

Medicinal Plants in Treating Hepatitis B Among Communities of Central Region of Ethiopia

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Purpose: In Ethiopia, most people rely heavily on traditional therapeutic plants that have been used for years. The practice of traditional medicines use to treat hepatitis is currently gaining popularity due to the limited availability and affordability of modern drugs. The aim of this study was, therefore, to assess the traditional medicinal plants use to treat viral hepatitis among communities of Central region of Ethiopia.

Methods: Data was collected from November 2018 to December 2021 in Central Ethiopia. An open-ended semi-structured interview was used among purposively selected herbalists, traditional medicine entrepreneurs, village heads, and patients visiting traditional healers for hepatitis treatments. A 5 mL blood sample was collected from patients who visited a traditional healers' clinic for hepatitis treatment and tested for HBsAg and HCV-antibody by using ELISA. Among HBsAg-positives, further nucleic acid test for HBV-DNA load was assessed to measure the effects of prescribed medicinal plants.

Results: Herbalists cited 24 plants that were used for hepatitis treatment; of which *Rumex nepalensis*, *Vangueria apiculata*, and *Solanum incanum* were the most frequently cited plants. Remedies were commonly prepared by crushing or powdering, mixing them with water, and taken orally. Forty-two individuals were diagnosed and treated as hepatitis patients by herbalists, of which eight of them were HBsAg-positive but no positives for anti-HCV ELISA. At the third and sixth months of viral load assessment among HBsAg-positive, serum HBV-DNA suppression was observed in three individuals treated with different combinations of frequently cited plants.

Conclusion: In this study, traditional healers used various plants to treat hepatitis. HBV-DNA suppressive activity was detected in three NAT-positive individuals who were treated by using a mixture of these frequently cited and highest preference-ranked plants. This suggests that these plants have antiviral properties and serve as a basis for more pharmacological research in the quest for new antiviral agents.

Keywords: medicinal plants, hepatitis, Central Ethiopia

Introduction

Viral hepatitis is a liver disease that causes inflammation and can lead to permanent damage.¹ Hepatitis B and C viruses, which cause chronic viral hepatitis, are the most widespread and serious public health issues, affecting millions of people and killing an estimated 1.5 million people each year globally.^{2,3} In developing countries, mainly in Sub-Saharan Africa, the burden of HBV infection remains disproportionately highly endemic (>8%) to intermediate (2–8%).^{4,5} Ethiopia is one of the developing countries with the highest hepatitis morbidity and mortality.^{6,7}

In Ethiopia, the use of traditional medicine has been practiced for centuries as a treatment for various diseases and remedies.^{8,9} The majority of Ethiopian people depend on traditional remedy for most of their health problems. Most of these therapies were traditional preparations made of medicinal plants basis and are sometimes the solitary source of healing for the majority of the people.^{10,11} Ethiopia is also domestic to various cultures, beliefs and languages that have

sequentially contributed to the great assortment of traditional understanding and exercise of the people to use therapeutic plants for various traditional treatment practices.^{12,13} Generally, therapeutic plants used for traditional remedy play an important role in the healthcare of the most of the people in Ethiopia.^{9,14}

Traditional healers use a variety of plants as a primary basis of remedy to treat different types of illnesses, including hepatitis, and these plants are an important part of Ethiopian culture.^{9,15} This widespread use of medicinal plants in Ethiopian cultures to heal these ailments, including hepatitis, is due to lack of modern medicines used to treat and cure some diseases including viral hepatitis.^{16–18} As a result, affected patients seek alternative remedies and visit traditional healer's treatment centers, particularly for herbal therapies due to their social acceptability, effectiveness, accessibility, and affordability since modern medicines used to treat some of diseases including viral hepatitis are expensive and out of reach for most people in developing countries.^{8,19–21} These traditional healers' treatment for hepatitis diseases varies from place to place and among traditional healers. In some instances, it is treated with a single plant preparation in places and in others with a concoction of two or more plants.^{22,23}

The usage of medicinal plants in Ethiopia is enormous, though the activities are poorly reported and difficult to access for modern study.¹⁸ As a result, the quest for plant-based antiviral hepatitis drugs in the country necessitates a comprehensive ethnobotanical investigation in various localities and districts to document the diverse information held by various ethnic groups and communities. In Ethiopia, there was limited studies in antihepatitis traditional medicinal plants, therefore, this study aimed to document and assess the practice of traditional therapeutic plants to treat viral hepatitis among communities of the Central region of Ethiopia.

