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Original Research

Qualitative Phytochemical Screening on Some Selected Medicinal Plants in Senan District, Northwestern Ethiopia

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Abstract

Plants are rich sources of phytochemicals, secondary metabolites like alkaloids, terpenoids, steroids, flavonoids, saponins, phenolics, glycosides, and others, found in various plant parts including leaves, fruits, roots, and stem bark. This study aimed to investigate the phytochemical components of *Ruta chalepensis*, *Vernonia amygdalina*, and *Zehneria scabra* species in Senan District, Northwestern Ethiopia. Methanol and petroleum ether extracts of selected plant parts were subjected to standard qualitative phytochemical screening. The analysis revealed the presence of several phytochemical compounds in both methanol and petroleum ether extracts of the aerial parts of *R. chalepensis* and the leaves of *V. amygdalina* and *Z. scabra*. Notably, methanolic extracts exhibited a higher abundance of phytochemicals due to the superior solubility of active compounds compared to petroleum ether. These findings highlight the potential of these plants as valuable sources of therapeutic agents. Traditionally, *R. chalepensis*, *V. amygdalina*, and *Z. scabra* have been employed to treat various ailments. While this study provides a foundation for further exploration, additional research, including quantitative analysis, compound characterization, isolation, and pharmacological evaluation, is necessary to fully unlock these plants' therapeutic potential.

Keywords: Ethnobotany, Medicinal plants, Phytochemical screening, Secondary bioactive chemicals

1. INTRODUCTION

Medicinal plants (MPs) have long been a cornerstone of healthcare systems worldwide, laying the foundation for modern medicine. Thus, plants are an essential component of life in many indigenous societies across the world as a readily and cheaply accessible alternative to allopathic medicines. Approximately 80% of the rural population in Sub-Saharan Africa relies on herbal remedies, often known as traditional medicine, for primary human and

veterinary healthcare due to the scarcity or high cost of pharmaceutical medicines (WHO, 2002). Naturally derived plant products with medicinal value play an important role in health care systems, both in humans and animals. This is evident with the growing interest in their utilization of a global perspective in the treatment of different ailments. In their crude form, medicinal plants contain a diverse structural array of compounds with varying chemotherapeutic relevance that are traditionally harnessed through various modes of preparation (Bahiru, 2006).

Phytochemicals accumulate in different parts of the plant, such as the roots, stems, leaves, flowers, fruits, or seeds (Adegoke *et al*., 2010; Gani *et al*., 2016). There are more than a thousand known and many unknown phytochemicals. It is well known that plants produce these chemicals to protect themselves, but recent research has demonstrated that many phytochemicals can also protect humans against ailments (Mohammed *et al*., 2014). Phytochemicals are biologically active, naturally occurring chemical compounds found in plants that provide health benefits for humans beyond those attributed to macronutrients and micronutrients. They protect plants from disease and damage and contribute to the plant's color, aroma, and flavor (Molyneux *et al*., 2007). In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, ultraviolet (UV) exposure, and pathogenic attack are called phytochemicals. A wide range of dietary phytochemicals are also found in fruits, vegetables, legumes, whole grains, nuts, seeds, fungi, herbs, and spices (WHO, 2002).

According to Getaneh and Girma (2014), phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Humans use secondary metabolites as medicines, flavorings, and recreational drugs in the recent past. Secondary metabolites have proven to be medicinal in nature. They have various protective and therapeutic effects that prevent ailments and maintain a state of well-being (Dias *et al*., 2012). Phytochemical screening results of different medicinal plants revealed that they contain therapeutically essential secondary metabolites, which could account for the antimicrobial or antibacterial activity of the plants (Jain *et al*., 2019). Phytochemicals of secondary metabolites include saponins, steroids, coumarins, alkaloids, terpenoids, flavonoids, phenolics, glycosides, tannins, carbohydrates, proteins, and amino acids.

Loliger (1991) suggested that phytochemicals have the potential to have a wide range of pharmacological and biological activities. Some instances of these activities include

antioxidant constituents (phenolic acid, hydrolyzable tannins, and flavonoids) of plant materials for the maintenance of health and protection against cancer, heart disease, and other diseases, as well as anti-carcinogenic and anti-mutagenic effects (Batiha *et al*., 2020). Therefore, the study was necessitated to detect the secondary metabolites or active ingredients of some commonly used potential medicinal plants in the Senan District.

