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Research Article

Phytochemical constituents of the roots of *Heliotropium verdcourtii* (Boraginaceae)

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Abstract: The medicinal value of medicinal plants lies in some bioactive constituents that produce a definite physiological action on the human body. *Heliotropium verdcourtii* is a deciduous shrub or small tree traditionally used in the treatment of various diseases including fever, dry cough, measles, convulsions, epilepsy, diarrhea, and other ailments. The chemical constituents of the roots of the plant were not investigated to date. The aim of the study was to investigate the phytochemicals present in the roots of *Heliotropium verdcourtii*. The freshly collected root of the plant was chopped and air dried under shade. The dried and finely grounded plant root was extracted through maceration with *n*-hexane, chloroform/methanol (v/v 1:1), and methanol successively. The extracts were subjected to qualitative phytochemical tests for screening the classes of secondary metabolites present in the plant. Compound isolation of the chloroform/methanol (v/v 1:1) extract was performed through silica gel chromatographic separation. The structures of all isolated compounds were determined by spectroscopic methods as well as comparison with previous reports in the literature. The yields of *n*-hexane, chloroform/methanol (v/v 1:1), and methanol extracts were 2.2 g (0.4%), 25 g (5.0%), and 19.8 g (4.0%), respectively. The qualitative phytochemical test of the extracts revealed the presence of flavonoids, terpenoids, phenolics, saponins, glycosides and alkaloids. Silica gel chromatographic separation afforded a mixture of three isomeric triterpenoids identified as α-amyrin, β-amyrin, and bauerenol. To the best of our knowledge these bioactive compounds were isolated from the root of this plant, for the first time.

1. INTRODUCTION

The use of herbs and medicinal plants for primary human health care is a universal phenomenon. Today, as much as 80% of the people in the world depend on traditional medicine as primary health care (Kimutai, 2017). There is therefore need to investigate such plants to understand their chemical constituents. The genus *Heliotropium* being a small tree or shrub comprises about 40 species and belongs to the family Boraginaceae (Weigend *et al.*, 2016). Many *Heliotropium* plants are mainly found being spread in tropical Asia, Africa, Australia,

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Europe, and Northern America (Gottschling & Hilger, 2004; Miller, 2003; Retief & Van Wyk, 2001). The bark, leaf juice, leaves, roots, seeds, stems, twigs and whole plant parts of *Heliotropium* are used as aphrodisiac, laxative, ethnoveterinary medicines, as traditional medicines, for ulcers and headaches, in treatment for schizophrenia, absorption of calcium, muscle protein, post-surgery recovery, sports injuries (Maroyi, 2021; Tidke *et al*., 2021).

Heliotropium verdcourtii is a deciduous shrub or small tree commonly found in the Savannah and secondary jungle of West Africa which includes Cameroon, Ghana, Gabon, Congo, and Nigeria (Ogundajo & Ashafa, 2017). The whole plant parts of *H. verdcourtii* are mainly used as aphrodisiac, laxative and ethnomedicines for gastro-intestinal problems, wounds, malaria, fever, typhoid, convulsions, epilepsy, toothache and respiratory infections (Jeruto *et al.*, 2011; Jeruto *et al.*, 2015; Li *et al.*, 2008; Maroyi, 2021; Oladunmoye & Kehinde, 2011). The presence of phenolic acids, lignans, flavonoids, nitrile glycosides, quinonoids, steroids, triterpenoids, and pyrrolizidine alkaloids was reported, in the genus *Heliotropium* (Jeruto *et al.*, 2011; Li *et al.*, 2010).

Phytochemistry of *H. verdcourtii* is characterized by anthraquinones, alkaloids, essential oils, flavonoids, fatty acids, glycosides, proanthocyanidins, phenols, pseudotannins, saponins, reducing sugars, tannins, steroids and terpenes (Ogundajo & Ashafa, 2017; Maroyi, 2021). Four triterpenoids namely, α-amyrin, β-amyrin, bauerenol, and a 12-13 epoxy ursane type pentacyclic triterpene were isolated from the leaves of the hexane extract (Chaluma *et al.*, 2018). Pharmacological activity tests of the extracts and the chemical constituents isolated from *H. verdcourtii* revealed antidiabetic, antibacterial, antihyperglycaemic and antioxidant activities (Maroyi, 2021).