Materials and Methods

Study Setting and Period

The study was done from November 2018 to December 2021 in three zonal or administrative districts of the Central Region of Ethiopia: namely Hadiya, Kambata, and Silte districts. The zones are located at 230 km from Addis Ababa, the capital city of the country. Most population are rural inhabitants with some living in zonal towns and other small towns with an average annual growth rate of 2.9%. According to the 2007 population census, the three zones have a total population of 3,062,431: Hadiya has a population of 1,231,196; Kambata has a population of 1,080,837 and Silte has a population of 750,398 with 251,264, 220,579 and 153,142 households, respectively.

Study Design and Population

A cross-sectional study was done to assess the traditional practice of therapeutic plants to heal viral hepatitis among communities of Central Region of Ethiopia. The study participants were taken from purposively selected traditional healers, traditional medicine entrepreneurs, village heads, and patients who were visiting traditional healers for treatments and diagnosed as hepatitis patients by traditional healers in the district.

Sample and Sampling Techniques

Traditional healers, village heads, traditional medicine entrepreneurs, and individuals who were visiting traditional healers for their treatments and diagnosed as hepatitis patients by traditional healers were purposefully included in the study. For that reason, purposively selected 52 herbalists, 9 traditional medicine entrepreneurs, 48 village heads, and 42 individuals who came to traditional treatment remedies and traditionally diagnosed hepatitis patients were included in the study. However, these individuals who came to traditional healer and not diagnosed as hepatitis cases by traditional healers were excluded.

Data Collection Process and Tools

The ethnobotanical survey consisted of an open, semi-structured interview in which the interviewee was asked to answer exhaustively the questions asked by the interviewer. The questionnaire incorporated the particular information of the informants including name of the respondent, age, gender, and address, knowledge of hepatitis and traditional healing practice. The questions were asked in their languages. The themes dealt with mainly relating to the scientific

and the local names, the parts used, time and plant material collecting season, its preparation, the dose, its route and the duration of the treatment, side effects, and antidotes used, and contraindications for these remedies. The interviewees concerned were mostly purposively selected herbalists, traditional medicine entrepreneurs, village heads, and patients who were visiting traditional healers' clinics for treatments and diagnosed as hepatitis patients by traditional healers. Field trips were made in the presence of the interviewee, to see the medicinal plants mentioned, to photograph them, and to collect specimens. A market survey was also used to collect additional ethnobotanical information concerning hepatitis treatment.

Collection of Plants' Specimen and Identification

Specimens of the documented therapeutic plants were collected from natural flora and home-gardens during the field gait. Voucher samples were labelled, dried up, and placed in Addis Ababa University (AAU) National Herbarium. The identification of the plants' species was done by botanists at National Herbarium of Addis Ababa University and the Aklilu Lemma Institute of Pathobiology.

Blood Sample Collection

Five-milliliter of blood samples were collected from each individuals who were visiting traditional healers for treatment and diagnosed as a hepatitis patient by traditional healers. The blood sample was collected before taking the treatment which was given by traditional healers. Separation of serum from collected whole blood was done and stored at deep freeze of temperature below -80°C and screened for HBV (HBsAg) and HCV (anti-HCV) infections by using ELISA assay of Beijing WANTAI Biological, China, following the manufacturer's instructions. From individuals whose blood samples were positive for ELISA test short assay or quantitative nucleic acid test, additional five-milliliter blood samples were collected on the third and sixth months after anti-hepatitis medicinal plants treatment and used for nucleic acid tests by using polymerase chain reaction (PCR) for treatment follow-up assessment.

Viral Load Quantifying

HBV-DNA detection was obtained in samples from people who tested positive for Hepatitis B surface antigen (ELISA) before starting typical anti-hepatitis medication, as well as three and six months later. Following the manufacturer's instructions, HBV DNA was extracted from 200 μL of plasma using the m2000sp, an automated sample preparation system designed to use magnetic micro-particle-based reagents for the purification of nucleic acids from samples (Abbott Laboratories, Abbott Park, IL, USA) with a lower quantification limit or sensitivity of 15 IU/mL for the 0.2 mL serum sample volume. Positive, negative, and high positive controls were employed. In the sample preparation technique, an internal controller DNA was supplied and processed alongside the calibrators, controls, and samples. During the extension and annealing steps, the presence of amplified HBV-DNA and an internal control was identified. The amplification cycle at which the Abbott m2000rt detected a normalized fluorescence signal was related to the log of the HBV DNA concentration in the original sample. An external calibration curve was then used to quantify each sample. About 10% of the samples were re-tested to ensure uniformity.

Data Entry and Analysis

MS Excel 2010 spreadsheet was used for data entry and determination of proportions, and percentiles, and to summarize the ethnobotanical data associated with the use of therapeutic plants for the treatment of hepatitis. A preference rating was carried out for the top seven medicinal plants that are used for the treatment of hepatitis. Ten informants were chosen at random from among traditional healers who reported using medicinal plants to treat hepatitis in order to determine the best favored species of medicinal plants and their level of efficacy. Seven medicinal plants that were chosen based on the frequency of citation order were given to the informants, who were then asked to rate each plant's preference on a scale of 1 to 7. The plant that was most favored received a score of 7, while the least favored species received a score of 1. Each species' value was added up, and the final score was used to establish each species' rank. This helped to agree the rank order of the most effective medicinal plants used by the public to heal hepatitis.