The community members are knowledgeable about recognition of medicinal plants and their usage to treat common ailments, and they continue to use herbal medicines to treat sickness as they have in the past (Wohlmouth *et al*., 2014). *Ruta chalepensis* (L.), *Vernonia amygdalina* (Del.), and *Zehneria scabra* (Linn. f.) Sond.) emerged as the most frequently cited MPs among the 120 species identified in the study area, according to community perceptions (Endesew, 2023). These plants were prioritized for qualitative phytochemical screening to identify potential bioactive compounds. This prioritization was based on their high-fidelity level and informant consensus factor, as well as supporting scientific literature. Following a comprehensive literature review, *V. amygdalina* has been identified as a plant with a long history of use in traditional medicine to treat a variety of ailments, including microbial infections such as anthrax, malaria, and intestinal parasites (Degu *et al*., 2024). Similarly, *R. chalepensis* used for the treatment of various health ailments, including inflammatory, central nervous system depressant, and antipyretic effects (Oshida *et al*., 2019; Megersa *et al*., 2021). Additionally, Z. *scabra* has demonstrated antidiarrheal and antisecretory properties (Tamiru *et al*., 2014). By transitioning traditional knowledge into modern scientific research, this study aims to both preserve valuable medicinal plants and contribute to the development of novel pharmaceuticals.

While numerous ethnobotanical studies have documented indigenous knowledge of medicinal plants, there remains a significant gap between traditional practices and rigorous scientific validation. Despite the anticipated phytochemical richness of many medicinal plants, limited experimental research has been conducted to substantiate their therapeutic claims. This study aims to bridge this gap by systematically documenting medicinally useful plants and their associated ethnobotanical knowledge, followed by rigorous experimental validation of their bioactive compounds.

2. MATERIALS AND METHODS

2.1. Study Area

The study area is located in Senan District, East Gojjam Zone, Amhara regional state. Senan District is one of the 22 districts in East Gojjam Zone. The district is 326 km away from Addis Ababa, the capital city of Ethiopia, 292 km away from Bahir Dar, the capital of the Amhara region, and 27 km from Debre Markos the capital city of East Gojjam Zone. The district is bordered on the south by Gozamen District, on the west by Machakel District, on the north by Bibugn District, and the east by Debay Tilat Gin District. The study site is geographically located at 10^{o} 25' 30" - 10^{o} 39' 0" N to 37^{o} 40' 15"-37^{o} 53' 0" E with an altitudinal range of 2300 - 4100 meters above sea level (m a.s.l). Plants with medicinal properties were gathered in Senan District Kebeles, while the phytochemical screening was carried out in the chemistry department, organic chemistry laboratory of Debre Markos University (DMU).

2.2. Materials for the Study and apparatus

A. Field apparatus

A global positioning system (GPS), cutter, polyethylene bag or plastic bag, and digital camera or smartphone were used as data collection materials in the field areas.

B. Laboratory apparatus

A blender (mixer/coffee grinder), a polyethylene bag, a spatula, a conical flask, an Erlenmeyer flask, an electrical shaker, a separatory funnel, filter paper (Whatman No. 1), a rotary evaporator, a drying oven, a refrigerator, an electronic balance, a volumetric flask, a beaker, a stir rod, a graduating cylinder, a test tube, a test tube rack, hose, a pipette, a dropper, and digital camera were used in the experiment.

Chemicals and reagents

The chemicals used for the experimental work of this study were methanol, petroleum ether, sulphuric acid (H_2SO_4), hydrochloric acid (HCl), ferric chloride ($FeCl_3$), Wagner's reagent, potassium iodide (KI), sodium hydroxide (NaOH), ammonia (NH_3), chloroform, and distilled water.

2.3. Experimental Methods for Phytochemical Investigation

Sample selection, collection, and preparation

In our study, three medicinal plant species with relatively high informant consensus value for

treating ailments in the study area were selected for further investigation of the most common phytochemicals (secondary metabolites) such as alkaloids, terpenoids, steroids, flavonoids, saponins, phenolics, and glycosides.

