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Research article

Chemical profiling of *Cannabis sativa* from eleven Tanzanian regions

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ABSTRACT

The aim of this research was to investigate the chemical profiles of *Cannabis sativa* from 11 Tanzanian regions using preliminary tests as well as instrumental analyses with GC-MS and LC-MS. Generally, all the seized samples tested positive for the presence of (Δ^9-THC. The preliminary test with Duquenois method followed by chloroform addition revealed the presence of Δ^9-tetrahydrocannabinol (Δ^9-THC) in all the samples. GC-MS analyses of the samples revealed the presence of nine cannabinoids including Δ^9-THC, Δ^8-THC, cannabidivarol, cannabidiol, Δ^9-tetrahydrocannabivarin (Δ^9-THCV), cannabichromene, cannabinol, caryophyllene, and cannabicouramaronone, whereas LC-MS chemical profiling revealed the presence 24 chemical substances, including 4 cannabinoids, 15 different types of drugs and 5 amino acids. The Pwani region had the highest percentage composition of Δ^9-THC (13.45%), the main psychoactive ingredient of *Cannabis sativa,* followed by Arusha (10.92%) and Singida (10.08%). The sample from Kilimanjaro had the lowest percentage of Δ^9-THC (6.72%). Apart from cannabinoids, the majority of other chemical substances were found in the Dar es Salaam region sample, which could be attributed to the fact that the city is the epicenter of business rather than the cultivation area, implying that the samples were obtained from different sources and blended as a single package.

1. Introduction

Cannabis sativa is a plant that belongs to the Cannabaceae family. It is thought to be the most commonly abused narcotic in the world, with 200 million users worldwide [1]. There are three primary plant species in the genus "Cannabis": C. ruderalis, *C. indica*, and C. sativa [2]. As a fibre-type of cannabis, C. sativa contains more Δ^9-tetrahydrocannabinol (Δ^9-THC) than cannabidiols (CBD), whereas *C. indica* which is a drug-type of cannabis, contains more CBD than THC. On the other hand, C. ruderalis possesses intermediate characteristics between the two. More than 500 secondary metabolites have been identified in *Cannabis sativa*, including cannabinoids and non-cannabinoids present in the plant's leaves, flowers, seeds, stems, and roots [3–5]. Cannabinoids constitute a group of C21 or C22 terpenophenolic compounds formed by alkylation reaction between alkyl resorcinol and a monoterpene unit [1]. Non-cannabinoids on the other hands constitute a variety of other secondary metabolites such as alkaloids, flavonoids, terpenes, non-cannabinoid phenols and so on [6–8].

Due to its dual status as a drug of abuse and a plant with both industrial and medical applications, *Cannabis sativa* is also seen as a contentious plant [9]. In the last ten years, 41 nations have approved the use of C. sativa or other cannabis-based products for

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therapeutic purposes. Apart from the therapeutic applications, the recreational use of Cannabis are being decriminalized and legalized. The recreational use of Cannabis was legalized in Canada in 2018 [10]. Some US states such as Colorado and California have also legalized the recreational use of Cannabis despite still being in the Schedule-I classification under the federal government [11]. However, many other nations still maintain legal restrictions against its use due to the presence of Δ^9-tetrahydrocannabinol (Δ^9-THC), which exhibits psychoactive effects [12]. The use of Cannabis use is associated to a number of negative side effects, including respiratory illnesses, car accidents, acute myocardial infarction, depression, anxiety, and other cannabis use disorders [13–17]. Therefore, caution should be used in the ongoing efforts to legalize cannabis usage for recreational purposes in numerous nations and states. Controlling the cultivation should meanwhile be exercised as a measure to minimize the possession and use of cannabis products for recreational purposes.

