

"Therapeutic Usage and Phytochemical Screening Study on some Selected Indigenous Medicinal Plants from Zegie and Lake Tana areas, Northwest Ethiopia"

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Abstract: Medicinal plants including *Achyranthes aspera*, *Brucea antidysentrica* and *Croton macrostachyus* are important in this study area are traditionally used to treat various illnesses. The objective was to assess the effectiveness of these traditionally used herbal agents and evaluate their preliminary phytochemical constituents. Detailed interviews about the therapeutic uses of the specific plants were presented for the traditional healers and users in the study area. The crude extracts by using water and ethanol solvents were performed from the plant materials and were further screened for phytochemical components by using standard procedures. The results from the traditional healers and users in the study area on the selected medicinal plants indicated the effectiveness of these herbal agents for some disease problems. Comparison of plant extracts in these species of herbal agents showed percentage yield variations with respect to the solvent type used and plant species. Plant extracts from *Achyranthes aspera* and *Brucea antidysentrica* showed the presence of flavonoides, carbohydrates and vitamin C but the absence of saponins and proteins (Peptides). *Croton macrostachyus* was found to have most of the screened phytochemical constituents (Including saponins, flavonoides, carbohydrates, free amino acids and vitamin C) except proteins. In conclusion, the herbal agents in the area indicated therapeutic effectiveness for various illnesses. The presence of the above mentioned phytoconstituents detected may be responsible for the therapeutic properties of these herbal agents.

Key words: Traditional healers • Indigenous plants • Phytochemical study • Zegie and Lake Tana areas

INTRODUCTION

Right from its beginning, the documentation of traditional knowledge, especially on the medicinal uses of plants, has provided many important drugs of modern day [1, 2]. Out of the total flowering plants reported from the world, more than 50,000 are used for medicinal purposes [3, 4]. Herbal medicines have been important sources of products for the developing countries in treating commonly occurring diseases and overcome the problems of resistance and side effects of the currently available antimicrobial agents. The World Health Organization (WHO) estimates that 80% of the people living in developing countries almost exclusively use the traditional medicines.

In Ethiopia, about 800 species of plants are used in the traditional health care system to treat nearly 300 mental and physical disorders. Ethiopia is rich in medical lore. The use of plants in religious ceremonies as well as for magic and medicinal purposes is very common and widespread. Based upon strong primitive roots, the art of native medicine is still widely practiced. While much of this lore is indigenous, yet there are strong indications of Hebrew and Egyptian as well as Greek and other Arabic influences. Traditional medicine still remains the main resource for a large majority (80%) of the people in Ethiopia for treating health problems and a traditional medical consultancy including the consumption of the medicinal plants has a much lower cost than modern medical attention [5-8]. The indigenous knowledge

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on usage of medicinal plants as folk remedies are getting lost owing to migration from rural to urban areas, industrialization, rapid loss of natural habitats and changes in life style.

The study of Ethiopian medicinal plants has not been realized as fully as that of China, India or other traditional communities elsewhere [9]. In Ethiopia, though there has been some organized ethno medicinal survey studies [10-12], there is limited preservation of medicinal herbs, scientific screening/confirmation and development of their therapeutic products. Ethnomedicinal surveys in Ethiopia and other parts of the world indicated that among many medicinal plants, some of the species identified in this study such as *Croton macrostachyus* (Euphorbiaceae), *Achyranthes aspera* (Amaranthaceae) and *Brucea antidysenterica* (Simaroubaceae) have been traditionally used for many other therapeutic roles.

Wound healing herbal extracts which are rich in saponins, flavonoids, proteins and free amino acids, vitamin C and carbohydrates promote blood clotting fight infection and accelerate the healing of wounds. Flavonoids have been deemed responsible for anti-inflammatory, anti-ulcerogenic, healing, antihypertensive and many other activities [13]. Plant products are potential wound healing agents and largely preferred because of their widespread availability, non-toxicity, absence of unwarranted side-effects and effectiveness as crude preparations.

