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Table of Contents: Volume 12 Number 30 25 December, 2018

ARTICLES

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Full Length Research Paper

Medicinal plant use and conservation practices by communities in the Togo Plateau Forest Reserve, Ghana

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The use of plants for medicine has constituted an important aspect of primary healthcare delivery system of people throughout the world for many centuries. Nonetheless, information on medicinal plants and their conservation are still lacking in some areas, including the Togo Plateau Forest Reserve in Ghana. This paper investigates the ethnobotany of the Togo Plateau Forest Reserve, Ghana, to document the traditional knowledge, uses and conservation of medicinal plants. Information on these plants was sought through a structured questionnaire administered to 384 registered members of the Ghana Federation of Traditional Medicine Practitioners' Association including certified traditional healers, traditional birth attendants (TBAs), vendors of herbal remedies, managers of herbal medicine centers, and local plant collectors from six communities within the catchment area of the Togo Plateau Forest Reserve. The study recorded 114 medicinal plant species, including 14 herbs, 6 lianas, 21 shrubs and 74 trees that are mostly collected from the wild. The most commonly cited medicinal plant species were *Azadirachta indica, Alstonia boonei, Morinda lucida*and *Nauclea latifolia*, mostly used to treat human conditions such as malaria, jaundice, rheumatism, andcough. Mostof the herbal medicines were prepared as decoctions and administered by drinking. Majority of the informants (75%) were within the age bracket of 41-60 years, with males (39%) out numbering the females (36%). Barks, roots and leaves were the most commonly used plant parts, and these were mostly collected by destructive methods. The unregulated collection of plant parts and harvesting methods may reduce plant species richness and abundance in the area, requiring sustained conservation efforts in order to benefit from them medicinally.

Key words: Medicinal plants, Togo Plateau forest reserve, plant diversity.

INTRODUCTION

The use of plants for medicine has constituted animportant aspect of primary healthcare delivery system of people throughout the world for many centuries (Agbovie et al., 2002; Jeruto et al., 2008; WHO, 2015).

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Currently, over 80% of the world's human populations rely on medicinal plants for their daily fight for better health (WHO, 2015). In Africa, in particular, medicinal plants are extensively (ca. 90%) used due to limited access to modern medical treatment, either resulting from lack of facilities or unavailability of hospital services (Satpong, 2000; Fyhrquist, 2007; Jeruto et al., 2008; Koduru et al., 2007). Studies show that about 46% of Ghanaians, for example, live in rural communities and depend largely on locally available plant resources for their health care needs (Fyhrquist, 2007).

The high demand for and dependence on traditional medicine (TM) for health care delivery in developing countries have resulted in the proliferation of traditional medicine dealers. According to the Ghana Federation of Traditional Medicine Practitioners Association (GHAFTRAM), there are more than 100,000 TM practitioners in Ghana of which 45,000 are registered. These practitioners are uniformly distributed throughout Ghana, resulting in approximately one TM practitioner for every 400 people, compared to one allopathic doctor for every 12000 people (WHO, 2015).

Several studies have documented the wide spectrum of application of medicinal plants among people (Abbiw, 1990; Addo-Fordjour et al., 2013; Jeyaprakash et al., 2011). Medicinal plants are commonly used to treat human conditions such asmalaria, jaundice, migraine, cough, rheumatism and gastrointestinal pain, among others (Addo-Fordjour et al., 2013; Jeyaprakash et al*.,* 2011). These human conditions are treated using diverse plant parts ranging from whole plant, roots, leaves and bark, with the latter being the most commonly used in herbal medicine preparation in Ghana (Addo-Fordjour et al., 2013; Asase et al., 2005; Ziblim et al., 2013) and elsewhere (Kakudidi, 2000; Kala, 2005; Kamatenesi-Mugisha et al., 2008).

Ecologists and conservationists have established that the harvesting methods often used for medicinal plants are critical to their survival (Balemie et al., 2004; Megersa et al., 2013). Destructive methods such as harvesting of the whole plant and roots, coupled with steady decline in customary laws that regulate the commercial collection of medicinal plant resources from the wild, are reportedly to cause the depletion of medicinal plant diversity as well as the indigenous knowledge associated with their conservation and use (Jeruto et al., 2008; Kamatenesi-Mugisha et al., 2008; Schippmann, 2001).

In this light, non-destructive methods of plant harvesting including consideration of the frequency, time, and season of harvesting which could affect natural regeneration (Abbiw 1990) are increasingly being encouraged. Researchers have also recommended harvesting of fruits and other aerial parts instead of the root, stem or the whole plant which could cause defoliation, debarking, root destruction and wounds (Abbiw, 1990; Jeruto, 2008). Extracting plant leaves also provides a more sustainable use strategy through rapid replacement by re-growt (Dold and Cocks, 2002).

Available records showed that intensive gathering of plants from the wild for medicinal purposes poses serious threat to Ghana's biodiversity (Addo-Fordjour et al., 2013; Asase et al., 2005; Bussmann et al., 2011; Megersa et al., 2013; Ziblim et al., 2013) as it increases the risk of local extinction of plant species in general (Ita and Offiong, 2013) and the scarcity of commonly used medicinal plants in particular (Bussmann et al., 2011; Kamatenesi-Mugisha and Bukenya-Ziraba, 2002; Megersa et al., 2013). Consequently, increasing commitment toward efficient controls and better conservation practices can help preserve medicinal plant diversity in Ghana.

The Togo Plateau Forest Reserve, covering a total land area of 14.763 hectares is the largest forest reserve in the Volta Region of Ghana. This reserve has been recognized as a biodiversity significant area due to its wide topographic range and high elevation (Abbiw, 1990; Agbovie et al., 2002) and thus provides a variety of medicinal plants to the surrounding communities.This has resulted in a high demand for and dependence on TM by the people for their healthcare needs leading to a proliferation of practitioners in the area, whose activities could pose a threat to the medicinal plants biodiversity on the plateau. Moreover, given the diverse plant species reportedly used for medicinal purposes in the area, targeting them for conservation can positively impact biodiversity conservation in the reserve generally. There is, therefore, an urgent need for information on medicinal plant usage and conservation practices by communities fringing the Togo Plateau Forest Reserve, but this is yet to be documented. The objective of this study is to document the traditional knowledge, uses and conservation of medicinal plants among the fringed communities in the Togo Plateau Forest Reserve.

MATERIALS AND METHODS

Study area

The Togo Plateau Forest Reserve was established by the British Colonial Administration in 1929, in the then Trans-Volta-Togoland and gazetted in 1931 as a forest reserve in Ghana. The reserve occupies an area of 14.763 ha, making it the largest reserve in the Volta Region of the country. It lies within longitudes 0°15′E and 0° 45′E and latitudes 6° 45′N and 7° 15′ N with elevation between 250 and 2680 m.a.s.l (Figure 1). The reserve is surrounded by several communities, most of which depend on medicinal plant resources mostly from the reserve for their healthcare needs. These communities include Hohoe, Alavanyo, Santrokofi and Akpafu, which are located within the HohoeMunicipality, as well as Bowiri and Nkonya both in the Biakoye District.

The Hohoe municipality has a total land area of 1,172 km^2, representing 5.6% of the land area of the Volta Region, and has Hohoe as its capital. The municipality lies in the wet semi-equatorial climatic zone, with annual rainfall of 1016-1210 mm and 4-5-month dry season between November and April. Temperatures are high throughout the year and range from 26 to about 32°C. The

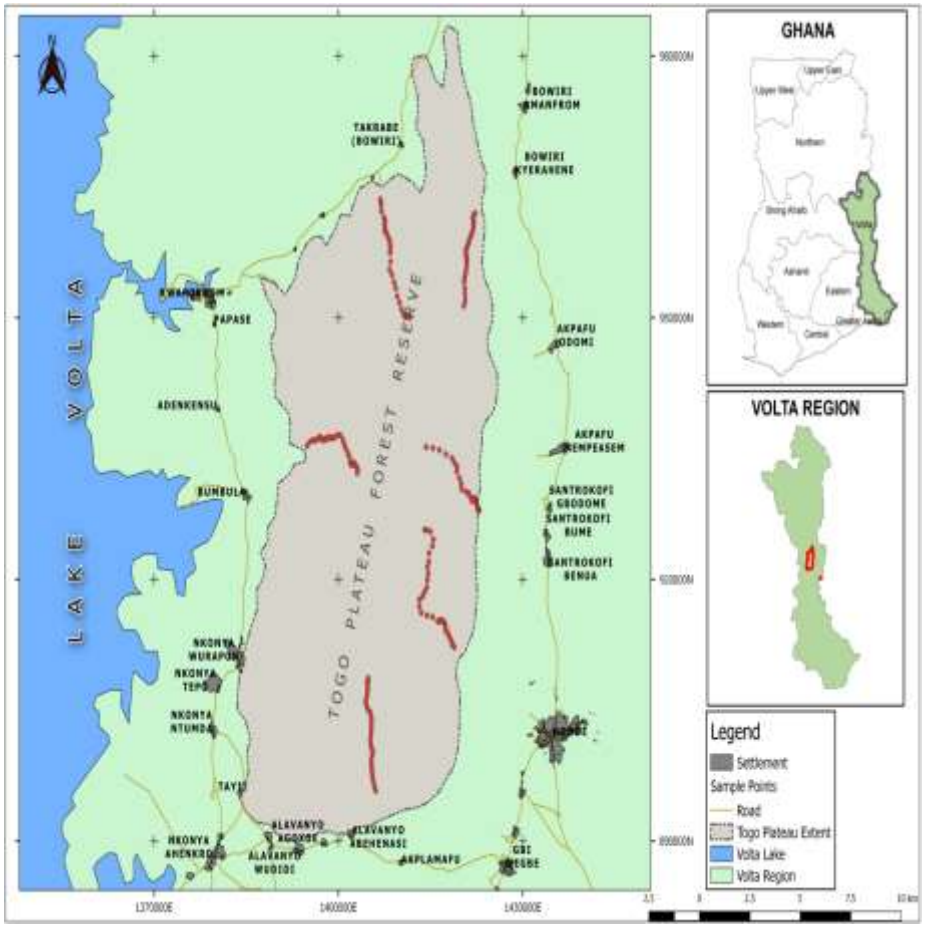

Figure 1. A map of the Togo Plateau Forest Reserve in the District Context.

population of the Municipality in 2010 was 172.950 (Ghana Statistical Service, 2010). Major economic activities include agriculture, forestry and fishery. The Biakoye District, on the other hand, has a total land area of 738.20 km^2, representing about 4.1% of the total land area of the region. The district capital is Nkonya Ahenkro.The district experiences the wet equatorial rainfall regime with its peak in July and September, respectively. The mean annual rainfall is about 1500 mm. There is a rather short dry season, which is characterized by the cool dry North-East trade winds from early December to mid-March. Temperatures vary between 22 and 34°C.The district is estimated to have 63,645 people (GSS, 2010). Major economic activities of the inhabitants include fishing, lumbering, carpentry, blacksmithing, distilling, palm oil extraction and gari processing.

Ethnobotanical study

Information on medicinal plant knowledge, use and their conservation status was sought through a structured questionnaire (Viertler, 2002) administered to a total of 384 people from six communities around the Togo Plateau Forest Reserve, Ghana. These informants (aged 30-70 years) were purposively and proportionately selected from each community in the order Hohoe (234 informant), Nkonya (42), Santrokofi (40), Bowiri (35), Alavanyo (23) and, Akpafu (10). The selection was done following consultation with opinion leaders, from the following category of medicinal plant dealers: certified traditional healers, traditional birth attendants (TBAs), vendors of herbal remedies, managers of herbal medicine centers, and local plant collectors. The selected informants were confirmed by GHAFTRAM as registered members. Information on respondents and medicinal plants of the study area including demographics (age, gender and education), growth form and plant parts used, condition before use and major uses constituted the checklist. Other data gathered included preparation methods, mode of administration, location and mode of plant collection, domestication status, and conservation status of these plants. Both local and scientific names of the plants were recorded with the help of experienced taxonomists. Scientific names were verified from the online resource “The Plant List” (2013, v1.1). The survey was conducted between September, 2017and June, 2018.

Data analysis

All medicinal plants encounted were compiled in a checklist including species same (local name, family, growth form, parts used, condition, and major uses). Descriptive statistics on the human conditions treated with the medicinal plants, the preparation methods, mode of administration and condition before use, availability, harvesting methods, and conservation status or efforts were also presented. The analyses were performed using MS Excel.

Table 1. Socio-demographic profile of respondents.

Description	Frequency (%)
Age	
30-40 years	69 (18)
41-50 years	150 (39)
51-60 years	138 (36)
61-70 years	27 (7)
Gender	229 (60)
Male	
Female	155 (40)
Education	
Basic	279 (73)
Secondary	67 (17)
Tertiary	38 (10)
Duration of practice	
1–5 years	63 (16)
6–10 years	169 (44)
11–15 years	110 (29)
16–20 years	42 (11)
Category of medicinal practice	
Certified traditional healers	114 (30)
Vendor of herbal remedies	102 (27)
Certified managers of herbal medicine centers	97 (25)
Certified traditional birth attendants	55 (14)
Local plant collectors	16 (4)

RESULTS

Socio-demographic characteristics of the studied communities

Majority of the informants (75%) were within the age bracket of 41-60 years, with males (39%) outnumbering the females (36%) (Table 1). Although all respondents had received formal education, only (17%) had reached the secondary school level or beyond. Results also indicated that all the respondents had knowledge of medicinal plants and their uses in the area, with (84%) of them claiming at least 6 years of experience as TM practitioners. Certified traditional healers constituted the largest category (30%) of practitioners identified in the study, followed by vendors of herbal medicine (27%), certified managers of herbal medicine centers (25%) and certified TBAs (14%). A small number of the respondents (4%) were classified as local plant collectors.

Medicinal plants identifiedin the study area

The study recorded 114 medicinal plant species, belonging to 42 families and distributed in four growth forms (14 herbs, six lianas, 21 shrubs and 74 trees) in the six communities surveyed (Table 2). Of the 42 families recorded, Leguminosae and Euphorbiaceae emerged as the most important medicinal plant families with 21 and 10 species, respectively, followed by Apocynaceae and Moraceae with seven species each. The remaining 38 families combined contributed 69 species. The medicinal plants identified in the study were reportedly used to treat about 75 human-related conditions (Table 2). Majority of the plants (51 species) were used to treat malaria, followed by jaundice (33), rheumatism (26), cough (25) and stomach pain (22) (Figure 2). Each of the remaining 70 conditions had 1-18 medicinal plant species cited as potential cure for it. The most commonly cited medicinal plant species were *Azadirachta indica* (256), *Alstonia boonei* (230), *Morinda lucida* (212) and *Nauclea latifolia* (193) (Figure 3).

The medicinal plants in the study area were collected from three sources, namely; forest reserve, garden and farmlands. Analysis revealed that (61%) of trees were collected from the forest reserve, (26%) from the farmland and 13% from the gardens. The distribution of

Table 2. Medicinalplants used by communities in the Togo Plateau Forest Reserve, Ghana withtheir family, growth form, parts used and major uses.