The essential parts of the three selected medicinal plant species, namely *R. chalepensis*, *V. amygdalina*, and *Z. scabra*, were collected from the study area. These plant species were employed in this experiment after they had been verified or validated by botanists. The parts of the selected plants were detached from the parent plant and washed with tap water to remove dust particles and other waste materials from their surface. The important plant parts were placed in a shaded and open place at room temperature for some days to dry in fresh air without exposure to sunlight or heat. The air-dried plants were chopped into small pieces and milled into a uniform powder with the help of a coffee grinder (Appendix 1), then packed in polyethylene bags and stored in a refrigerator until used for extraction.

Extraction procedure

Using methanol and petroleum ether, the powder from three eligible plant species sections was extracted through maceration. By dissolving and weakening the plant's cell wall, maceration aims to release the soluble phytoconstituents (Handa *et al*., 2008). A hundred grams (100g) of dry powder (ground material) from chosen plants were mixed with five - hundred milliliters (500 mL) of the solvents at room temperature for each extraction procedure. Following a 72-hour period of shaking the combinations using an electrical shaker, the mixture was filtered via filter paper. Following filtering, the mixture was concentrated using a rotary evaporator set at a temperature lower than the extracting solvent's boiling point (methanol and petroleum ether) while operating at a lower pressure (Appendix 1). For later usage, the extracted samples were either left in the air or refrigerated at 4°C.

Phytochemical analysis/screening test

Phytochemical analysis of the methanol and petroleum ether crude extracts of selected plants was done (Appendix 1) using the following standard methods:

Test for alkaloids (Wagner's reagent test)

Extracts were dissolved individually in diluted HCl and filtered. Then the filtrates were treated with Wagner's reagent (iodine in KI). The formation of a brown or reddish precipitate indicates the presence of alkaloids (Sochor *et al*., 2010).

Test for terpenoids (Salkowski's test)

Two (2 mL) of chloroform was added to the plant extract (0.5g) in a test tube. Then concentrated H_2SO_4 was added to this mixture, which resulted in a reddish-brown interface, confirming the presence of terpenoids (Perez, 2001).

Test for steroids (Salkowski's test)

Two (2 mL) of the extract, chloroform, and 1 mL of concentrated H_2SO_4 were added carefully along the sides of the test tubes. A red color was produced in the solvent layer, which confirms the presence of steroids (Banu and Cathrine, 2015).

Test for flavonoids

Two (2 mL) of each solvent extract was treated with 2 mL of dilute NH_3 solution and a few drops of concentrated H_2SO_4. A formation of yellow color indicates the presence of flavonoids (Sochor *et al*., 2010).

Test for saponins

A small amount (2 mL) of each of the samples in a test tube and 2 mL of distilled water were added and vigorously shaken for 15 minutes. The formation of one centimeter (cm) of foam confirms a positive result (Sochor *et al*., 2010).

Test for phenols (Ferric chloride test)

Ten (10 mL) of an alcoholic solution of the extract, 2 mL of distilled water followed by drops of a 10% aqueous $FeCl_3$ solution were added. The formation of a blue or green color indicates the presence of phenols (Shrestha *et al*., 2015).

Test for glycosides

A small amount (2 mL) of alcoholic extract was dissolved in 1 mL of water and the aqueous NaOH solution was added. The formation of a yellow color indicates the presence of glycosides (Shrestha *et al*., 2015).

Data analysis for phytochemical test

The phytochemical tests were organized and analyzed qualitatively. As the most published articles recognized the phytochemical components were recorded as a plus (+) sign for positive results for the tested bioactive compounds and a minus (–) sign for the negative result for tested results.

3. RESULTS

The results of qualitative phytochemical analysis of methanol and petroleum ether extracts of *Ruta chalepensis* aerial parts, *Vernonia amygdalina*, and *Zehneria scabra* leaves were summarized in tabulation and figures (Table 1, Figure 1-6).

Table 1. Phytochemical constituents of the selected medicinal plants

Phytochemical constituents	**Type of test (reagent)**	**Color**	**Test result**					
			R. chalpensis		*V. amygdalina*		*Z. scabra*	
			ME	PEE	ME	PEE	ME	PEE
Alkaloids	Wagner's reagent Test	Brown/ Reddish	+ +	+	++	++	+	+
Terpenoids	Salkowski's test	Reddish Brown	+ +	-	+	+	++	+
Steroids	Salkowski's test	Red	-	-	-	-	++	-
Flavonoids	Alkaline reagent Test	Yellow	+	-	+	-	++	+
Saponins	Foam test	White foam	+	+	++	+	++	-
Phenols	Ferric chloride test	Blue-black (blue or green)	+ +	-	++	-	++	-
Glycosides	NaOH test	Yellow	+ +	+	+	-	++	+