Tanzania is one of the nations that forbids and criminalizes the use of *Cannabis sativa* [18]. Studies on Tanzania's illicit drug market revealed that, between 2011 and 2016, 10219.8 kg of *Cannabis sativa* were seized, making it the most prevalent narcotic in the country [19]. This is attributed to the presence of dense forests in the country, which makes illegal cultivation easy [18]. The extent of *Cannabis* production in Tanzania is demonstrated by the sheer number of its seizures [20]. Controlling the possession and use of *Cannabis sativa* is still a global challenge, particularly in terms of locating the cultivation areas [21].

This study aimed to investigate the variability of chemical composition of *Cannabis sativa* that were seized from various regions of Tanzania. It is well known that different geographical, hence climatic conditions such as temperature and humidity, as well as the nature of the soil exerts an effect on the type and quantity of secondary metabolites present in plants. Taking advantage of this understood fact, the study was conducted to establish the pattern of chemical composition between samples from different regions. The rationale for this study was that by monitoring the chemical composition of the seized samples in relation to where it originated from, it could be possible to predict the origin of that sample, therefore take control measure against cultivation. However, this investigation was conducted as a baseline study involving only the seized samples from eleven selected regions. The need for further systematic study which involves cultivation of the plants in different regions with different climatic conditions and types of soil is evident. While the variation of secondary metabolites is obviously expected, such study can help to establish database that could be useful to track down the seized samples as to whether it originate from local or international trafficking, hence take the appropriate measures.

This study was carried out by using three methods: Duquenois-Levine test, GC-MS and LC-MS. Duquenois-Levine test using the Duquenois-Levine reagent was carried out as a preliminary test to ascertain the presence of Δ^9-THC prior to further instrumental analysis. Duquenois reagent consists of a mixture of ethanol, acetaldehyde and vanillin, which reacts with the Δ^9-THC present in cannabis sample and form a purple colored bilayer, indicating the positive results for Δ^9-THC. When exposed to acidic conditions by