Species Name	Local Name	Family	Growth form	Parts used	Condition	Major uses
Justiciaflava (Forssk.) Vahl	Ligbetovi	Acanthaceae	Herb	L,B	Fresh	dysentery
Cyanthulaprostrata (L.) Blume	Tsiofoganu	Amaranthaceae	Herb	W	Fresh	pain relief, heart human conditions
Lanneaacida A. Rich.	Kuntunkuri	Anacardiaceae	Tree	W	Fresh	Convulsion
Annonasenegalensis Pers.	Mampihege	Annonaceae	Tree	R	Dry	malaria, impotence, whitlow, chest pains, cough, leprosy
*Monodoratenuifolia*Benth	Yiku	Annonaceae	Tree	B,S	Fresh	malaria, dysentery/ diarrhoea, migraine
*Uvariadoringii*Diels	Gbanagbana	Annonaceae	Liana	L,B,R	Fresh	jaundice, menstrual problems, piles, sore eye, malaria
Uvariachamae P. Beauv.	Gbanagbana	Annonaceae	Shrub	L,B	Dry	malaria, jaundice, yellow fever, piles, sore eye,
Xylopiaaethiopica (Dunal) A. Rich	Etso	Annonaceae	Tree	L,S	Fresh	colds, coughs, piles, anaemia
*Alstoniaboonei*De Wild.	Siaketekre	Apocynaceae	Tree	B	Dry	jaundice, malaria, ashma, cough
Baisseamultiflora A. DC.	Agordati	Apocynaceae	Liana	L	Dry	skin irritations, malaria, ashma, fractures/ dislocation
Funtumia elastica (Preuss) Stapf	Okae	Apocynaceae	Tree	B	Dry	Piles
Holarrhenaflorimbunda (G. Don) T.Durand&Schinz	Aforkpati	Apocynaceae	Shrub	L,R	Fresh	diabetes, jaundice,breastmilk production, urinary infections, aid delivery in pregnant women
Landolphiadulcis (Sabine ex G.Don) Pichon		Apocynaceae	Herb	L	Fresh	indigestion, fatigue, toothache/tooth decay
*Rauvolfiavomitoria*Afzel	Dodemakpowoe	Apocynaceae	Tree	L,B	Dry	leprosy, rheumatism, fractures/ dislocations, measles
Strophanthussarmentosus DC.	Amatsiga	Apocynaceae	Liana	L,R	Fresh	sore eye, body pain
*Voacangaafricana*Stapf	Ofuruma	Apocynaceae	Tree	W	Fresh	body pains, haenia, cancer, wounds
Vernoniaamygdalina Del.	Egborti	Asteraceae	Shrub	L	Fresh	gastrointestinal pains
Newbouldialaevis (P. Beauv.) Seem.	Kpotiyia	Bignoniaceae	Shrub	R	Fresh	fractures/dislocations
*Spathodiacampanolata*P.Beauv.	Adatsigolo	Bignoniaceae	Herb	B	Dry	appetizer, backache, bladder trouble/kidney human conditions.
*Adansoniadigitata*L.	Adidoti	Bombacaceae	Tree	W	Fresh	kidney/ bladder human conditions, diuretic, diarrhea
*Bombaxbuonopozense*P. Beauv.	Okuo	Bombacaceae	Tree	L	Fresh	placental expulsion, childbirth, breastmilk production
Ceibapentandra (L.) Gaertn	Ewuti	Bombacaceae	Tree	B	Dry	belly pains, haenia
*Canariumscheweinfurtii*Engl.	Dunorviwo	Burseraceae	Tree	B	Fresh	chest pains, cough/ whooping cough, piles, jaundice.
Celtis mildbraedii Engl.		Cannabaceae	Tree	L,B	Fresh	rheumatism, dewormer, abscesses, headache, measles
*Myrianthusarboreus*Beauv.	Nyankuma	Cecropiaceae	Tree	B	Dry	migraine

Table 2. Contd.

Maytenussenegalensis (Lam.) Exell		Celastraceae	Shrub	W	Fresh	ulcers, wounds, gastrointestinal disorders
*Combretummicranta*G.Don	Aveto	Combretaceae	Shrub	L,B	Fresh	rheumatism, jaundice, herpatitis, bruises, sprains.
Pteleopsissuberosa Engl. & Diels		Combretaceae	Shrub	B	Dry	clean uterus after delivery/miscariage/abortion
Terminaliamacroptera Guill. & Perr	Petni	Combretaceae	Tree	R	Fresh	gastrointestinal disorders
*Bursocarpuscoccineus*Schumach	Awennade	Commelinaceae	Tree	L,R	Dry	hypertension, measles, impotence
Palisotahirsuta (Thunb.) K.Schum.	Klugbogbo	Commelinaceae	Herb	L	Fresh	belly pain
*Cnestisferruginea*Varl. Ex DC.	Akitase	Connaraceae	Liana	L	Fresh	Dysentery
Costusafer Ker Gawl.*Costusafer*	Eyra	Costaceae	Herb	L	Dry	aids early walking in infants.
*Diospyrosmadagascariense*Gürke	Kusibiri	Ebenaceae	Tree	B	Fresh	rheumatism, headache, sexual weakness, induce abortion
Alcorniacardifolia (Shumach. &Thonn.) Müll.Arg.	Avovlo	Euphorbiaceae	Herb	L,B	Dry	sore eye, colds, gastrointestinal and liver disorders, rheumatism, dysentery
*Brideliaferruginea*Benth.	Asaraba	Euphorbiaceae	Tree	B,R	Fresh	diuretic, aphrodisiac, rheumatism, dysentery
Drypetesparvifolia (Müll.Arg.) Pax&K.Hoffm.	Katrika	Euphorbiaceae	Tree	R	Fresh	catarrh
Mallotusoppositifolia (Geiseler) Müll.Arg.	Satadua	Euphorbiaceae	Shrub	L	Fresh	Migraine
Margaritariadiscoides (Baill.) Webster	Pepea	Euphorbiaceae	Tree	B	Dry	abscesses, intestinal worms, induce abortion.
Microdesmiapuberula . Hook. F	Ofumai	Euphorbiaceae	Shrub	L,B	Fresh	expel all forms of intestinal worms
Phyllanthustogoensis Brunel & Roux	Kpavidetume	Euphorbiaceae	Liana	L	Fresh	typhoid fever, jaundice, malaria
Ricinodendronheudelotii (Baill.) Pierre ex Heckel	wamma	Euphorbiaceae	Tree	R	Fresh	anaemia, infertility
Securinegavirosa (Roxb. Ex Wille.) Pax.EtHoffn	Nkanna	Euphorbiaceae	Tree	R	Dry	relieve pain
*Uapacatogoensis*Baill.	Kontannini	Euphorbiaceae	Tree	B,R	Dry	stimulant against fatigue, sterility, jaundice
*Garcinia kola*Heckel	Tweapea	Guttiferae	Tree	S	Dry	chest pains, migraine
*Hymenocardiaacida*Tul.		Hymenocardiaceae	Tree	R	Dry	malaria, colds
*Hoslundiaoppositа*Vahl	Akotadzeveti	Lamiaceae	Shrub	L	Fresh	stimulate liver and increase bile production
Napoleonavogelii Hook. & Planch.	Esia	Lecythidaceae	Tree	R	Dry	ashma, cough/whooping cough
Petersianthusmacrocarpus (P.Beauv.) Liben		Lecythidaceae	Tree	B,R	Fresh	bronchial trouble, general cases of cancer
Afzeliaafricana Pers.	wokpa	Leg-Cae	Tree	B	Dry	gastrointestinal pains, piles, pneumonia
*Berliniaoccidentalis*Keay	Kwatafom	Leg-Cae	Tree	B	Fresh	jaundice
Cassia sieberiana D.C	Agbobladzoe	Leg-Cae	Tree	R	Dry	kidney human conditions/ bladder trouble/ chest pains, aphrodisiac,

Table 2. Contd.

Danielliaoliveri (Rolfe) Hutch. &Dalziel	Sopi	Leg-Cae	Tree	B,R	Fresh	jaundice, burns, migraine, cough, headaches, stiffness, belly pains, anxiety, insanity
*Dialiumguineense*Willd.	Atortoe	Leg-Cae	Tree	R,Sh	Fresh	ashma, body weakness, jaundice, tooth decay, headache
Erythrophloemsuaveolus (Guill. &Perr.) Brenan	Potrodom	Leg-Cae	Tree	B	Fresh	anaesthetic, scabies, chicken pox, leprosy, guinea worm, rheumatism, migraine
Griffoniasimplicifolia (DC.) Baill.	Kagya	Leg-Cae	Shrub	L	Fresh	kidney/bladder human conditions
Sennasiamea (Lam.) (Lam) H.S.Irwin		Leg-Cae	Tree	R	Dry	malaria, jaundice, sore throat
*Acacia kamerunensis*Gand.	Nnwere	Leg-Mim	Shrub	B,R	Fresh	kidney /bladder human conditions, fever, colds, aphrodisiac
Albiziazygia (DC.) J.F Macbr.	Toziwa	Leg-Mim	Tree	B	Dry	purgative, intestinal worms, bronchitis, cough
*Entadaabyssinica*Steud. Ex A. Rich		Leg-Mim	Tree	L	Fresh	malaria, rheumatism, stomach wounds, cough
Parkia bicolor A. Chev.	Dawadawa	Leg-Mim	Tree	B	Fresh	bronchitis, cough, abscesses
Piptadestiastrumafricanum (Hook. f.) Brenan	Yewoye	Leg-Mim	Tree	R	Dry	abortion
Tetrapleuratetraptera (Schum&Thonn.) Taub	Prekese	Leg-Mim	Tree	F	Dry	Malaria
Abrusprecatorius L.	Dedekude	Leg-Pap	Herb	L,F	Fresh	inflamation of small and large intestine
Amphimaspterocarpoides Harms	Yaya	Leg-Pap	Tree	R	Dry	blood tonic
*Baphianitida*Lodd	Toti/Odzori	Leg-Pap	Tree	R	Fresh	gastrointestinal pains
Lonchocarpuscyanescens (Schum. &Thonn.) Benth	Santa	Leg-Pap	Shrub	R	Dry	rheumatism, aphrodisiac, childhood diarrhoea, blood tonic.
Milletiazechiana Harms	Amatike	Leg-Pap	Tree	B	Dry	bronchial trouble
Pterocarpusmalbraedii Harms	Hote	Leg-Pap	Tree	L,R	Fresh	body weakness, dysentery, cough, bronchitis, rashes
Tamarindusindica L.	Tamarind	Leg-Pap	Tree	R	Fresh	snake bite, asthma
*Anthocleistadjalonensis*A. Chev.	Gboloba	Loganiaceae	Tree	B	Dry	rheumatism, joint pains
Azadirachtaindica A. Juss.	Liliti	Meliaceae	Tree	B,R	Dry	malaria, jaundice, body weakness
Khayaivorensis A. Chev.	Logo	Meliaceae	Tree	B	Fresh	blood supply, gastrointestinal pains, rheumatism
Khayasenegalensis (Desr.) A. Juss.	Logo	Meliaceae	Tree	L,B	Dry	malaria,anaemia, jaundice, gastrointestinal pains
Trichiliaheudelotii Planch. exOliv.	Tandro	Meliaceae	Tree	B	Fresh	malaria, gastrointestinal pains, cough, jaundice
*Antiaristoxicaria*Lesch.	Loko	Moraceae	Tree	B	Dry	cough/whooping cough
*Ficusexasperata*Vahl.	Tsatsaflala	Moraceae	Tree	L,B	Fresh	migraine, cough
Ficusingens Del.		Moraceae	Tree	R	Fresh	menstrual disorders
Leucaenaglauca(L.) Benth.	Odzimtsui	Moraceae	Tree	R	Dry	prevent miscarriage, body weakness
Milliciaexcelsa (Welw.) C. C Berg	Odum	Moraceae	Tree	B	Fresh	headache, malaria
*Treculiaafricana*Decne		Moraceae	Tree	R	Fresh	skin irritations

Table 2. Contd.

Trilepisiummadagascariense DC.	Dzekludzi	Moraceae	Tree	B	Dry	cough/ whooping cough
Pycnanthusangolensis (Welw.) Warb.	kpornugbordeti	Myristicaceae	Tree	L,B	Fresh	appetizer, digestive tonic
*Eugenia calophylloides*DC.	Pepra	Myrtaceae	Shrub	S	Dry	dewormer
Lophiralanceolata Banks ex C.F.Gaertn.	Azobe	Ochnaceae	Tree	R	Fresh	migraine, headache, backache, jaundice
*Olaxsuscorpioides*Oliv.	Ahoohendedua	Olacaceae	Shrub	L,R	Dry	jaundice, yellow fever, malaria, aphrodisiac
Adenialobata (Jacq.) Engl.	Damalia	Passifloraceae	Herb	B	Dry	aphrodisiac
*Piper guineense*Schumach. &Thonn.	Kale	Piperaceae	Tree	L,B	Dry	malaria, jaundice, yellow fever, piles, body pains
Cymbopogoncitratus (DC.) Stapt.	Tigbe	Poaceae	Herb	L	Fresh	malaria, hypertension
*Securidacalongependuculata*Fresen.	Kpaliga		Tree	B,R	Fresh	leprosy, sore eye, rheumatism, migraine, snake bite
Crossopterixfabrifuga(Afzel. Ex G.Don) Benth.	Pakyisie	Rubiaceae	Shrub	L,B	Dry	sterility, aphrodisiac, breast milk production, chest pain, cough/whooping cough, gastrointestinal pain
*Morindalucida*Benth.	Venamakpa	Rubiaceae	Tree	R	Fresh	Malaria
*Nauclealatifolia*Sm.	Egbesi/Nyimo	Rubiaceae	Tree	R	Fresh	sexual weakness
Pavettacorymbosa(DC.) F. N. Williams	Kronko	Rubiaceae	Tree	L,B	Fresh	syphilis, jaundice, cough, malaria, rheumatism, bronchitis
*Rothmanialongiflora*Salisb.	samankube	Rubiaceae	Shrub	L,B	Fresh	prevent miscarriage, relief pain,
Cytrusaurantifolia (Christm.) Swingle	Mumoe/Donti	Rutaceae	Tree	R	Fresh	urine retention, gonorrhoea, syphilis, bilharziasis
Clausenaanisata (Willd) Hook.f. exBenth.	Eyira/Amuti	Rutaceae	Shrub	L,R	Fresh	sore eye, measles, cough, sore throat, headache, piles, heart human conditions, rheumatism, body pains
*Zanthoxylumleprieurii*Guill. & Perr.	Exedza	Rutaceae	Tree	L,B,R	Fresh	appetizer, migraine, haenia, rheumatism
Zanthoxylumzanthoxyloides (Lam.) Waterm.	Xeti/Xeke	Rutaceae	Tree	B,R	Fresh	sore eye, induce pregnancy, gastrointestinal pains, impotence,abscesses, rheumatism, paralysis
*Allophylusafricanus*P. Beauv.	(Kotamenyati)	Sapindaceae	Tree	R	Fresh	migraine, fracture/dislocation/sprains, rheumatism
Blighiasapida K.D. Koenig	Akye	Sapindaceae	Tree	R,B	Fresh	jaundice, migraine, haenia
Cardiospermumgrandiflorum Sw.	Tooto	Sapindaceae	Shrub	B,R	Fresh	sore eye, appetizer
Deinbolliapinnata (Poir.) Schumach. &Thonn.	Potoke	Sapindaceae	Shrub	L,R	Fresh	aphrodisiac, ashma
Lecaniodiscuscapanoides Planch. Ex Benth.	Dwindwera	Sapindaceae	Tree	B,R	Fresh	jaundice, migraine, fractures
Paulliniapinnata L.	Adifiehotsui	Sapindaceae	Herb	W	Fresh	fractures/dislocations, abscesses ,malaria, yellow fever
Manilkaramultinervis (Baker) Dubard	Yiku	Sapotaceae	Tree	B	Fresh	Cough
*Vitellariaparadoxa*Gaertn. F.	Yorkuti	Sapotaceae	Tree	R,S	Fresh	diabetes, stroke, waist pain, fracture

Table 2. Contd.

*Cola gigantea*L.	Bisiti	Sterculiaceae	Tree	B	Fresh	waist pains, piles
Pterygotamacrocarpa K. Schum.	kyereye	Sterculiaceae	Tree	L	Fresh	flatulence
*Sterculiatragacantha*Lindl.	Sofo	Sterculiaceae	Tree	R,Sh	Fresh	boils, malaria, expel intestional worms, whitlow
GrewiaPubescens	Yuatega	Tiliaceae	Shrub	L	Fresh	measles
Tremaorientalis (Linn.) Blume	Wadzawadza	Ulmaceae	Tree	L,B	Fresh	appetizer, haenia, sterility, high blood pressure,
Centellaasiastica (L.)	Gatigati	Umbelliferae	Herb	L	Fresh	Leprosy
*Clerodendrumumbellatum*Poir.		Verbenaceae	Liana	L	Fresh	belly pain
Vitexdoniana Sweet	Eforti	Verbenaceae	Tree	R,B	Fresh	colds, chicken pox, leprosy, sterility

NB: Bark (B), root (R), leaf (L), seed (S), shoot (Sh), whole plant (W), fruit (F).

shrubs and herbs followed a similar pattern as the trees, with majority collected from forests. Medicinal lianas were collected from two sources, namely; forest (80%) and farmland (20%) (Table 3).

Considering the domestication status, 65% of the tree species was collected from the wild while 35% was cultivated. From the shrubs, 68% were wild species whilst 32% were cultivated; 53% of the herbs were wild species whilst 47% were cultivated (Table 3). Different plant parts were used for the preparation of the traditional medicines (e.g., barks, roots, leaves, seeds and fruits). Of these, the bark (54 species) was more widely used than the roots (48) and leaves (44), but a few species however, the medicines are derived from the whole plant (6), seeds (5), fruits (3) and shoots (2) respectively (Figure 4).