Ethical Approval and Consent to Participate

The principles outlined in the Declaration of Helsinki were followed in the conduct of the study. Prior to collecting data, the study protocol was reviewed and approved by the ethical review committees of the Southern Nation Nationalities and People's Regional State Health Bureau (Ref No. PN37-/86/19743) and the Aklilu Lemma Institute of Pathobiology, Addis Ababa University (Ref No. ALIPB IRB/002/2011/2018). After being asked about their voluntariness and provided with comprehensive information about the study's goals, potential hazards, and significance in their languages, the participants were asked to provide signed, and informed permission. The participants received assurances of their complete autonomy to choose whether or not to engage in the study. After completing their herbal treatment, those who still tested positive for nucleic acids were counseled and connected to medical facilities for further care. Every piece of information gathered for the study was kept private.

Results

Socio-Demographic Characteristics of the Study Participants

A total of 48 village heads consisting of 30 (62.5%) males and 18 (37.5%) females; 9 traditional medicine entrepreneurs consisting of 7 (77.7%) males and 2 (22.3%) females; 52 traditional herbalists consisting of 37 (71.2%) males and 15 (28.8%) females; and 42 individuals diagnosed as hepatitis patients by traditional healers based on signs and symptoms of the disease. Of the hepatitis-diagnosed individuals, 17 (40.5%) were males and 25 (59.5%) were females with ages ranging from 25 to 86 with mean age \pm SD = 47 \pm 15.4 years).

Anti-Hepatitis Medicinal Plants Documented

It was reported that residents of the study districts employed a total of 24 medicinal plants from 22 families to treat hepatitis. Three medicinal plants from the *Asteraceae* family and one plant from each other represented the other families. The most frequently cited anti-hepatitis medicinal plants were *R. nepalensis*, *V. apiculata*, *S. incanum*, and *C. macrostachyus*. *R. nepalensis* was reported by 12 informants, *V. apiculata* by 11 informants, *S. incanum* by 10 informants, and *C. macrostachyus* by 9 informants (Table 1).

Methods of Medicinal Remedy Preparation

The primary methods of remedy preparations were shown to be crushing (37.5%), squeezing (29.2%), and powdering (20.3%). Majority (79.2%) of the anti-hepatitis plants were prepared fresh while some (12.5%) were used either fresh or dried, and small proportions (8.3%) were reported to be dried and kept for future uses. Majority (71.0%) of the healers used mixtures of different species to treat hepatitis. Plant parts were collected when required, and there was no fixed time needed for its collection. Additives like tea, milk, coffee, honey, and sugar were used in most (78.0%) preparations of the remedy.

Dosage Preparation and Route of Administration

In this study, all remedies were administered orally. In most cases, the prepared therapies or remedies were given once a day and completed within one week. In some cases, remedies could be extended beyond one month until recovery from the ailment evidenced by the absence or disappearance of the signs and symptoms. Dosages were estimated using spoons, glasses, and tea or coffee cups (Table 1). Remedy prescription was reported to be based on the patient's age, the degree and duration of the illness, the presence of other diseases, and the experiences of traditional healers. Children were given lower doses than adults; physically strong patients took a higher dose or amount than weak patients.

The absence of several adverse effects of traditional remedies or medicines after administration was often mentioned by the traditional healers, but some preparations from the plants *R. nepalensis*, *V. amygdalina*, and *P. falcatus* were indicated to have some adverse or side effects characterized by abdominal pain, vomiting, and diarrhea. Treatment with *O. rochetiana* and *A. abyssinicus* was reported to rarely cause loss of consciousness. The majority (66.0%) of the traditional healers indicated that they used milk, "tella", and meat soup with butter as antidotes to mitigate the adverse effects of some remedies. Most (81.5%) of the healers reported that milk, honey, coffee, sugar, or "tella" were used as additives for most of the remedies to make them more palatable and/or improve their effectiveness.