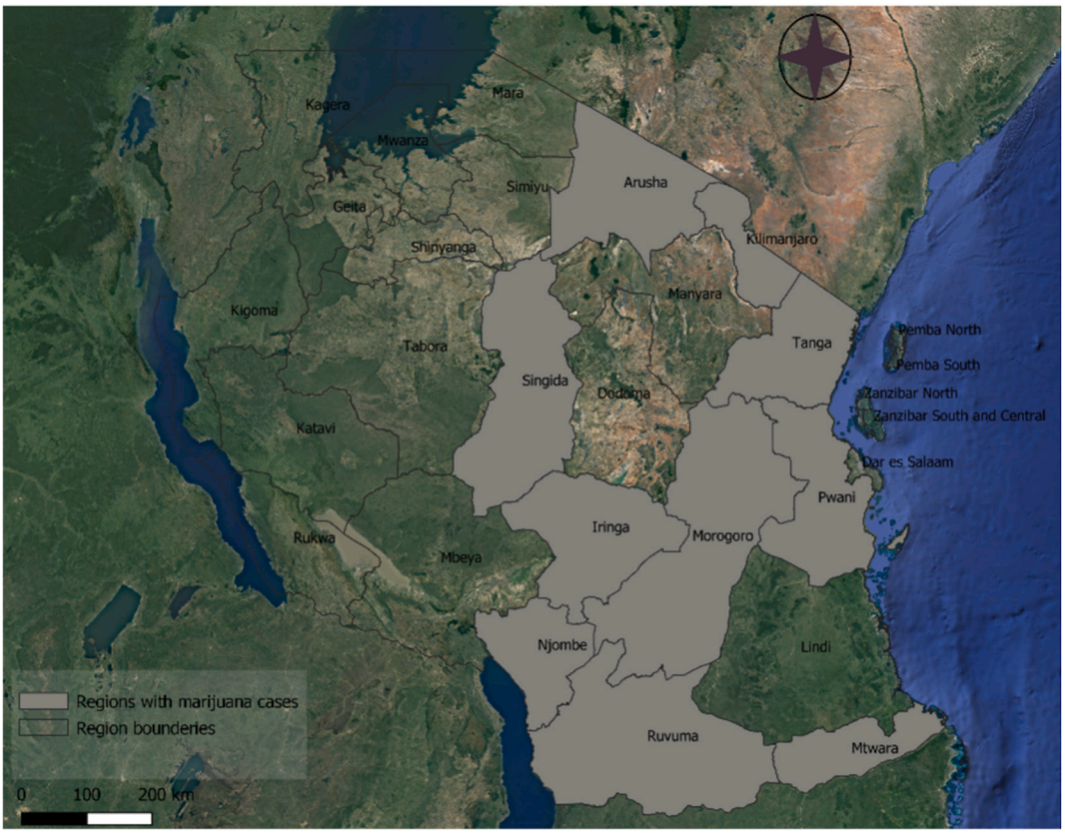

Fig. 1. Tanzanian map showing the regions where the *Cannabis* samples were confiscated.

using concentrated HCl, the Duquenois reagent reacts at the para position of the free phenol group of Δ^9-THC. However, considering that there are varieties of other compounds that consists of a phenol group at the free para position, it is possible to get false positive results. To overcome such a limitation, a modification is performed by addition of chloroform in which only molecules with long aliphatic chains can penetrate the chloroform layer due to the presence of five-carbon chain at the C3 [22].

Studies on Cannabis using one of the newer omics biotechnology fields, metabolomics, which analyzes the structure and functions of the whole makeup of a given biological function at the metabolic level, have provided a picture of every metabolite as well as insight into the metabolic response to a biological situation or experimental manipulation [23]. The polymorphic plant species produces a wide range of bioactive metabolites, with cannabinoids and terpenoids being the major classes of phytochemicals [23]. By means of metabolomics, researchers can now use semi-quantitative and quantitative chemical mediators that make up a particular phenotype to complement genomic and protein-level analyses of diseases [23,24].

Additionally, metabolomic research has enabled the taxonomy of cannabis varieties in terms of chemovars, the evaluation of novel cannabis-based sources of bioactivity in medicine, the development of novel food products, and the optimization of cannabis cultivation to boost yield and potency [23]. Metabolomics is a useful tool for the pharmaceutical, preventative healthcare, and agricultural industries, among others, because of its non-invasiveness and close connection to the phenotype. Metabolomics has already made it possible to make well-informed decisions, which has advanced cannabis research and development in the areas of biomarker discovery [24,25].

2. Materials and methods

2.1. Materials

The *Cannabis sativa* leaves and seed samples utilized in this study were obtained from eleven regions of mainland Tanzania as confiscated samples by the Tanzania Police Force (TPF) and other law enforcement authorities, from farms, during transportation and/or drug marketplace (Fig. 1). These regions were selected based on the availability of *Cannabis sativa* seizures, whereas Dar es Salaam was solely selected not as a cultivation area, but as a prominent market. Five samples were taken from each of the eleven regions that were chosen, and merged to form one sample for analysis as a single replicate. The *Cannabis sativa* seizure collection as a whole was dried out by storing it in a shed at room temperature. All drug seizure exhibits were then stored at the Government Chemist Laboratory Authority (GCLA) in Dar es Salaam for further experiments.

2.2. Methods

2.2.1. General analytical scheme

Cannabis sativa L. was identified using its microscopic characteristics whereby a binocular microscope was used in the process. The screening and confirmatory methods suggested in the literature were used for the forensic drug testing [26–28]. Only the reagents and solvents of analytical grade were employed in this work. The subsections that follow contain more information regarding the screening tests and the confirmation of the compounds.

2.2.2. Sample preparation

2.2.2.1. Duquenois-Levine test. To prepare the Duquenois-Levine reagent, 2.0 g of Vanillin was mixed with 2.5 mL of acetaldehyde, and the mixture added to 95% ethanol to make 100 mL of the reagent solution. 0.25 g of dried, pulverized and homogenized *Cannabis sativa* leaves were mixed with 1 mL of Duquenois-Levine reagent, shaken followed by addition of conc. HCl and vortexed for 2 min, followed by addition of 1 mL of chloroform. The mixture was then left to settle for 5 min.