H. verdcourtii is a plant highly distributed in Ethiopia, where it is locally named *Game* in Amharic, *Hulaga*, in Afan Oromo, and *Gidincho* in Sidama. It is a highly used as hedge plant in Ethiopia. Traditionally it is used for the treatment of various diseases like toothache, dysentery, tetanus, skin diseases and gastric ulcers (Chaluma *et al.*, 2018). The decoction of the leaves of *H. verdcourtii* is used to improve the quality and quantity of milk products of livestock in Ethiopia (Bezabih *et al.*, 2017). In Kenya an infusion and sap of the leaf is used to treat fever and as laxative agent, respectively. The root juice is used for healing wounds (Maundu and Tengnäs, 2005).

Despite the traditional use of the plant's roots against various life threatening diseases, there are few scientific reports dealing only with the phytochemical screening and biological activities of the root of *H. verdcourtii*. Recent reviews on the species, however, show that leaves of the plant are extensively studied, phytochemically (Maroyi, 2021). Hence this paper presents the results of the isolation and identification of chemical constituents from the roots extract of *H. verdcourtii* of Ethiopian origin.

2. MATERIAL and METHODS

2.1. Experimental

Grant thermostatic bath shaker (GLS-400) was used in the course of maceration of plant material. TLC spots were detected by a UV-2550 (SHIMADZU) UV-Vis spectrometer (Shimadzu, Kyoto, Japan). Column chromatography (CC) was performed with column size 3 cm × 30 cm packed with silica gel 60, size 0.063-0.200 mm (70-230 mesh ASTM). Thin layer chromatography (TLC) was performed on aluminum sheets, silica gel 60 F_{254}, and layer thickness 0.2 mm (Merck). NMR spectrum data was generated with 400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR, TMS as internal standard and $CDCl_3$ as solvent with the chemical shifts reported in parts per million (ppm).

2.2. Collection and Preparation of Plant Materials

H. verdcourtii specimens (leaves, flowers, seeds, stems) were collected on October, 2021 from Rufo Waeno Kebele, Aleta Chuko Woreda, Sidama Region, Ethiopia, for authentication of the plant. The plant was authenticated by botanist Retta Regassa Department of Plant Science, Hawassa College of Teachers Training, Hawassa, Ethiopia and a specimen was stored in the Herbarium of Hawassa College of Teachers Training with voucher no: HHF/0021-24. In addition to the aforementioned morphological parts, the roots were also collected for phytochemical analysis. The plant material was prepared in such a way that the root of *H. verdcourtii* was washed with tap water to remove soil particles and other foreign materials, and air dried in a shade for three weeks. The air-dried root was pulverized into powder using electric grinder. The pulverized plant material was kept in sealed plastic container and put on a dry cup board until used for extraction.

2.3. Extraction

The pulverized root (500 g) of *H. verdcourtii* was soaked in *n*-hexane (1.5 L) in a 5 liter Erlenmeyer flask, at room temperature. The flask was shaken for 72 h on an orbital shaker. The solution was filtered and the filtrate was concentrated under vacuum at 36 to 38 °C. The marc was further extracted with $CHCl_3$:MeOH (*v/v* 1:1) and methanol successively, likewise. The extracts were put in refrigerator until used for further analysis.

2.4. Phytochemical Screening Tests

The *n*-hexane, $CHCl_3$:MeOH (*v/v* 1:1), and methanol extracts were subjected to qualitative phytochemical screening tests for the presence of the classes of secondary metabolites including alkaloids, terpenoids, flavonoids, phenolics, glycosides, and saponins, following standard procedures (Harborne, 1973; Parekh & Chands, 2008).