Table 1 Medicinal Plants Identified in Treating Hepatitis B in the Study Districts

| No. | Scientific Name | Family Name | Local Name | Part Used | Habit | Route of Administration | Remedies Preparation | Citation Frequency | Voucher No. |
|-----|---|---------------|------------------|----------------------|-------|-------------------------|---|--------------------|-------------|
| 1 | <i>Rumex nepalensis</i> Spreng. | Polygonaceae | Shisho | Root | H | Oral | Fresh root washed, crushed, and boiled with water and taken with honey/sugar and ½ to 1 water glass is taken daily for 3 days to 1 month with minimal dose. | 13 | GB-01-2018 |
| 2 | <i>Vangueria apiculata</i> K.Schum. | Rubiaceae | Gerawa | Leaf | Sh | Oral | Fresh leaf squeezed and mixed with cold water infusion and one coffee cup drunk daily for 2–5 days. | 11 | GB-02-2018 |
| 3 | <i>Solanum incanum</i> L. | Solanaceae | Achongara | Root | Sh | Oral | The Juice of the crushed root is boiled in water and taken with butter and sugar at night for 2 days to 2 weeks. | 10 | GB-03-2018 |
| 4 | <i>Croton macrostachyus</i> Del. | Euphorbiaceae | Mesana | Stem bark | T | Oral | Squeezed fresh stem/ bark juice mixed with milk or “tella” and taken for 2 days. | 8 | GB-04-2018 |
| 5 | <i>Allophylus abyssinicus</i> (Hochst) Radlk | Sapindaceae | Ta’a | Stem bark | T | Oral | Squeezed fresh stem/ bark juice mixed with milk or “tella” and taken once. | 7 | GB-05-2018 |
| 6 | <i>Podocarpus falcatus</i> (Thunb.) R. B. ex. Mirb. | Podocarpaceae | Digiba | Leaf | T | Oral | Fresh leaf crushed and boiled with water and taken with milk/sugar and 1 water glass is taken daily for 3–5 days. | 8 | GB-06-2018 |
| 7 | <i>Vernonia amygdalina</i> Del. | Asteraceae | Heba | Leaf | Sh | Oral | Juice of crushed fresh leaf is taken for 2 days. | 4 | GB-07-2018 |
| 8 | <i>Ficus sur</i> Forssk. | Moraceae | Oda’a | Sap by removing bark | T | Oral | Fresh sap/latex taken with milk/“tella” once per week for 3 weeks. | 3 | GB-08-2018 |
| 9 | <i>Brucea antidysenterica</i> JF. Mill. | Simaroubaceae | Aballo/ Chironta | Root | Sh | Oral | Powdered root boiled with water and taken with milk daily for 4 days. | 3 | GB-09-2018 |
| 10 | <i>Dicliptera laxata</i> CB. Clarke | Acanthaceae | Omoro | Whole part | H | Oral | Fresh whole part crushed and mixed with water and taken daily | 1 | GB-10-2018 |
| 11 | <i>Clausena anisata</i> (Willd.) Benth. | Rutaceae | Bahit-haqa | Leaf | Sh | Oral | Fresh leaf crushed with water infusion and 1 water glass is taken for every other day for 5 days | 2 | GB-11-2018 |
| 12 | <i>Rosmarinus officinalis</i> L. | Lamiaceae | Sigamexibasha | Whole part | H | Oral | Powdered root boiled with water and taken with sugar daily | 1 | GB-12-2018 |

(Continued)

Table 1 (Continued).

| No. | Scientific Name | Family Name | Local Name | Part Used | Habit | Route of Administration | Remedies Preparation | Citation Frequency | Voucher No. |
|-----|--|----------------|----------------|------------|-------|-------------------------|--|--------------------|-------------|
| 13 | <i>Vernonia auriculifera</i> Hiern | Asteraceae | Barawa | Root | Sh | Oral | Crushed root with water infusion and taken daily | 2 | GB-13-2018 |
| 14 | <i>Achyranthes aspera</i> L. | Amaranthaceae | Kechaba | Root | H | Oral | Fresh root crushed and boiled with water and 1 cup is taken daily for 3 days with milk/sugar/coffee | 2 | GB-14-2018 |
| 15 | <i>Pycnostachys abyssinica</i> Fresen. | Lamiaceae | Bobanqa | Root | Sh | Oral | Root powder mixed with water and taken weekly for 4 weeks | 4 | GB-15-2018 |
| 16 | <i>Eucalyptus camaldulensis</i> Dehnh. | Myrtaceae | Qadal-barizafa | Sap | T | Oral | Fresh sap/latex taken with milk once per week for 2 weeks | 3 | GB-16-2018 |
| 17 | <i>Momordica foetida</i> Schumach. | Cucurbitaceae | Wari-rebuta | Whole part | H | Oral | Squeezed fresh stem/bark juice mixed with tea taking and taken once a day for 3 days | 2 | GB-17-2018 |
| 18 | <i>Ekebergia capensis</i> Sparrm. | Meliaceae | Ololla | Stem Bark | T | Oral | Squeezed fresh stem/bark juice mixed with milk and taken every other day for 3 days | 3 | GB-18-2019 |
| 19 | <i>Dovyalis abyssinica</i> (A. Rich.) Warb | Flacourtiaceae | Koshima | Root | Sh | Oral | Powdered root mixed with hot water infusion and drunk with milk daily for 5 days | 1 | GB-19-2019 |
| 20 | <i>Cordia africana</i> Lam. | Boraginaceae | Wedesha | Leaf | T | Oral | Fresh leaf boiled in water taken with coffee once a day | 4 | GB-20-2019 |
| 21 | <i>Carduus nyassanus</i> (S. Moore) R.E. Fr. | Asteraceae | Hali'utta | Root | H | Oral | The root powder is mixed with "tea/milk" and one coffee cup is taken daily for 1 week | 6 | GB-21-2019 |
| 22 | <i>Smilax aspera</i> L. | Smilacaceae | Harira | Root | Sh | Oral | Powdered root mixed with cold water infusion and one water glass mixed with milk is drunk daily for 3 days | 5 | GB-22-2019 |
| 23 | <i>Olinia rochetiana</i> A. Juss. | Oliniaceae | Guna | Leaf | T | Oral | Crushed juice of the fresh leaf is taken daily for 3 days | 4 | GB-23-2019 |
| 24 | <i>Carissa spinarum</i> L. | Apocynaceae | Atura | Root | Sh | Oral | Root powder mixed with water and is taken weekly for one month | 7 | GB-24-2019 |