The powerful anti-haemorrhagic effects of plant derived extracts is also associated with their rich contents in triterpenoid saponins, polysaccharides, coumarins and flavonoids which have a powerful anti-haemorrhagic effects [14]. Flavonoids are also known to be involved in vascular protection, by decreasing the vascular permeability and inhibitory activity of several enzymes such as metalloproteinases by chelating ions such as zinc.

The traditional therapeutic practices with local herbal agents in Zegie and Lake Tana areas has been studied by Teklehaymanot and Gidey [10] though it lacks scientific confirmation on the phytochemical constituents, effectiveness of therapeutic plant extracts, side effects and development of the active plant extracts. Considering the rich diversity of plants in Zegie and Lake Tana areas, it is necessary to screen plants for their medicinal importance. Thus, the present investigation was designed to survey the traditional therapeutic usage by local healers, extract from the

selected plant specimens by using different solvents, evaluate the extract results and describe some unique features of these selected plant extracts (Phytochemical components) and their putative association with potential therapeutic effects in the day-to-day practice of traditional medicine.

MATERIALS AND METHODS

Study Area: The therapeutic and phytochemical study were conducted on the commonly used indigenous herbal plants of Zegie and Lake Tana monasteries. Lake Tana which is the largest lake in Ethiopia and source of Blue Nile is found in North West high lands of Ethiopia, particularly in the Amhara National Regional State (ANRS). It is situated with the altitude of 1830 meters above sea level and covers 3200 km². The Lake Tana is the source of Blue Nile, the only river which drains Lake Tana from South East corner at Bahir Dar town. The Lake Tana environment has a distinct seasonal pattern, particularly with respect to dry and wet periods. The water temperature fluctuates between 19°C during winter January to March and to 24°C during May-June.

Zegie Peninsula (11° 43' N, 37° 20' E) is located at 600 km Northwest of Addis Ababa in the country's northwestern highlands, at an altitude of approximately 1800 meters above sea level as it shown below on Figure 1. It is partly surrounded by Lake Tana. Zegie Peninsula is about three hours motorboat drive or 37 km on land from Bahir Dar. The residents are Amhara people and speak the country's official language Amharic. Tankwas (Papyrus boats) of ancient design, manufactured on the shores of Lake Tana, are the alternative forms of transport for the local people between Zegie and Bahir Dar. There are seven monasteries on the peninsula (The churches and monasteries of Zegie Peninsula at the south western side of Lake Tana include Ura Kidanemihret, Azwa Mariam, Mehal -Zegie, Ghiorgis and Yeganda Teklehaymanot). The monasteries of Zegie are different from the island monasteries in that they are not highly isolated from the local communities. They are also surrounded by the residents who are leading a non-monastic life. This can be reached from Bahir Dar City mainly by boat. There are also other several monasteries on Lake Tana and some of them include Kibran Gebrial, Narga Selassie, Daga Estifanos, Tana Cherkos, Kirstos-Samra and others [15].

