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Chemical and microbiological hazards of Khat (*Catha edulis*) from field to chewing in Ethiopia

Beyene Wondafrash Ademe^{1,*}, Leon Brimer², Anders Dalsgaard² and Tefera Belachew³¹ Department of Nutrition and Dietetics, Health Sciences Institute, Jimma University, Ethiopia.² Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark.³ Department of Nutrition and Dietetics, Health Sciences Institute, Jimma University, Ethiopia.

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Abstract

Catha edulis of the plant family *Celastraceae* commonly named as Khat in Ethiopia is a stimulant plant containing amphetamine-like substances. Fresh Khat leaves described to contain some 60 different constituents. Consumers chew leaves and masticate it for hours. Some users make a paste from dried leaf powder, mixing with sugar and water which is easily chewed and swallowed. In some areas, dried leaves are used as a tea, and also crushed and rolled into cigarettes. Less attention has been directed toward possible chemical and microbial contamination and risky pathways. The aims of this study are 1. Identify the actual Khat production-consumption process from farm to use. 2. Detect chemical and microbial contamination risk of consumable Khat leaf in process 3. Assess the microbiological qualities chewable Khat leaf. Descriptive statistics from the main dataset, for Khat consumption, showed that out of 1169, 564 household heads were Khat consumers and out of these consumers, 347 (62.52%) consume daily. Majority of the pilot study participants (retailers and consumers) confirmed that pesticide chemicals are sprayed on Khat bushes and trees, and Khat farmers disclosed the use of Dichlorodiphenyltrichloroethane (DDT) and Malathion on their farm. The mean CFU/gm was 1.69×10^5 which indicate poor hygiene /handling. Our observation showed that the processes from farming, Transporting, collection, distribution (whole-sellers, retailers) to consumption indicate a potential risk for Khat leaf to become contaminated with Chemicals/ microorganisms.

Keywords: Khat production and consumption Flowchart; DDT; Malathion; Microorganisms; CFU

1. Introduction

Catha edulis Forsk of the plant family *Celastraceae* (Spindle-tree) commonly named as Khat in Ethiopia is an amphetamine-like stimulant plant widely consumed in Eastern Africa and southern Arabian region [1, 2]. According to a recent phylogeography study, the primary sources of wild and cultivated Khat genotypes centres of origin are Ethiopia and Kenya [3], from where it spread to countries such as Yemen, Uganda, and Madagascar [4]. Fresh Khat leaves could contain around 60 secondary substances [5]. Main constituents are cathine (S, S-(+) - nor-pseudoephedrine) and cathinone (S- (-)- α -amino-propiofenone) [6]. Around 400 million people in the Horn of Africa and the Arabian Peninsula are using Khat leaves and Khat chewing has been a social custom for centuries [7].

Humans have for centuries used a variety of sedatives, narcotics, hallucinogens, antidepressants, and stimulant herbs and Khat is among them. More research outputs are available about the pharmacologic, physiological and psychological effect of *Catha edulis*. The adverse effects of Khat use and abuse such as oral, gastrointestinal, cardiovascular, neurological, psychiatric, hepatotoxicity, cytotoxicity have also been studied [8]. Khat's social status and recreational

* Corresponding author: Beyene Wondafrash Ademe E-mail: beyenewondafrash@gmail.com

use as a traditional herb have hindered the knowledge about its harm in terms of morbidity and mortality and less attention has been directed towards possible chemical and microbial contamination of chewable Khat leaf.

