

Research Article

Identification of Functional Groups and Phytochemical of Crude Extracts of *Ocimum Sauve* Roots Using Molecular Absorption Spectroscopic Techniques

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Abstract

Traditional medicines have been used in health maintenance, disease prevention and treatment in both developed and developing countries. From those the one is *ocimum sauve*. *Ocimum sauve* was used to treat a herpes simplex traditionally in North shoa people, of Ethiopia. The main objective of the present study is to determine bioactive compounds of *ocimum sauve* and indicate its functional group by using Fourier Transform Infra-Red spectroscopy. The study area was North shoa district Oromia regional state of Ethiopia where thus plant specimens were collected. The root of *ocimum sauve* were dried in shaded region, ground into a powder and extracted with different solvents. The root crude extracts of *ocimum sauve* was tested for phytochemicals screening. The phytochemicals screening of the root extract of *Ocimum sauve* indicates that the presence of bioactive compound such as alkaloids, saponins, flavonoids, phenols, steroids, glycosides, terpenoids and tannins compounds and some functional groups are present like alcohol, alkane, alkenes, alkynes and aromatic compounds functional groups. This plant is used in North shoa society, Oromia regional state of Ethiopia to treat herpes simplex. The result of this work supplements the traditional knowledge of the people for the traditional medicinal uses of this plant. Further research is recommended to isolate and identify pure compounds of different parts of this plant.

Keywords

Ocimum Sauve, Crud Extract, Phytochemical, FT-IR

1. Introduction

In both developed and developing countries different indigenous people use different plants for the treatment of different diseases. Those plants were called medicinal plants. These medicinal plants are unique for particular organism or classes of organisms [15]. Medicinal plants have been used for contributions health care for humans and animals in developing countries like Ethiopia. [12] “Research on plants with medicinal properties and identification of the chemical

components responsible for their activities have justified the ancient traditional healing wisdom and have proven the enduring healing potential of many plant medicines [2].” Wild plants have always been a major source of primary health care and other necessities of daily life for local communities throughout the world, an indication that medicinal plants can provide the best alternative source to obtain a variety of drugs [14]. The use of medicinal plant as herbal

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remedies is a part of traditional heritage in rural area of North Shoa zone especially among the forest habitats. Thus, the present study aimed at investigating phytochemical, functional groups and antibacterial activity in *ocimum sauve* willds roots in North Shoa zone of Oromia regional state of Ethiopia. This *ocimum sauve* will leaves herbs medicine is used to treat herpes simplex" Mich" diseases.

"Ocimum sauve is a seasonal, ruderal and anthropophilic plant which is sometimes cultivated [9]." "This aromatic shrub grows specifically at high altitudes [10]." It is a culinary medicinal plant that assigned to the family lamiaceae, which contains aromatic valued for their medical and culinary uses. It is commonly called scent leaf. Ocimum Sauve is a branched erect shrub which grows about 50-100 cm in height. The branches and steam of ocimum sauve are villous or sub-glabrous which turn red-brown when dry.

It is geographical distribution stretches from tropical Asia to tropical west and East Africa where it is found wide spread on mountain us and open waste areas of the world [9]."



Figure 1. Picture of *Ocimum sauve* willds herbal medicine.

1.1. Statement of the Problem

For the great achievement of modern medicine traditional medicinal plants plays an important role for the contribution of healthcare. Ethiopia, is a one of the countries which intensively use plants for traditional medicine for treatment of various health problems. People from North Shoa Zone form Oromia Regional State of Ethiopia use different plants for medicinal purposes. Among the plants is *ocimum sauve*. "The research conducted around Debre Libanos monastery for the survey on the knowledge and uses of medicinal plant reported that the ocimum sauve willds is used to treat herpes simplex, headache, intestinal illness and parasites [11]." "Another survey conducted round Fiche districts North shoa zone of Oromia regional state on traditional medicine indicates that there are some medicinal plants that have been used as traditional medicine around Fiche districts [1]." "Sahalie and co-workers [6] have reported chemical composition and anti-bacterial activity of leaves extracts of ocimum lami-

ifolium as treatments for urinary tract infections." The research findings on the sought traditional plant so far were survey of the knowledge and practices of the indigenous people of using the plant for medicinal purposes [13]. However, none of the literature addressed in their work about phytochemicals screening and functional groups of this plant which are considered to the treatment of the medicine identified. As ocimum lamiifolium belongs to the same family with ocimum sauve, one expects that the reported chemical composition could be addressed in species ocimum sauve. Therefore, this study investigates phytochemicals and functional group of the crude extracts of roots of ocimum sauve willds as treatment of herpes simplex.

