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Extraction and Phytochemical Screening of Secondary Metabolites from Leonotis Ocymifolia (Bokolu) plant on areal part

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Article

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Abstract

Nowadays, the uses of medicinal plants are more diverse to prevent human health care, in which medicinal plants are powerful inhibition of microorganisms cause disease. The study is conducted to assess extraction, phytochemical screening and methods of extracting secondary metabolites of a traditionally used medicinal plant, Leonotis ocymifolia. The plant has been extracted using 80% aqueous ethanol by maceration technique, and 6.0 g of grey ethanol crude were obtained. The crude ethanol suspended in water and partitioned within different polarity of solvents (petroleum ether, ethylene acetate, and n-buthanol), respectively. During partitioning, 2.15 g of ethylene acetate were collected after concentrated using a rotary evaporator and few grams of petroleum ether were obtained. The study revealed phytochemical components like alkaloids, flavonoids, phenols, tannins, glycosides, terpeniods and steroids.

Introduction

Medicinal plants are now more focused than ever because they have the capability of producing many undeniable benefits to people, especially in the line of medicine and pharmacological study. The medicinal power of these plants lies in phytochemical constituents that cause definite pharmacological actions on growth of pathogens [1]. Phytochemicals are natural compounds produced in plants such as alkaloids, glycosides, flavonoids, tannins, steroids, terpenoids and phenols are of secondary metabolites of plant that serves as defensives agents of microorganism's [2]. Around worldwide, including Ethiopia traditional medicinal plant is more applicable to treat disease and infection starting from ancient time. The use of traditional medicine in Ethiopia is based on indigenous knowledge and has long been practiced in the treatment of various diseases [3]. For centuries, people have relied heavily on traditional medicine to treat a variety of physical and mental ailments. Approximately 80% of people in the country still rely on traditional medicine as their primary healthcare system [4], and more than 95% consists of traditional herbal medicines.

In Ethiopia, many plant species used as a medicinal plant and *Leonotis ocymifolia* is typically used plant almost all part of population of Ethiopia [5]*. Leonotis ocymifolia* is minaret flower (flowering plant), shrub and has along white corolla covered by orange rufous hair which is belong to Lamiaceae family [6]. In Ethiopia, it is known as Bokolu, and it is good traditional medicinal plant for many diseases. The plant is reasonably drought-resistant and wind tolerant. Unlike the similar *Leonotus leonuris,* in the *ocymifolia,* the tubular flowers are bolder and larger [7]. *Leonotis ocymifolia* is folk medicine which is used to treat mich and other belief in Ethiopia [2,8]. In society, the plant is used for the diarrheal, headache, cold (influenza), cattle doweling, stomach worm and malaria in some part of Ethiopia specially oromia region[Ethiomedicine]. The main objective of this study was to investigate in vitro isolation of secondary metabolites based on different polarity of solvent and phytochemical screening of secondary metabolites.

Results and Discussion

Extraction yields

6.0 g of grey crude was produced from the maceration of 100g of *L. ocymifolia* plant powder. The process of partitioning the ethanol crude involved suspending (dissolving) the crude extracted in an aqueous solution, starting with the least polar solvent (petroleum ether), increasing the polarity of the solvents, and generating a separating layer due to the immiscibility of the two solvents. Following the partitioning of the ethanol crude into ethylene acetate, and n-buthanol crude, respectively, 2.15 g of yellowish ethylene acetate crude and 1.80 g of n-buthanol crude were ultimately produced. However, measuring petroleum ether is extremely difficult.

Partition of Petroleum ether

Ethanol crude extracts dissolve in aqueous solution and are partitioned within petroleum ether. Due to the immiscibility of the two solvents, a phase boundary is formed.

The upper layer (grey color) suspended over the aqueous solution (Figure 1) is petroleum ether crude solution and is carefully separated into a different container. The petroleum ether solution is concentrated by exposure at room temperature, and very few grams of crude were obtained.

Partition of Ethylene acetate

Ethylene acetate also partitioned within the aqueous ethanol solution, and phase boundary is formed in which the yellowish solution floated over aqueous.

The upper layer (yellowish) solution (Figure 2) is ethylene acetate, and the two layers separated. The ethylene crude solution is concentrated, and 2.15g of crude collected. In this partition, relatively high mass proportion of secondary metabolites revealed.

Partition of n-buthanol

Finally, the aqueous ethanol partitioned within n-butanol (Figure 3), and 1.80 g of crude collected.

The remaining aqueous solution discarded because of primary metabolites. Ethanol solvents can extracts from least polar compound to very more polar (primary metabolites). Therefore, partitioning is used to remove these primary metabolites from crude extract and narrow down the range of secondary metabolites based on their polarities.