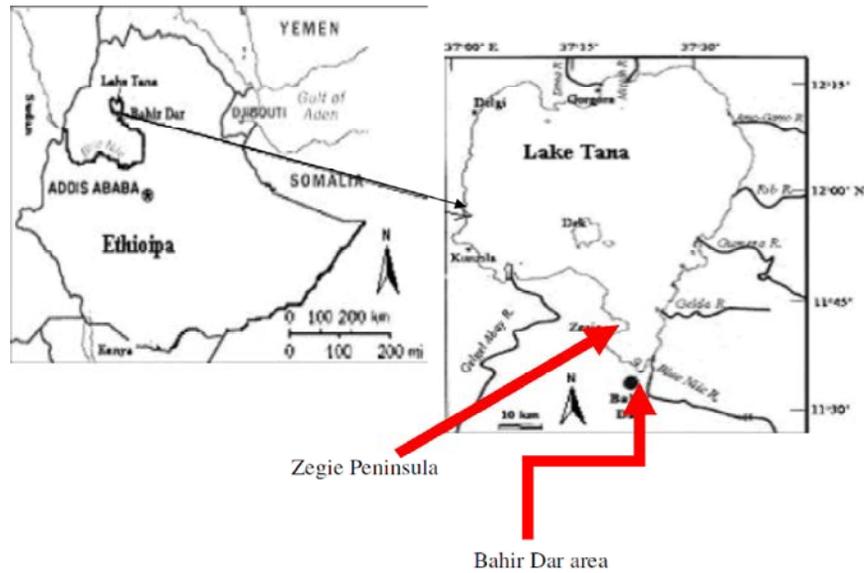


Fig. 1: Location of Zegie Peninsula on Lake Tana in Ethiopia (Source: ANRS Tourist Information Center, 2005).



Fig. 2: A picture showing Zegie Peninsula (main study area) and its forest view along side of Lake Tana.

Plant material Collection, Botanical Identification and Preparation of Plant Extracts:

Some selected medicinal plants (*Croton macrostachyus*, *Achyranthes aspera* and *Brucea antidysenterica*) for specific illness problems based on the interview results from traditional healers were collected from Zegie (as shown on Figure 2 below) and Lake Tana areas in association with the local healers. The botanical identification of the herbal agents were made taking the specimens to the National Herbarium of Addis Ababa University, Faculty of Science, Department of Plant Biology and Biodiversity and the specimens were stored with voucher numbers there. The botanical identification of the selected medicinal plants was described below on Table 1.

Table 1: The botanical identification of the plant specimens conducted for this study.

Collection no.	Botanical (Species)	Name Family Name
1.	<i>Achyranthes aspera</i> L.	Amaranthaceae
2.	<i>Croton macrostachyus</i> Del.	Euphorbiaceae
3.	<i>Brucea antidysenterica</i> JF. Mill.	Simaroubaceae

Study Design: Qualitative study with traditional healers followed by phytochemical screening experiments were conducted to implement this study.

Interviews with Traditional Healers: For the qualitative study, discussion (interview) with traditional healers and people were conducted about the therapeutic use and effectiveness of medicinal plants especially for those some selected herbs for this study for specific disease problems and the data was documented.

Preparation of Plant Extracts: After the botanical authentication is made for the plant specimens, plant materials (mainly leaf) were collected from the study area and washed under running tap water to remove adhering dust and then air dried under shade and then powdered into small fractions by using a grinding mill. The plant extracts from the plant materials (leaf) were prepared by using organic and inorganic solvents (ethanol and distilled water) [16, 17].

About 100 grams (g) of powdered material of the plant samples were taken in a large, clean beaker/flask and soaked in 500 milliliters (ml) of 99% ethanol. The beakers were sealed with aluminum foil and kept for a period of one week accompanying occasional shaking and stirring. The solution was filtered twice, firstly with clean cloth (four fold) and then with Whatman's filters paper

(Whatman No.1) and finally the filtrates were concentrated by using a rotary evaporator at 40°C and then to dryness by keeping it in hot air oven (40°C). The condensed crude extracts were weighed and placed in a refrigerator at -4°C with glass bottles until use. The yields of different plant extracts were measured in percent (%) weight-by weight (w/w) related to the dried material used.

On the other hand, about 100 g of each powdered sample of the herbs were extracted by soaking in 300ml of distilled water in a beaker, stirred and boiled (15-20 minutes in water bath) and then left overnight. Thereafter, the solutions were filtered using filter paper (Whatman No. 1) and the filtrates were concentrated with a rotary evaporator and then up to dryness by keeping it in hot air oven as mentioned above for ethanol extract. The condensed extracts were weighed and placed in a refrigerator at -4°C until use. The yields of different plant extracts with distilled water as a solvent were also measured in % w/w (related to the dried material used).