Abbreviations: T, tree; Sh, Shrub; H, Herb; GB, Gizachew Beykaso.

Traditional Knowledge and Its Transfer Modality in the Districts

Most (85.0%) of the respondents reported that if they had hepatitis or were diagnosed with hepatitis, they would prefer traditional herbal treatments to modern medications because they believed that they would not get better medications for hepatitis in modern health services. About 82.0% of them believed that traditional healers could cure hepatitis with medication, and they could easily access it at a low price. Traditional healers used to diagnose hepatitis mainly based on jaundice and others such as fatigue, joint pain, fever, nausea, vomiting, and loss of appetite, abdominal pain, and dark urine. In these treated patients, traditional healers reported improvements in these symptoms after the treatment period.

Most (84.6%) of the medicinal plants were cited by traditional healers of age greater than forty-six years. On average, significantly higher numbers (74.0%) of medicinal plants were reported by men than women. The majority (79.6%) of healers reported that they acquired the indigenous knowledge from their parents, while some (11.4%) stated that they gained it from their tribal local elders and few (9.0%) mentioned acquiring the knowledge by other means such as through observation and self-trial. Of the traditional healers who used to transfer their knowledge on medicinal plant use, almost all (96.0%) reported doing it orally to their family members. All healers kept their knowledge secret, exposing it only to their sons or close family members in their old age. Of the total interviewed healers, 79.6% reported that they preferred to transfer their knowledge to their trusted eldest sons, but few (9.0%) reported transferring it to any member of their family. All healers reported that they transferred their knowledge without any payment to their trusted eldest sons and some other members of their family. However, all healers reported that they were not willing to share their knowledge with people outside their family circle.

Preference Ranking

Seven medicinal plants that were described to be the most useful plants to treat hepatitis were ranked by each of 10 randomly selected traditional healers based on their perceived level of importance. Accordingly, *R. nepalensis* was found to be the most preferred medicinal plant to treat hepatitis followed by *S. incanum* and *V. apiculata* (Table 2).

Serological Results

A total of forty-two individuals were diagnosed as hepatitis patients by traditional healers for anti-hepatitis herbal treatments. Eight (19.1%) out of the forty-two individuals who were diagnosed as hepatitis patients by traditional healers were found to be positive for HBsAg, but all (100%) individuals' blood samples were negative for the anti-HCV ELISA test. The eight individuals who tested positive for HBsAg before the initiation of the anti-hepatitis medicinal plants treatment was also tested positive for HBsAg at three and six months after the initiation of anti-hepatitis medicinal plants treatment (Table 3).

HBV Viral Load Assessment

HBV DNA was detected in all eight (100%) blood samples that tested positive for HBsAg before the initiation of the antihepatitis medicinal plants' treatment. HBV DNA was also detected in seven (87.5%) of the HBsAg-positive