Key: ME = Methanol extract, PEE = Petroleum ether extract, (-) = indicates absent/not present, (+) = indicates present, and (++) = highly present

In the present study, the presence of several phytochemicals was investigated in three medicinal plant species. These phytochemicals or secondary metabolites, such as alkaloids, terpenoids, steroids, flavonoids, saponins, phenols, and glycosides. These bioactive components of the extracts are believed to exhibit many biological activities that are responsible for pharmacological purposes and protect from most chronic illnesses (Sagwan *et al*., 2010; Rajurkar and Gaikwad, 2012). The finding showed the presence of most of the phytochemical components in methanolic extract when compared to petroleum ether solvents. A phytochemical screening test helps in isolating and characterizing the chemical components present in the plant extracts; understanding these components is necessary to comprehend herbal medicines and their preparations, and ultimately to ascertain the true worth of traditional remedies.

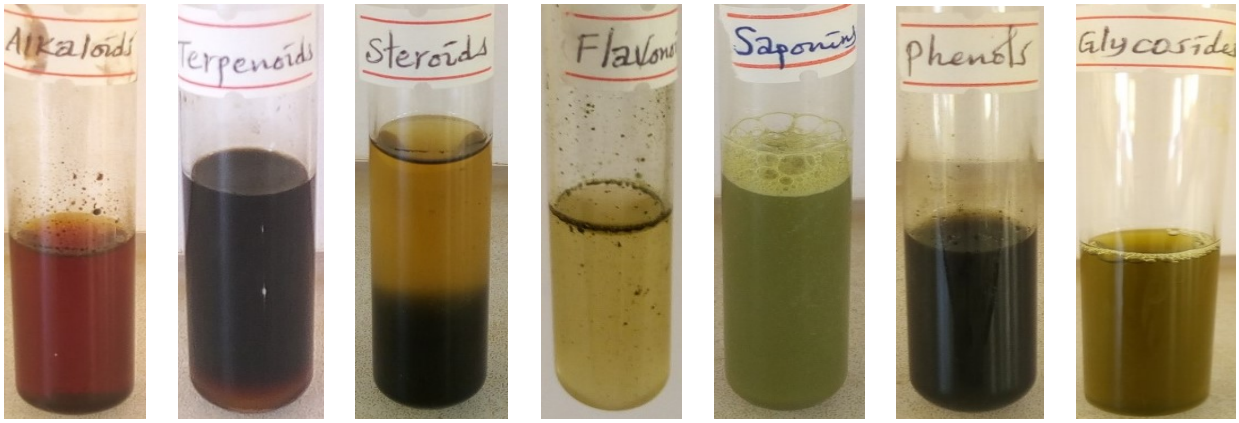


Figure 1. Methanol extract of *R. chalpensis* aerial part

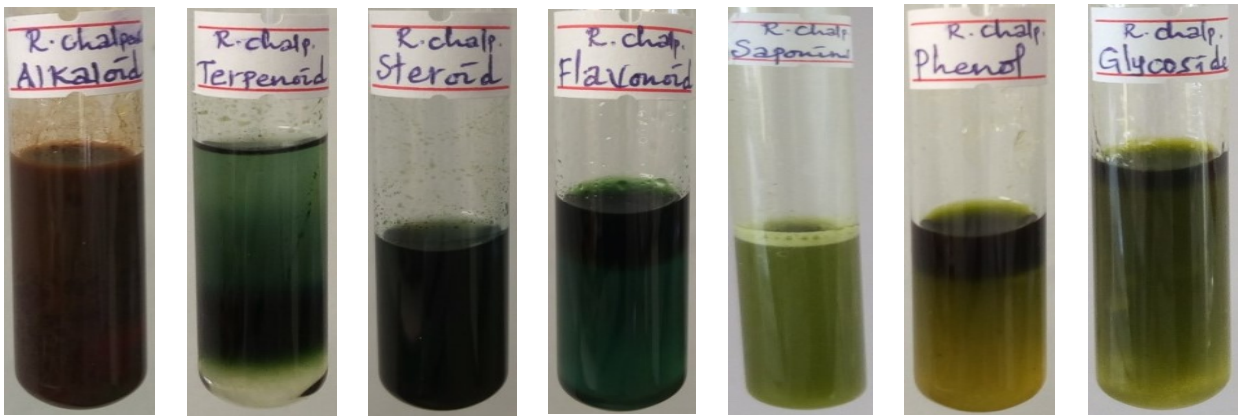


Figure 2. Petroleum ether extract of *R. chalpensis* aerial parts

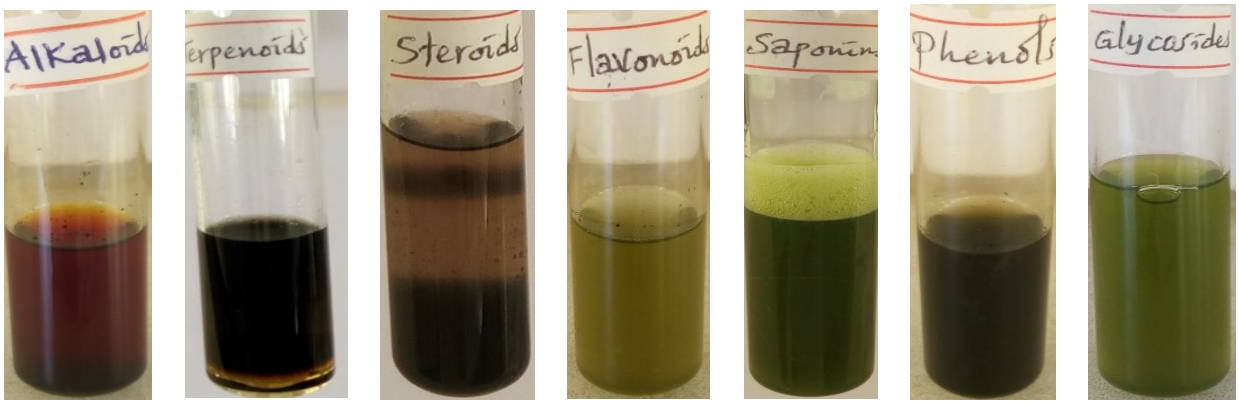


Figure 3. Methanol extract of *V. amygdalina* leaves

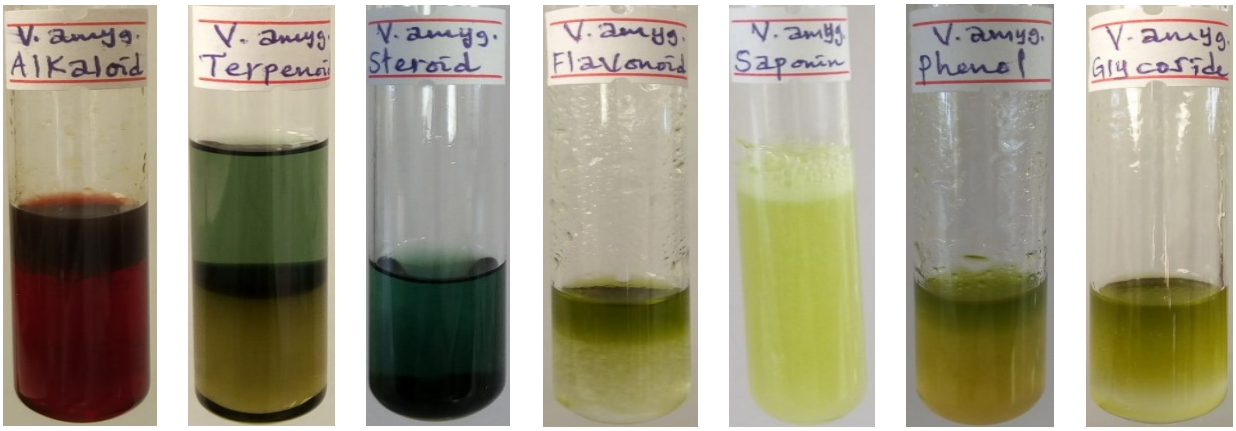


Figure 4. Petroleum ether extract of *V. amygdalina* leaves