Preliminary Phytochemical Screening: Preliminary phytochemical studies for the presence of active principles (flavonoids, saponins, proteins, free amino acids, carbohydrates and vitamin C) in the selected plants was conducted by using appropriate analytical methods which followed standard procedures [13, 18, 19]. Hence extracts of the plant samples were tested for the presence of active principles such as saponins, flavonoids, proteins, free amino acids, carbohydrate and vitamin C as follows:

Test for Saponins [13]: Foam Test – Test solution was mixed with water and shaken and observed for the formation of froth, which is stable for 15 minutes for a positive result.

Test for Flavonoids [19]: Ferric chloride test – Test solution when treated with few drops of freshly prepared ferric chloride solution (10%) would result in the formation of blackish red/green, blue or violet color indicating the presence of flavonoids.

Alkaline reagent test/NaOH test – Test solution was treated with sodium hydroxide (NaOH) solution and showed an increase in the intensity of yellow color which became colorless on addition of few drops of dilute hydrochloric acid (HCl), indicating the presence of flavonoids.

Lead acetate solution test – Test solution when treated with few drops of lead acetate (10%) solution would result in the formation of yellow precipitate for a positive result.

Test for Proteins [18]: Biuret test – Test solution was treated with 10% NaOH solution and two drops of 0.1% copper sulphate solution and observed for the formation of violet/pink color.

Test for Free Amino Acids [18]: Ninhydrin test – Test solution when boiled with 0.5% solution of ninhydrin reagent, would result in the formation of purple color suggesting the presence of free amino acids.

Test for Carbohydrate [18, 19]: Benedict's test – Test solution was mixed with few drops of Benedict's reagent (Alkaline solution containing cupric citrate complex) and boiled in water bath, observed for the formation of reddish brown precipitate to show a positive result for the presence of carbohydrate.

Molisch test- Test solution was treated with 10% Molisch reagent and concentrated sulfuric acid added, the formation of ring suggests a positive result.

Test for Vitamin C [13]: Dinitrophenyl hydrazine (DNPH) test – Test solution was treated with DNPH dissolved in concentrated sulphuric acid. The formation of yellow precipitate would suggest the presence of vitamin C.

Data Collection: Qualitative and quantitative data was collected from herbalists and laboratory experiments (plant extraction and phytochemical screening procedures).

Ethical Clearance: Ethical clearance was obtained from the Biotechnology Research Institute of Bahir Dar University (BDU). All the traditional healers and study participants were informed about the purpose of the study and finally their verbal consent was obtained before interviewing and plant specimen collection. The information provided by each respondent was kept confidential.

Data Analysis: Quantitative data was presented and the results were expressed in % and means \pm standard error (SE). Qualitative data from traditional healers and on phytochemical test results were presented as well. Statistical tests like independent t-test and univariate analysis of variance were used to compare the % plant extracts for solvent type used and plant species differences, respectively, by using the statistical software SPSS 19. In all the statistical tests, a confidence level of 95% and $p < 0.05$ was considered significant.

Table 2: Some selected medicinal plants list and their therapeutic uses by local healers and users in the study areas of Zegie and Lake Tana

Sr. No.	Botanical (Species) name	Family	Therapeutic uses for disease problems (including local names of the illnesses)	Plant parts used and therapeutic preparations
1	<i>Brucea antidysenterica</i> JF. Mill.	Simaroubaceae	Dysentery ('Kuruba'), haemorrhoids, weight loss, fever, itwhing and diarrhea ('Bullad')	-Juice of leaf is taken orally in the morning -Fruit or leaf powder mixed with milk is taken orally for three days -Leaf/fruit powder mixed with honey and fermented for seven days is taken orally until cure
2	<i>Croton macrostachyus</i> Del.	Euphorbiaceae	Wounds ('Kusil'/ 'Ekek'), dysentery/diarrhea ('Kuruba'), stomach disorder), fungal infections of skin/ <i>Tineaversicolor</i> ('Quaqucha')	-Dressing with the powders of leaves mixed with butter -Leaves are eaten with wat/food or drinking leaf powder mixed with water -Rubbing and covering with leaves at the affected area
3	<i>Achyranthes aspera</i> L.	Amaranthaceae	Blood clotting and <i>Herpes zoster</i> ('Shererit')	-Dressing with fresh leaves -Chewing fresh leaves

