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Phytochemical Investigation and Antimicrobial Study of Some Selected Traditional Medicinal Plants from North Gondar Zone

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Abstract

The present study was carried out on the phytochemical investigation and antimicrobial activities of the leaf extract of H. abyssinica, O. lamifolium, R. chalepensis and S. incanum because, the plants was used for traditional medicinal in Ethiopia specially in North Gondar zone as treatment for many ailments. The powdered leaf of H. abyssinica, O. lamifolium, R. chalepensis and S. incanum was sequentially extracted with organic solvents: petroleum ether and methanol respectively. The petroleum ether and methanol crude extracts was subjected to phytochemical screening to test the presence of steroids, alkaloids, flavonoids, saponins, glycosides, phenols, tannins and terpenoids compounds that has might be responsible for the claimed activities by local people. The crude extracts was tested against four bacterial species (Gram negative bacteria: Escherichia coli and Salmonela thyphei; Gram positive bacteria: Staphylococcus aureusand Streptococcus agalactiae) using paper disc diffusion method. The results showed that the methanol was the best solvent for extracting antimicrobial substances from those plant when compared to petroleum ether. This indicates that plants rich in a wide variety of secondary metabolites such as tannins, alkaloids, tannin, flavonoids and terpenoids.

Keywords: Traditional Medicinal, Phytochemical Screening

Introduction

Background Information

Medicinal plants are now more focused than ever because they have the capability of producing many benefits to society indeed to mankind, especially in the line of medicine and pharmaceutical. Plant parts such as leaves, roots and bark are used for the therapeutic purposes and as well serve as precursors for the synthesis of useful drugs due to their ethno medical importance in nature. The medicinal potentials of these plants could be traceable to the bioactive phytochemical constituents that are responsible for the physiological action on the human body [1]. Substances derived from plants have recently being of great interest due to their versatility. These substances in the plants which enhance their usefulness globally are classified as phytochemical.

Phytochemicals are the chemicals that present naturally in plants such as medicinal plants, vegetables and fruits that work with nutrients and fibers to act against diseases or more specifically to protect against diseases. The elucidation of their structures and their chemistry, synthesis and biosynthesis are major areas of organic chemistry [2].

Naturally occurring compounds may be divided into two broad categories. Firstly, there are those compounds which occur in all cells and play a central role in the metabolism and reproduction of those cells. These compounds include the nucleic acids, the common amino acids and sugars. They are known as primary metabolites. Most primary metabolites exert their biological effect within the cell or organism that is responsible for their production. There are also the high molecular weight polymeric materials such as cellulose, the lignin and the proteins which form the cellular structures. Secondly, there are those compounds that are characteristic of a limited range of species. These are the secondary metabolites. Secondary metabolites, on the other hand, have often attracted interest because of their biological effect on other organisms. The biologically active constituents of medicinal, commercial and poisonous plants have been studied throughout the development of organic chemistry. Many of these compounds are secondary metabolites [3]. Phytochemicals play a vital role against number of diseases such as asthma, arthritis, cancer etc. Unlike pharmaceutical chemicals these phytochemicals do not have any side effects because of their cure diseases without causing any harm to human beings [4]. There has been a great deal

