* methanol and ethyl acetate leaves extracts exhibited much higher antibacterial activity against the tested pathogenic bacteria (Table 3).

The chloroform leaves extract of *B. abyssinica* inhibited the growth of *Escherichia coli* with value of 15*mm* at the 25mg/ml and had much higher concentration to the rest of bacterial strains. The chloroform fractions exhibited moderate antimicrobial activity in both bacterial species. Conversely, the petroleum ether extracts exhibited less activity against bacterial species.

Drug resistance among bacterial species is a serious problem in public health thus the discovery and development of new antimicrobial drugs from plants is among the most exciting areas of pharmacological research [10]. The present study extends the efforts of discovering drug templates from Ethiopia medicinal plants. Extracts from the leaves of *B. abyssinica* were evaluated against four pathogenic bacteria species. The results of evaluating antibacterial activities of *B. abyssinic*a extracts revealed that *B. abyssinic*a contains secondary metabolites with antibacterial activity against Gram positive bacteria and Gram negative bacteria.

Methanol and ethyl acetate leaves extract *B. abyssinica* exhibited high antibacterial against all tested bacteria which implies that polar secondary metabolites are responsible for the activity. Previous report on the leaves of *B. abyssinica* has established the presence of Flavonol glycosides, Xanthone and mangiferin [11]. Flavonol glycosides have been reported to possess high antimicrobial sensitivity to some bacterial strains than gentamycin [12]. It is therefore possible that Flavonol glycosides in the B. abyssinica leaves methanolic extract are responsible for the antibacterial activity displayed. Flavonol glycosides from *B. abyssinica* are therefore potential source of bactericides.

CONCLUSION

The present study revealed the presence of alkaloids, flavonoids, triterpenoids, tannis, steroids, phytosterols, coumerins, sterols, terpenoids, phenols and anthraquinones constituents. It is evident that the extract of *B. abyssinica* contains secondary metabolites which possess remarkable antibacterial activities. Thus, bactericides of great value could be developed from antimicrobial secondary metabolites from *B. abyssinica* as alternative medicines to manage pathogenic bacteria.

Acknowledgment

The authors are grateful to acknowledge Debre Tabor University for providing facilities required to carrying this work. Department of Chemistry, Bahir Dari University is duly acknowledged for providing laboratory Equipment.

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Citation: Mathewos Anza, et al (2015). Phytochemical Screening and Antibacterial Activity of Leaves Extract of Bersama abyssinica. J. of Advanced Botany and Zoology, V3I2. DOI: 10.15297/JABZ.V3I2.07.