2.5. Compound Isolation and Structure Elucidation

Solvent selection for the chromatographic separation was performed by various proportions of solvent mixtures of *n*-hexane, chloroform, dichloromethane, ethyl acetate, and methanol. The *n*-hexane-ethyl acetate solvent system showed better TLC profile and it was chosen for silica gel column chromatographic separation. Silica gel slurry of *n*-hexane was used for packing the column in order to achieve list polarity to the mobile phase. The $CHCl_3$:MeOH (v/v 1:1) extract (15 g) was adsorbed on silica gel and was added to the column. Separation of the components through column chromatography was conducted with increasing polarity in *n*-hexane-ethyl acetate solvent systems of various proportions. 22 fractions of each 30 ml were collected and the components in each fraction were analyzed by TLC. The fractions (on TLC) were concentrated using rotary evaporator. The concentrated and dried compounds were put in vial and stored in a refrigerator until sent for spectral analysis. Fraction number 11 (*n*-hexane: EtOAc; 4:1) resulted in a white solid compound (Rf = 0.57) with minor impurity. This was further purified by washing with *n*-hexane and resulted in 250 mg of white solid compound. The structure elucidations of the isolated chemical constituents were determined by generating ^{1}H- and ^{13}C-NMR data and by comparing the experimental spectroscopic data with previous reports in the literature.

3. RESULTS and DISCUSSION

3.1. Extraction Yield

Phytochemical investigation of specimens of plant origin is needed to increase the amount of chemical constituents and to maintain their activities (Aziz *et al.*, 2003). Obtaining high extract yield is an important step in the course of secondary metabolite investigation and detection of biologically active compounds. Choice of appropriate extraction method is also essential for the tweaking of phytochemical constituents leaving out avoidable materials with the aid of the

solvents. Further selection of suitable extraction process and optimization of various parameters are very important for up scaling from bench scale to large scale phytochemical analysis. The most commonly used extraction techniques include conventional techniques such as maceration, percolation, infusion, decoction, hot continuous extraction etc. In this study cold maceration technique is used. The extraction yield of the plant material is presented in Table 1.

Table 1. Percent yield of the crude extracts.

Extract	Mass of extract (g)	Yield (%)
n-Hexane	2.2	0.4
$CHCl_3$:MeOH (v/v; 1:1)	25	5.0
Methanol	19.8	4.0

Extraction solvent choice needs to be based on the plant material matrix properties, chemical properties of the secondary metabolites, matrix-metabolite interaction, efficiency and desired properties (Ishida *et al.*, 2001; Hayouni *et al.*, 2007). The extractability of solvents depends on compound solubility in the solvent, the mass transfer and the strength of matrix interaction with heat and mass diffusion rate (Dhanani *et al.*, 2017). The extraction solvent choice also depends on what natural compounds or classes of natural compounds one is looking for. In this study, however, the focus was performing total phytochemical analysis of the plant roots. The high yield of the $CHCl_3$:MeOH (*v/v* 1:1) extract suggests that constituents in the plant specimen are moderately polar.

3.2. Phytochemical Screening

Plants of the genus *Heliotropium* are rich in bioactive constituents such as phenolic acids, lignans, flavonoids, nitrile glycosides, quinonoids, steroids, triterpenoids, and pyrrolizidine alkaloid (Jeruto *et al.*, 2011; Li *et al.*, 2008). The previous report on phytochemical screening of the leaf extracts of *H. verdcourtii* shows the presence of alkaloids, saponins, glycosides, terpenoids, anthraquinones, phenolics, and flavonoids (Ogundajo & Ashafa, 2017). In this study, the result of the qualitative phytochemical test of the extracts revealed the presence of flavonoids, terpenoids, phenolics, saponins, glycosides and alkaloids which is in agreement with previous works.

The classes of secondary metabolites found in the plant have the following biological activities. Flavonoids are known by antioxidative, free radical scavenging, coronary heart disease prevention, hepatoprotective, anti-inflammatory, anticancer and antiviral activities (Kumar & Pandey, 2013). Saponins are known for their biological activities such as antimicrobial, antifungal, anti-inflammatory, antiviral, antioxidant, anticancer, and immunomodulatory effects (Juang & Liang, 2020). Glycosides are known to possess remarkable therapeutic potential and pharmacological activities. Analgesic, anti-inflammatory, cardiotonic, antibacterial, antifungal, antiviral, and anticancer effects are some of the pharmacological activities (Soto-Blanco, 2022). Terpenoids have antimicrobial and antidiarrheal activities (Prashant *et al.*, 2011). Phenolic constituents exhibit antibiotic, antimicrobial, and antidiarrheal activities (Jacob and Burri, 1996; Prashant *et al.*, 2011). Alkaloids exhibit a wide range of activities. They are not only biosynthesized in nature against herbivores but also decrease bacterial or fungal influx (Adamski *et al.*, 2020). They are therefore constituents that have high prospective in medicine, plant defense, veterinary, or toxicology. The presence of such classes of secondary metabolites supports the ethnomedicinal use of the species.