Methods of preparation and modes of administration of medicinal plants

The most prevalent methods of medicinal plants preparation among thecommunities in the study area were decoction (51%), pounded (14%), powder (12%) and boil (10%). Other medicicines were seldomly prepared as infusion (4%), concoction (3%), crushed/mashed (2%), balm/ ointment (1%) and with juice, tea, tincture and poultice all accounting forless than (1%) (Table 4). With respect to the mode of administration of the drugs, 59% of the respondents reported that the medicines are taken orally. Other modes of administration include rubbing (10%), bathing (7%) and vapour bathing (6%). Few remedy preparations were applied through inhale vapour, drop on eye, ear, nose, sniffed, massage, chewed/swallowed and sit-bath (1–5%) (Table 4).

Medicinal plants and conservation practices

Most of the medicinal plants harvesting methods adopted by the respondents in the study area are destructive (81%) (Table 5). Most people collected the plant parts (59%) for medicine instead of the whole plant (11%) or individual plants (30%). On the availability of medicinal plants, 64% of the people noted that the plants were difficult or very difficult to find these days (Table 5). On whether the reserve was neccessary or not, majority of the people (45%) responded in the affirmative (Table 5). On the feasibility of conserving the medicinal plants, only 36% of the respondents agreed this was possible. The rest either did not agree (43%) or did not even appreciate the benefit associated with it (21%). Half (50%) of the respondent could not tell whether or not any conservation efforts had been made to save the Plateau from total degradation, although 31% acknowledged the initiation of such efforts either by the government or the community.

DISCUSSION

Demographic characteristics of the respondents

The study results confirmed the view that knowledge on medicinal uses of plants is often confined to the elderly (Abbiw, 1990; Addo-Fordjour et al., 2013; Jeyaprakash et al., 2011). This observation might be due to the fact that majority of the people in the study communities mostly spend their early years schooling and only

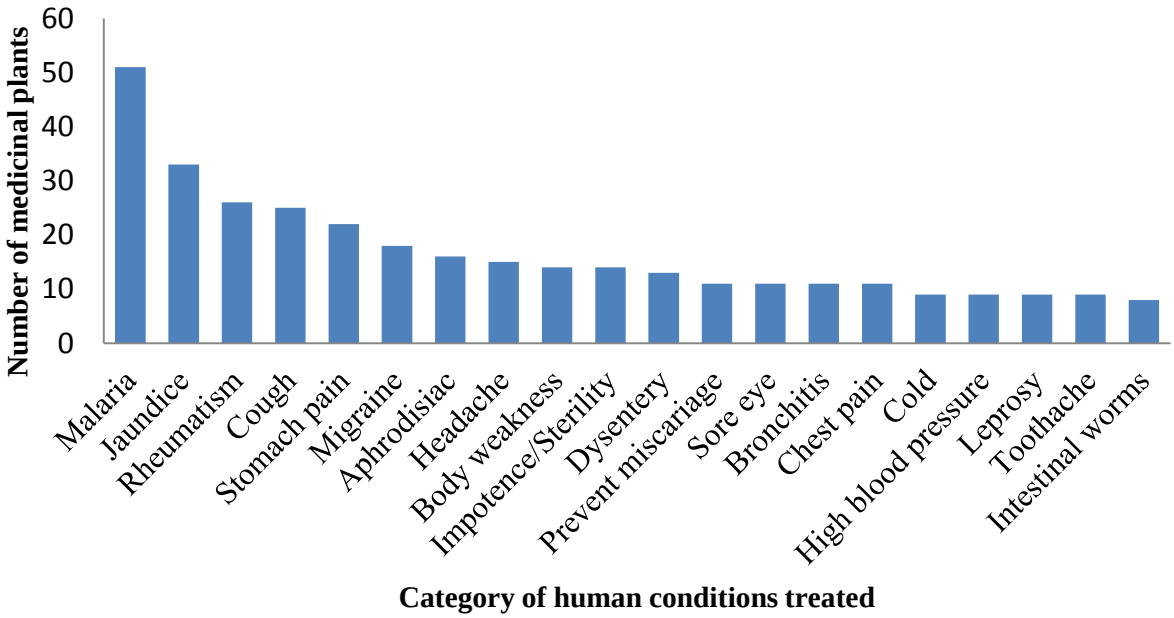

Figure 2. Human conditions cured using the recorded medicinal plants of the study area.

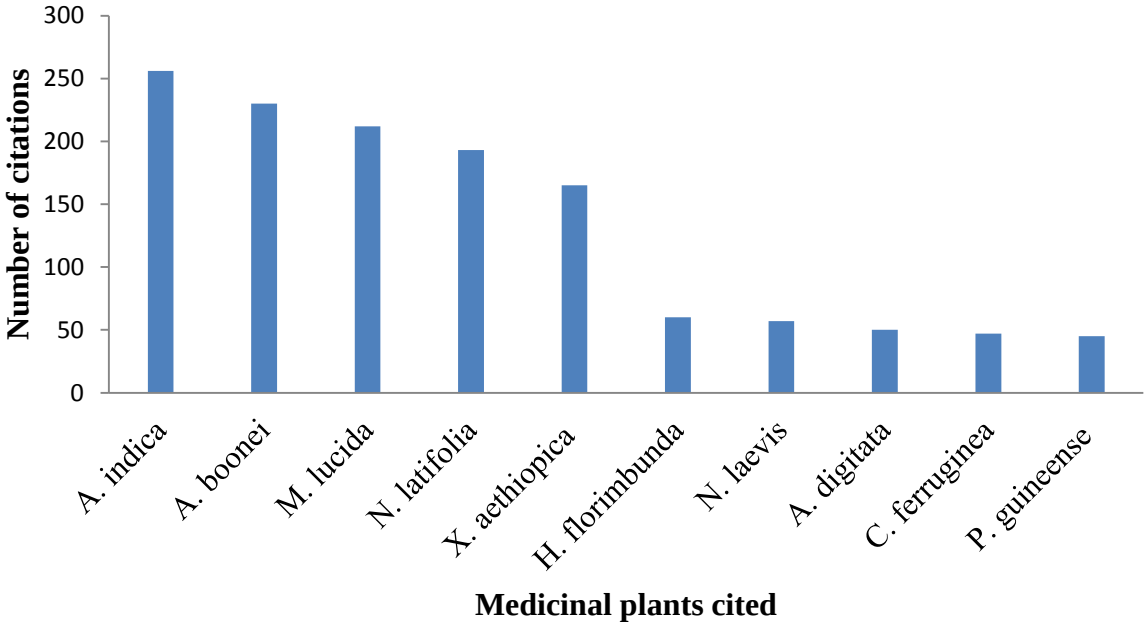

Figure 3. Most important medicinal plants recorded in the study area.

resort to traditional medicine practice if they do not find jobs after school. The younger generation may also be ignorant about the vast medicinal resources available in their surroundings and/or are typically more inclined toward market resources (Addo-Fordjour et al., 2013).

The male dominance in the traditional medicinal practice over their female counterparts in this study could be linked to gender roles. The hectic nature of medicinal plants collection and processing appears to discourage many women from the practice. The few women encountered were mostly into vendorship (Abbiw, 1990; Addo-Fordjour et al., 2013).The educational status of respondents helps to ascertain their mindset towards conservation of medicinal plant species. People who have higher/tertiary education are believed to have a potential knowledge of conservation of medicinal plants

Table 3. Relationships among growth forms, collection location, and domestication status of medicinal plants (values are percentages).

Growth form	Medicinal	Collection location			Domestication status	
	Uses	Forest	Farmland	Garden	Wild	Cultivated
Tree	62	61	26	13	65	35
Shrubs	20	62	25	13	68	32
Herbs	13	53	31	16	53	47
Liana	5	80	20	-	80	20

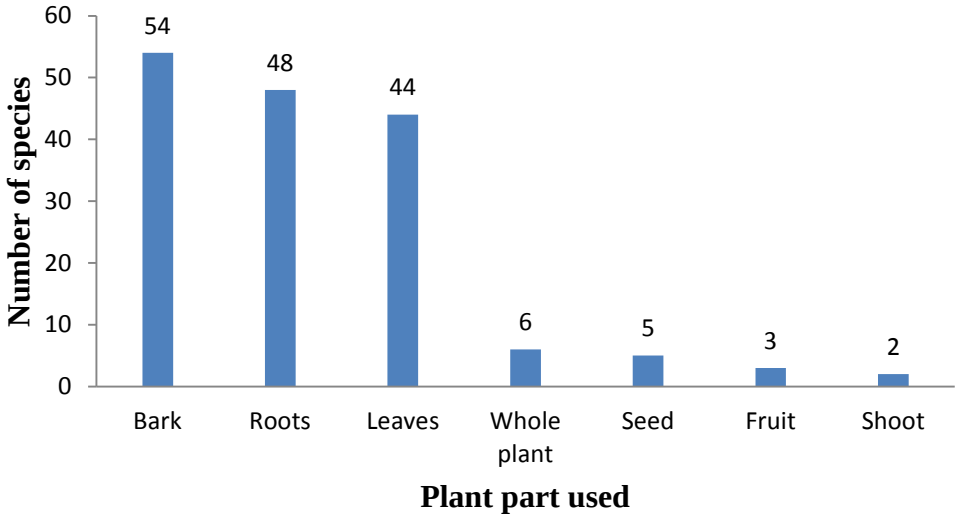

Figure 4. Plant parts used for the treatment of human conditions.

and their use in curing human ailments than those with no or low level of education (Abbiw, 1990; Addo-Fordjour et al., 2013). Thus, the generally low level of education in the area (with 73% ending at the basic level) might have accounted for the limited knowledge on conservation of the medicinal plant species (Abbiw, 1990).

Medicinal plants use in the study area

Medicinal plant species richness (114) recorded by the communities around the Togo Plateau Forest Reserve is within the range recorded by severalsimilar studies. For example, Jeruto et al. (2008) recorded 115 species in the Nandi Forest in Kenya, Okello et al. (2009) recorded 107 species as used by the Sabaot people around Mt. Elgon, Ndegwa (2012) recorded 119 species as used by the Ogiek people in the East Mau forest, and Megersa et al. (2013) also recorded 126 species in WayuTuka District, West Ethiopia. Other studies, however, recorded less than 100 species. For example, e.g., Amri and Kisangau (2012) recorded 82 species in Kimboza Forest Reserve in Morogoro, Tanzania, Ziblim et al. (2013) recorded 47 species in the Northern Region, Ghana whereas Addo-Fordjour et al. (2013) had 52 species in the Aparapi Forest Reserve also in Ghana. The generally high number of medicinal plants species listed in this study confirms the vast knowledge of the peopleon medicinal plants in the area and their applications to treat human conditions.

The herbal preparations made from these medicinal plants were mostly used to treat malaria (51 species), jaundice (33), rheumatism (26), cough (25) and stomach pain (22), among others. This is evidenced in most of the informants citing plant species like *Azadirachta indica, Alstonia boonei, Morinda lucida, Nauclea latifolia*, etc. for the treatment of malaria, consistent with a number of previous studies (Addo-Fordjour et al., 2013; Asase et al., 2005; Jeyaprakash et al., 2011).

The greater contribution of the families Leguminosae, Euphorbiaceae, Apocynaceae and Moraceae to the medicinal plant species in this study confirms many similar studies (Abbiw, 1990; Asase et al., 2005). The more frequent use of trees for medicinal purpose by these communities relative to lianas, herbs and shrubs is also well documented by many studies (Amri and Kisangau, 2012; Jeruto et al., 2008; Megersa et al., 2013; Ndegwa, 2012; Okello et al., 2009).

Leguminosae happened to have most abundant and widely distributed component species on the plateau from

Table 4. Method of preparation and mode of administration of medicinal plants.

Description	Frequency (%)
Method of preparation	
Decoction	196(51)
Pounded	55(14)
Powder	46(12)
Boil	37(10)
Infusion	15(4)
Concoction	12(3)
Crushed/Mashed	10(2)
Balm/Ointment	5(1)
Juice	4(0.9)
Tea	2(0.6)
Tincture	1(0.3)
Poultice	1(0.3)
Mode of administration	
Drink	227(59)
Rub	38(10)
Bath	28(7)
Vapour bath	23(6)
Inhale vapour	20(5)
Drop on eye, ear, nose	19(5)
Sniffed	12(3)
Massage	8(2)
Chewed/Swallowed	6(2)
Sitz-bath	3(1)

the inventory study, probably explaining why the people are more familiar with plants from this family and their uses.

The study communities are very close to the reserve and this makes assessing it for medicinal plants an easy task. This has accounted for most of the plants listed being wild species (Abbiw, 1990; Addo-Fordjour et al., 2013; Datta et al., 2014; Ziblim et al., 2013). This practice of collecting most of the medicinal plants from the reserve by these communities is a threat to the survival of the reserve as it is now visibly seen that the wild stock of the reserve is eroded at the base. The practice is against the recommendations made by WHO (2015) that medicinal plant materials should be cultivated in farmlands and gardens to ensure their continual supply and therefore reduce the pressure on wild species. Countries like China have cultivated between 100–250 species of medicinal plants (Schippmann et al., 2002). In Africa, however, South Africa is the only country with records ofcultivation of medicinal plants, and even that an estimated 99% of the 400–550 species currently sold for use in traditional medicine originate from wild sources and only 1% are cultivated (Kakudidi et al., 2000).

The collection and use of plant barks and roots for medicinal purposes by the communities are not compatible with international conservation policies (Bussmann et al., 2011; Kamatenesi-Mugisha et al., 2000; Kamatenesi-Mugisha and Bukenya-Ziraba, 2002; Ziblim et al., 2013), as such practices can affect the survival of plants or may even remove their gene pool from the population. For that matter, harvesting of these plant parts would require careful monitoring to allow sufficient time for regeneration of the medicinal plants. The low level of education of the informants might be the reason why most of them are not adhering to the basic scientific principles of medicinal plants harvesting in a sustainable way.

Method of preparation and mode ofadministrationof medicinal plants

The use of different preparation methods of medicinal plants (e.g., decoction, pounded, powder, boil, infusion, concoction) and modes of administration (either internally or externally) to cure human conditions agree with many similar studies around forest-fringed communities (Abbiw, 1990).

The *decoction* was obtained by boiling the plant parts in water until the volume of the water reduced to minimum

Table 5. Conservation practices in relation to medicinal plant use in the TPFR area, Ghana.

Description	Frequency (%)
Mode of plant collection	
Only part collected	227(59)
One/Few individuals collected	117(30)
Whole plant	40(11)
Plant harvesting methods	
Destructive	312(81)
Non destructive	72(19)
Plant availability	
Easy to find	138(36)
Difficult to find	209(54)
Very difficult to find	37(10)
Is the reserve necessary?	
Yes	174(45)
No	118(31)
I don't know	92(24)
Can these medicinal plants be conserved?	
No	165(43)
Yes	139(36)
I don't know	80(21)
Any conservation efforts made by Government/Community?	
I don't know	191(50)
Yes	118(31)
No	75(19)

or required amount. The *powder* was prepared by grinding or pounding of the dried plant parts. The *inhalation* was done by burning of plant parts and inhaling the smoke through the nose or mouth. *Poultices* were done by crushing the plant part and mixing with a little hot water and apply directly over the area. *Rubbing* was done by crushing the plant part andmixing with water or processed as ointment/liniment and rubbed on body.
Tincture was prepared by placing the plant part into alcohol and leaving it to steep for a few days in a tightly sealed container. *Infusion* was done by steeping the plant part in cold water/hot water often overnight, the mixture is then strained.

The herbal remedies commonly used by the studied communities including drinking, rubbing, bathing,vapour bathing and inhalation are in keeping with findings of previous studies in Ghana (Abbiw, 1990; Addo-Fordjour et al., 2013; Asase et al., 2005; Ziblim et al., 2013). The frequent citation of malaria as the human conditions mostly treated with the medicinal plants in this study points to the widespread nature of these human conditions in the country and the urgent need to reverse this trend not only in the study area but also in other rural areas.