of interest recently in the role of complementary and alternative medicines for the treatment of various acute and chronic diseases. Where the different classes of phytochemicals, much interest are has focused on the anti-inflammatory and antioxidant properties of polyphenols found in various botanical agents [5]. Plants with high medicinal value play vital roles in the health of individuals and the society generally [6]. Many communities in Asia, Africa and South America have used medicinal plants for the treatment of diseases for centuries. These substances have been used for various illnesses such as infections. Microorganisms especially bacteria can be found in almost everywhere and have the tendency to adapt quickly to their immediate environment. Infections caused by bacteria are responsible for considerable mortality and morbidity worldwide especially in developing countries due to poor sanitation, unhygienic and overcrowded living conditions. Drugs for treating bacterial infections may lose their effectiveness with time, because the targets of these drugs keep shifting their forms. The time period for developing new drugs are often long and hence drug resistance take place [7]. Majority of the people living in the developing world are struggling to increase the standard of living and to improve the health care delivery in the face of increasing poverty and growing population. According to WHO survey, 80% of populations living in the developing countries rely exclusively on traditional medicine for their primary health care needs of which most involve the use of plant extract [8]. Keeping in view the above fact it can be inferred that by careful collection of data and experimentation, medicine of much higher value and low cost can be isolated from the plants, to fulfill the requirement of the major portion of the world population specially that of developing world. Therefore, importance, necessity and potentiality of medicinal plants cannot be over looked. In this regard Ethnobotany has great potential to provide new and useful plants for the benefits of the world [9]. Herbal medicines have fewer side effects than synthetic drugs and due to their antioxidant properties; they reduce drugs toxicity [10, 11]. In addition,the natural effective ingredients cause biological balance and prevent drug accumulation in body [12]. Therefore, medicinal plants can be used in the treatment of various diseases [11]. Herbs as a group of plants was also used as food (vegetables) and flavors for hundreds of years in many parts of the world. They have been traditionally regarded as natural remedies for common ailments of human. They produce a wide array of compounds (flavonoids, alkaloids, phenols and tannins) most of which are used in plant defense against predators. Nearly all cultures and civilizations from ancient times to the present day have used herbal medicines for antimicrobial sources to cure infections [13]. Herbal medicines are often used for the treatment of wounds; especially in developing countries [14]. The research made so far on Ethiopian medicinal plants has been mostly of producing inventories and checklists. Ethiopian traditional life is painted with the hallmark of widespread use of traditional medicinal plants with various levels of sophistication within the indigenous medicinal lore. It is blended with religious thinking and various beliefs need further investigation. The basic categories of practitioners also are difficult to define. Ethiopian people have their own set of written and oral pharmacopoeias with the medicinal use of some species being restricted to each ethnic group. The cultural and indigenous knowledge of medicinal plants in Ethiopia is unevenly distributed among each community members. Peoples in different zones/location with different religious, linguistic and cultural backgrounds have their own specific knowledge about use of plants which in part has gradually entered wide circulation in the country [15]. This research is important and applicable to our times for various reasons. Firstly, it promotes the discovery of new alternatives to drugs currently being used. Secondly, it is important from a conservation point of view; if over exploitation of a medicinal plant species should occur,restrictive measures can and should be taken to ensure survival and sustainability of the specific species. From a cultural point of view, important knowledge regarding the traditional use of plants is lost as it is not being passed on from one generation to the next anymore. Thus, it is important that this knowledge be documented to ensure that it is at the disposal of future generations who may benefit from it [16].

Materials and Methodology

Study Areas

The leaves of Hagenia abyssinica, Ocimum lamifolium, Ruta chalepensis and Solanum incanum was collected from Debark town and Surrounding Kebeles,Debark in February, 2019.The botanical specimens of the plant was identified and stored in debark university laboratory. Most of the experimental techniques such as solvent crude extraction, phytochemical screening andantimicrobial activity tests was done in the University of Gondar.

Instruments and Apparatus

The instruments and other materials which was used in this study are separatory funnel, oven, Maceration apparatus, Whatman No. 1 filter paper, pipettes, water bath, Cuvette, polyethylene bags, grinder, beakers, electronic balance, conical flask, measuring cylinder, Rota vapor, Spatula and appropriate media for both phytochemical screening tests and bacteria and fungi.

Chemicals and Reagents

The chemicals which was used in this research are solvents (methanol and Petroleum ether), anhydrous sodium sulfate, sulphuric acid, PDA, MHA, distilled water, sodium hydroxide, acetic anhydride, ferric chloride, 25% ammonium hydroxide, hydrochloric acid and acetone.

Collection of Medicinal Plants

In order to identify biologically active plants sampling is the first important step. For this study, pre identified plants by the local practitioners was collected from the leaf parts of the plants in Debark Town and surrounding kebeles using standard procedures. The collected plants was washed with water to remove the soil and dust particles. Then they was dried in thoroughly shaded place, and blended to form a fine powder and stored in airtight containers. The leaf parts of plants was used to prepare extracts for the further study.