3.3. Isolated Compounds and Their Structure Elucidation

Fractionation of the chloroform:methanol (*v/v* 1:1) extract resulted in a white solid compound with Rf = 0.57 (*n*-hexane-EtOAc 4:1). The ^{1}H-, ^{13}C-NMR, and DEPT-135 spectral data, however, revealed the white solid compound being a mixture of three compounds (isomers) where purification through silica gel column chromatography and recrystallization was not successful. The experimental spectroscopic data was compared with spectroscopic data reported in literature (Carothers *et al.*, 2018; Chaluma *et al.*, 2018; Liliana *et al.*, 2012; Mesfin, 2018; Raga *et al.*, 2013; Sathish *et al.*, 2017) for structure elucidation.

3.3.1. *Compound 1*

^{1}H-NMR spectrum: δ 5.12 (1H, t *J* = 6.62 Hz) is a proton attached to sp^2 hybridized carbon (H-12). δ 3.16 (1H, t *J* = 6.71 Hz) is a characteristic peak of a proton attached to a carbon atom bearing a hydroxyl group (H-3). δ 0.80 (H-25, s, H-30, d *J* = 6.70 Hz), 0.81 (H-24, s), 0.86 (H-29, d *J* = 6.54 Hz), 0.88 (H-26, s), 0.92 (H-23, s), 1.00 (H-28, s) and 1.06 (H-27, s) are eight aliphatic methyl signals. Moreover, the remaining proton signals for five methine and eighteen methylene protons were observed in the aliphatic region.

^{13}C-NMR spectrum: The signals at δ 38.0 (C-1), 27.3 (C-2), 18.4 (C-6), 32.9 (C-7), 23.3 (C-11), 28.8 (C-15), 26.6 (C-16), 31.3 (C-21), and 41.5 (C-22) are methylene (CH_2) carbons, 79.2 (C-3), 55.2 (C-5), 47.7 (C-9), 124.4 (C-12), 59.0 (C-18), 39.6 (C-19), 39.7 (C-20) are methine (CH) carbons and 28.2 (C-23), 15.6 (C-24), 15.6 (C-25), 16.8 (C-26), 23.3 (C-27), 28.1 (C-28), 17.4 (C-29), 21.3 (C-30) are methyl (CH_3) carbons. The absence of peaks on DEPT-135 spectrum at δ 38.8 (C-4), 41.2 (C-8), 36.9 (C-10), 139.6 (C-13), 42.0 (C-14) and 33.8 (C-17) confirms these peaks belong to quaternary carbon atoms. The two olefinic peaks at δ 124.4 and 139.7 were for C-12 and C-13 of which the latter is sp^2 hybridized quaternary carbon, respectively. The signal at δ 79.2 belongs to sp^3 hybridized oxygenated methine carbon (C-3).

3.3.2. *Compound 2*

The ^{1}H- and ^{13}C-NMR spectral data of this compound is similar to **compound 1** except some differences in the ^{13}C-NMR spectra. The ^{13}C-NMR spectra peaks at δ 145.3 and 121.7 belong to the olefinic carbons (C-13 and C-12) and δ 40.0 and 37.7 are C-20 and C-19 respectively. The difference in the chemical shift of the C-20 and C-19 is due to the shift of the methyl groups towards C-20 which resulted in an increase in C-20 and a decrease in C-19 chemical shift values. The chemical shift at C-3 also showed a slight shift and appeared at δ 79.0 which also distinguishes this compound from **compound 1**.

The experimental spectroscopic data of **compound 1** was in good agreement with reported data for (3*β*)-urs-12-en-3-ol commonly known as *α*-amyrin, viminalol, or *α*-amyrinol (Liliana *et al.*, 2012; Sathish *et al.*, 2017; Chaluma *et al.*, 2018). *α*-Amyrin (**1**) (1, Figure 1) is a triterpene which possesses a double bond between C-12 and C-13 where the hydrogen at the 3*β* position is replaced by a hydroxyl. It is a hydride derivative of pentacyclic triterpene known as ursane.