Medicinal plants and conservation practices

The unsustainable harvesting method employed by the respondents in the study area is a major concern as these activities can cause genetic erosion from the population and a general decline of biodiversity (Balemie et al., 2004; Megersa et al., 2013). Other threats to medicinal plants availability reported by the informants include drought, deforestation and firewood collection. Addressing some of these issues would require the domestication of some medicinal plant species which the indigenous people can easily fall on for their traditional remedies. This will reduce the pressure on the wild species. However, opinion on the need for the reserve by the people of the study area was very high. This is good because such reserves provide numerous benefits to

man, including the maintenance of global and regional temperatures within appreciable range through the sequestration of carbon, protection of endangered species by serving as habitats, provision of food, supply of oxygen, etc. (Akerele et al., 1991; Balemie et al., 2004; Bodeker, 2002; Bussmann et al., 2011; Kamatenesi-Mugisha et al., 2000; Kamatenesi-Mugisha and Bukenya-Ziraba, 2002; Megersa et al., 2013).

The negative view of conservation of the reserve by some of the informants, citing restricted access to forest products, which serve as the source of their livelihood sustainance and restricted access to medicinal plants leading to loss of traditional botanical/medicinal knowledge is quite a serious issue (Akerele et al., 1991; Balemie et al., 2004; Bodeker, 2002; Kamatenesi-Mugisha et al., 2000; Megersa et al., 2013).

In this regard, the establishment of by-laws by the Ghana Wildlife Society to regulate collection of medicinal plants from the reserve is a step in the right direction. There should be conscious effort by both the government and the community towards the conservation of biodiversity of the plateau.

Conclusion

The study results demonstrated a rich diversity of medicinal plants used to treat a variety of ailments by communities around the Togo Plateau Forest Reserve. Leguminosae is the most represented medicinal plant family in the study area. This is evident from the floristic inventory, which recorded most of the tree species from this family on the landscape. The component species should therefore be targetedand managed sustainably. Crucially, most of the medicinal plants were collected from the reserve, exposing it to the danger of deforestation. Domestication (cultivation of medicinal plants in farmlands and gardens) therefore is the obvious management choice as it could help reduce the pressure on the wild species stock (Bodeker, 2002). The local people have very little knowledge on conservation issues; hence there is an urgent need to include them in resources utilization and management of the reserve. Also there is the need for workshops to be organised periodically by resource persons from either the Ghana Federation of Traditional Medicine Practitioners' Association or Ghana Forestry Services Division to educate these plant poachers on appropriate conservation measures for plant exploitation.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Evaluation of cytotoxic and antitumor activity of perillaldehyde 1,2-epoxide

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The study aimed to evaluate cytotoxicity and antitumor activity of perillaldehyde 1,2-epoxide (PE), a *p*-menthane monoterpene derivative against four human tumor cell lines ovarian cancer (OVCAR-8), colon carcinoma (HCT-116), glioblastoma (SF-295) and leukemia (HL-60) using the colorimetric MTT assay. PE showed a high degree of inhibition of cell proliferation (GI = 95.66 to 99.71%) and IC_{50} 16.14 µM (± 1.86), 23.61 µM (± 1.13), 21.99 µM (± 2.64) and, 9.70 µM (± 1.01) against tumor cells, respectively. Then, *in vivo* antitumor activity of the PE was assessed in sarcoma 180-bearing mice. Tumor growth inhibition rates were 33.4, 56.4 and 66.6% at doses of 100 and 200 mg/kg/day for the PE and 25 mg/kg/day for 5-FU intraperitoneal treatments, respectively. Toxicological effects related to the spleen, kidneys, liver, and hematological were investigated in mice submitted to treatment. Furthermore, histopathological analyses of these organs were absent of any morphological changes in the animals treated with PE. The viability of HL-60 cells was affected by perillaldehyde 1, 2-epoxide after an exposure period of 72 h when analyzed by trypan blue exclusion. PE reduced the number of viable cells associated with an increase in non-viable cells, which contributes to the increased number of dead cells in the morphological analysis. The incorporation of ethidium bromide/acridine orange, the treated cells suggests cytotoxicity via apoptosis and necrosis. So on the results, we conclude that PE presents cytotoxic and antitumoral activity through apoptotic and necrotic processes.

Key words: Essential oils, *p*-menthanes, natural products, cytotoxicity, antitumor activity, sarcoma 180.

INTRODUCTION

Cancer is the second most common cause of death in the world, surpassed only by cardiovascular disease

and is also known as malignant tumor characterized by abnormal growth and proliferation of cells (Amaral et al., 2015; Polu et al., 2015). It is a frightful disease because the patient suffers pain, disfigurement, and loss of many physiological processes. Cancer may be uncontrollable and incurable and may occur at any time at any age in any part of the body (Umadevi et al., 2013).

Natural products, especially constituents of essential oils from medicinal plants have been successful in treating various disorders in traditional medicine (Chinta et al., 2015; Sousa, 2015). Compounds of natural origin have provided new and potential leads to cancer chemotherapy, and many of them are the drug of choice in cancer treatment (Polu et al., 2015). Natural source products have offered many useful anticancer agents in current use, such as the plant-derived drugs vinblastine, irinotecan, topotecan, etoposide, and paclitaxel (Bhanot et al., 2011; Qurishi et al., 2011).

Sources are still available in abundance and offer the best possibilities of finding substances of therapeutic interest (Butler, 2008). Although there are several cancer therapies, an ideal anticancer drug has not been discovered, and numerous side effects limit treatment. However, research into new drugs has revealed a variety of new chemical structures and potent biological activities that are of interest in the context of cancer treatment (Carvalho et al., 2015).

In fact, several compounds derived from natural products are in phase clinical trials mainly for cancer treatment, such as the monoterpene *S*-(-)-perillyl alcohol which shows cytotoxic and antitumor activity in various experimental models (Andrade et al., 2015; Andrade et al., 2016). Perillyl alcohol, a naturally occurring monoterpene found in essential oils of peppermint and lavender has been widely researched it is useful agents against a variety of human tumor cell lines (Andrade et al., 2015; Chen et al., 2015; Garcia et al., 2015).

A study performed by Andrade and collaborators (Andrade et al., 2015) was demonstrated the cytotoxicity of seventeen analogous compounds of perillyl alcohol having featured (-)-8,9-perillaldehyde epoxide, (-)-perillaldehyde, (+)-limonene 1,2-epoxide and, (-)-8-hydroxycarvotanacetone.

In this context, the development of new derived compounds from natural plants and their analogues for anticancer and antitumor activities, and for this, the significant challenge is to synthesize, isolate and characterize novel derivatives based on bioactivity and mechanisms of action of these compounds. However, there is a continuing need for research and development of new anticancer drugs, drug combinations and chemotherapy strategies, by methodical and scientific exploration of an enormous pool of synthetic, biological and natural products (Umadevi et al., 2013). Therefore, considering the perillyl alcohol anticancer bioactivity, this study aimed to evaluate its chemical analogue, perillaldehyde 1,2-epoxide, on antitumor activity, toxicology effects and cytotoxicity mechanism (Figure 1).

MATERIALS AND METHODS

Preparation of perillaldehyde 1,2-epoxide

A solution of perylaldehyde (4.5 g; 30 mmols) in MeOH (60 ml) was added to a solution of H_2O_2 (30%, 15.3 ml, 150 mmols). Then an aqueous solution KOH 6 mol/L (5.0 ml, 30 mmol) was added slowly, drop by drop, and kept at 0°C (ice bath), as described by Tantanak and collaborators (Tantanak et al., 1998).

The reaction medium remained under stirring for 4 h at the same temperature. Subsequently, it was removed from the ice bath, and an aqueous phase was extracted with CH_2Cl_2 (50 ml), and the combined organic phases washed twice with distilled water (50 ml) and water stripped with anhydrous Na_2SO_4. Then the material obtained was concentrated on a rotary evaporator, and the product purified by silica gel column chromatography (hexane/ethyl acetate 9:1). Perillaldehyde 1,2-epoxide was obtained with a 71.6% (4.77 mmol) yield. Perillaldehyde 1,2-epoxide is a *p*-menthane cyclic compound that contains an epoxide ring. It has a molecular formula of $C_{10}H_{14}O_2$, molar volume of 143.1 ± 3.0 cm^3/mol, surface tension of 29.6 ± 3.0 dyn/cm, and density of 1.161 ± 0.06 g/cm^3.

The compound perillaldehyde 1,2-epoxide was analyzed by infrared, ^1H and ^{13}C NMR. The ^1H- and ^{13}C-NMR measurements were obtained with a Mercury-Varian spectrometer (Palo Alto, CA, USA) operating at 200 MHz (for ^1H), and 50 MHz (for ^{13}C). The infrared spectra were recorded on a Bomen Michelson model 102 FTIR (Bomen, Chicago, IL, USA) and the most intense or representative bands reported (in cm^{-1}). IR (KBr) ν_{max}: 3020, 2980, 1725, 1670, 900 cm^{-1}; ^1H-NMR ($CDCl_3$): δ 8.83 (s, 1H); 4,68 (d, J= 12,4 Hz, 2H); 3.52 - 3.38 (m, 1H); 2.72 - 1.68 (m, 7H); 1.67 (s, 3H); ^{13}C-NMR ($CDCl_3$): δ 199.1, 148.0, 109.6, 63.5, 57.3, 36.8, 29.9, 25.2, 20.7, 19.5 (CAS 90926-06-0).

Evaluation of the cytotoxic effect of perillaldehyde 1,2-epoxide human cancer cell lines

Cell lines and MTT assay

For cytotoxicity assays, four human cancer cell lines HCT-116(colon carcinoma), OVCAR-8 (ovarian adenocarcinoma), SF-295 (glioblastoma), and HL-60 (Promyelocytic leukemia) were acquired from National Cancer Institute, Bethesda, MD, USA and evaluated for monoterpene perillaldehyde 1,2-epoxide. Cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 µg/ml streptomycin, and 100 U/ml penicillin, and incubated at 37°C in a 5% CO_2 atmosphere.The MTT method, cytotoxic described by Mossman (1983), was used to evaluate the activity of the monoterpene perillaldehyde 1,2-epoxide against four human cancer cell lines. For the experiments, the cells were

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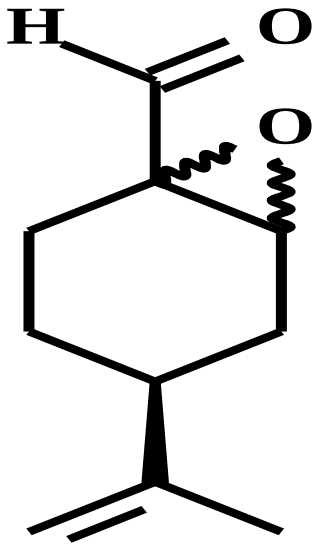

Figure 1. Chemical structure of perillaldehyde 1,2-epoxide.

placed in 96-well plates (0.1×10^6 cells/ml in 100 µl medium). After 3 days(72 h) of incubation, the perillaldehyde 1,2-epoxide dissolved in dimethyl sulfoxide (DMSO 0.7%), at a final concentration of 10.60 µM, was added to each well and incubated for 3 days (72 h) at 37°C in a 5% CO_2 atmosphere (three independent experiments, performed in triplicate).

DMSO at 1% was used as negative control and doxorubicin at 0.55 µM was used as positive control (purity > 98%; Sigma Chemical Co., St. Louis, MO, USA). At the end of incubation, the plates were centrifuged at 5000 rpm for 10 min, and the supernatants were removed. A 150 µl of an aqueous MTT solution containing 0.5 mg/ml MTT was added to each well and incubated for three hours at 37°C in a 5% CO_2 atmosphere. Cell viability was measured by the ability of cells valves to reduce the yellow dye 3-(4,5- dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT; Sigma Chemical Co., St. Louis, MO, USA) to a purple formazan product.

After incubation, the precipitate was dissolved in 150 µl DMSO, and absorbance 595nm was measured using a multiplate reader (DTX 880 Multimode Detector, Beckman Coulter Inc.). The absorbance values of these tests will be expressed as cell growth inhibition percentage (GI%) by the following formula: [GI% = 100 − [(T/NC) × 100%]. NC is the Absorbance of the negative control, and T is the Absorbance of test compound (perillaldehyde 1,2-epoxide).

The median inhibitory concentration able to induce 50% of maximal effect (IC_{50}) of the perillaldehyde 1,2-epoxide was determined after evaluation of GI%. All cells were retested using the same protocol above with a varying concentration of compound (0 to 150.60 µM) (dos Santos Júnior et al., 2010; Ribeiro et al., 2012).

Trypan blue dye exclusion assay

The human cancer cell line HL-60 was used at a concentration of 0.3×10^6 cells/ml, incubated for 3 days (72 h) with perillaldehyde 1,2-epoxide (4.85, 9.70 and 19.40 µM) and examined on an inverted microscope. At the end of incubation 90 µl was withdrawn from the cell suspension and added to 10 µl of trypan blue. The cells were differentiated into viable and non-viable and counted in a Neubauer chamber. A Doxorrubicin 0.55 µM was used as positive control (Veras et al., 2004).

Elucidation of cell death

Morphological analyses using a fluorescence microscope

The human cancer cell line HL-60 was used at a 0.3×10^6 cells/ml, incubated for 1 day (72 h) with perillaldehyde 1,2-epoxide (4.85, 9.70 and 19.40 µM). The cell suspension was transferred to an eppendorf tube and centrifuged for 5 min at low speed (145 × g). The supernatant was discarded, and the cells were resuspended in 20 µl of PBS solution. Then 1 µl of aqueous acridine orange/ethidium bromide solution (AO/EB, 100 µg/ml) was added to each tube, and an aliquot of these cells transferred to a slide and mounted with a coverslip and then brought to a fluorescence microscope for observation of cellular events (apoptosis and necrosis). Doxorubicin (0.55 µM) was used as positive control (Geng et al., 2005).

Morphological analysis using an optical microscope

The HL-60 cell line, plated at a concentration of 0.3×10^6 cells/ml, was incubated for 72 h with the compound (4.85, 9.70 and 19.40 µM) and examined under a microscope inverted. After that, 50 µl of cell suspension was added to the slide of the centrifuge (Cytospin™), to observe the morphology of the treated cells. After cell adhesion to the blade, fixing was done with methanol for 1 min and was first used hematoxylin staining, followed by eosin. The morphological changes were observed under optical microscope. Doxorubicin (0.55 µM) was used as positive control (Veras et al., 2004).

Hemolytic assay

To evaluate hemolytic activity, blood was collected from three mice of Swiss orbital path (anesthetized with isoflurane 1.5%) and diluted 1:30 in saline (0.85% NaCl + 10 mM $CaCl_2$). The erythrocytes were washed two times in saline by centrifugation (15 g / 3 min.), resuspended in saline to obtain a suspension of erythrocytes 2% and the assay was performed in a 96-well plate (Jimenez et al., 2003). The single concentration of compound (500 µg/ml) was added to the suspension of red blood cells (Bezerra et al., 2005; Kang et al., 2009; Pita et al., 2012). Mixtures were incubated on a mixer for 60 min and then centrifuged at 875 × g for 5 min. Triton X-100 (1%) was used as the positive control. The absorbance of the supernatants was determined at 540 nm using spectrophotometrically. Triton X-100 (1%) was used as the positive control.