Phytochemical Screening

Phytochemical screening of main secondary metabolites such as alkaloids, flavonoids, steroids, saponins, tannins, terpenoids, and phenols was studied on petroleum ether, ethylene acetate, and n-butanol crude partitions. In the ethylene acetate partition, terpenoids, phenols, flavonoids, and steroids were present, whereas phenols, glycosides, and tannins were present in the n-butanol partition (Table 1).

Table1: Table of Phytochemical Screening

s/no	components	petroleum ether	ethylene acetate	n- buthanol
1	alkaloids	+	-	-
2	flavonoids	-	+	-
3	phenols	-	+	+
4	glycosides	-	-	+
5	terpenoids	-	+	-
6	saponins	-	-	+
7	tannins	-	-	-
8	steroids	-	+	-

In general, the extraction of the aerial parts of *Leonotis ocymifolia* in this study revealed secondary metabolites like alkaloids, flavonoids, phenols, glycosides, terpinoids, tannins, and steroids and the result similar within [3]. In contrast, saponins are not present in any of the crude oil partitions, and components are indicated by the "+" sign, while "-" denotes the absence of a component (Table 1). Table 1 show that the sugar component (primary metabolites) remained in the aqueous solution while nearly all of the secondary metabolites were detected in both solvents (ethyl acetate and n-butanol partition). The reason for this is that highly polar chemicals are attracted to water as a solvent.

Material and methodology

Plant collection

In December 2024, the mature and healthy *Leonotis ocymifolia* plants were gathered from a family garden in Goba woreda. Located in the Bale zone of the Oromia region, southeast of Addis Ababa, Ethiopia, lies the Goba woreda. The plant was authenticated by Botanist Reta Regassa, and a voucher specimen with the number BD00130/24 was deposited at the HU Herbarium, Hawassa University, for future reference. A sufficient number of plant sections will be collected, and the newly collected plants will undergo three rounds of washing in the laboratory, first with tap water to get rid of dust and then with distilled water to rinse. Next, after being cut with a knife into small pieces, it dries in the shade.

Chemical and Material

Chemicals: distilled water, ethanol (80%), ethyl acetate (AR), NaOH ,silica gel (mesh 200-400), petroleum ether(AR), naphthol, iodine, KI, HCl(37%), chloroform(AR), H_2SO_4 (98%), $FeCl_3$, methanol (80%).

Material: Erlenmeyer flask, water bath shaker (SHZ -82), rotary evaporator (RE100-S), vacuum pump, column chromatography, TLC, dropper, spatula, electronic balance, Buchner funnel, watch man filter paper, iron stand up, electrical drier, Knife, separating funnel.

Extraction

The plant's dried sections were ground using an electrical mill, and 100g of finely ground *Leonotis ocymifolia* powder was soaked in 80% aqueous ethanol in 1:8 ratios for three days. The plant was then shaken for about 36 hours at 200°C using a water bath shaker (SHZ-82). Then watch man filter paper was used to separate the liquid. Grey ethanol crude was obtained by using a rotary evaporator (RE100-S) to concentrate the filtered liquid. Ultimately, ethanol crude was divided into petroleum ether and suspended in distilled water using a separating funnel. The two components were then carefully separated. Subsequently, the remaining aqueous solution was cautiously separated into separate containers after being gradually divided into ethylene acetate and n-buthanol. Ultimately, a rotary evaporator (RE100-S) was used to concentrate each partitioned solution independently.

Phytochemicals screening

Petroleum ether crude, ethylene acetate, and n-butanol solutions were used to test the phytochemical components. Using standard procedure, this is done to determine whether secondary metabolites are present or absent [9,10].

Test for alkaloids

Few drops of Mayer’s reagent were added to 1ml of extract. A yellowish or white precipitate was formed, indicating the presence of alkaloids [10].

Test for flavonoids

Two to three drops of sodium hydroxide were added to 2 mL of extract. Initially, a deep yellow color appeared but it gradually became colorless by adding few drops of dilute HCL, indicating that flavonoids were present[10].

Test for phenols

Two milliliters of 5% neutral ferric chloride solution were added to 1 ml of extract, the dark blue coloring indicating the presence of phenolic compounds [10].

Test for glycosides

Few drops of Molisch's reagent will be added to 2 ml of extract and will be shaken well. 2ml of conc. H_2SO_4 will be added on the sides of the test tube. A reddish violet ring appeared at the junction of two layers immediately indicated [10].