Table 2 Preference Ranking Exercise on Six Selected Plant Species Used to Treat Hepatitis

| Medicinal Plants | | Key Informants (Coded A to J) | | | | | | | | | | Total Score | Rank |
|-------------------------------|------------------|-------------------------------|---|---|---|---|---|---|---|---|---|-------------|------|
| Botanical Name | Local Names | A | B | C | D | E | F | G | H | I | J | | |
| <i>Rumex nepalensis</i> | Shisho | 7 | 6 | 7 | 6 | 6 | 7 | 7 | 6 | 7 | 7 | 66 | 1st |
| <i>Solanum incanum</i> | Achongera/Maheta | 5 | 7 | 6 | 7 | 7 | 4 | 5 | 7 | 6 | 6 | 60 | 2nd |
| <i>Vangueria apiculata</i> | Garawa | 6 | 5 | 4 | 5 | 4 | 6 | 4 | 4 | 5 | 5 | 48 | 3rd |
| <i>Croton macrostachyus</i> | Mesana | 3 | 4 | 5 | 3 | 5 | 5 | 6 | 5 | 4 | 4 | 44 | 4th |
| <i>Allophylus abyssinicus</i> | Ta'a | 4 | 3 | 3 | 1 | 1 | 2 | 3 | 1 | 2 | 3 | 23 | 5th |
| <i>Podocarpus falcatus</i> | Digiba | 1 | 2 | 2 | 4 | 3 | 3 | 1 | 2 | 1 | 1 | 20 | 6th |
| <i>Brucea antidysenterica</i> | Chironta | 2 | 1 | 1 | 2 | 2 | 1 | 2 | 3 | 3 | 2 | 19 | 7th |

Table 3 Progression of the HBV Viral Loads in Hepatitis Patients Treated with the Different Plant Combinations in Central Ethiopia

| Patients | Types of Plants | Preparation and Treatment Duration |
|-----------|---|---|
| Patient 1 | Combination of <i>V. apiculata</i> and <i>C. s nyassanus</i> | Squeezed fresh leaf of <i>V. apiculata</i> and powder root of <i>C. nyassanus</i> mixed with water drunk and one coffee cup with sugar milk is drunk once a day for three consecutive days |
| Patient 2 | Combination of <i>R. nepalensis</i> and <i>S. incanum</i> | Crushed fresh roots of <i>R. nepalensis</i> and <i>S. incanum</i> boiled with water and one water glass of the juice is taken with honey and butter two times per week for one month |
| Patient 3 | Combination of <i>R. nepalensis</i> , <i>C. macrostachyus</i> and <i>V. apiculata</i> | Crushed fresh roots of <i>R. nepalensis</i> , squeezed fresh stem bark juice of <i>C. macrostachyus</i> , and squeezed fresh leaf of <i>V. apiculata</i> are boiled and mixed with milk and one water glass is taken per week for three weeks |
| Patient 4 | Combination of <i>S. aspera</i> and <i>S. incanum</i> | Powder of root of <i>S. Aspera</i> and crushed fresh root of <i>S. incanum</i> are boiled in water and one coffee cup is taken with butter and sugar at night weekly for one month |
| Patient 5 | Combination of <i>R. nepalensis</i> and <i>V. apiculata</i> | Crushed fresh roots of <i>R. nepalensis</i> and squeezed fresh stem bark juice of <i>V. apiculata</i> are mixed with milk and taken once a week for two weeks |
| Patient 6 | Combination of <i>C. spinarum</i> and <i>C. macrostachyus</i> | Powdered root of <i>C. spinarum</i> and squeezed fresh stem bark juice is mixed with water and milk, and taken two times weekly for two weeks |
| Patient 7 | Combination of <i>C. macrostachyus</i> and <i>S. incanum</i> | Powdered root of <i>C. macrostachyus</i> and crushed root juice of <i>S. incanum</i> boiled with water are taken with sugar at night daily for 4 days |
| Patient 8 | <i>A. abyssinicus</i> | Squeezed fresh stem bark juice of <i>A. abyssinicus</i> is mixed with milk and one water glass is taken once |

individuals' second samples at the third month of initiation of medicinal plants treatment and five (62.5%) individuals out of eight HBsAg-positive individuals' third samples at six months of anti-hepatitis medicinal plants treatment. All blood samples taken before the initiation, at the third, and six months after initiation of the traditional antihepatitis medicinal plant were found positive for HbsAg (Table 4).

In one individual (Patient 2) treated with medicinal plants of crushed fresh root extracts of *R. nepalensis* and *S. incanum* boiled in water, HBV-DNA suppression was observed during the third and sixth months of viral load assessment. Similarly, at six months of viral load evaluation, HBV-DNA suppression was also observed in individuals who were treated with medicinal plants