Research findings indicated illness and death case reports associated with Khat consumption. However, further study should be done to ascertain the exact role of Khat chewing in such pathologies. Death due to Endocarditis caused by bacterium *Gemella morbillorum* [9], blood infection with bacteria *Campylobacter fetus*, duodenal ulcer due to *Helicobacter pylori* colonization [10, 11], severe liver fibrosis and cirrhosis [12], severe liver injury from Khat abuse resulting in liver transplantation [13], infection from *Fasciola hepatica* (parasitic flatworm) [14], high incidence of oral squamous cell carcinoma [15], a decrease in cell viability and an increase in cell apoptosis, alteration of the effectiveness of immune activity of peripheral blood mononuclear cells (PBMCs) [16], jaundice, coma and death from toxicity [17], increased risk of *Mycobacterium tuberculosis* infection through its active ingredient (cathinone) macrophage immune modulation action [18].

During harvesting Khat leaves and shoots of the shrub are handpicked, huddled and wrapped up with banana leaves and are often kept in moistened conditions for up to 48 hours before consumption [19]. Farmers and traders deliver the leaves to the point of sale carried by humans, animals or open tracks in unhygienic condition. Usually, young fresh leaves that are valued for their stimulant potency are picked in the morning and chewed on the same day. During chewing, leaves are placed in the buccal fold and masticated for several hours. Some users make a paste from dried leaf powder, mixing with sugar and water, which is easily chewed and swallowed. In some areas, dried leaves are used as a tea. Leaves also crushed and rolled into cigarettes [20, 21]. In Kenya, efforts are made to promote Khat as tea-sachets, bottled juice as well as an ingredient in wine [21, 22]

Khat farmers in Ethiopia use organophosphate pesticides, such as Malathion, Diazinon, and organochlorine-DDT for pest control, and users could ingest pesticides sprayed on Khat field. There were death reports that were due to poisoning following the ingestion of pesticides used on the Khat tree. Diazinon levels from Khat analysis, grown from South West Ethiopia (Jimma) detected 173.9-751.4 µg/kg, which is high compared to the level of 10 µg/kg set for vegetables and fruit by the European Union (EU) [23].

Although the 1970 commitment and 2001 “Stockholm Convention on Persistent Organic Pollutants” banned DDT [24], Khat farmers confirmed that DDT is also sprayed on their Khat plant, accessed during outdoor spray for *Anopheles* mosquito eradication [25] or from chemical shops. Khat analysis from Eastern Ethiopia (Hararge) localities also showed that the average residue Level of DDT observed in three Khat samples were 1224, 1372 and 1163 µg/Kg which is much higher than the EU M Maximum Residue Levels (MR)L for citrus fruits, vegetables and sugar plants (10µg/Kg). From Southwest Ethiopia, near Jimma town (Badabuna), Diazinon was also detected in all the Khat samples with an average of 500µg/Kg (range 200–800 µg /kg) which were above the set for vegetables by EU [26].

Mycotoxins producing toxigenic fungi were also isolated from Khat leaf. A study was done in Yemen (around Sana'a), from 30 samples of chewable Khat leaves, and eleven mycotoxigenic fungi from which *Aspergillus*, *Alternaria*, *Penicillium*, and *Cladosporium* were the most frequent genera. This finding was considered to be a threat to public health [27]

It is well-established that the contamination of raw vegetables with various pathogens is associated with human gastrointestinal disease outbreaks, particularly bacterial pathogens of the *Enterobacteriaceae* family [28]. Microbial pathogens, spoilage bacteria, fungi, and parasites found on any ready-to-eat vegetables could also be present on Khat leaves posing an infection risk to the Khat consumers [29]. Khat leaf laboratory analysis in Ethiopia (Hawassa: the capital city of SNNP) from 51 Khat samples, 29 had aerobic mesophilic counts with the mean count of 7.2×10^5 cfu/g. *Enterobacteriaceae* and bacterial spores had mean counts of around 104 CFU/g and *Staphylococcus sp.*, *Bacillus cereus*, and yeasts and moulds had counts of 103 CFU/g. according to this finding Khat consumption was considered hazardous [30].

This paper is not about the wider phenomenon of morbidity and mortality linked to Khat consumption. But by revealing case reports, actual practices and processes of the plant cultivation and use, it is hoped that this information will be noted by agricultural extension experts, Khat using communities, and health professionals.