Test for terpenoids

About 5 mL of extract will be dissolved and mixed with 2 ml of chloroform, then 3 mL of concentrated H_2SO4 were added and reddish brown coloration at the interface wills confirmed the presence of terpenoides [10].

Test for tannins

0.1% $FeCl_3$ solution added to few ml of extract and the blue, blue-black, green or blue-green color which confirmed the presence of tannins [10].

Test for sponins

A drop of Na_2CO_3 solution was added to 5 ml of extract in a test tube. After vigorous shaking, it was left to rest for five minutes. Foam formation indicated the presence of saponins [10].

Test for steroids

2 ml of chloroform and concentrated H_2SO_4 were added with the 5 ml aqueous plant crude extract. In the lower chloroform layer red color appeared that indicated the presence of steroids [10].

Conclusion

According to the results, the plant is rich within secondary metabolites and further experiments are required to comprehensively, characterized bioactive component and explore their antimicrobial activities.

Declarations

Data availability

The data and material used to support the findings of this study are available main body text and are additional available from the corresponding author upon request.

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Author contributions

Defar Bekele: conceptualization, writing (original draft), Conceived and designed the experiments methods; performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Validation, Writing (review and editing), preparing figures, collecting data, validation investigation, and Formal analysis.

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Competing interests

The author declares no competing interests.