1.2. Objectives of the Study

1.2.1. General Objective

The general objective of this research was to identify functional groups and phytochemicals in ocimum sauve.

1.2.2. Specific Objectives of the Study

The specific objectives of the study are:

1. To carry out crude extraction of the ocimum sauve root.
2. Investigation of phytochemicals on crude extracts ocimum sauve root.
3. Identify some functional group of the compound using FT-IR spectroscopy spectrum

2. Materials and Methods

2.1. Chemicals and Materials

2.1.1. Chemicals

The solvents such as n- hexane, dichloromethane and methanol (Hind) are used for gradient extraction for Ocimum sauve root extract and to investigate phytochemicals reagent like Dragendroff's, dilute sodium hydroxide, ferric chloride, concentrated hydrochloric acid, concentrated sulphuric acid glacial acetic acid, acetic anhydride, chloroform (Germany) CDCl_3 and DCl_3 were used. All chemicals are analytical grade. The chemicals used were purchased from Ran chem. Co. Ltd. Agents in Addis Ababa, Ethiopia.

2.1.2. Apparatus and Instruments

The apparatus used for the study were Rotary evaporator (Heidolph, UK) for concentration of crude extracts, Grant (GLS 400) thermostatic bath shaker (for maceration of plant materials), Oven (model N50L, GENLAB, WIDNES, ENGLAND) for drying the wet material like beaker conical flask, Analytical Balance ADAM (AFP-110L) for measuring plant material. Glass wears like beaker, UNICAM UV 300 and Measuring cylinder, conical flask (for maceration plant mate-

rial), Buchner funnel (for filtration purpose), and whatmann filter paper. Infrared (IR) spectra were obtained from Perkin Elmer BX infrared spectrometer ($400 - 4000\text{ cm}^{-1}$). At Adama science and technology University, Department of Biology, spectroscopic analysis were carried out.

2.2. Methods

2.2.1. Collection of Plant Materials

The root of *Ocimum sauve* was collected by digging the ground in May 2012 E. C from Dera Woreda, North Shoa Zone of Oromia Regional State. Then the roots of *ocimum sauve* willds was washed through running and ionized water to remove some soil particles since the sample was obtained under the ground. The sample of plant material was authenticated by botanist at Wolaita Sodo University, Department of Biology and 800 g of the sample was taken for laboratory.

2.2.2. Sample Preparation

The collected plant morphological parts of roots was chopped into small pieces and air- dried under shade on a plastic material for two weeks and milled to suitable size with a grinding machine at Wolaita Sodo University College of natural and computational science Department of chemistry, chemistry laboratory. 520 grams of plant material was

prepared, and the prepared sample was stored under refrigerator below 4°C until was used for extraction.

2.2.3. Extraction

The basic operation included steps, such as pre-washing, drying of plant materials or freeze drying, grinding to obtain a homogenous sample and often improving the kinetics of analytic extraction and also increasing the contact of sample surface with the solvent system. Proper actions must be taken to assure that potential active constituents are not lost, distorted or destroyed during the preparation of the extract from plant samples. If the plant was selected on the basis of traditional use.

Then it is needed to prepare the extract as described by the traditional healer in order to mimic as closely as possible the traditional 'herbal' drug [3]. The selection of solvent system largely depends on the specific nature of the bioactive compound being targeted. The root of *Ocimum sauve* 520 g was extracted with n-hexane, dichloromethane/methanol (1:1) and methanol and using maceration techniques for 48 hrs with continuous shaking. The crude extracts of each solvent were stored in hood until it's become dry and to determine the weight and safe for further analysis. General schematic flow chart indicates general extraction techniques.