1.1. Objective

The objective of this study was to identify and evaluate chemical and microbiological hazards along the chain of Khat production in the field to human consumption.

2. Material and methods

2.1. Study area

The pilot study was conducted in Jimma Town, located 350 km southwest of the capital Addis Ababa. The town is divided into 17 kebeles (smallest administrative units in Ethiopia). According to the 2012 Central Statistical Agency census report [31], the projected total population of the town is 134, 040. The town is generally characterized by a warm and humid climate.

2.2. Method

This study was undertaken within Empowering New Generations to Improve Nutrition and Economic Opportunities' (ENGINE) project sites which envision bringing sustainable improvement in the nutritional status of rural households in Ethiopia. We reviewed literature evidence and case reports on Khat use related health outcomes; i.e. pesticides contaminants and microbiological contaminations. A baseline study conducted to understand the Khat production-consumption process so as to account for chemical and microbial analysis of consumable Khat leaf. Piloting was done from five Khat farm sites, five Khat retailers and five consumers respectively. In addition, five chemical shops in Jimma town were visited and interviewed. Observation guide from production to consumption was used to explore Khat cultivation, processing and consumption practices related risks by using a flow diagram. The Hazard Analysis Critical Control Points (HACCP) approach was used to identify the hazards of chemical and microbiological risky pathways from farm to use. HACCP helps to determine the corrective measures for situations where critical control points are beyond control limits [32] Estimated daily intake (EDI) of pesticides from consumable Khat leaf was assessed by extrapolating pesticides database [33] and from previous studies conducted in Ethiopia on pesticide residues from Khat leaf.

For the microbial quality of consumable Khat leaf study, a total of 10 bunch (locally named as *Zurba*) of fresh chewable Khat leaves were purchased and collected in sterile plastic bags from 10 retail market points of Jimma town (southwest Ethiopia), and directly brought to the laboratory, processed and analyzed for their microbial load. From each of the 10 sample lot, 5gm of fresh chewable Khat leaves were mixed with 100 ml of 1% buffered peptone water in a sterile plastic bag; shaken and squeezed intermittently for over 2-3 minutes to obtain a homogenized stock solution and to find viable cells from the leaf surface. Dilutions were done taking 1ml from the stock. 100 µl diluted solution from each Serial (10^{-1} to 10^{-7}) were subcultured on indicative agars Rapid E. coli plates (7 duplicated plates and 1 control). The 15 plates were incubated overnight at 37°C. The colony was counted by colony counter (magnifier). The microbiological qualities of sampled chewable Khat leaf were assessed as the Total Viable Count (TVC) which measures only viable CFU cells of large group of bacteria that could contain certain pathogenic bacteria, which are relatively easy to measure as a group and their presence in chewable Khat is likely to indicate the presence of pathogenic bacteria as Indicator organisms.

Table 1 Methodology summary

Research Objectives	Study area	Sample size	Sampling method	Study design	Study variables	Data source	Main outcome	Analytic method
To identify chemical and microbial exposure associated with Khat production and consumption processes and practices	Jimma town Khay sale points and nearby rural farms	10 Khat samples, 5 Khat farm, 5 Khat retailers and 5 Consumers	Convenient sampling, observation guide and process flowchart	Khat supernatant lab.culture, Baseline assessment & HACCP model approach (cross-sectional)	Observation & flowchart variables (chemical shops, leaf picking & preparation, storage, transport, manure usage); CFU culture; pesticide reference values (ADI, ARfD, MRLs)	Khat retail points, interviews and pesticides databases	Chemical and microbial risks of Khat production and consumption	Quantifying the lab culture and Hazard identification

2.3. Study Ethics

Ethical approval was obtained from the ethical clearance committee of Institute of health science of Jimma University institutional review board. After receiving ethical clearance, informed verbal consent was obtained from each study participant. Personal identifying information was not included in the questionnaire, and the observations and interviews remained confidential.