Evaluation of *in vivo* antitumor activity and toxicological analyses

In vivo antitumor activity assay

To evaluate the *in vivo* antitumor activity, 60 male mice, weighing 26–31 g were used; they were purchased from bioterium of the Federal University of Sergipe, Brazil. The animals were maintained under laboratory conditions of temperature, humidity, and light with food and water *ad libitum*. The experimental protocol was submitted and approved by the Animal Care and Use Committee at the Federal University of Sergipe (CEPA: 16/2014). Ten-day-old sarcoma 180 ascites tumor cells (2 x 10^6 cell / 500 µl) were implanted subcutaneously into the left axillary region of the experimental mice (Bezerra et al., 2006). The animals were divided into 6 groups of 10 animals in polypropylene cages. One day after inoculation, was administered intraperitoneally in group one 5% DMSO (negative control-NC), groups two, three, four and five perillaldehyde 1,2-epoxide as doses de 25, 50, 100, 200 mg/kg/day respectively dissolved in 5% DMSO (test compounds) and group six 5-fluorouracil (5-FU, purity > 99%; Sigma Chemical Co.) 25 mg/kg/day (positive control). 72 h after the last day of treatment under isoflurane inhalation (1.5%, calibrated vaporizer) anaesthesia, peripheral blood samples were collected from the retro-orbital plexus (toxicological analyses), the animals were euthanized by cervical dislocation, and the tumors, livers, spleens, and kidneys were excised and weighed. Inhibition of tumor growth (%) was expressed by the following equation (Bezerra et al., 2006):

$$(\%) = [(NC - TC) / NC] \times 100$$

NC = mean tumor weight of the negative control group
TC = mean tumor weight of the test compound treated group

Systemic toxicological analyses

Control groups (group one and six) and the groups that showed antitumor activity *in vivo* against sarcoma 180 were submitted to an evaluation by six toxicological parameters: variation in body mass, organ weights, liver, renal and hematologic parameters and histopathological analyses. First, the mice were weighed at the beginning and end of the experiment, and the animals were observed for signs of abnormalities throughout the study. Second, the livers, kidneys, and spleens were removed and weighed after euthanasia. Third and fourth parameters using obtained plasma peripheral blood samples of the mice and Clinical Chemistry® kits (Abbott; Architect C 8000) were evaluated liver function measured by aspartate aminotransferase (AST) and alanine aminotransferase (ALT), and renal function measured by urea and creatinine. The fifth parameter was hematological analysis, an aliquot of blood from each animal was placed in EDTA, and total leucocyte counts were determined by standard manual procedures using optical microscopy. The last parameter was performed after 10% formaldehyde fixation, the spleens, liver, and kidneys were dehydrated in alcohol, diaphanized in xylene and paraffin-embedded. Subsequently, 5-µm-thick histological sections were obtained and stained with hematoxylin and eosin. Histological analyses were performed under optical microscopy (Amaral et al., 2016; Dória et al., 2016). The mice were weighed at the beginning and end of the experiment, and they were observed for signs of abnormalities throughout the study. At the end of the investigation, the animals were anesthetized with ether anesthesia, and peripheral blood samples by retro-orbital plexus were collected.

For hematological analysis, an aliquot of blood from each animal was placed in EDTA, and total leucocyte counts were determined by standard manual procedures using optical microscopy. Serum samples were obtained to evaluate liver function (aspartate aminotransferase (AST) and alanine aminotransferase (ALT)), and renal function (urea and creatinine) using Clinical Chemistry® kits (Abbott; Architect C 8000). Then, the animals were sacrificed in a CO_2 chamber, and the spleens, liver, and kidneys were weighted and fixed with 10% formaldehyde. The organs were dehydrated in alcohol, diaphanized in xylene and paraffin-embedded. Subsequently, 5-µm-thick histological sections were obtained and stained with hematoxylin and eosin. Histological analyses were performed under optical microscopy (Amaral et al., 2016; Dória et al., 2016).

Statistical analysis

The results were expressed as the mean ± SEM, and the differences between the experimental groups were analyzed using ANOVA of unidirectional analysis of variance followed by the *Student Newman-Keuls* test. Significant values of $p < 0.05$ were considered. All statistical analyses were performed using GraphPad program® (Intuitive Software for Science, San Diego, CA, USA).

RESULTS AND DISCUSSION

Cytotoxic effect of perillaldehyde 1,2-epoxide tumor cell in culture

MTT assay

Compounds of natural origin have provided new and potential leads to cancer chemotherapy, and many of them are the drug of choice in cancer treatment (Prakash et al., 2013). Natural products are important sources of chemical structures, and these will be used as templates for construction of new compounds with improved biological properties (Mann, 2002). Following this trend, the perillaldehyde 1,2-epoxide from the evaluation of the cytotoxicity of structurally correlated *p*-menthane

Table 1. Cell growth inhibition percentage (GI %) and values concentration able to inhibit 50% of cell growth (IC_{50}) of monoterpene perillaldehyde 1,2-epoxide against four tumor cell lines.

Cells line	Histotype		Perillaldehyde 1,2-epoxide	Doxorubicin
HCT-116	Colon carcinoma	GI %	99.46 ± 1.54	99.24 ± 0.15
		IC_{50} µM	16.14 ± 1,86	0.02 ± 0.02
OVCAR-8	Ovarian adenocarcinoma	GI %	99.37 ± 0.30	100 ± 0.63
		IC_{50} µM	23.61 ± 1.13	1.95 ± 0.64
SF-295	Glioblastoma	GI %	95.66 ± 5.06	99.57 ± 0.31
		IC_{50} µM	21.99 ± 2.64	0.44 ± 0.11
HL-60	Promyelocytic leukemia	GI %	99.71 ± 2.43	100 ± 0.26
		IC_{50} µM	9.70 ± 1.01	0.04 ± 0.02

Data are presented as GI values in % and IC_{50} values in µM with their mean ± SEM. The data were collected in from three independent experiments, performed in triplicate and measured by the MTT assay after 72 hours of incubation. Doxorubicin was used as positive control.

derivatives described by Andrade and collaborators was developed (Andrade et al., 2015).

Following the criteria of American National Cancer Institute to discovering new anticancer drugs, were selected four human cancer cell lines to investigate cytotoxicity of perillaldehyde 1,2-epoxide: colon carcinoma (HCT-116), ovarian adenocarcinoma (OVCAR-8), glioblastoma (SF-295) and promyelocytic leucemia (HL-60) (dos Santos Júnior et al., 2010; Ribeiro et al., 2012). The compound was first used in a single concentration of 150.60 µM and evaluated according to the cell growth inhibition percentage (GI%) as: without cytotoxicity, low cytotoxicity (GI 1 - 50%), moderate cytotoxicity (GI 51 – 75%) and high cytotoxicity (GI > 75%) (Mahmoud et al., 2011). The GI% values are presented as the mean ± SD of three replicates measured by MTT assay after 72 h of incubation. The results showed that the compound perillaldehyde 1,2-epoxide demonstred high cytotoxicity activity, with GI > 95% for four lines human tumor cells used (Table 1).

Based on a similar study performed by Andrade et al. (2015), with 18 derivative compounds of perillyl alcohol, compound perillaldehyde 8,9-epoxide was the *p*-menthane derivative with the highest cytotoxic activity for the human cancer cell lines HCT-116, OVCAR-8, and SF-295. Comparing the perillaldehyde 1,2-epoxide with the perillaldehyde 8,9-epoxide, position isomers with high cytotoxicity *in vitro*, both substances have a skeleton *p*-menthane containing an aldehyde group and an epoxide group in its chemical structure. The replacement of the hydroxyl group with the aldehyde group and adding the epoxide group the structure of perillyl alcohol (GI = 95.82, 91.68, 90, and 92%, respectively) resulted in an increasein GI% perillaldehyde 1,2-epoxide (GI = 99.46, 99.37 and 95.66%, respectively) and perillaldehyde 8,9-epoxide (GI = 98.64, 96.32 and 99.89%, respectively). This result suggests that the presence of these two functional groups in the compounds may be contributing to this higher biological effect.

Thus, the compound in this study was promoted for the determination of median inhibitory concentration able to produce 50% of maximal effect (CI_{50}) to verify the potency of the compound. For this, were used the same cell lines and measurement methods used to determine the GI%, varying only the concentration of compound between 0 to 150.60 µM. The perillaldehyde 1,2-epoxide exhibits values IC_{50} in the range of 9.70 to 21.99 µM in the HCT-116 and SF-295 cell lines, respectively. Doxorubicin, used as the positive control, showed IC_{50} values ranging from 0.02 to 1.95 µM for HCT-116 and OVCAR-8 cell lines, respectively (Table 1). For this test, considered as a promising candidate for antineoplastic activity, substances that present lower IC_{50} values or equal to 24.10 µM (Suffness and Pezzuto, 1990). Therefore, the compound perillaldehyde 1,2-epoxide is a potential candidate for anticancer activity and eligible for progression of studies.

Trypan blue dye exclusion assay

The perillaldehyde 1,2-epoxide showed higher cytotoxicity front leukemic cell line HL-60 (GI=99.71%±2.43). Faced with this result, three concentrations of perillaldehyde 1,2-epoxide, ½ IC_{50} (4.85 µM), IC_{50} (9.70 µM) and 2 × IC_{50} (19.40 µM) were chosen

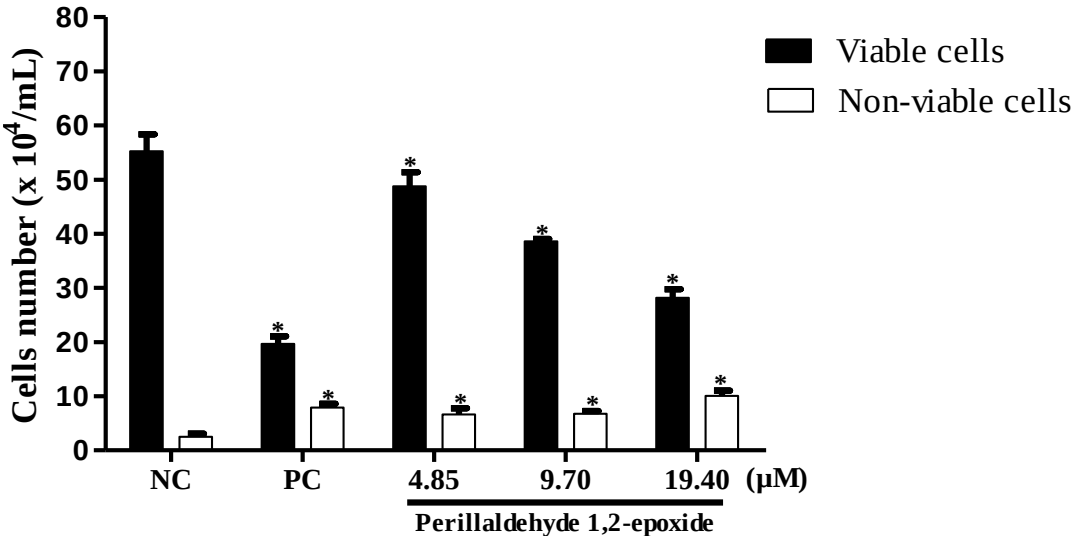

Figure 2. Effect of perilladehyde 1,2-epoxide on leukemic (HL-60) cell viability measured by the trypan blue dye exclusion after 72 h of incubation. The negative control (NC) was treated with 0.1% DMSO used for diluting the test compound. Doxorubicin, 0.55 µM was used as the positive control (PC). Data are presented as mean values ± S.E.M. of two independent experiments performed in duplicate. * $p < 0.05$ compared with the negative control by ANOVA followed by *Student-Newman-Keuls* test.

against HL-60 for evaluation of antiproliferative effects. The analysis of cell viability in HL-60 leukemic cell lines was performed by trypan blue exclusion after 72 h of exposure. Trypan blue exclusion test allows separately quantify the viable cells of cells killed by the test compound (Barros et al., 2013). Perilladehyde 1,2-epoxide caused a significant reduction in the number of viable cells ($p < 0.05$) and increase in the number of non-viable cells ($p < 0.05$) at the concentrations of 4.85, 9.70 and 19.40 µM (Figure 2). Studies have shown that compounds can interact with MTT, inhibiting the reduction of MTT and may produce a false positive result. Therefore, the use of other viability assays, such as trypan blue exclusion is indicated before progression of the studies (Trevisi et al., 2006; Pita et al., 2012). Then, we confirm the cytotoxic activity of perilladehyde 1,2-epoxide front HL-60 by two methods (MTT and Trypan blue exclusion).

Elucidation of cell death

Morphological analyses using a fluorescence and light microscopy

For the identification of the cell death process induced by perilladehyde 1,2-epoxide against HL-60 two assays, acridine orange/ethidium bromide was used and analysed by fluorescence microscopy and optical microscopy using hematoxylin-eosin coloration. After 72 h of incubation of the cells with compound at concentrations of 4.85, 9.70 and 19.40 µM and acridine orange/ethidium bromide stained, was observed as a result of a reduction in the number of viable cells and increase of cell death by apoptosis statistically significant ($p < 0.05$). This effect was observed when compared to the negative control group at all three concentrations of the compound tested (Figure 3).

In the morphological analysis by hematoxylin-eosin coloration of treated cells with perilladehyde 1,2-epoxide, in all concentrations tested there were signs of death by apoptosis as was also observed in the test with acridine orange/ethidium bromide (Figure 4). Other compounds structurally similar to the perilladehyde 1,2-epoxide as carvone (Aydın et al., 2015), carvacrol (Jaafari et al., 2009), limoneno (Sahin et al., 1999), perillyl alcohol (Clark, 2006) and perillic acid (Yeruva et al., 2007) also induce cell death by apoptosis in tumor cell lines. Cell death processes have well defined morphologic characteristics. Apoptosis includes cells with pyknotic appearance, condensation of chromatin, the fragmentation of the nucleus and the shedding of apoptotic bodies, vacuoles containing cytoplasm and intact organelles (Nikoletopoulou et al., 2013). Apoptosis is the cell death mechanism better known, used as an essential target for cancer therapy, by anticancer drugs such as the colcichine, taxanes and vinca alkaloids (Su et al., 2013; Topham and Taylor, 2013).

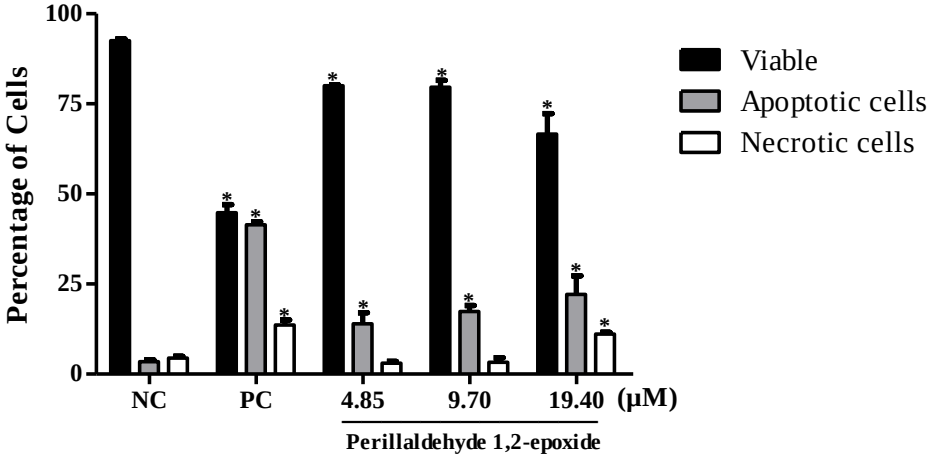

Figure 3. Identification of cell death induced by perilladehyde 1,2-epoxide in cancer cell line (HL-60), using acridine orange/ethidium bromide after one day (72 h) of incubation. Viable cells were identified with a black bar, apoptotic cells were detected with gray bar, and necrosis cells were exposed with a white bar. The negative control (NC) was treated with 0.1% DMSO used for diluting the test compound. Doxorubicin, 0.55 µM was used as the positive control (PC). Data are presented as the mean values ± standard error of the mean of three independent experiments performed in duplicate.

When concentration of 19.40 µM of perilladehyde 1,2-epoxide was used, concomitantly to cell death by apoptosis, was observed cell death by necrosis in tests performed with acridine orange/ethidium bromide and hematoxylin-eosin (Figures 3 and 4). Several treatments for malignant neoplasms can induce cell death by activation of necrosis to include photodynamic treatment (PDT), by the alkylation of agents harmful to DNA, and several other chemical compounds or substances such as apoptolidine, β-lapachone and honokil also appear to promote the death of cancer cells by necrosis (Zong and Thompson, 2006). Morphologically, the necrotic cells are characterized by the swelling of organelles, rupture of the plasma membrane (cell lysis), the nucleus becomes distended (nucleus intact) and usually followed by inflammatory reactions (Nikoletopoulou et al., 2013). Necrosis appears to be a limiting factor for increasing the concentration of the compound, but additional testing with higher levels has to be performed for more conclusions that are accurate.

Thus, the compound perilladehyde 1,2-epoxide can be considered a promising candidate for evaluation *in vivo* antitumor activity, by present cytotoxic activity predominant cell death by apoptosis, being subject to necrotic cell death at the highest concentration.

Evaluation of hemolytic activity

The evaluation of the hemolytic effect of perillaldehyde 1,2-epoxide was performed using erythrocytes Swiss mice, according to the methodology described by Costa-Lotufo and collaborators (Costa-Lotufo et al., 2002). This technique allows evaluating the potential of test compound in causing damage to the plasma membrane of the cell, either by the formation of pores or by the full rupture of it. However, this assay, we observed the absence of hemolytic activity in the tested concentration 500 µg/ml (Data not presented). These results suggest a possible selectivity for perillaldehyde 1,2-epoxide and enabling progression of studies related to the anticancer activity.