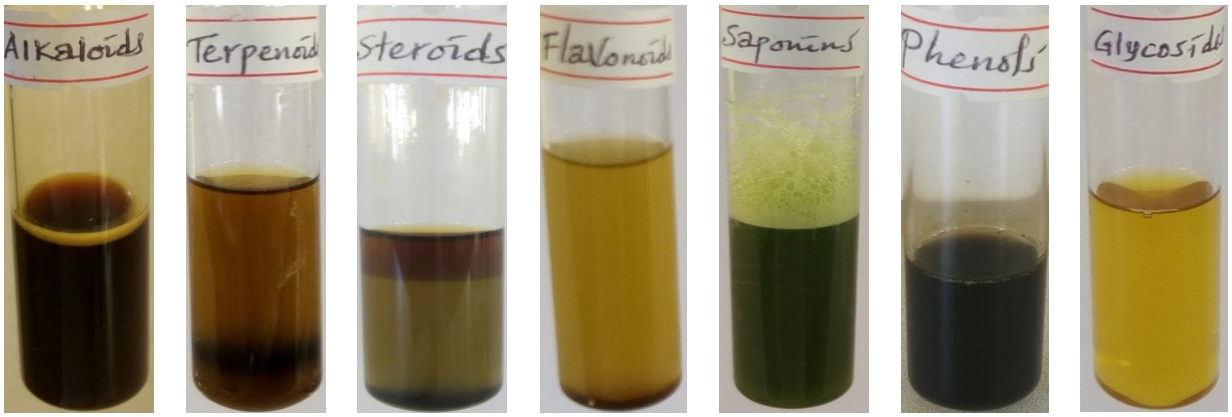


Figure 5. Methanol extract of *Z. scabra* leaves

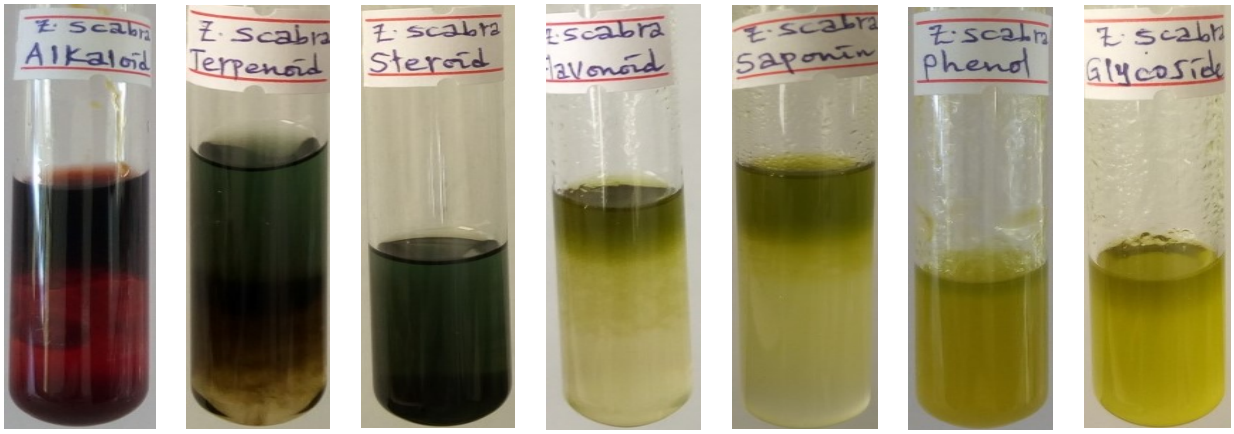


Figure 6. Petroleum ether extract of *Z. scabra* leaves

4. DISCUSSION

In the present study, the presence of several phytochemicals such as alkaloids, terpenoids, steroids, flavonoids, saponins, phenols, and glycosides was investigated in three (*R. chalepensis*, *V. amygdalina*, and *Z. scabra*) selected medicinal plant species. These plant extracts were subjected to a phytochemical screening technique to identify the major secondary metabolites or bioactive compounds using qualitative chemical tests that involved color changes as a result of interactions with several standard reagents.