The spectroscopic data of **compound 2** agrees with previously reported data of *β*-amyrin (**2**) (2, Figure 1) (Chaluma *et al.*, 2018; Liliana *et al.*, 2012; Sathish *et al.*, 2017).

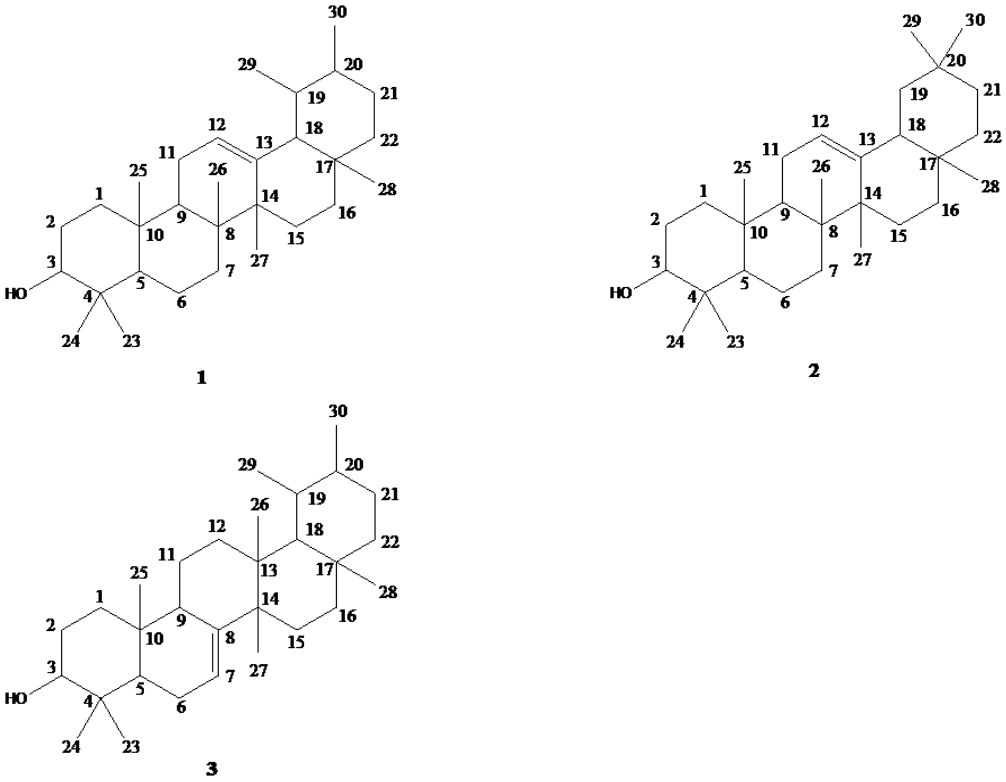

Figure 1. Structures of α-amyrin (**1**), β-amyrin (**2**), and bauerenol (**3**), respectively.

3.3.3. *Compound 3*

^{1}H-NMR spectrum: the chemical shift at 5.41 (1H, t J = 7.1 Hz) characterizes olefinic proton. The chemical shift at 3.24 (1H, t J = 6.70 Hz) is the characteristic peak of methine proton attached to a carbon atom bearing hydroxyl functional group. The signals at δ 2.19, 1.54, 1.27, and 1.14 belong to the methine (CH) protons. The signals at δ 2.16, 1.97, 1.64, 1.61, 1.60, 1.54, 1.50, 1.49, 1.48, 1.43, 1.19, 1.18, 1.14, and 1.09 are methylene (CH_2) protons. The signals at δ 1.05, 1.02, 0.97, 0.96, 0.95, 0.90, 0.84, and 0.75 are methyl protons.