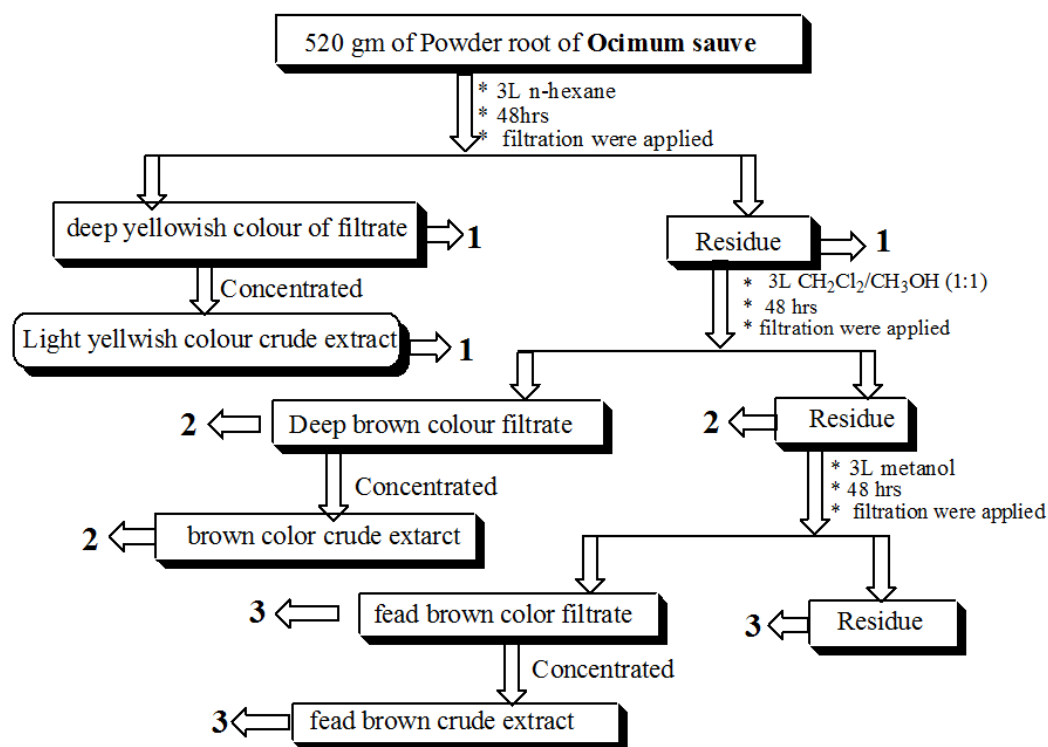


Figure 2. General outline for the extraction of root of *Ocimum sauve*.

2.3. Phytochemical Screening

Phytochemical analysis for various secondary metabolites of the crude extracts of ocimum saue was expressed by using methods described below. The groups of phytochemical constituents that would be expected to investigate are alkaloids, flavonoids, tannins, phenols, steroid, saponins, terpenoids and glycosides.

Test for Alkaloids

Using a top loading weigh balance, 0.1 g of crude extracts of ocimum saue were dissolved individually in dilute hydrochloric acid and filtered by using filter paper. Then the filtrate was tested for alkaloids using dragend ocimum saue crude extract reagent was added. A prominent yellow precipitate shows presence of alkaloids compound in root crude extract of ocimum saue. [4].

Test for Flavanoids

Ferric chloride test: Ocimum saue root crude extract solution of 0.1 g was treated with 3-4 drops of Ferric chloride solution. Formation of a blackish red color shows flavonoids presence [5].

Test for phenols

The 0.1 g extracts were treated with 3-4 drops of ferric chlorides solution. Formation of green or bluish black color indicates the presence of phenols [5].

Test for Glycosides

Ocimum saue root crude extract of 0.1 g was added in test tube, 2 ml and 1 ml glacial acetic acid and 3 drops of ferric chloride solution respectively were added. The contents were heated and cooled then transferred to a test tube containing 2 ml concentrated sulphuric acid. Formation of a brown ring at the interface (presence of deoxy sugar characteristic of cardenolides) and observation of pale green color in the upper layer (steroidal nucleus) shows glycoside presence [5].

Test for Terpenoids

Ocimum saue root crude extract of 0.1 g was mixed in 2 ml chloroform and then 3 ml concentrated sulphuric acid was added. At the interface a reddish-brown color shows terpenoids presence [7].

Test for Tannins

Ferric Chloride Test: Ocimum saue root crude extracts dried powder sample 0.1 g was boiled in 10 ml distilled water on a hot plate and the extract was filtered. Two milliliter portion of filtrate was measured and 3 drops of 0.1% ferric

chloride solution was added. Formation of a green color solution shows tannins presence [5].

Test for Saponins

Froth test: From crude extracts 0.1 g were dissolved in 20 ml distilled water and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins [5].

Test for Steroids

Liebermann Burchard reaction: Measured 200 mg ocimum saue root crude extract solution in 10 ml chloroform solution, 3 drops of acetic anhydride and Sulphuric acid solutions were added slowly from side of test tube. Observation of a brown ring at the junction of the two layers and the upper layer turns green separating the liquids shows steroids presence [5].

2.4. Solution Preparation for FT-IR Instrument

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. Crude extract of Dichloromethane/ Methanol (1; 1) were selected to determine the functional group based on this 0.1 g crude extract were placed in 100 ml DMSO, (Dimethyl sulfoxide) solvent, the solution were prepared in conical flask, then the solution was filtered by using filter paper, the filtered solution subjected in to Infrared (IR) spectra were obtained from Perkin Elmer BX infrared spectrometer ($400 - 4000\text{ cm}^{-1}$), depending this the following data were recorded from the instrument.