The finding showed that the extract of *R. chalepensis* aerial parts treated with methanol had all the tested compounds, such as alkaloids, terpenoids, flavonoids, saponins, phenols, and glycosides, except steroids. Based on these findings, this plant has great importance as an efficient source of therapeutic agents. Thus, *R. chalepensis* has been utilized to cure various ailments. This correlated with the findings of other studies (Alemayehu *et al*., 2019; Gedlu, 2022). In addition, Megersa *et al*. (2021) reported the presence of alkaloids, flavonoids, saponins, and phenols. To the contrary, Degu *et al*. (2020) reported the presence of steroids in hydromethanol crude extracts of the plant leaves in addition to all test compounds. Gizaw and Yibralign (2022) reported the presence of steroids in the aerial parts (leaves and fruits) of *R. chalepensis* in addition to all test compounds. However, the extract of this plant within petroleum ether had alkaloids, saponins, and glycosides from those tested compounds. This result agreed with the findings of Megersa *et al*. (2021) and Gedlu (2022), but Rahmani and Ouahrani (2022) claimed that saponins and glycosides were absent.

The study indicates that the methanolic extract of *V. amygdalina* leaves consisted of all essential test compounds like alkaloids, terpenoids, flavonoids, saponins, phenols, and glycosides except steroids. The presence of these bioactive compounds is believed to be responsible for their pharmacological activities. This result is similar to the findings of previous studies (Bihonegn *et al*., 2019; Sharew, 2021). Furthermore, Degu *et al*. (2020) reported the presence of alkaloids, saponins, and the absence of steroids in hydromethanol extracts of *V. amygdalina* leaves. On the contrary, Abate and Mengistu (2018) reported only steroids present in the tested compounds. However, the petroleum ether extract of these plant leaves had only alkaloids, terpenoids, and saponins as compared to methanol extracts.

With regard to the extract of *Z. scabra* leaf treated with methanol, it also contained all-important tested compounds including, alkaloids, terpenoids, steroids, flavonoids, saponins, phenols, and glycosides. The results reveal that the presence of these phytochemical constituents enhances the medicinal value of the plant. This result is in line with other studies that reported the presence of bioactive compounds within the leaves of the plant (Alebachew, 2018; Bayu *et al*., 2018; Abebe and Hunie, 2022). In addition, Belay and Makonnen (2020) reported the presence of all tested compounds within a 70% ethanolic extract. On the contrary, Talema (2020) reported only the presence of saponins, and Tamiru *et al*. (2014) reported the absence of alkaloids and steroids in the 80%

methanolic extract of *Z. scabra* leaves. However, the leaf extract of this plant in petroleum ether contains alkaloids, terpenoids, flavonoids, and glycosides from those tested compounds. To the contrary, Alebachew (2018) revealed the absence of alkaloids, terpenoids, and glycosides but agreed with only the presence of flavonoids in the leaf extracts.

Generally, the differences in the reports of many of the studies could be due to the variation in time of plant collection, climate condition, nutrient or mineral content, methods of extraction, type of test (reagent), amount of solvent used, and other factors. The majority of bioactive components or secondary metabolites were extracted from methanol extracts rather than petroleum ether. This may be due to the high solubility of active compounds and the nature of polarity among solvents.

The phytochemical investigations help find chemical ingredients in the plant that may help to locate the source of pharmacologically active principles against several infections. The phytochemicals present in different extracts exhibit a number of biological activities and have a great contribution to fighting or healing various ailments when used as a medication or drug. Hence, the inclusiveness of all the above-tested bioactive compounds in a medicinal plant increases the efficacy of treating different ailments. This suggests that the extract of those plants has a broad spectrum of antimicrobial activities.

5. CONCLUSIONS

The present study revealed the wide use of medicinal plants in Ethiopian communities due to many plants containing different types of phytochemical substances possessing pharmacological properties. The *R. chalepensis*, *V. amygdalina*, and *Z. scabra* were the medicinal plant species in the study area for the traditional treatment of various ailments. Thus, qualitative phytochemical investigation was employed in such plants to identify their secondary metabolites that contributed to the healing potential of ailments. It has been found that most of the phytochemical compounds are present in all the studied plants, with variations in the chemical constituents of each solvent (methanol and petroleum ether) extract. The presence of phytochemicals in those plants can make, a potential drug. To highlight the research's conclusions, more investigation is required to quantify, isolate, characterize, and examine the specific compound's biological activity (such as pharmacological testing and antibacterial effects) in preparation for drug development.

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Competing interests

We have no competing interests.

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Appendix 1: Pictorial expressions of the study related to the phytochemical analysis of methanol and petroleum ether extracts of *R. chalepensis* aerial parts, *V. amygdalina* and *Z. scabra* leaves.



Figure A1: Images illustrating some aspects of the laboratory (lab.) activity in DMU