3. Results

Descriptive statistics from the main dataset, for Khat consumption, showed that out of 1169, 564 household heads were Khat consumers and out of these consumers, Majority, 347 (62.52%) chewers consume daily. Most, 313 (57.30%) consume for social setting or community social activities followed by 313 (56.4%) who reported to enhance working capacity and 165(29.73%) referred to religious reasons.

The baseline survey from Khat production farm sites demonstrates that most Khat plantation plots are located around the homesteads where manures, human and animal wastes are used as fertilizers. Leaves and sprouts were hand-picked, sealed with banana leaves, and transported by open vehicles, animals or humans to point of sale. We confirmed that Khat leaf for consumption is not washed or otherwise cleaned before being chewed. Our observation showed that the processes from farming, collection, Transporting, distribution (whole-sellers, retailers) to consumption indicate a potential risk for Khat leaf to become contaminated with Chemicals/ microorganisms. (Table 2)

Table 2 Observations/measurements of Khat production/consumption practices

Variable	Indicator	Method
Khat farm	Cultivation, nursery and type of agricultural inputs used	Observation
Local pesticide retail shops	Type and availability of chemicals	Observation Interview
Khat leaf collection/picking	Hand picking, storage area & container, hygiene conditions	Observation
Personal hygiene and Hygienic conditions during consumption	Hand washing habit, availability of water for hand washing	Observation
Khat Leaf preparation for sale	Place of preparation, Steps of preparation and activities. Hygienic conditions.	Observation
Transportation to auction/retail sites	Transport means (human, animal, and vehicle) and activities. Hygienic conditions.	Observation
Storage conditions	Storage materials and processes, time of storage. Hygienic conditions.	Observation
Weed, compost and manure use	Ways of the application during Khat cultivation	Observation
Conditions at the distribution points	Sale points and conditions, possible contamination points (refuse dump near sale points, animals around vendors, roadsides with dust)	Observation
Khat chewing sessions	Place of session, leaf serving procedure, amount of leaf, group of consumers, groundnuts and drinks served. Hygienic conditions.	Observation
Supernatant culture for Microbial quality assessment	CFU from edible Khat leaf samples at 10 retail sites	Sampling & Measurement

As shown in the flowchart (Figure 1), Khat Farming, collecting, preparation, transporting, retail and consumption contains hazards for the end-users (consumers). Chemicals such as DDT and Malathion are sprayed for pest control at different seasons. A variety of pesticides were on sale from chemical shops. There are individuals who own sprayer jar with pesticides and enter a contract with Khat farmers to do the spraying.