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Figures

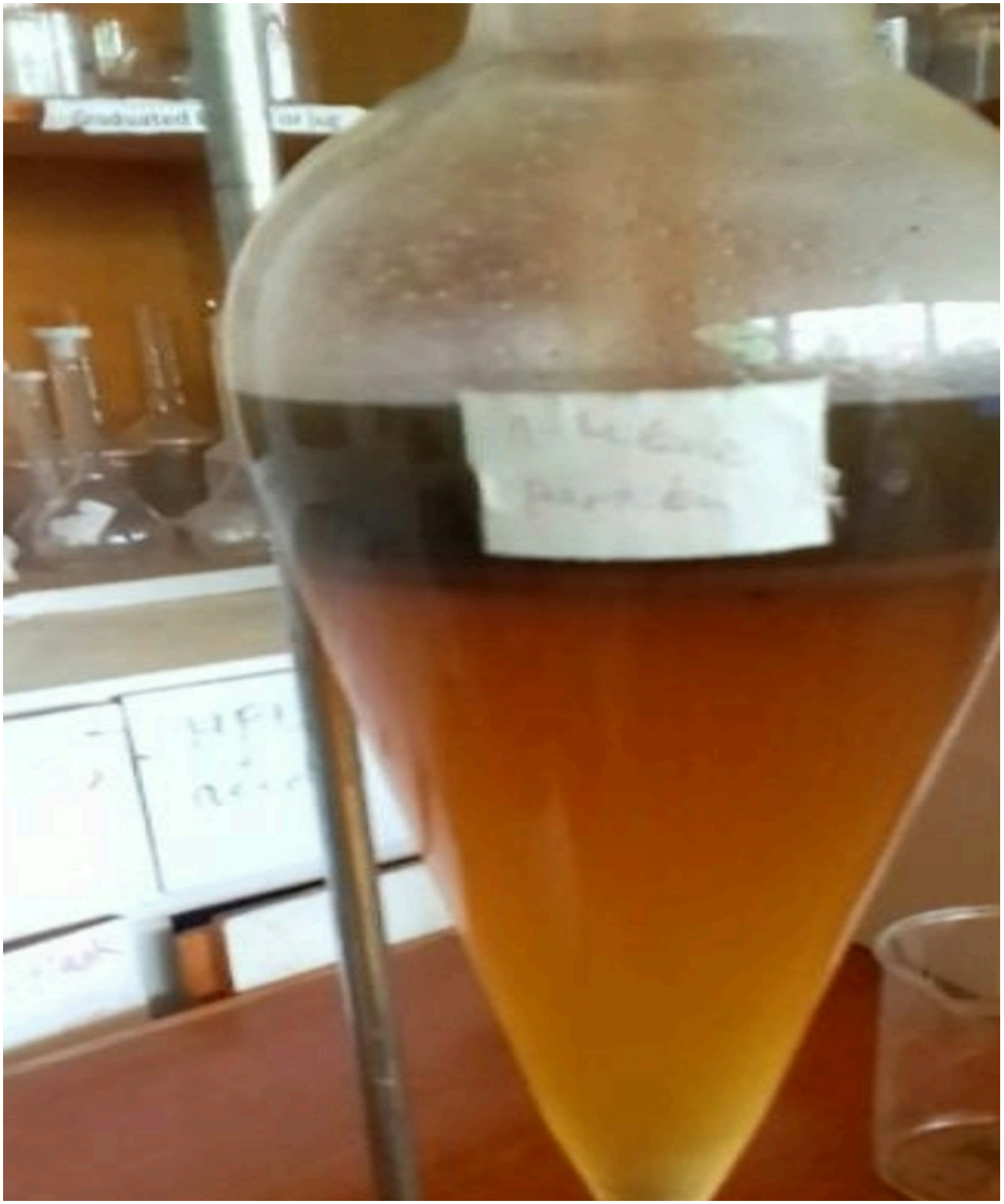

Figure 1

partition by petroleum ether

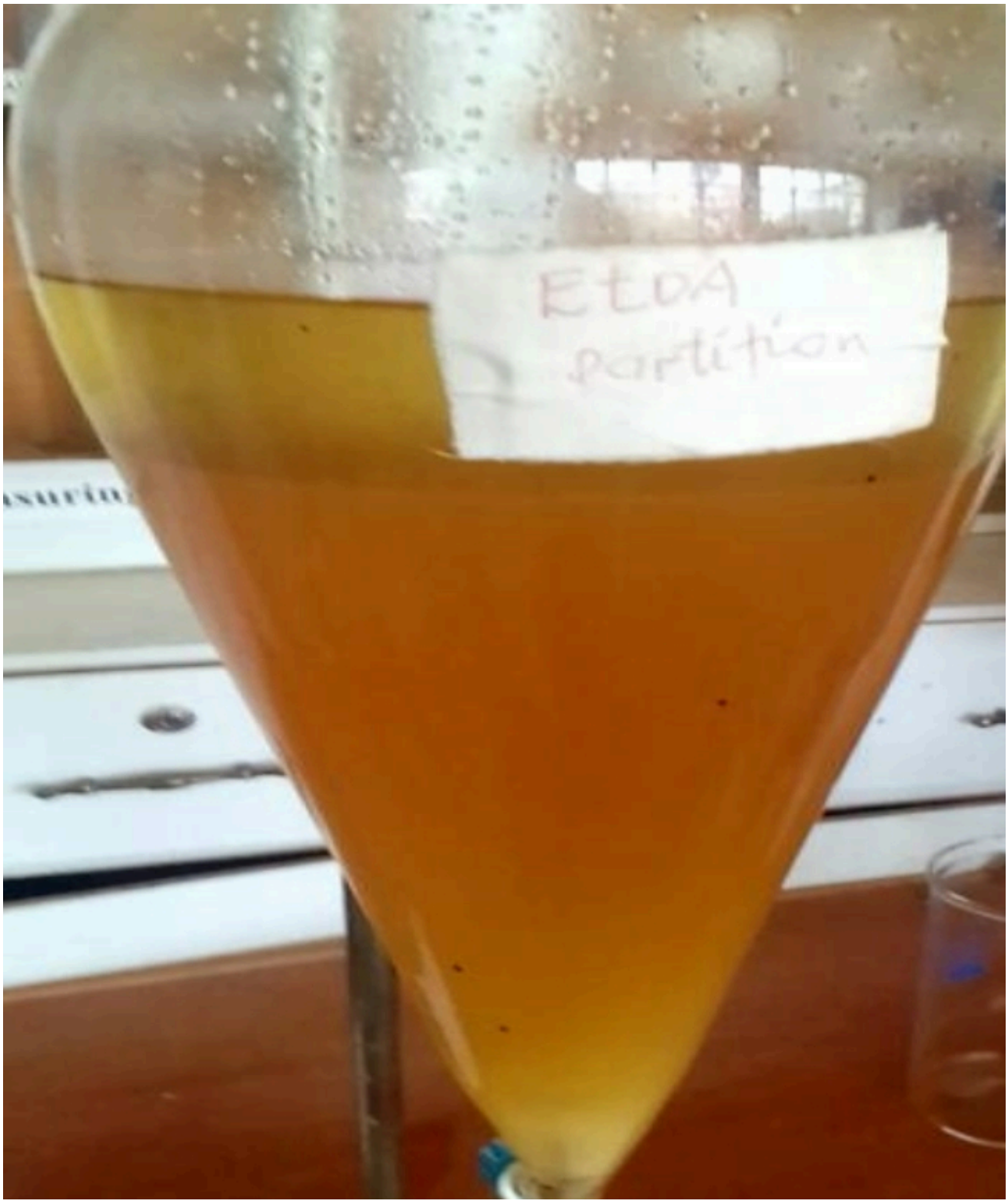

Figure 2

partition by ethylene acetate

Figure 3

partition by n-butanol