Table 3: The percent weight extracts of different plant species by using ethanol and water solvents

Plant species	Solvent used	% w extracted from	
		100gm sample (Mean ±SE)	P-value
<i>Croton macrostachyus</i>	Ethanol	6.63±0.546	0.02*
	Water	4.40±0.264	
<i>Achyranthes aspera</i>	Ethanol	2.90±0.152	0.06
	Water	2.67±0.067	
<i>Brucea antidysenterica</i>	Ethanol	4.10±0.261	0.058
	Water	3.37±0.088	

*P<0.05 indicates significant differences!

RESULTS AND DISCUSSION

Interview Results on the Selected Medicinal Plants from Traditional Healers and Users: Detailed interviews and discussion results from seven traditional healers and about ten herbal medicine users in the study area about locally used (specifically on the selected) medicinal plants explained or indicated the effectiveness of these herbal agents for various illnesses. The qualitative result was presented as follows on Table 2.

Plant Extraction Results and Extract Percentage Yields:

The percent extracts found from the different plant materials in this study was compared with the solvent types used (ethanol vs distilled water). The % extract from *Croton macrostachyus* with ethanol was higher than that of water (6.63±0.546 vs 4.40±0.264) and the difference was statistically significant (P<0.05). Ethanol extracts also showed higher % yields for both *Achyranthes aspera* and *Brucea antidysenterica* but the differences were not statistically significant (P>0.05) as shown on Table 3 below.

Comparison of ethanolic extracts in the three species of herbal agents showed the highest % yields from *Croton macrostachyus* (6.63±0.546), followed by *Brucea antidysenterica* (4.10±0.261) and the least was found from *Achyranthes aspera* (2.90±0.152) and the difference was statistically significant (P<0.05). Whereas, the % extracts with distilled water as a solvent did not show significant differences among the three herbal agents (P>0.05) as shown on Table 4 below.

Preliminary Phytochemical Analysis on the Crude Extracts of Different Medicinal Plants:

Extracts were tested for the presence of active principles such as saponins, flavonoids, proteins, free amino acids, carbohydrates and vitamin C. The phytochemical screening in the present study has revealed the presence of saponins (in *Croton macrostachyus*), flavonoids (in *Croton macrostachyus*, *Achyranthes aspera* and *Brucea antidysenterica*), carbohydrates (in *Croton macrostachyus*, *Achyranthes aspera* and *Brucea antidysenterica*), free amino acids (in *Croton macrostachyus* and *Brucea antidysenterica*) and vitamin C (in *Croton macrostachyus*, *Achyranthes aspera* and

Table 4: Percent weight extracts with different solvents of the selected medicinal plants in this study

Extraction solvents	Plant species%	wt extracted	P-value
from 100gm sample (Mean±SE)			
Ethanol extract	<i>Croton macrostachyus</i>	6.63±0.546	0.03*
	<i>Achyranthes aspera</i>	2.90±0.152	
	<i>Brucea antidysentrica</i>	4.10±0.261	
Water extract	<i>Croton macrostachyus</i>	4.40±0.264	0.08
	<i>Achyranthes aspera</i>	2.67±0.067	
	<i>Brucea antidysentrica</i>	3.37±0.088	

*P<0.05 indicates significant differences!

Table 5: Phytochemical screenings conducted and results on the selected medicinal plant leaf extracts with ethanol and water solvents

Phytochemical tests performed	Extracts of plant species and solvents used					
	<i>Croton macrostachyus</i>		<i>Achyranthes aspera</i>		<i>Brucea antidysentrica</i>	
	Ethanol	Water	Ethanol	Water	Ethanol	Water
1.Saponins test	+	+	-	-	-	-
2.Flavonoides test						
• Ferric chloride test	+	+	+	+	+	+
• NaOH test	+	+	+	+	+	+
• Lead acetate Test	+	+	-	-	+	+
3.Buired test	-	-	-	-	-	-
4. Carbohydrate tests						
• Molisch test	+	+	+	+	+	+
• Benedicts test	+	+	+	+	+	+
5.Test for free amino acids	+	+	-	-	+	+
6. Test for vitamin C	+	+	+	+	+	+

* All tests were performed with two replicates, the plus signs (+) indicate positive results and the minus signs (-) indicate negative results!