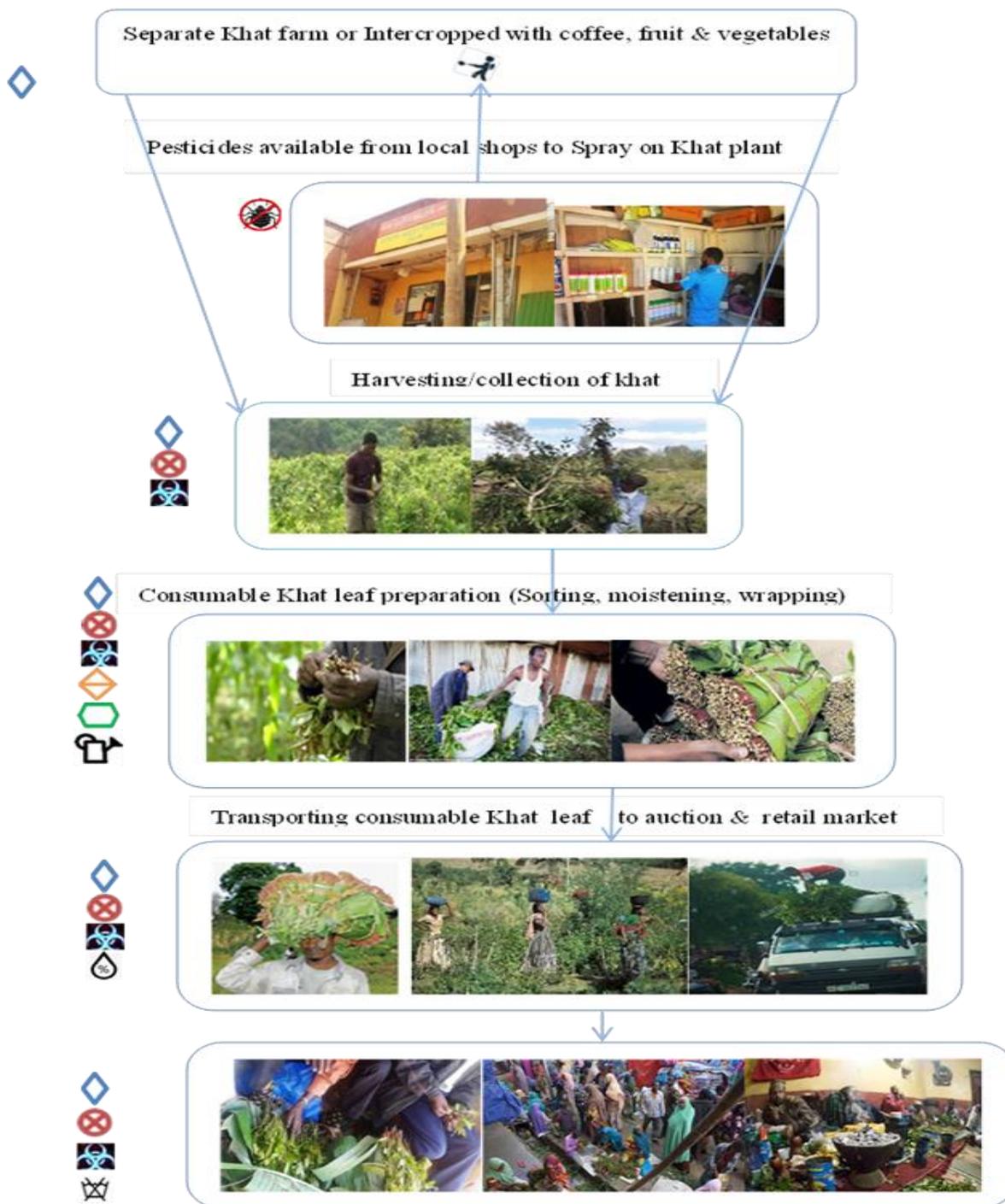


Figure 1 Khat Farming, collecting, preparation, transporting, retail and consumption flow chart

Legend for figure 1

-  Initial contamination with waste manures (human & animal) & plant debris
-  Available Local chemical shops

-  Pesticides spray-on Khat
-  Hands contamination
-  Environmental contamination
-  Wrapping with broadleaves (Banana, false banana)
-  Sorting by hand with body contact and surrounding contamination
-  Moistening Khat leaves to prevent from drying
-  Unhygienic transportation (humid, contaminated)
-  Consumable Khat leaves & sprouts never washed before chewing

At chemical shops available in Jimma Town we observed various pesticides stocks such as Malathion 50 Emulsifiable Concentrate, Endosulphan 35%, Chlorpyrifos 48, 2 Emulsifiable Concentrate and DDT (concealed from the shelf to be offered on request); Herbicides such as 2, 4-D-dimethylamine salt, Glyphosate 48%SL; Fungicides such as Curazate® RWP, Trust cymocop® 439.5 WP, Matco.8-64, and Indofil M-45 are also on the shelf.

DDT products are also available in chemical shops in our research site (Jimma town). (Figure 2) Our pilot interview and observations in Khat fields verified that DDT is sprayed on Khat trees and bushes. Customers are farmers from outside the town and shopkeepers' recommend/advice which chemicals to purchase for Khat spray.