Antitumor activity *in vivo* and toxicological analyses

Tumor Sarcoma 180

Being the perillaldehyde 1, 2-epoxide cytotoxic against four human cancer cell lines (IC_{50} < 4,0 µg/ml) and having a predominant cell death of apoptosis, we decided to investigate the possible *in vivo* antitumor activity of the test compound. Based on the use of experimental tumors for the identification of new products with potential anticancer, the antitumor activity of perillaldehyde 8,9-epoxide was performed using sarcoma 180 tumors (original Swiss mice tumor, transplantable, and well-characterized experimental model) and treated by intraperitoneal route once a day for seven consecutive days (Britto et al., 2012; Yang et al., 2016).

The activity of perillaldehyde 1,2-epoxide on animals transplanted with sarcoma 180 tumor cells is presented in

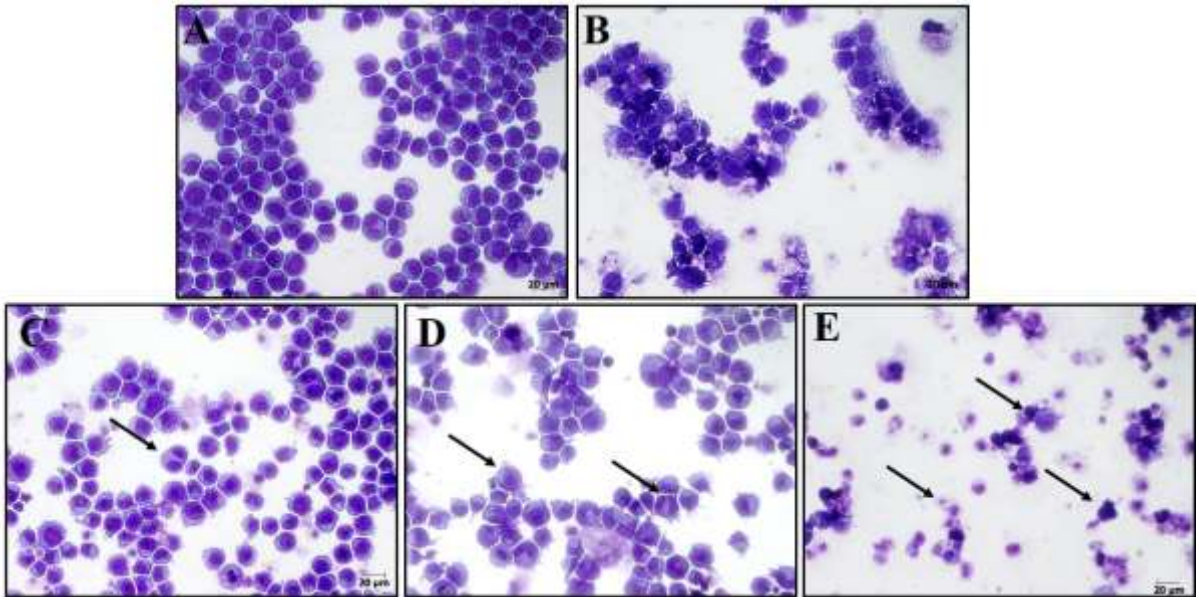

Figure 4. Morphological analyses of the effect of perilladehyde 1,2-epoxide on HL-60 cancer cell line after 72 h of incubation, using optical microscopy. Figure A (negative control) was treated with the vehicle (0.1% DMSO), Figure B (positive control) was treated with doxorubicin (0.55 µM) and Figure C, D, and E were treated with perilladehyde 1,2-epoxide in concentrations of 0.8, 1.6, 3.2 µg/ml respectively. Black arrows show a reduction of cell volume; the nucleus becomes distended, nuclear fragmentation and cellular debris.

Figure 5. The mean tumor mass was 1.62 ± 0.05 g, 1.48 ± 0.07 g, 1.22 ± 0.16 g and 0.83 ± 0.15 g for animals treated with compost test at doses of 25, 50, 100, and 200 mg/kg/day, respectively**.** The NC group presented mean tumor mass of 1.76 ± 0.08 g. Statistically significant alterations ($p < 0.05$) were observed in mice treated with perillaldehyde 1,2-epoxide at concentrations of 100 and 200 mg/kg/day when compared with NC group, with tumor mass growth inhibition rates of 33.44 and 56.39%, respectively. The test compound at doses of 25 and 50 mg/kg/day showed no statistically significant alterations ($p > 0.05$) compared with NC group. The PC group presented tumor mass growth inhibition rates of 66.38% and mean tumor mass of 0.51 ± 0.06 g, statistically significant when compared with NC group.

When comparing the PC group to the groups treated with test compound, statistical alterations were observed for the groups 25, 50 and 100 mg/kg/day ($p < 0.05$) and no statistically significant changes for the group 200 mg/kg/day ($p > 0.05$). These results indicate that dose of 100 and 200 mg/kg/day the perillaldehyde 1,2-epoxide has antitumor activity *in vivo* against the sarcoma 180 with more significant action at the highest dose.

Other monoterpenes *p*-menthane as perillaldehyde 8,9-epoxide and perillyl alcohol were also submitted to evaluation of antitumor activity *in vivo* using the same experimental tumor model. Both compounds were tested at doses of 100 and 200 mg/kg/day, demonstrating tumor mass growth inhibition rates of 38.4 and 58.7% for the perillaldehyde 8,9-epoxide and 35.3 and 45.4% for the perillyl alcohol, respectively (Andrade et al., 2016). Compared to the data obtained in this study with those obtained by Andrade and collaborators (Andrade et al., 2016) it was observed proximity between tumor mass growth inhibition rates and therefore a similar antitumor *in vivo* potency. The alcohol perillyl has been studied quite a long time to be able to inhibit the growth of tumor cells in cell culture and exert cancer preventive and therapeutic activity in a variety of animal tumor models.

Furthermore, the perillyl alcohol has been successfully used intranasally in the treatment of patients with malignant brain tumors (Chen et al., 2015). Thus, the results so far found with perillaldehyde 1,2-epoxide are promising for progression of studies.

Toxicological analyses

It is known that most of the currently used anticancer drugs are cytotoxic to tumor cells, but they also have non-specific action, because they affect healthy cells, leading to an undesirable side effect (Sun and Peng, 2008). Therefore we decided to evaluate toxicological characteristics of mice with tumor sarcoma 180

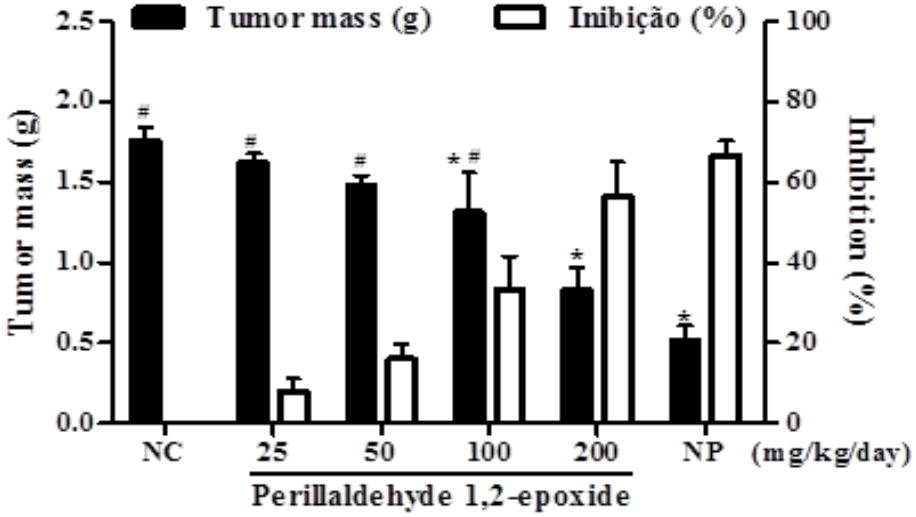

Figure 5. Investigation of antitumor activity of perillaldehyde 1,2-epoxide (25, 50, 100, and 200 mg/kg/day, i.p.) in mice transplanted with experimental tumor sarcoma 180**.** Tumor mass (g) was identified with black bars and inhibition (%) of tumor growth with white bars. The negative control (NC) was treated with 5%, i.p. (diluiting the test compound). The positive control (PC) was administered with 5-Fluorouracil at 25 mg/kg/day, i.p. Data are presented as a mean ± standard error of the mean (*n* = 10 mice/group). *p < 0.05 compared with the NC group, and #p < 0.05 compared with the PC group by variance analysis, followed by the *Student-Newman-Keuls* test.

undergoing treatment with perillaldehyde 1,2-epoxide at doses of 100 and 200 mg/kg/day, to assess the cost and benefits of intervention. For this, some toxicological parameters were evaluated as variation in body mass, organ weights (liver, spleen, and kidney), liver (AST and ALT), renal (urea and creatinine), hematologic (total leukocytes) parameters and histopathological analyses (Dória et al*.*, 2016).

There was no significant change (p > 0.05) in organ weights (liver, spleen and kidney), liver (AST and ALT) and renal (urea and creatinine) parameters in the groups treated with perillaldehyde 1,2-epoxide at doses of 100 and 200 mg/kg/day when compared with NC group (Table 2). These data are significant since many anticancer drugs cause changes in renal function, liver function and spleen organ volume as a result of the lack of specificity of antineoplastic, inducing severe adverse effects (Bezerra et al*.*, 2008; Amaral et al*.*, 2016).

When analyzing the variation in body mass and total leukocyte count, a significant reduction in body mass (p < 0.05) and a decrease in total leukocytes were observed in the groups treated with perillaldehyde 1,2-epoxide at one dose of 200 mg/kg/day compared to NC group. The group administered with compost test at a dose of 100 mg/kg/day no significant change (p > 0.05) the mass variation but significant decrease in total leukocytes (p < 0.05). These data can be considered a limiting factor for the administration of higher doses of test compound but does not prevent the advancement of studies because adverse effects such as body weight loss and leukopenia are common in some marketed antineoplastics (Cao et al*.*, 1998; Zamagni et al*.*, 1998). An example of these adverse effects is observed in the use of 5-FU 25 mg/kg/day as a positive control in the present paper, where a significant reduction (p < 0.05) in the variation of body weight, spleen weight and some leukocytes was checked.

Histopathology is a test used to aid in data biochemical and microscopic analysis, detecting any morphological change or characteristic lesion in the evaluated tissues. Histopathological examination was performed on organs, liver, spleen, and kidney of animals with sarcoma 180 and all tissue structures are substantially preserved in treated groups with perillaldehyde 1,2-epoxide at a dose of 100 and 200 mg/kg/day (Data not presented)**.**

Conclusion

Therefore, the present study indicates that the perillaldehyde 1,2-epoxide exhibits high cytotoxic activity against the human cancer cell lines HCT-116, OVCAR-8, SF-295 and HL-60 with the possible predominant process of cell death by apoptosis and *in vivo* antitumor effects

Table 2. Toxicological parameters of the evaluation of the monoterpene perillaldehyde 1,2-epoxide.

Parameter	Treatments (mg/kg/day)			
	DMSO	5-FU	Perillaldehyde 1,2-epoxide	
	5%	25	100	200
Body weight (g)	1.10 ± 0.27	-3.60 ± 0.84*****	1.40 ± 0.87	-1.20 ± 0.40*
Liver (g/100g body weight)	4.86 ± 0.08	4.63 ± 0.12	4.64 ± 0.10	4.89 ± 0.23
Spleen (g/100g body weight)	0.51 ± 0.02	0.34 ± 0.06*****	0.45 ± 0.02	0.48 ± 0.03
Kidney (g/100g body weight)	1.10 ± 0.04	1.13 ± 0.03	1.07 ± 0.03	1.13 ± 0.02
AST (U/L)	262.31 ± 20.62	227.63 ± 25.64	240.02 ± 13.81	248.00 ± 13.33
ALT (U/L)	58.00 ± 2.77	51.60 ± 2.48	47.00 ± 1.87	54.40 ± 3.50
Urea (mg/dL)	56.60 ± 3.72	44.40 ± 3.34	45.20 ± 2.65	45.50 ± 5.33
Creatinine (mg/dL)	0.38 ± 0.01	0.37 ± 0.01	0.35 ± 0.01	0.37 ± 0.03
Total leukocytes (x10^3 cells/µl)	11.45 ± 0.21	2.50 ± 0.31*****	6.60 ± 0.73*****	8.00 ± 1.01*****

Data are presented as mean values ± SEM, n = 10 animals/group with tumor sarcoma 180. AST: aspartate aminotransferase; ALT: alanine aminotransferase. 5% DMSO, i.p. was used as the negative control (NC). 5-Fluorouracil at 25 mg/kg/day, i.p. was used as the positive control (NP).* $p < 0.05$ for all groups compared with the negative control using analysis of variance, followed by the *Student-Newman-Keuls* test.

without presenting substantial toxicity in experimental tumor model sarcoma 180. It is possible to continue the study for applicability of this compound in other tumor models or as prototypes for the development of antineoplastic agents.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Diversity and use of wild edible plants by migratory shepherds in the Himachal Pradesh of the Western Himalayas, India

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The present study was carried out in Himachal Pradesh of the Western Himalayas, India to obtain information on the wild edible plants used by the migratory shepherds. The shepherds started their migration in July from Chitkul, Rakchham, Batseri, Sangla and Kamru of district Kinnaur (Himachal Pradesh). Questionnaire for the survey, personal field visits and participatory observations were used to collect information about the use of various plants by the migratory shepherds. The shepherds move in a group of 5 to 6 comprising their own family members and size of the flock (of sheep and goats) varied from 654 to 990. The migration route followed from their villages to Churdhar ranges (mid hills) and to Sirmour ranges (low hills). It was observed that in all 50 species were used by shepherds enroute from high hills to low hills. In high hills, 23 species, in mid hills 31 species and in low hills 34 species were found to be used as livelihood source. Some of the plants, besides being used as fruits and vegetables, are also used as herbal tea (bark of *Betula utilis*) and condiments. *Morchella esculenta* was found to be one of the delicacies used for food in their tribe. The documentation of plant resources and the indigenous knowledge of shepherds highlighted in the present study is a step in raising awareness about the importance of these edible plants and their further conservation.

Key words: Edible plants, sheep, goats, shepherds, seasonal migration, Himachal Pradesh.

INTRODUCTION

Forest constitutes an important resource in the mountain of the Himalayas. The Indian Himalayan region (IHR) occupies about 18% of the total geographical area of India and is the world's youngest mountain range. It exhibits a diverse topography, climate, ecology, and land use pattern and is known for rich biodiversity in the foot hills and the mountainous region of the Himalayas throughout. The rich floral composition is extensively utilized in various forms including medicine, food, fuel, fodder, fiber and timber by the inhabitants (Misri, 1995).

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The Himalaya also comprises diverse human groups, which differ in terms of language, culture, tradition, religion and pattern of resources use (Sharma et al., 2005).

Himachal Pradesh, a north Indian state, lies in western part of the Himalayan range with variation in altitude, rainfall and temperature. Average elevations above mean sea levels vary from 350 to 7000 m and the climate is typically temperate. It has a forest cover of 26% (FSI, 2013). Himachal Pradesh is home to a sizeable tribal population like the Gaddis, Pangwals, Kinnauras, Lahulis, Bhots and Gujjars. Nomadic graziers use sub-alpine and alpine pastures for rearing their livestock. Due to ever-increasing demand of animal products, the livestock population has increased manifold, thereby increasing pressure on these pastures (Suri, 2014).

Nomadic tribes rearing sheep and goats move their livestock throughout the year in search of fodder and pastures. They move from high hills to low hills and vice versa and move throughout the year, leaving for low hills and plains with the commencement of winter season and returning to their villages in summer season. Shepherds move from high hills in the month of July towards mid hills, and finally by September-October reach low hills and plains where they settle temporally upto March, and again start their return journey for high hills.The routes followed are century's old. Their duration of stay both during migration and reverse migration often differs. The carvans generally move along the roads for convenience and avoid traffic. Every year the movement of shepherds is in the early morning and the routes of migration and reverse migration differ and there is no set pattern to the variation (Suri, 2014).