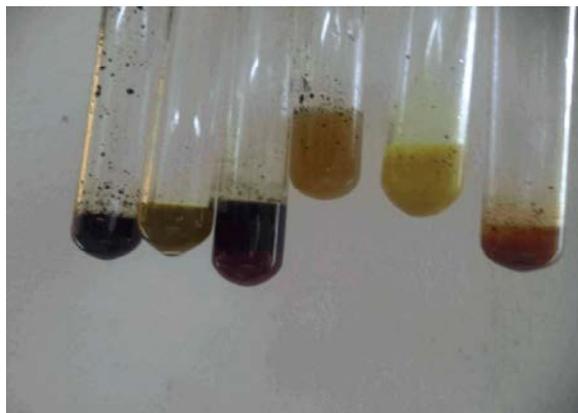


Fig. 3: Characteristic pictures from the phytochemical screening tests on the selected medicinal plant leaf extracts during this study.

Brucea antidysentrica) in their leaf extracts. The present study also indicated the absence of free amino acids in *Achyranthes aspera* and proteins (peptides) in all the three leaf extracts of the studied herbal agents. The results for these preliminary tests were presented on Table 5 and Figure 3.

DISCUSSION

The interview and discussion data on the traditional use of herbal agents and these currently studied medicinal plants for treating various illnesses by local healers and people in the study area indicated their effectiveness which was in accordance with other previous studies conducted [10-12]. Besides, the associated low side effects were also explained despite further studies are required.

The yield of medicinal plant extracts which contain the bioactive metabolites vary considerably with plant species and the method or solvent used for extraction in this study.

It is plausible that the ethanolic extracts were generally more potent than the aqueous extracts probably because the active principles in the plant dissolved more readily in and were better extracted by a less polar solvent (ethanol) than water. This is in agreement with many other literatures [13, 20, 21] who reported existence of differences in% yield and the activities of extracts obtained from the same morphological part of a plant using different solvents. For instance, the

methanolic extract of the fruits of *Tetrapleuratetrapterawas* found more higher or potent than the aqueous extract [22].

The preliminary phytochemical screenings conducted on the selected medicinal plants in this study indicated the presence of various active principles including saponins, flavonoides, carbohydrates, free amino acids and vitamin C which may be associated with the effectiveness of these herbal agents for healing specific disease problems. For example, flavonoids are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers [13, 14].

The preliminary phytochemical screening tests may be useful in the detection of the bioactive principles and subsequently may lead to drug discovery and development from these natural products. Furthermore, these tests may facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds [23, 24].

CONCLUSION

The percent yield of phytochemical constituents which are bioactive metabolites in a plant extracts vary considerably with plant species and the method/solvent of extraction in this study. In this solvent extraction of the selected medicinal plantsethanolic extracts were generallymore higher than the aqueous extracts probably because the active principles in the plant dissolved more readily in and were better extracted by a less polar solvent (ethanol) than water.

The presence of different phytoconstituents (mainly saponins, flavonoides, carbohydrates, free amino acids and vitamin C) in the different indigenous medicinal plant extracts may be responsible for the therapeuticproperties of these herbal agents by traditional healers. Meanwhile, *Croton macrostachyus* among the three studied herbal agents was found to have most of the screened phytochemical constituents (including saponins, flavonoides, carbohydrates, free amino acids and vitamin C) except proteins/peptides were absent.

Further research including in vivo toxicity and effectiveness/pharmacological studies should be conducted to screen and develop potential and cost-effective therapeutic products from these herbal agents against various disease problems.

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