Figure 2 Pesticide retail shops from the research site (shop; Malathion; Chlorpyrifos 48E.C; Endosulphan 35%; 2-4-D-dimethylamine salt). The photo was taken by the author, October 2016.

Local retailers and habitual consumers confirmed that 'Khat leaves which are sprayed with DDT appear clean and shiny; causes burning sensation on the lips and mouth while chewing and the twig and leaves have no signs of insect bite'. (Fig 3)



Figure 3 Field observations during Pesticide spray-on Khat plant and sign of pesticides sprayed leaf demonstrated by the retailer... The photo was taken by the author, October 2016.

Retailers had knowledge of the use of pesticides on Khat. Khat traders confirmed that Khat was neither washed nor otherwise cleaned before being delivered for sale or during consumption. The reason behind this assertion was the

assumption to keep the natural flavour of the chewable Khat. Consumable fresh Khat leaf is delivered, from whole sellers or individual farmers, daily from 11am to early evenings. (Figure 4) Depending on popularly used Khat variety, 500g wrapped bundles, priced between USD2-5.



Figure 4 Unhygienic conditions from farm site, sampling retail spot, and local Khat market in Jimma Town. The photo was taken by the author, December/2016.

The primary Khat customers were predominantly adult males. Whole fresh leaves and buds are chewed immediately after purchase without cleaning. Even though consumers know about chemical spray on Khat and even differentiate the sprayed ones from not sprayed they showed no concern about the health risks of Khat use.

During baseline/pilot information collection process the practice of the chemical application on Khat bush/tree was witnessed. Khat farmers had access to pesticides from local shops or directly from sprayers. DDT and Malathion were the most frequently applied pesticides. We observed that Khat plant is intercropped with other perennial and seasonal crops and pesticides are sprayed on all plants indiscriminately which implies the risk of contamination on other crops as well.

As shown in (Table 4) the concentration of pesticide in the Khat leaf (mg/kg) is taken from published articles or set to be equivalent to MRLs database for other crops [34]. Based on the baseline information and previous investigations [35] the average fresh weight of Khat consumption is 0.15kg/day. For an adult having a bodyweight of 60 kg the mean value for the daily intakes of a given pesticide can be calculated as follows;

$$EID = \frac{Conc.P \times W}{C.Avg.Wt}$$

Where,

EID= Estimated daily intake (EDI) in mg/kgbw/day

Conc.P = concentration of pesticide in Khat leaf (mg/kg)

W= Weight of Khat chewed in kg

C.Avg.Wt= average wt. of consumers

Accordingly, DDT residue exposure is calculated to be 0.003mg/kg BW/day. Compared to ADI &MRLs set values it is lower. However, this may not guarantee safety, and the fact that (a) Khat is a permanent crop and is sprayed frequently (2-3times per year) (b) Habitual chewers use it on daily basis, for longer hours over the years, (c) Khat is consumed fresh-raw with no washing or any sort of cleaning, and (d) DDT is a persistent organic pollutant, bio-accumulative and toxic it can create adverse effects upon Khat chewers and the natural environment. On the other hand, a study from western Ethiopia localities measured 1.3mg/kg of Khat DDT residue which is higher than MRLs set values. Diazinon residue exposure is calculated to be 0.02mg/kg BW/day. Compared to ADI set values (0.0002 mg/kg BW/day) it is a hundred times higher. A study was done around Southwest Ethiopia (Jimma) town revealed Diazinon level of 0.75 mg/kg of Khat which is higher than MRLs set values (0.1 mg/kg for blackberries & 0.5 mg/kg for Lettuce.