Table 4 The Serological HBV HBsAg and HBV-DNA at the Different Time Duration

| | HBsAg and Serum Levels of HBV-DNA at Different Time Duration IU/mL | | | | | |
|-----------|--|------------------|-------------------------------|-------------------|-------------------------------|-------------------|
| | T1 (Before Treatment) | | T2 (At 3 months of Treatment) | | T3 (At 6 months of Treatment) | |
| | HbsAg | HBV-DNA IU/mL | HbsAg | HBV-DNA IU/mL | HbsAg | HBV-DNA IU/mL |
| Patient 1 | + | 25,897 IU/mL | + | 21,991 IU/mL | + | 18,311 IU/mL |
| Patient 2 | + | 109,194 IU/mL | + | Not detected | + | Not detected |
| Patient 3 | + | 3,070 IU/mL | + | 162 IU/mL | + | Not detected |
| Patient 4 | + | 597,708,763IU/mL | + | 797,063,253 IU/mL | + | 932,510,210 IU/mL |
| Patient 5 | + | 12,769,956 IU/mL | + | 3,732 IU/mL | + | Not detected |
| Patient 6 | + | 2,273 IU/mL | + | 1,994 IU/mL | + | 3,050 IU/MI |
| Patient 7 | + | 17,955 IU/mL | + | 412 IU/mL | + | 97 IU/mL |
| Patient 8 | + | 9,582 IU/mL | + | 9,458 IU/mL | + | 9,582 IU/mL |

Abbreviations: T1, Time or before treatment; T2, Time 2 or at 3 months of treatment; T3, Time 3 or at 6 months of treatment.

extract of three plants combined including *R. nepalensis*, *C. macrostachyus*, and *V. apiculata*, (Patient 3) and a combination of two plants (*R. nepalensis* mixed with *V. apiculata*) (Patient 5). In addition to this, the serum HBV-DNA suppression effects were also observed during viral load assessment in individuals treated with the combination of *R. nepalensis*, *C. macrostachyus*, and *V. apiculata*, and a combination of *B. antidysenterica* with *S. incanum* (Table 4). In serum HBV-DNA-positive individuals, suppression of HBV-DNA level was observed after treatment with different herbal medicines, commonly with combination of *R. nepalensis* by traditional healers.

Discussion

According to the respondents' beliefs in the districts, the preference for traditional medicine in treating hepatitis was a result of the lack of better medications for hepatitis in modern healthcare facilities; however, the identity of medicines used to treat hepatitis is unknown except to the healers, who probably pass their knowledge through generations in the family line. This is similar with study findings done in Ethiopia and other countries.^{12,24,25}

In the study area, traditional healers largely transfer knowledge of medicinal plants use orally to a selected family member, especially to the trusted elder sons, when they get very old. This indicates that traditional medicinal healers need their knowledge to be held in secret and thus there is a great danger of losing it. Traditional healers reported that they strongly believed traditional medicine was effective if done within a family or with close relatives and when it was kept secret; if not medicinal plants would lose their healing power. The traditional healer's strong belief in keeping the knowledge secret could be due to the fear of losing societal recognition and the reputation that traditional healers have earned through time. This is also supported by other similar findings from Ethiopia and other countries.^{12,26,27}

Most of the knowledge was owned by older people who were responsible for maintaining the well-being of their communities. The fact that traditional medical practice in the study area is dominated by old people indicates the extent of danger the transfer of knowledge is facing. Comparable results were reported in other studies conducted in Ethiopia and elsewhere in Africa.^{23,28} It was reported that considerably greater numbers of medicinal plants species were reported by males than females. The small number of females serving as respondents might be because the knowledge is mostly transferred through the male line. Similar results were reported in studies conducted elsewhere in Ethiopia and other countries in Africa.^{22,29}

Some of the traditional medicinal plants species used to treat hepatitis in this study are also used elsewhere in Ethiopia and other countries for the same purpose. These include *C. macrostachyus*,³⁰ *C. africana*,³¹ *B. antidysenterica*, *M. foetida*,³² *Ficus sur*,³³ *A. aspera*,³² and *R. nepalensis*.³⁴ The reason for the similarity in use may be due to the existence of biologically active compounds that are effective against hepatitis. As shown by preference ranking exercises, *R. nepalensis* followed by *S. incanum* and *V. apiculata* were the most preferred plants by traditional healers to treat hepatitis, possibly indicating their high healing potential in the treatment of the disease. This finding is also supported by other similar findings reported elsewhere in Ethiopia.^{35–37}

The finding of this study indicated that more than one species of the medicinal plants were commonly used by traditional medicinal healers to prepare therapies. This could be attributed to the synergistic or additive effects that they possibly have for the duration of treatment. This is supported by other similar study findings reported elsewhere in Ethiopia.^{38–40} However, a study done in other countries indicated that most preparations were prepared from a single healing plant species.^{41,42} This could be explained by the socio-cultural differences of traditional healers. The most of antihepatitis medications in the study area were prepared as of freshly harvested plant parts. These remedies were prepared by boiling in water, and the healers commonly believed that additives like milk, sugar, honey, tea, coffee could improve palatability and also potentiate the effects of prescribed remedies which are also similar with the findings done somewhere else in Ethiopia.^{43–47}