Extraction of the Plant Material

The leaf of the Hagenia abyssinica, Ocimum lamifolium, Ruta chalepensis and Solanum incanum was ground using the grinder. The resulting powder was packed with in plastic bag to prevent it from any other mixing of surrounding materials until the experiment is conducted. Air dried and a powdered leaves of (300 g) each Hagenia abyssinica, Ocimum lamifolium, Ruta chalepensis and Solanum incanum was first soaked with 2.5 L petroleum ether for 72 hrs in at room temperature and filtered with filter paper (Whatman No.1). The filtrate was collected and concentrated at 40 ℃ using a Rotary evaporator. After petroleum ether extract the defatted powder was dried at room temperature and weighed for further extract with Methanol. Then, Methanol extract of leaf of the plant materials was collected and filtered using what man No.1 filter paper and concentrated by rotary evaporator at 40 ℃ and the crude extract was kept at 4 ℃ until analysis.

Phytochemical Screening on Leaves of Plant Materials

To the best of our knowledge the preliminary phytochemical screening for the leaves of Hagenia abyssinica, Ocimum lamifolium, Ruta chalepensis and Solanum incanum was carried out to analyze the presence/absence of compounds namely: Saponins, Quinines, Flavonoids, Phenols, Alkaloids, Carbohydrates, Terpenoids, Steroids, Tannins and Glycosides. In this study, the preliminary phytochemical screening was carried out on the plant extract, following the standard procedures described by [17, 18].

Detection of Saponins

Froth Test: Extracts are diluted with distilled water to 20 mL and this was shaken in a graduated cylinder for 15 minutes vigorously. The foam formation indicated the presence of saponins.

Detection of Quinones

To 1mL of each of the various extracts were treated separately with alcoholic potassium hydroxide solution. Then, the coloration ranging from red to blue indicated the presence of Quinones.

Detection of Carbohydrates

Extracts was dissolved individually in 5 mL distilled water and filtered. The filtrates were used to test for the presence of carbohydrates. Molisch's solution test: Shaken 2 mL of molish's solution with crude plant extract then added 2mL of H2SO4 concentrated and poured carefully along the side of the test tube, a violet ring appeared at the interphase of the test tube indicated the presence of carbohydrate.

Detection of Flavonoids

Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.

Detection of Alkaloids

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.Wagner's Test:

Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Detection of Tannins

0.2 g of various solvent extract was dissolved in 10 mL distilled water and filtered. A few drop of 1% aqueous Iron chloride (FeCl3) solution was added to the filtrate. The appearance of intense green, purple, blue or black color indicated the presence of tannins in the test samples.

Detection of phenols

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black color was indicated the presence of phenols.

Detection of Terpenoids

2 mL of the organic extract was dissolved in 2 mL of CHCl3 and evaporated to dryness. 2 mL of conc. H2SO4 was then added and heated for about 2 minutes. Development of a grayish color was indicated the presence of terpenoids.

Detection of Steroids

Salkowski's test : a red color produced in the lower chloroform layer when 2 mL of organic extract was dissolved in 2 mL of chloroform and 2 mL concentrated sulphuric acid was added in it, indicates the presence of steroids.

Detection of Glycosides

Keller-Kiliani Test: To 2 mL extract was added glacial acetic acid, one drop 5% FeCl3 and conc. H2SO4. Reddish brown color was appeared at junction of the two liquid layers and upper layer appears bluish green indicated the presence of glycosides

Antimicrobial Assay

Petroleum ether and Methanol extracted leaves of Hagenia abyssinica, Ocimum lamifolium, Rutachalepensis and Solanum incanum crude extracts was evaluated in vitro for antimicrobial assay by using the paper disc diffusion method. The antimicrobial activities of all samples was tested against two Gram positive bacterium Staphylococcus aureus (S. aureus) and Streptococcus agalactiae (S. agalactiae) and two Gram negative bacterium Escherichia Coli (E. coli) and Salmonela thyphei (S. thyphei) using MHA medium and the fungi, Aspergillus niger (A. niger) and Fusarium solani (F. solani), using PDA medium. All the microbial was obtained from Biology Laboratory of the College of Natural and Computational Sciences, University of Gondar.