Table 4 Estimated Khat pesticide exposure extrapolated from thresholds for the common pesticides exposure (Values from European Union (EU) & FAO Codex Alimentarius Pesticides database)

Common pesticides sprayed on Khat	Reference values from European Union (EU) & Codex Alimentarius Pesticides database		Estimated (calculated) exposure
DDT	ADI ¹	0.01 mg/kg bw/day	Not calculated
	ARfD ²	Not approved	Not calculated
	MRLs ²	0.2 mg/kg for carrot	Not calculated
		0.1 mg/kg for cereals	
	Measured mean from 3 sites (Ethiopia)	1.3mg/kg of Khat	1.3mg/kg Khat*0.15kg Khat/60kgbw =0.003mg/kg bw/day
Malathion	ADI* ¹	0.03 mg/kg bw/day	Not calculated
	ARFD* ²	0.3 mg/kg bw	Not calculated
	MRLs* ²	1.0 mg/kg for spices, fruits	Not calculated
	ADI* ¹	0.0002 mg/kg/day	Not calculated
	ARFD* ²	0.025 mg/kg bw/day	Not calculated
	Diazinon	MRLs* ²	0.5 mg/kg for lettuce
0.1 mg/kg for blackberries			
Measured interval from Jimma Ethiopia		0.17-0.75mg/kg of Khat	0.75 mg/kg Khat *0.15 kg Khat /60 kgbw =0.02mg/kgbw/day (calculated from the Max.value)

BW: body weight

¹Values from European Union (EU) pesticide database²Values from Codex alimentarius pesticide database

Acceptable Daily Intake (ADI): An estimate of the amount of a substance in food or drinking water that can be consumed daily over a lifetime without presenting an appreciable risk to health (WHO, 1987). It is derived from long-term animal (in vivo) studies (NOAEL).

Acute Reference Dose (ARfD): An estimate of the amount of a substance in food or drinking Water that can be ingested over a short period of time, usually during one meal or one day, Without appreciable health risk to the consumer on the basis of all known facts at the time of evaluation. It has decreased margin of safety (JMPPR, 2002)

No Observed Adverse Effect Level (NOAEL): It is the value taken from tests with animals or epidemiological data to determine the TDI Identified in the critical studies. This value is then divided by a safety factor (usually 100) which is intended to take into account the different levels of sensitivity between humans and animals

Tolerable daily intake (TDI): The daily amount of a chemical that has been assessed safe for human being on long-term basis (usually whole lifetime)

A maximum residue level (MRL): The highest level of a pesticide residue that is legally tolerated in or on food or feed when pesticides are applied correctly (Good Agricultural Practice)

Application of pesticides applied on Khat extends to other crops, and it is likely that the actual daily intake may be higher due to additional exposure through other food consumption. This directs the need for awareness creation among the people in the community and the need for launching community-based education programs through local health care and agricultural professionals, educators, development agents, administrative bodies and researchers.

Laboratory analysis for microbial contamination of chewable Khat showed that there is infection risk to the consumers. From 10 Khat sample lots, we have determined the presence of coliform as an indicator of microbiological quality. Indicator organisms (CFU) were isolated from all the 3 consecutive serial dilutions of the 10 samples of 30 RAPID'E.coli2 plates; ranging from 15-389cfu/100µl, 8-50cfu/100µl, and 1-19cfu/100µl with mean of 136cfu/100µl, 28cfu/100µl and 9cfu/100µl respectively. Similarly, the mean CFU/gm was 1.69×10^5 . The result showed higher microbial loads which are an indication of poor hygiene or handling practices or unhygienic condition of processing, handling, and consumption of chewable Khat leaves.