3. Results and Discussions

3.1. Phytochemical Screening Analysis

Phytochemical examinations revealed that, Ocimum saue root crude extract contains steroids, alkaloids, saponins, glycosides, tannins, terpenoids, phenols and flavanoids phytochemical compounds with different concentration as seen in (Table 2). Bioactive secondary metabolites have been utilized as natural medicines and plants containing those compounds have been used as medicinal plants and are prescribed in many recipes as forms of crude drugs [8]. The result of the tests was presented Table 1.

Table 1. Phytochemical Analysis of Ocimum saue root extracts in this work.

Bio-active components	Reagent used	Crude extract					
		n-hexane		Dichloromethane/methanol		Methanol	
		Result	Observed color	Result	Observed color	Result	Observed color
Alkaloids	Dragendroff's reagent	+-	Weak red color	++	Red color	+-	Weak red color

Bio-active components	Reagent used	Crude extract					
		n-hexane		Dichloromethane/methanol		Methanol	
		Result	Observed color	Result	Observed color	Result	Observed color
Saponins	Distilled water	--	Non foam formation	++	Strong-foam formation	+--	Weak foam formation
Flavonoids	Alkaline reagent	---	Colorless	++	Intense yellow color	---	Colorless
Phenols	Ferric chloride	---	Colorless	++	Fade-green color	+--	Weak Bluish black color
Steroids	Liebermann Buchard rxn	++	Blue-green color	+++	Blue-green color	+++	Blue-green color
Glycosides	Keller-Killani test	++	Reddish brown color	+++	Deep reddish brown color	++	reddish brown color
Terpenoids	Sulphuric acid	++	Reddish brown color	+++	Deep reddish brown color	++	Reddish brown color
Tannins	Ferric chloride	---	Colorless	+++	Brownish green	+	Brownish green

Key: Weak (+--), Moderate (+), Strong (++), Very Strong (+++), Absent (--)

From the above Table 1 observed that, n-hexane crude extracts indicate the presence of alkaloids, steroids, glycosides and terpenoids, whereas flavonoids, phenols, Saponins and tannins are absent. Crude extract of dichloromethane/methanol (1:1) indicates the presence of alkaloids, saponins, flavonoids, phenols, steroids, glycosides, terpenoids and tannins. Crude extract of methanol indicates the presence of alkaloids, saponins, phenols, steroids, glycosides, terpenoids and tannins. In the present study, the crude extracts of *ocimum sauve root* revealed the presence of alkaloids, saponins, flavonoids, phenols, steroids, glycosides, terpenoids and tannins. This plant is used traditionally in North shoa zone of Oromia regional state of Ethiopia to the treatments of herps simplex which is called viral diseases. Therefore, due to the presence of these phytochemical constitutes the root crude extracts of *ocimum sauve* has good anti biological activities.

3.2. FT-IR Data Analysis

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The root crude extracts of *ocimum sauve* was passed into the FTIR and the functional groups of the components are separate based on its peak ratio. The IR spectrum of crude extract of Dichloromethane/Methanol *Ocimum sauve* plant material exhibits characteristic intensity band at different peak values. The results of *ocimum sauve* root crude extract FT-IR analysis confirmed the presence of Alcohols (1193 cm^{-1} , 1143 cm^{-1}), Aldehydes (2666 cm^{-1}), Alkenes (1946 cm^{-1}), Alkane (2922 cm^{-1}), Aromatic compounds (952 cm^{-1} , 895 cm^{-1} , 862 cm^{-1} , 763 cm^{-1}) and Ketones with Four member cyclic (1781 cm^{-1} , 1722 cm^{-1}) as it indicates in Figure 2. The detail description of the functional groups which are obtained from the result of FT-IR is explained briefly in Table 2.

Table 2. IR spectrum on crude extract of dichloromethane/ methanol on *Ocimum sauve* plant material.