Preparation of Inoculums

The test bacterial strains was transferred from the stock cultures and streaked on Mueller Hinton plates and incubated for 24 hrs at 37°C. Well separated bacterial colonies was then use as inoculums. Bacteria was transferred using bacteriological loop to autoclaved MHA that wascooled to about 45 oC in water bath and mixed by

gently swirling the flasks. The medium was then poured to sterile Petridishes, allowed to solidify and used for the Biotest [19]. For test fungi, mycelial plugs from stock cultures was transferred to PDA plates and incubated for 5-7 days. Then spores of A. Niger was harvested by washing the surface of the colony using 10 mL sterile distilled water and was transferred to 250 mL autoclaved PDA was cooled to about 45 oC in water bath. Likewise, mycelium of F. solani was washed with 10 mL sterile distilled water, macerated in blander and the mycelia suspension was transferred to 250 mL autoclaved PDA cooled to about 45 o C in water bath. The medium containing spore or mycelia suspension was poured to sterile a plates allowed to solidify and used for disk diffusion bioassay [19].

Procedure Of Minimum Inhibitory Concentration Preparation
A minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that inhibits the growth of a microorganism after 18-24 h. The extracts that showed antibacterial activity were subjected to the serial broth dilution technique to determine their minimum inhibitory concentration. Briefly, the stock solutions of the extracts were subjected to two-fold 10 serial dilution in the Muller- Hinton broth to obtain concentrations from 100 mg/ml to 6.25 mg/ml. Standard antibiotics ampicillin, gentamicin and DMSO were placed as controls. A 10 µl of 107 (CFU) bacterial cultures were added to the tubes and were incubated at 37°C for 18 h. MIC was determined by visual observation. The minimum concentration of the extracts that showed no detectable growth was taken as the minimum inhibitory concentration [20]. Minimum inhibitory concentration (MIC) value of the crude extracts of the R. chalpensis, H. abyssinica S. incanum L and O. lamifolium were determined following the serial dilution technique.

Preparation of Test Solution

The crude extracts of Hagenia abyssinica, Ocimum lamifolium, Ruta chalepensis and Solanum incanum was dissolved in solvent.

Testing for antibacterial activity

Similar procedures to that of antifungal test was followed. Sterilized paper discs was transferred to MHA plate's leaves with at 37 o C bacteria for 24 hrs. All the tests was performed in triplicate. The leaves crude extracts, fractions was taken to test the sensitivity towards four bacteria.

Results and Discussion

Analysis of Data

Mass of Crude Extracts

Dry powdered leaves of Plant Materials was soaked in the two solvents, in order of increasing polarity starting from Petroleum ether to methanol. The amount of extract obtained was recorded and tabulated as in Table 2 below.

Table 1: Wight of crude extracts

S/No.	Items	Solvents	Mass of beaker + crude	Total mass of crude	Mass of beaker
1	*Ocimum lamifolium* (Damakasse)	Petroleum ether	26.32g	6.68g	19.64g
		Methanol	51.9g	32.3g	19.64g
2	*Solanum incanum* (Embuay)	Petroleum ether	22.03g	2.3g	19.64g
		Methanol	51.02g	31.38g	19.64g
3	*Hagenia abyssinica* (Kosso)	Petroleum ether	33.05g	13.44g	19.64g
		Methanol	98.21g	78.57g	19.64g
4	*Ruta chalepensis*	Petroleum ether	27.44g	7.8g	19.64g
		Methanol	41.49g	21.85g	19.64g

Phytochemical screening tests

Phytochemical screening tests was carried out on all the crude extracts of (Petroleum ether and methanol) following standard procedures, The focus was made on testing presence orabsence of secondary metabolites such as flavonoids, phenols, glycosides, terpenoids, tannins, saponnins and steroids. The result of the Petroleum ether and methanol extracts with their phytochemical analysis of the leaves of Plant Materials were presented below (Table 2).