Table 5 Colony forming units in the 3 serial dilutions and mean CFU/g

Dilution1 cfu/100µl	Dilution2 cfu/100µl	Dilution3 cfu/100µl	D1-D3	CFU/g	Deviation	SD
192	26	9	227	2.28E+05	2.28E+05	3.50E+09
139	34	6	179	1.80E+05	1.80E+05	1.20E+08
74	12	3	89	8.94E+04	8.94E+04	6.32E+09
389	35	19	443	4.45E+05	4.45E+05	7.63E+10
316	50	12	378	3.80E+05	3.80E+05	4.45E+10
24	8	1	33	3.32E+04	3.32E+04	1.84E+10
93	24	19	136	1.37E+05	1.37E+05	1.04E+09
73	14	5	92	9.24E+04	9.24E+04	5.85E+09
45	16	14	75	7.53E+04	7.53E+04	8.75E+09
15	8	6	29	2.91E+04	2.91E+04	1.95E+10
<u>Mean CFU</u>				<u>1.69E+05</u>	SMSQ	1.84E+10
					SD	1.36E+05

4. Discussion

In many Khat production areas in Ethiopia, its production and consumption are integrated into the culture, economy, and livelihoods of rural households. Consumers chew its leaves, buds and soft stems frequently and for a long hour as raw, without any processing, washing or cleaning it before consuming [35].

Our baseline study showed that during Khat production and consumption process there is no even minimal processing of any kind. Khat leaves are handpicked, sorted and wrapped by broad leaves or grasses. After transported under unhygienic conditions the sprouts and leaves are retailed at open markets and verandas. In such processes contamination at every step was evident. Risk (R=P*I) assessment as an outcome of the interaction between probability and Impact (likelihood) [35], can be used to evaluate the health and safety impact of chemical and microbial risks of Khat chewing. But the Khat issue does not exist in isolation. A holistic approach by understanding the interaction among psychological, social, institutional, cultural, and economic aspects of Khat agriculture and use should be understood. These interactions may amplify or mitigate public responses to the risk events. Discussions of event identification and risk response are beyond the scope of this paper.

Studies showed that Khat consumption practice was associated with unacceptable levels of microbial and chemical contamination. Wrong application of pesticides, particularly on Khat plant, which is consumed regularly and raw could lead to serious health effects on consumers. To assess consumer exposure to pesticide and the level of pesticide consumption risk, there should be made an investigation on the Acceptable Daily Intakes (mg/kgbw) (ADI), Acute Reference Dose (mg/kgbw) (ARFD), and whether pesticide concentration exceeds the toxicological threshold or the MRLs (mg/kg Khat consumed) for such routine exposure [36, 37].

Vegetables and their leaf ecology provide nutrients, moisture and favourable pH for microbial pathogen species of *Shigella*, *Salmonella*, *Aeromonas* and *Yersinia* together with *Clostridium botulinum*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, which can cause food-borne illness. Minimal processing of vegetables (sanitizing, washing, freezing trimming) is often challenged by the microflora density, diversity and growth, not only on the surface but also by biofilm formation deep in the cellular structure of their veins, cuticle, depression of the stomata, or in the intercellular junction [38].

The purpose of the laboratory culture was to assess the microbiological quality of chewable Khat leaf. Following the standard operating procedure Indicator organisms (CFU) were isolated from all the 10 samples of 30 RAPID'E.coli2 plates (100%). A colony could arise from one cell or several thousand cells and CFU can be influenced by the colony morphology and colony density.

5. Conclusion

Microbial pathogens (bacteria, virus, fungi, and parasites) and pesticide residues can be present on chewable Khat leaves and health risk to the consumers could be imminent. This Study showed that Khat consumption practice was associated with unacceptable levels of microbial and chemical contamination. Habitual consumers shall be aware of the risk of Khat chewing from an infectious risk perspective and possible chemical contaminants exposure side effects as well.

Compliance with ethical standards

Acknowledgments

We are grateful to Khat farmers, retailers and chemical shopkeepers for providing the necessary information.

Disclosure of conflict of interest

The authors declare that they have no competing interests.

Statement of ethical approval

Ethical approval was obtained from the ethical clearance committee of Institute of health science of Jimma University institutional review board. After receiving ethical clearance, informed verbal consent was obtained from each study participant. Personal identifying information was not included in the questionnaire, and the observations and interviews remained confidential.

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