Although in the state of Himachal Pradesh, it is very difficult to make an exact estimate of the migratory goat and sheep population it has been reported that these constitute about 70% of the total goat and sheep (Misri, 1998). The recent trend of nomadic herders settling in hospitable climates has brought agriculture and other occupations to front and shepherding taking the secondary position (Biswas and Rao, 2016). Wild edible plants have supported human populations in all inhabited continents (Khyade et al., 2009) and the relationship between the surroundings and their indigenous people form the subject of ethnobotany dealing with the study of plants used by people for medicine, clothing and food (Jain and De, 1996). The majority of ethnobotanical surveys and reviews focus on medicinal plants. Native knowledge of wild plants is important for sustained utilization of these edible plant species (Kapoor, 1978; Arora, 1981; Jain, 1987; Jasmine et al., 2007) and the availability of enormous diversity of wild plants has attracted attention of researchers over time (Joshi et al., 2018). However, there is still paucity of detailed information and documentation of wild edible plants in the country and Himachal Pradesh is no exception to this; and the available information is sparse and scattered (Arora and Pandey, 1996).

In the recent past, prominence of wild plants in food and other nutraceutical uses has gained importance and, as a result, there is extractive pressure on these plant resources. Extraction of these edible plants (like *Picrorhiza kurrooa*, *Rhododendron arboretum*, *Bauhinia variegata, Cannabis sativa*, *Morchella esculenta*, *Ficus palmata, Dioscorea deltidea*, *Zanthoxylem armatum*, *Asparagus filcinus, Juglans regia, Ephedra gerardiana,* medicinal and aromatic plants, etc.) is already popular even amongst urban people. This is now putting increased market pressures on these species with the rural people realizing the supplementary income generation and nutritional potential of these plants (Jana and Chauhan, 1998). Therefore, there has been a revival of interest in survey, identification and documentation of wild edible plants during the last few decades (Jasmine, 2007).

Furthermore, the shepherds who are always on the move throughout the year are mostly dependent upon the wild food and fruits available as they move and no much studies have been conducted to know about the use of wild plants as food by migratory shepherds in Himachal Pradesh. With this aim in view the present studies were conducted to know the wild edible plants used by shepherds during migration. It was also aimed to analyze the migration route of shepherds from upper regions to the foot hills of the Himalayas.

MATERIALS AND METHODS

Himachal Pradesh is one of the most fascinating mountainous state of India (Figure 1). Nestled in the lap of the Western Himalaya, Himachal Pradesh is located almost in the center of the Himalayan mountain range and is a land of remarkable bio-geographical diversity (Kayastha, 1971). The present study is undertaken in Himachal Pradesh situated between 30°22'40''-33°12'40'' N latitude and 75°45'55''-79°04'20''E longitude, covering an area of 55,675 sq Km. Physiographycally, the state consists of three discrete regions, the outer Himalaya, mid hills and greater Himalaya. The outer Himalaya, also called Shivalik hills, ranges from 350 to 1,500 m a.m.s.l. The mid hills cover an area up to 3,500 m. The greater Himalaya also called as high altitude alpine zone generally starts from an elevation of 3,510 m and above. It includes higher altitude areas of Kinnaur, Kullu, Lahul-Spiti districts and Pangi valley of Chamba district. Because of varied altitudinal variations and climatic conditions, the state is enriched by diverse plant species, which include around 3,400 species of flowering plants ranging from tropical to alpine zone (Kaur and Sharma, 2004). The unique feature of Himachal Pradesh is the presence of fodder and fruit trees, shrub and herbs throughout the state (Thakur and Puri, 2016).

The present study documents the use of wild edible foods (plants and / or plant parts) by the tribal migratory shepherds of Kinnaur district in Himachal Pradesh. A total of five field surveys were carried out taking into account the migratory route of the shepherds from high alpine region to low foot hills. In the higher reaches of Kinnaur district the Kinnaure (shepherds) started movement from

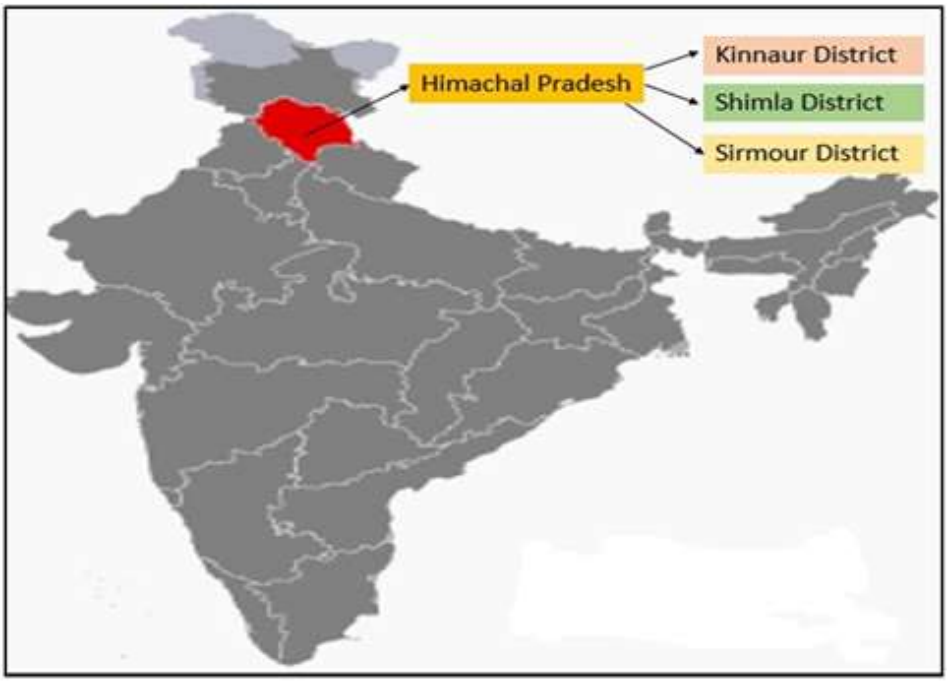

Figure 1. Google map of India showing study sites in Hamachi Pradesh.

five different villages of Kinnaur and they were Chitkul, Rakchham, Batseri, Sangla, and Kamru. Besides the place of origin of shepherds surveys were done for four other sites namely–Chopal, (mid hills),Churdhar (mid hills), Renukaji (low hills) and Poanta Sahib (low hills). These four places were their part of migratory route (Figure 2). The migratory shepherd's groups were randomly selected and interviewed during field trips shown in Figure 4 (Khanna and Ramesh, 2000).

Shepherds migration from Chitkul (3,450 m), Rakchham (3,100 m), Batseri (2,700 m), Sangla (2,600 m), and Kamru (2,700 m) starts in the month of July. These sites situated at high altitude remain cut-off from rest of the world for about 3-4 months due to heavy snowfall and harsh environment conditions during winters. The information on wild edible plants was collected using a pretested questionnaire, through participatory observation and discussion during July 2017 to August 2018. The specimens of edible plants being used by shepherds were collected, dried and mounted on herbarium sheets, with label information describing when and where they were collected. Plants were identified either in the field itself or with the help of experts from Botanical Survey of India, and Forest Research Institute, Dehradun, Uttarakhand. Vouchers of plants were places in the herbarium of the Shoolini University, Solan (BSI, 1996).

RESULTS AND DISCUSSION

Harsh environmental conditions and non-availability of feed cause seasonal migration of shepherds. It is a traditional process in the tribes of higher Himalayan region. It was observed that most of the shepherds start migration from their villages in the month of July and August and in October there is no migration as the winters sets in. The shepherds move in a group of 5-6 comprising their own family members (Table 1). The migratory flock includes both sheep and goats and size of the flock is huge and varied from 654 to 990 (Table 1). Irrespective of their origin of migration the shepherds move first to grazing sites in Chopal in Shimla district. The shepherds also take along with them 2-4 horses (local hardy breeds) for carrying provisions and tents (Kumaravelu et al., 2008).

Often four to five dogs also accompany the shepherds and, in fact, these dogs are trained in protecting the sheep and goats from wild animals and also keep the flock together. The disparity of flock size generally is an indicator of status of farmer's livestock holding capacity. The present study also indicated that shepherds having high number of flock are comparatively well off compared to those with less number (Table 1). Many studies have reported that flock size is directly associated with migration distance, flock with larger size travel longer distance as compared to small sized flocked (Kumaravelu et al., 2008; Balamurugan et al., 2012). In our study it was found that irrespective of flock size, the shepherds travel same distance. In the second stage, the shepherds then move to Churdhar ranges and from here to Renukaji in Sirmour district. The routes of migration are generally fixed and proper permission is obtained from the authorities for the purpose. Finally, in the months of September-October they reach the low hills in Poanta-

Figure 2. Satellite map showing migratory routes of shepherds from their originated villages Chikul, Batseri Sangla and Kamru (high hills) to Chopal and Churdhar (Mid hills) to the final destination Renukaji and Paonta Sahib (Loe hills).

Table 1. Basic information of the shepherd's caravan in the study area.

S/N	Particulars	Study sites				
		I Chitkul (3,450 m)	II Rakchham (3,100 m)	III Batseri- (2,700 m)	IV Kamru (2,700 m)	V Sangla (2,600 m)
1	Group size (No.)	5	5	6	5	6
2	Average family income (all sources, Rs. Lakh/annum)	3	2	4	3	2
3	Horses	3	2	4	2	2
4	Dogs	3	2	4	2	3
5	Flock size (sheep & goats)	780	640	990	654	712
6	Migration period			July-August		

* 1 US $ = Rs70.

Sahib in Sirmour district and temporarally settle here upto the month of March, and start their return journey to their respective places by end March (spring season). Similar study on seasonal migration of Bakkarwals and Gujjars tribes from high hills to low hills has been carried out in Jammu and Kashmir (Suri, 2014).

The present studies revealed that the livelihood of shepherd's family is dependent either getting food and fruits from the forests / trees on the path they transect and selling the meat and milk products from their herds. The wild plants not only serve as their food but also for their livestock. It was observed that during their migration from upper hills to lower hills, a total of 50 species (Figure 3) were being used by the migratory shepherds and a few of these belonged to the same families, all these plants are integral part of shepherd's diet during migration (Tables 2 to 5). The collection of various plants and plant parts varied from plant to plant, depending upon their availability and usability; both as those (1) consumed raw and (11) used after cooking. During their seasonal migration the shepherds are much dependent on forest products for their requirements of fruits, vegetables and medicines (Thakur and Puri, 2016).

As the shepherds move from their place of origin (high

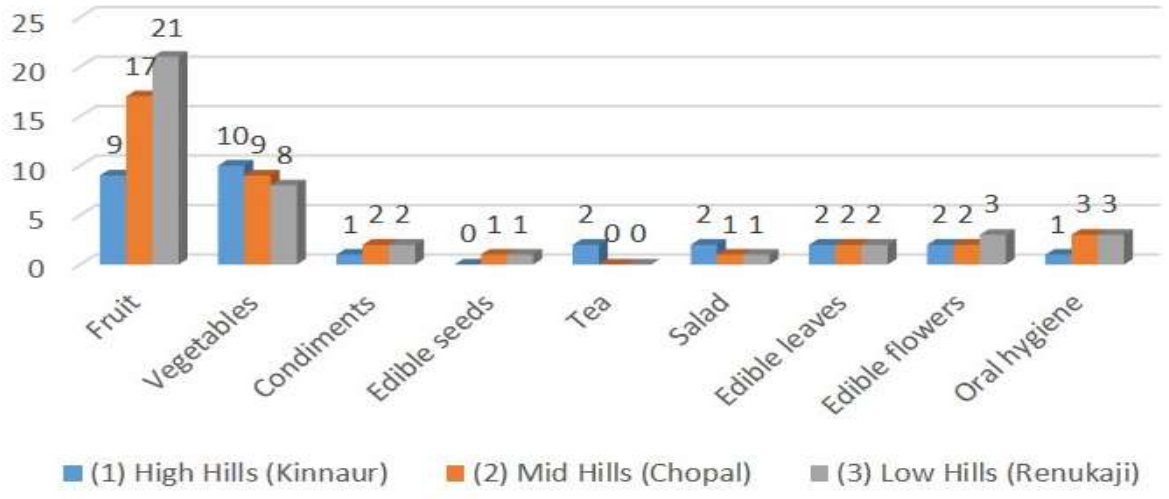

Figure 3. Number of wild edible species used by shepherds for food purpose in the study.

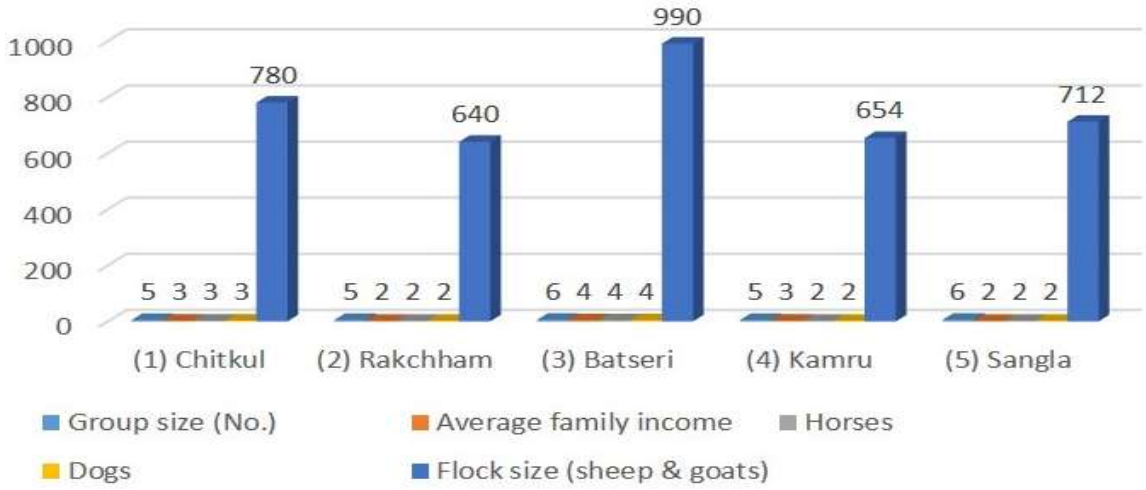

Figure 4. Basic information of the shepherds caravan in the study area.

hills) towards the lower hills the plant species varies with altitude. In the higher hills 23 species (herbs, shrubs and trees) were found to be consumed by the shepherds (Table 2). Interestingly, the shepherds informed that their preference for food is *Morchella esculenta* (Fungi) as it is one of the delicacy in food in their tribe. Similarly, in the mid hills 31 plant species were found to be taken as food as the shepherds move towards Shimla hills (Table 3). It was observed that some edible species like *Chenopodium album*, *Cannabis sativa*, *Dioscorea deltodea*, *Hippophae salicifolia*, *Juglans regia*, *Myrsine africana*, *Oxalis corniculata*, *Rumex hastatus* and *Urtica palviflora* were also present in higher hill regions (Table 3). However, there are many others (numbering 22) which are new to their diet. From mid hills as the shepherds move towards lower hills (Poanta-Sahib), they encounter 34 type of edible species (Table 4). Among these it was observed that 50% of these species are used as fruit. The use of many of these edible species has been already reported in many surveys from different parts of Himachal Pradesh (Monika et al., 2016; Thakur and Puri, 2016).

It is evident from Tables 2 to 4 that most of the plants or their parts are being used as fruits and vegetables. While the fruits are often consumed as raw, vegetables

Table 2. Wild edible food plants consumed by the shepherds during migration in the high hills of Himachal Pradesh of the Western Himalayas, India.