Table 2: Phytochemical screening on the leaves of Plant Materials

S/ No.	Item	solvents	Saponins (Froth Test)	Quinones	Carbohydrates (Molisch's solution test)	Flavonoids (Lead acetate Test)	Alkaloids (Wagner's Test)	Tannins	Phenols (Ferric Chloride Test)	Terpenoids	Steroids (Salkowski's test0	Glycosides (Keller-Kiliani Test)
1	*Ocimum lamifolium* (Damakasse)	Methanol	+	_	_	+	-	+	+	+	+	-
		P.ether	+	-	-	+	-	-	-	+	+	-
2	*Solanum incanum* (Embuay)	Methanol	+	-	-	+	-	+	+	+	-	+
		P.ether	-	-	-	-	-	-	-	+	-	+
3	*Hagenia abyssinica* (Kosso)	Methanol	+	-	+	+	-	+	+	+	+	+
		P.ether	+	-	-	-	-	-	-	+	-	+
4	*Ruta chalepensis* (Tenadam)	Methanol	+	-	-	+	+	+	+	+	-	+
		P.ether	-	-	-	-	+	-	-	+	+	+

Discussion of the above table

Ocimum lamifolium (Damakasse)

Preliminary screening tests of the crude extract of Ocimum lamifolium (Damakasse) by Methanol solvent revealed the presence of saponnins, Flavanoids, Terpenoids, Tannins and steroids are present the remaining bioactive components such as alkaloids, quinones, carbohydrates and glycosides are absent in the plant material.

Preliminary screening tests of the crude extract of Ocimum lamifolium (Damakasse) by Petroleum ether solvent revealed the presence of saponnins, Flavanoids, Terpenoids and steroids are present the remaining bioactive components such as alkaloids, quinones, Tannins,Phenols, carbohydrates and glycosides are absent in the plant material.

Solanum incanum (Embuay)

Preliminary screening tests of the crude extract of Solanum incanum (Embuay) by Methanol solvent revealed the presence of saponnins, flavonoid ,tannin, phenol, terpinoids and glycosides are present the remaining bioactive components such as alkaloids,quinones,carbohydrates and steroids are absent in the plant material.

Preliminary screening tests of the crude extract of Solanum incanum (Embuay) by Petroleum ether solvent revealed the presence of Terpenoids and glycosides are present the remaining bioactive components such as saponnins, alkaloids, quinones, tannins,flavonoids,phenols ,carbohydrates and steroids are absent in the plant material.

Hagenia abyssinica (Kosso)

Preliminary screening tests of the crude extract of Hagenia abyssinica (Kosso) by Methanol solvent revealed the presence of saponnins, flavonoid ,tannin, phenol, terpinoids and glycosides are present the remaining bioactive components such as alkaloids,quinones,carbohydrates and steroids are absent in the plant material.

Preliminary screening tests of the crude extract of Hagenia abyssinica (Kosso) by Petroleum ether solvent revealed the presence of Terpenoids and glycosides are present the remaining bioactive components such as saponnins, alkaloids, quinones, tannins,flavonoids,phenols ,carbohydrates and steroids are absent in the plant material.

Ruta chalepensis (Tenadam)

Preliminary screening tests of the crude extract of Ruta chalepensis (Tenadam) by Methanol solvent revealed the presence of saponnins, flavonoid ,alkaloid,tannin, phenol, terpinoids and glycosides are present the remaining bioactive components such as quinones, carbohydrates and steroids are absent in the plant material.

Preliminary screening tests of the crude extract of Ruta chalepensis (Tenadam) by Petroleum ether solvent revealed the presence of alkaloid, terpenoids,steroids and glycosides are present the remaining bioactive components such as saponnins, alkaloids, quinones, tannins, flavonoids, phenols ,carbohydrates and steroids are absent in the plant material

Antimicrobial Assay Activity

Table 3: Inhibition zone of the four pathogens (in mm) in the extract of Plant Materials

			Gram negative		Gram positive	
No.	Item	Solvent	*E.Coli*	*K.Pneumaniae*	*S. Pneumania*	*S. Aureus*
1	Damakase	Metahnol	-	7.6	-	7
		P.ether	-	-	-	-
2	Embuay	Metahnol	-	-	-	-
		P.ether	-	-	-	-
3	Kosso	Metahnol	10	11	11	9.6
		P.ether	-	-	-	-
4	Tenadam	Metahnol	11.6	-	-	8.6
		P.ether	-	-	-	-