Class of compound	Absorption, cm^{-1}	Intensity	Assignment (IR table)
Alkanes	2922 cm^{-1}	Sharp peak	C-H stretch
Aldehydes	2666 cm^{-1}	multi peak	H-C=O stretch
Alkenes	1946 cm^{-1}	Sharp peak	C=C asymmetric stretch
Ketones	1781 cm^{-1}	Sharp peak	C=O stretch
Four member cyclic	1722 cm^{-1}	Sharp peak	RR'C=O, C=O stretch

Class of compound	Absorption, cm^{-1}	Intensity	Assignment (IR table)
Alcohols	1193 cm^{-1}	Multi, Sharp peak	C-O stretch, Ar-O-H
	1143 cm^{-1}	Multi, Sharp peak	RR'R''C-OH (3°) C-O stretch
	952 cm^{-1}	Sharp peak	C-H bending
Aromatic Compounds	895 cm^{-1}	Sharp peak	C-H bending
	862 cm^{-1}	Sharp peak	C-H bending
	763 cm^{-1}	Sharp peak	C-H bending

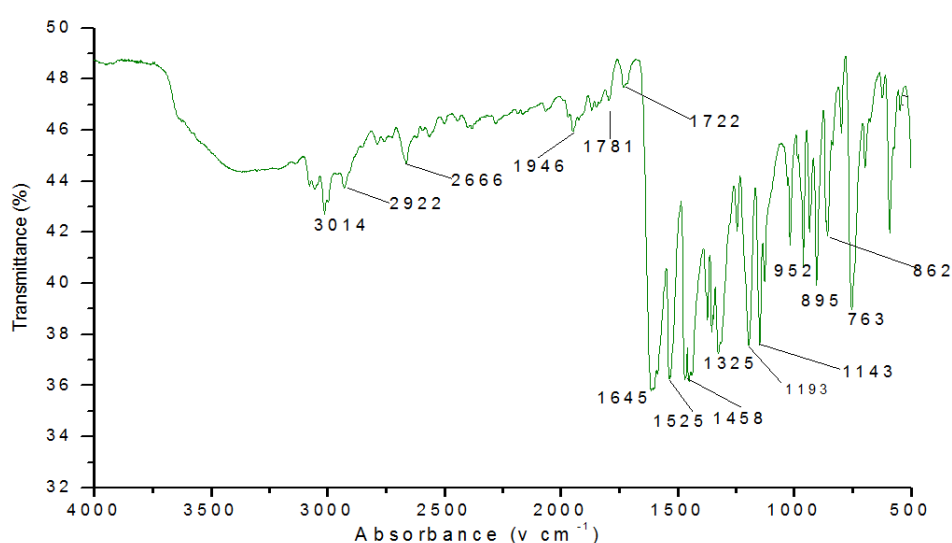


Figure 3. FT-IR spectrum for dichloromethane/methanol crude extracts.

From the spectra we can see clearly that although they show each absorption spectrum of various components, each band represents some characteristic absorption peaks of functional groups in the sample. By using the FT-IR spectrum, the results of the present study spectrum also revealed the functional constituents present in the crude extracts of *ocimum sauve* root. Many workers applied the FTIR spectrum as a tool for differentiating, classifying and discriminating closely related plants. The results of the present study also supplemented the previous observations and provided the functional groups at root of *ocimum sauve* crude extract. Therefore, the present work on *Ocimum sauve* root crude extract displayed novel phytochemical markers as use full analytical tool to check and identify the medicinally important plant.

4. Conclusion and Recommendation

4.1. Conclusion

As the presence work indicates that, the methanol reagent has produced more crude extract but dichloromethane/methanol (1:1) combination reagent phytochemical profile is stronger than other reagents.

The identification of phytochemicals in *Ocimum sauve* root crude extracts showed all the eight phytochemicals have been observed in both methanol and its combination with dichloromethane. The presence of these phytochemicals indicates that these plants were used as good treatments for herpes simplex, because these phytochemicals have famous biological activities. The FT-IR spectrum obtained from dichloromethane/methanol crude extracts indicates that the presence of Alkanes, Aldehydes, Ketone, Alkenes, Alcohols and Aromatic compounds functional groups. Due to the presence of these functional groups *Ocimum sauve* exhibit good antimicrobial activity with reference to standard.

4.2. Recommendation

Over the years, medicinal plants were used for the management and treatment of several diseases and illness especially in developing countries like Ethiopia. However, the efficacies of these plants are yet to be known. The *Ocimum sauve* is widely used in rural areas of North shoa zone for management and treatment of various diseases and ailments. From the present work, it can be recommended that the root crude extracts of *ocimum sauve* was used to treat different

diseases especially for the treatment of herpes simplex due to the presence of phytochemicals such as alkaloids, flavonoids, phenol, terpenoids, saponin, steroids and glycoside. For further understanding about the use of the plant for the treatment of different disease the bioactivity of the pure compounds from these plants as well as the use of these compounds instead of their synthetic counterparts should be considered for further studies.

Abbreviations

FT-IR Fourier Transform Infra -Red
IR Infra-Red

Author Contributions

Dereje Ketema Beyene is the sole author. The author read and approved the final manuscript.

Conflicts of Interest

The author declares no conflicts of interest.

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