^{13}C-NMR spectrum: The two peaks at δ 145.3 and 116.4 are olefinic carbons (C-8 and C-7). The peaks at δ 37.7 (C-16), 36.9 (C-1), 32.4 (C-12), 31.5 (C-22), 29.2 (C-21), 28.9 (C-15), 27.7 (C-2), 24.2 (C-6), 16.9 (C-11), are methylene (CH_2) carbons. The peaks at δ 77.2 (C-3), 54.9 (C-18), 50.4 (C-5), 48.2 (C-9), 35.3 (C-19), 32.0 (C-20) are methine (CH) carbons and the peaks at δ 39.9 (C-28), 27.6 (C-23), 25.7 (C-29), 23.7 (C-26), 22.7 (C-27), 22.5 (C-30), 14.7 (C-24), and 13.0 (C-25), are methyl (CH_3) carbons. The peaks at δ 41.5 (C-14), 38.8 (C-4), 37.7 (C-13), 35.3 (C-10) and 32.0 (C-17) are quaternary carbons.

DEPT-135 spectrum: the upward peaks at δ 116.4 (C-7), 77.2 (C-3), 54.9 (C-18), 50.4 (C-5), 48.2 (C-9), 35.3 (C-19), 32.0 (C-20) are methine (CH) carbons whereas the peaks at δ 39.9 (C-28), 27.6 (C-23), 25.7 (C-29), 23.7 (C-26), 22.7 (C-27), 22.5 (C-30), 14.7 (C-24), and 13.0 (C-25), are methyl (CH_3) carbons. The downward peaks at δ 37.7 (C-16), 36.9 (C-1), 32.4 (C-12), 31.5 (C-22), 29.2 (C-21), 28.9 (C-15), 27.7 (C-2), 24.2 (C-6), 16.9 (C-11), are methylene (CH_2) carbons. The absence of peaks at δ 145.3 (C-8), 41.5 (C-14), 38.8 (C-4), 37.7 (C-13), 35.3 (C-10) and 32.0 (C-17) implies they belong to quaternary carbons.

The above ^{1}H- and ^{13}C-NMR data were in good agreement with the reported data for bauerenol (**3**) (3, Figure 1) (Carothers *et al.*, 2018; Chaluma *et al.*, 2018; Mesfin, 2018; Raga *et al.*, 2013; Sathish *et al.*, 2017).

Ursane type triterpenes are widely distributed in the plant kingdom, as aglycones or in combined forms, and have several biological activities. *α*-amyrin is usually found in oleo-resin of the various species of *Bursera* or *Protium* of the Burseraceae family. It exhibits several biological activities *in vitro* and *in vivo* conditions against several health-related conditions, such as microbial, inflammation, cancer cells, and viral and fungal infections (Liliana *et al.*, 2012). Bauerenol, on the other hand, showed cytotoxic and apoptotic potential against human HepG2 cancer cells and it is also anti-*Trypanosoma brucei* agent (Carothers *et al.*, 2018). In addition to this bauerenol prevents migration, proliferation and invasion of retinoblastoma cells through induction of autophagy, apoptosis and cell cycle arrest (Chen *et al.*, 2022). *β*-Amyrin possesses anti-inflammatory, anti-fibrotic, and anti-apoptotic effects on dimethyl nitrosamine–induced hepatic fibrosis in male rats (Thirupathi *et al.*, 2017). The presence of *α*-amyrin might have caused the root of the species to have the traditional medicinal effects as wound healing (Maundu & Tengnäs, 2005).

4. CONCLUSION

In this study, phytochemical screening and compound isolation were carried out on the root of *H. verdcourtii*. The classes of secondary metabolite screening test of the *n*-hexane extract revealed the presence of terpenoids, flavonoids, and glycosides, alkaloids, saponins, and phenolics. The presence of these bioactive constituents is significant as they may account for the wide scope ethnomedicinal use of the species. Silica gel column chromatographic separation of the chloroform/methanol (*v/v* 1:1) extract has led to the isolation of the mixture of three biologically active ursane type pentacyclic triterpenes identified as *α*-amyrin, *β*-amyrin, and bauerenol. The presence of *α*-amyrin might be the cause of the root to have a wound healing property. This is the first report of the isolation of the aforementioned chemical constituents from the root of *H. verdcourtii*, of Ethiopian origin.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Tegene Tesfaye Tole: Methodology, Supervision, Visualization, Formal Analysis, and Writing original draft. **Habtamu Hailu Feso**: Investigation, Resources, Visualization, Formal Analysis. **Legesse Adane**: Methodology, Supervision, and Validation.

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