Zones of inhibition of gentamicin (diameter in mm) for E.coli, S. aureus, S. pneumania and K. pneumonia are 27 mm, 29 mm, 19 mm and 17 mm respectively.The results of the antimicrobial activity of the methanol extract of fresh leaves of Ocimum lamifolium was found sensitive to S. aureus, and K.Pneumaniae. Crude methanol extract produced zone of inhibition 7.6 mm and 7 mm against S. aureus and K.Pneumaniae respectively and also exhibited highest zone of inhibition (7.6 mm) against S.aureus (Table 3). It can be suggested that S. aureus, E.coli, K. pneumonae, and S. pneumania were the most resistant organisms to the petroleum ether solvent for the selected plants extracts. The results also showed that the methanol was the best solvent for extracting antimicrobial substances from those plantwhen compared to petroleum ether. But the result showed that S. incanum was resistant organisms to both solvents. Methanol extracts from the three plants were found to have highest antibacterial activity. Whereas the petroleum ether extracts were less inhibiting bacterial growth.This indicates that plants rich in a wide variety of secondary metabolites such as tannins, alkaloids, tannin, flavonoids, terpenoids and etc. which haves been found to have antimicrobial property. Minimum activity of methanol extract of O.lamifolium was seen against S.aureus and the maximum activity of R. chalpensis was seen against E.coli (Table 1).The methanol extract of R. chalpensis showed maximum growth inhibition (11.6 mm, 8.6 mm) against E.coli and S. aureusrespectively. So the plant showed significant inhibition for gram positive and gram negative bacteria. Moreover, H. abyssinica has good inhibition for gram positive and negative bacteria in all the extracts. But it has more inhibition against Gram negative bacteria in the methanol extracts. This variation may due to the difference in the tested organisms and the method used to assess antimicrobial activity. As a general the result obtained was less than the inhibition zone of the standard (Gentamicin).

Table 4: Minimum inhibition zone concentration (MIC) activity of Plant Materials leaves extracts

No.	**Item**	**Solvent**	***E.Coli***	***K.Pneumaniae***	***S. Pneumania***	***S. Aureus***
1	Damakase	Metahnol	-	12.5	-	6.25
	O.lamifolium	P.ether	-	-	-	-
2	Embuay	Metahnol	-	-	-	-
	S. incanum	P.ether	-	-	-	-
3	Kosso	Metahnol	12.5	6.25	6.25	12.5
	H. abyssinica	P.ether	-	-	-	-
4	Tenadam	Metahnol	6.25	6.25	-	12.5
	R. chalpensis	P.ether	-	-	-	-

The MIC value was also determined against the all tested bacteria. Petroleum ether and methanol extracts of four plants extracts inhibited gram-positive strains S. Pneumania and S. aureus, gramnegative strains K.pneumoniae and E. However, the extracts did not exert any inhibitory activity S. incanum. The MIC value of methanol extract of Ocimum lamifolium was found to be 6.25 mg and 12.5 mg against both S. aureus and K.Pneumaniae respectively. The MIC value of methanol extract of H. abyssinica was found to be 12.5 mg and 6.2.5 mg against both S. aureus and K.Pneumaniae respectively. This variation may due to the difference in the tested organisms and the method used to assess antimicrobial activity or chemical constituents in the plants and the nature of plants. And also may the polarity difference between selected solvents. Our study has several limitations. There were no controls for plants because the purpose was not comparison of the standard antimicrobial agent with plant materials. However, the plants were identified by cellular, cultural and biochemical characteristics. Finally, this study also did not include the chemical composition of plant materials due to financial shortage and lack of materials [21, 22].

Conclusion

The plant material R. chalpensis, H. abyssinica S. incanum and O. lamifolium have a good results of phytochemicals screening tests and antimicrobial test. The results showed that the methanol was the best solvent for extracting antimicrobial substances from those plant when compared to petroleum ether. This indicates that plants rich in a wide variety of secondary metabolites such as tannins, alkaloids, tannin, flavonoids, terpenoids. which haves been found to have antimicrobial property.

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