S/N	Plant name	Family	Voucher no.	Part used	Habit	Uses
1	*Asparagus filcinus* Buch.-Ham. ex D.Don	Asparagaceae	SUBMS/BO 750	Young shoots	Herb	Young shoots used as vegetable
2	*Berberis lyceum* Royle	Berberidaceae	SUBMS/BOT-659	Fruits	Shrub	Fruits are edible
3	*Berginia ciliate* (Royle) Raizada.	Saxifragaceae	SUBMS/BOT-352	Leaves	Herb	Leaves are used as vegetable
4	*Betula utilis* D.Don.	Betulaceae	SUBMS/BOT-387	Bark	Tree	Bark used in tea
5	*Chenopodium album* L.	Chenopodiaceae	SUBMS/BOT-628	Young shoots	Herb	Young shoots are used as vegetable
6	*Cannabis sativa* L.	Cannabinaceae	SUBMS/BOT-658	Seeds	Herb	Roasted seeds are used as condiments
7	*Celtis tetrandra* Roxb.	Cannabaceae	SUBMS/BOT-378	Fruits	Tree	Fruits are edible
8	*Dioscorea deltidea* Wall. ex Griseb.	Dioscoreaceae	SUBMS/BOT-661	Tuber	Herb	Tuber used as vegetables
9	*Euphorbia hirta* L.	Euphorbiaceae	SUBMS/BOT-662	Leaves	Herb	Leaves used as vegetable
10	*Ephedra gerardiana* Wall. ex Florin.	Ephedraceae	SUBMS/BOT-422	Ripe Fruits	Shrub	Ripe fruits are edible
11	*Hippophae salicifolia* D.Don.	Elaeagnaceae	SUBMS/BOT-425	Fruits	Shrub	Fruits are edible
12	*Juglans regia* L.	Juglandaceae	SUBMS/BOT-687	Fruits	Tree	Fruits are edible, Twigs used as tooth brush
13	*Morchella esculenta*Fr.	Morchellaceae	SUBMS/BOT-446	Whole plant	Fungi	Used as vegetable (a delicacy and an important source of income)
14	*Myrsine africana* L.	Myrsinaceae	SUBMS/BOT-690	Fruits	Shrub	Fruits are edible
15	*Oxalis corniculata* L.	Oxalidaceae	SUBMS/BOT-343	Leaves	Herb	Leaves taken as salad or cooked as vegetable
16	*Oxalis acetosella* L.	Oxalidaceae	SUBMS/BOT-386	Fruits, Leaves	Herb	Fruits are edible; and leaves are cooked
17	*Polygonum capitatum* Buch,-Ham. ex D.Don.	Polygonaceae	SUBMS/BOT-339	Fruits	Herb	Fruits are edible
18	*Prunus armeniaca* L.	Rosaceae	SUBMS/BOT-619	Fruits	Tree	Fruits are edible
19	*Rumex hastatus* D. Don.	Polygonaceae	SUBMS/BOT-689	Aerial leaves	Herb	Aerial leaves are edible
20	*Stellaria media* L.	Caryophyllaceae	SUBMS/BOT-404	Fresh leaves	Herb	Fresh leaves are used as vegetable
21	*Taxus wallichiana* Zucc.	Taxaceae	SUBMS/BOT-390	Stem Bark, Leaves	Tree	Bark and leaves are used for making tea
22	*Thymus serphyllum* L.	Lamiaceae	SUBMS/BOT-461	Leaves	Herb	Leaves used as cooked food
23	*Urtica palviflora* Roxb.	Urticaceae	SUBMS/BOT-687	Tender, Shoot, Inflorescence	Herb	Used as vegetable

Table 3. Wild edible food plants consumed by the shepherds during migration in the mid hills of Shimla and Sirmour districts (HP) of the Western Himalayas.

S/N	Plant name	Family	Voucher no.	Part used	Habit	Uses
1	*Amaranthus viridis* L.	Amaranthaceae	SUBMS/BOT-751	Whole plant	Herb	Young leaves are cooked and used as vegetable/saag
2	*Bauhinia variegata* L.	Fabaceae	SUBMS/BOT-637	Flowers, buds	Tree	Flower buds eaten as vegetable, petals used in a curd preparation
3	*Berberis asiatica* Roxb. ex DC.	Berberidaceae	SUBMS/BOT-691	Fruits	Shrub	Fruits are edible
4	*Berberis vulgaris* L.	Berberidaceae	SUBMS/BOT-445	Fruits	Shrub	Fruits are edible
5	*Berginia ciliate* (Royle) Raizada.	Saxifragaceae	SUBMS/BOT-752	Leaves	Herb	Leaves are used as vegetable SUBMS/BOT-692

Table 3. Contd.

6	*Chenopodium album* L.	Chenopodiaceae	SUBMS/BOT-693	Young shoots	Herb	Young shoots are used as vegetable
7	*Cannabis sativa* L.	Cannabinaceae	SUBMS/BOT-695	Seeds	Herb	Roasted seeds are used as condiments
8	*Coriaria nepalensis* Wall.	Coriariaceae	SUBMS/BOT-698	Fruits	Shrub	Ripen fruit are edible
9	*Celtis tetrandra* Roxb.	Cannabaceae	SUBMS/BOT-699	Fruits	Tree	Fruits are edible
10	*Carissa opaca* Stapfex. ex Haines	Apocynaceae	SUBMS/BOT-700	Fruits	Shrub	Fruits are edible
11	*Dioscorea deltidea* Wall. ex Griseb.	Dioscoreaceae	SUBMS/BOT-701	Tuber	Herb	Edible vegetable
12	*Dabregesia hypoleuca* (Hochst.) Wedd.	Urticaceae	SUBMS/BOT-702	Fruits	Shrub	Fruits are edible
13	*Ficus palmate* Forsk.	Moraceae	SUBMS/BOT-703	Fruits	Tree	Fruits are edible
14	*Grewia optiva* Drumm. ex Burret.	Tiliaceaea	SUBMS/BOT-704	Fruits	Tree	Ripen fruits are edible
15	*Hippopha esalicifolia* D.Don.	Elaeagnaceae	SUBMS/BOT-705	Fruits	Shrub	Fruits are edible
16	*Juglans regia* L.	Juglandaceae	SUBMS/BOT-706	Fruits	Tree	Fruits are edible, Twigs used as tooth brush
17	*Myrsine africana*L.	Primulaceae	SUBMS/BOT-707	Fruits	Shrub	Fruits are edible
18	*Oxalis corniculata* L.	Oxalidaceae	SUBMS/BOT-708	Leaves	Herb	Leaves taken as salad or cooked as vegetable
19	*Pinus roxburghii* Sarg.	Pinaceae	SUBMS/BOT-709	Seeds	Tree	Seeds are edible
20	*Prunus armeniaca* L.	Rosaceae	SUBMS/BOT-710	Fruits	Tree	Fruits are edible
21	*Prunus cerasoides* D.Don.	Rosaceae	SUBMS/BOT-711	Fruits	Tree	Fruits are edible
22	*Pyracantha crenulata* (D.Don) M. Roem.	Rosaceae	SUBMS/BOT-712	Fruits	Tree	Fruits are edible
23	*Punica granatum* L.	Lythraceae	SUBMS/BOT-713	Fruits	Tree	Directly consumed
24	*Rhododendron arboretum* Sm.	Ericaceae	SUBMS/BOT-714	Flowers, Buds	Shrub	Flowers, buds are used as vegetable
25	*Rhus palviflora* Roxb.	Anacardiaceae	SUBMS/BOT-715	Fruits	Shrub	Fruits are edible
26	*Rubus ellipticus* Sm.	Rosaceae	SUBMS/BOT-716	Fruits	Shrub	Fruits are edible
27	*Rumex hastatus* D. Don.	Polygonaceae	SUBMS/BOT-717	Aerial parts and leaves	Herb	Aerial parts; leaves are edible
28	*Urtica palviflora* Roxb.	Urticaceae	SUBMS/BOT-697	Tender, Shoot, Inflorescence	Herb	Used as vegetable
29	*Urtica dioica* L.	Urticaceae	SUBMS/BOT-673	Leaves, Shoot	Herb	Used as vegetables
30	*Vitex negundo* L.	Verbenaceae	SUBMS/BOT-718	Flowers	Shrub	Flowers are edible
31	*Zanthoxylum armatum* DC.	Rutaceae	SUBMS/BOT-696	Dried fruits and seeds	Shrub	Dried fruits and seeds as condiment and for oral hygiene

are used after cooking and in some cases these are also used as salad. Number of plants being used for collection of fruits ranged from 9 in high hills to 21 in the low hills (Table 5). In the high hills, some parts of the plants were also used as herbal tea and further for oral hygiene. Number of species used for oral hygiene is relatively higher in low and mid hills than in high hills. Other uses include the use of plants or plant parts as condiments or as edible seeds/nuts. Ethno-botanical uses of wild plants and plant products obtained from the environment without any cost have been already reported from many states of India (Tambe and Rawat, 2009).

The shepherds during migration generally move along the roadside and rarely adopt bridal pathways or shortcuts. For their own stay they use makeshift tents and shift tents frequently within 5 to 6 days. There is always scarcity of food and fodder for themselves and livestock. For this they explore adjoining areas, particularly degraded

Table 4. Wild edible food plants consumed by the shepherds during migration towards the low hills of Sirmour district.

S/N	Plant name/Family	Family	Voucher no.	Part used	Habit	Uses
1	*Achyranthus bidentata* Blume.	Amaranthaceae	SUBMS/BOT-719	Leaves	Herb	Leaves are used as vegetable
2	*Achyranthes aspera* L.	Amaranthaceae	SUBMS/BOT-720	Whole plant	Herb	Edible, young leaves are cooked and used as vegetable/ saag
3	*Amaranthus viridis* L.	Amaranthaceae	SUBMS/BOT-721	Whole plant	Herb	Young leaves are cooked and used as vegetable/saag
4	*Bauhinia variegata* L.	Fabaceae	SUBMS/BOT-401	Flowers, buds	Tree	Flower buds eaten as vegetable, Petals used in a curd preparation
5	*Berberis umbellata* Wall.	Berberidaceae	SUBMS/BOT-438	Fruits	Shrub	Fruits are edible
6	*Berginia ciliate* (Royle) Raizada.	Saxifragaceae	SUBMS/BOT-722	Leaves	Herb	Leaves are used as vegetable
7	*Celtis australis* L.	Cannabaceae	SUBMS/BOT-379	Fruits	Tree	Ripen fruits are edible
8	*Cannabis sativa* L.	Cannabinaceae	SUBMS/BOT-723	Seeds	Herb	Roasted seeds are used as condiments
9	*Coriaria nepalensis* Wall.	Coriariaceae	SUBMS/BOT-724	Fruits	Shrub	Ripen fruits are edible
10	*Celtis tetrandra* Roxb.	Cannabaceae	SUBMS/BOT-725	Fruits	Tree	Fruits are edible
11	*Carissa opaca* Stapf ex. Haines.	Apocynaceae	SUBMS/BOT-726	Fruits	Shrub	Fruits are edible
12	*Dabregesia hypoleuca* (Hochst.) Wedd.	Urticaceae	SUBMS/BOT-727	Fruits	Shrub	Fruits are edible
13	*Ficus palmate* Forsk.	Moraceae	SUBMS/BOT-728	Fruits	Tree	Fruits are edible
14	*Grewia optiva* Drumm. ex Burret.	Tiliaceae	SUBMS/BOT-729	Fruits	Tree	Ripen fruits are edible
15	*Hippophae salicifolia* D.Don.	Elaeagnaceae	SUBMS/BOT-730	Fruits	Shrub	Fruits are edible
16	*Juglans regia* L.	Juglandaceae	SUBMS/BOT-731	Fruits	Tree	Fruits are edible, Twigs used as tooth brush
17	*Myrica esculenta* Buch,- Ham. ex D. Don.	Myricaceae	SUBMS/BOT-732	Fruits	Tree	Fruits are edible
18	*Myrsine africana* L.	Primulaceae	SUBMS/BOT-733	Fruits	Shrub	Fruits are edible
19	*Oxalis corniculata* L.	Oxalidaceae	SUBMS/BOT-734	Leaves	Herb	Leaves taken as salad or cooked as vegetable
20	*Polygonum capitatum* Buch,-Ham. ex D.Don.	Polygonaceae	SUBMS/BOT-735	Fruits	Herb	Fruits are edible
21	*Pinus roxburghii* Sarg.	Pinaceae	SUBMS/BOT-736	Seeds	Tree	Seeds are edible
22	*Prunus armeniaca* L.	Rosaceae	SUBMS/BOT-737	Fruits	Tree	Fruits are edible
23	*Prunus cerasoides* D.Don.	Rosaceae	SUBMS/BOT-738	Fruits	Tree	Fruits are edible
24	*Pyracantha crenulata* (D.Don) M. Roem.	Rosaceae	SUBMS/BOT-739	Fruits	Tree	Fruits are edible
25	*Pyrus pashia* Buch.- Ham. ex D.Don.	Rosaceae	SUBMS/BOT-740	Fruits	Tree	Fruits are edible
26	*Punica granatum* L.	Lythraceae	SUBMS/BOT-741	Fruits	Tree	Directly consumed
27	*Rhododendron arboretum* Sm.	Ericaceae	SUBMS/BOT-742	Flowers, Buds	Shrub	Flowers , buds are used as vegetable
28	*Rhus palviflora* Roxb.	Anacardiaceae	SUBMS/BOT-743	Fruits	Shrub	Fruits are edible
29	*Rubus ellipticus* Sm.	Rosaceae	SUBMS/BOT-744	Fruits	Shrub	Fruits are edible
30	*Rumex hastatus* D. Don.	Polygonaceae	SUBMS/BOT-745	Aerial parts; leaves	Herb	Aerial parts; leaves are edible
31	*Solanum nigrum* L.	Solanaceae	SUBMS/BOT-746	Fruits	Herb	Fruits are edible
32	*Urtica dioica* L.	Urticaceae	SUBMS/BOT-747	Leaves, Shoot	Herb	Used as vegetables
33	*Vitex negundo* L.	Verbenaceae	SUBMS/BOT-748	Flowers	Shrub	Flowers are edible
34	*Zanthoxylum armatum* DC.	Rutaceae	SUBMS/BOT-749	Dried fruits and seeds	Shrub	Dried fruits and seeds as condiment and for oral hygiene

Table 5. Number of wild edible species used by shepherds for food purposes in the study area.

S/N	Plant /plant parts used as	Altitude range and plants (No.)		
		High Hills (Kinnaur-2,320 m to 6,816 m)	Mid Hills (Chopal-2,550 m; Churdhar-3,647 m)	Low Hills (Renukaji-672 m; Poanta Sahib-398 m)
		Number of species		
1	Fruit	9	17	21
2	Vegetables	10	9	8
3	Condiments	1	2	2
4	Edible seeds	0	1	1
5	Tea	2	0	0
6	Salads	2	1	1
7	Edible leaves	2	2	2
8	Edible flowers (including use in preparation as raita)	2	2	3
9	Oral hygiene (including use as tooth brush)	1	3	3

lands, allow fields and village commons. It was interesting to note that their migration patterns closely mirrors the seasonal availability of natural food and fodder. Tambe and Rawat (2009) also observed in Khangchendzonga National Park that migration movements match with seasonal fodder resource availability. Shepherds during enroute migration face constraints like food, fodder, water deficit, veterinary facilities, wild animals, predators and sometimes road accidents of their livestock. Such constraints have also been reported by many previous studies (Rao et al., 2011; Suresh et al., 2011; Kaintura et al., 2017). The livelihood of shepherds and their family members is also met through selling of meat (of sheep and goat) and dairy products (milk, yoghurt, butter etc.).

Conclusions

The shepherds are very close to nature as they spend most of their time in forests and pastures with their livestock. They move with their livestock in search of quality grazing lands; and while on the move during migration they depend on local wild edible plants as their food. Unfortunately, deforestation activities and the changing climatic conditions have made availability of wild edible plants as a scarce resource to the migratory shepherds. Plants and plant products play an important role in the lives of these shepherds. The critical review of the past work done and the results of this survey suggest that wild edible plants are very important for migratory shepherds living in tribal areas in Himachal Pradesh. It is also emphasized that sufficient interest has not been put in conserving and promoting traditional wild edible plants. The need is to adopt large scale plantation of these wild edible plants within the forests as well as along roadsides so that the migratory shepherds are benefitted.

RECOMMENDATION

This study is an approach to promote the wild edible plants that are richly existing in the rural regions to the global level. Wild edible plants are easy to available from our surroundings without any cost and provide substantial health and economic assistances to those who depend on them. It is now clear that efforts to conserve wild edible plants and preserve traditional food systems and farming practices need to be enhanced and combined.

Future work

Further research focusing on these wild edible plants might give information regarding the bioactive compounds to fight diseases in an effective manner. For future public awareness and community based management needs to be encouraged. Research on indigenous wild edible plants should also be taken up and disseminate the results so to have diversity in diet.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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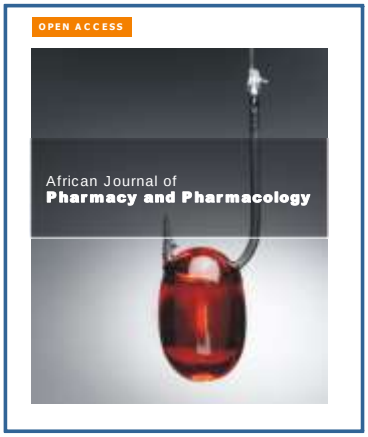

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