Most traditional healers were found to have inconsistent knowledge of dosage and use of antidotes while prescribing medicinal preparations to their patients. This could be due to the experiences and socio-cultural differences of traditional healers. Most of these medicinal preparations were reported to have no serious adverse effects except for certain minor effects like abdominal pain, diarrhea, and vomiting. The low toxicity of the remedying preparations of the healing plant species is reflected in the high tendency of the community to use them. This is consistent with other studies done in Ethiopia and some other countries.^{47,48} The prescribed differences in dosage, duration, the unit of measurement, and time

for treatment of hepatitis were also determined by age, physical fitness, and the presence or absence of any other diseases. The finding is in consistent with the result of different ethnobotanical studies done somewhere else in Ethiopia that have also discussed the lack of standardization and precision as a shortcoming of the traditional healthcare system.^{33,43}

Forty-two individuals were traditionally diagnosed as hepatitis patients for herbal treatment by traditional herbalists tested for HBsAg and anti-HCV ELISA. Eight (19.1%) of these individuals were positive for HBsAg but no positives for the anti-HCV ELISA test. This may explain the fact that most of the individuals who were diagnosed as hepatitis patients and treated for hepatitis by traditional medicine were not actual viral hepatitis patients, rather they may be non-viral hepatitis or misdiagnosed as hepatitis patients by traditional herbalists. The result is also in similar with other findings of different ethnobotanical studies done in somewhere else in Ethiopia and other countries, which have a lack of missed diagnosis and precision as a shortcoming of the traditional healers' treatment.^{8,9,49}

In some HBV-DNA-positive individuals, the suppression of serum levels of HBV-DNA was observed after treatment with a combination of different extracts of herbal medicines by traditional healers. The suppression effects were detected in individuals treated with the combination of *R. nepalensis* and *S. incanum*, and the combination of *R. nepalensis* and *A. abyssinicus*. Similarly, the serum HBV-DNA suppression effects were also observed in individuals treated with the combination of *R. nepalensis*, *C. macrostachyus*, and *V. apiculata*, and the combination of *B. antidysenterica* and *S. incanum*. These suppression effects observed by extracts of these plants might be associated with the presence of chemical ingredients that have antihepatitis properties. The result is also supported by the findings of other studies done somewhere else.⁵⁰ More other studies also reported the antihepatitis properties of *R. nepalensis*,⁵¹ *S. incanum*,⁴² and *A. abyssinicus*.⁵⁰

Serum HBV-DNA-positive patients treated with these plants, particularly when combined with *R. nepalensis*, showed serum viral suppression effects in this finding. This indicates *R. nepalensis* has an action of antihepatitis properties even though the mechanisms of its action and ingredients were not studied. There are also other studies in agreement with this finding that *R. nepalensis* has antihepatitis properties.⁵¹

Conclusion

This study established that the communities have been using different traditional therapeutic plant species and the associated indigenous knowledge as a primary choice while contemporary health services are expanding in the Districts. The reported new antihepatitis traditional therapeutic plant types in this study have confirmed the call for further on ethnobotanical and other scientific research studies. The use of these plants to treat hepatitis is still needed by the communities as a result of the lack of substitutes for better medication by modern healthcare programs in health facilities. This study also established that indigenous knowledge transfer is declining after generation to generation as the main method of transmission is oral. Therefore, this study recommends the urgent documentation of the medicinal plants' knowledge before it is completely lost. Increased use-value recorded and higher preference ranking scores on the mentioned therapeutic plant species would empower upcoming pharmaceutical investigations and conservation practices.

In some HBV-DNA positive patients treated with the combination of frequently sited and highest preference ranked plants revealed viral suppression activities, which partly explains some antiviral properties of the plants and is used as a basis for further pharmacological investigations in the effort of the search for new and locally affordable anti-hepatitis viral agents beside isolating and identifying their active principles. Pharmaco-chemical investigations are required to assess the efficacy and safety of all claimed therapeutic plants. The priority to assess bioassay and toxicity investigations should, however, be taken to therapeutic plants that have the highest preference score and HBV DNA suppressive effects before they are recommended for broader use and advance pharmacological analysis. Further efforts should also be taken by concerned bodies to lead into the results of any future studies on the efficacy and safety of the reported therapeutic plants back to the owners of the knowledge.

Data Sharing Statement

The data and resources used to provide the conclusions of this study are available upon rational request from the corresponding author.

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Author Contributions

Significant contributions were made by each author to the conception and design, data collection, analysis, and interpretation processes; writing of the article or substantial intellectual content revisions; reading and approval of the completed manuscript before it was submitted to the current journal; and acceptance of responsibility for all aspects of the work.

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Disclosure

In accordance with the authors, they have no competing interests with this work.

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