2.2.2.2. GC-MS and LC-MS analyses. Extraction for Gas Chromatography-Mass Spectrometry (GC-MS) analysis was performed by adding 5 mL of methanol/dichloromethane (1:9) mixture to 0.25 g of powdered sample, whereas extraction for LC-MS analysis was done by adding 5 mL of methanol (99.9%, HPLC grade) to 0.25 g of the powdered sample. The mixtures were vortexed for 2 min to allow proper mixing and left to settle for 24 h. The mixtures were then sonicated for 15 min followed by 30 min of centrifugation at 1400 rpm. The resulting extracts of the sample were collected for instrumental analyses.

For GC-MS 50 μL of extract were added to 950 μL of the mobile phase to make 1000 μL of the solution and shaken to allow proper mixing. The sample solution was then stored in a refrigerator at about −5 °C, ready for analysis. GC-MS analysis was done using an Agilent 7890B series Gas Chromatography-Mass spectrometer, model G4513A, operating in EI mode (MS) at 70 eV, with Agilent J&W HP-5ms UI (15 m × 0.25 mm x 0.25 μm) analytical column. Heating was conducted from 25 °C to 70 °C using a heating rate of 25 °C per minute and maintained for 3 min, followed by further heating at 6 °C per minute to 180 °C for 13 min. Helium was used as the carrier gas while the ion source and interface temperatures in MS were 280 °C and 300 °C, respectively. Identification of cannabinoids was done by Triple Quadrupole Mass Detector (QQQ) with Quadrupole temperature of Q1 equals to 150 °C of spectrometer Agilent 7000D series.

For Liquid Chromatography-Mass Spectrometry (LC-MS), 50 μL of the extracts was added to 950 μL of mobile phase (water/acetonitrile, 1:1 v/v) to make 1000 μL of the solution and shaken to allow proper mixing. The solution was stored in a refrigerator at a temperature of −5 °C for LC-MS analysis. LC-MS analysis was conducted by using a ThermoFisher Scientific U300, coupled with Mass

spectrometer of Q-Exactive type with ionization of APCI type connected with +VE/-VE. A 3 kV electrospray ES/APCI voltage used, with a scan type of MS2 and collision energy of 45 eV. The composition of the eluent used was 0.1% formic acid in acetonitrile, and the elution was carried out for 0–2 min. The Accuser RP-MS column with dimensions of 100 mm × 4.6 mm × 2.6 μ was used, with a column temperature of 35 °C and a flow rate of 300 μL/min. Nitrogen was used as a collision gas, with a mass resolution of 17,500 and a scan range/time of 120–780 *m/z*. Measurements were performed on each sample for a total of 30 min.

3. Results

Both the microscopic characteristics and the Duquenois–Levine test results confirmed that the samples seized from the 11 regions were *Cannabis sativa*. The positive Duquenois–Levine test (Fig. 2) indicates the presence of Δ^9-tetrahydrocannabinol, an active ingredient in *Cannabis sativa* and demonstrated that the seized samples were genuine or contained *Cannabis sativa* as one of the components in a mixture.

It should be noted that the reaction between Duquenois reagent and a sample containing Δ^9 –THC forms a purple colour. Therefore, the formation of purple colour in Fig. 2 indicates that the sample contained the Δ^9 –THC. In Fig. 2(a) dark purple colour was observed after addition of HCl, except for sample 3 from Mtwara. After further addition of chloroform (Fig. 2(b)), clear purple solution was formed. The formation of purple colour following the addition of HCl may give force positive results due to the fact that there are many other compounds that contain phenol group and capable of reacting at the free para position. Therefore, the formation of clear purple colored solution following the addition of chloroform confirm the presence of Δ^9 –THC because only the molecules that contain the phenol group and long aliphatic chain are able to penetrate the chloroform layer and react.

In Fig. 2(b), the third test tube, which contained the sample from Mtwara region, formed a yellowish solution in the chloroform layer. This is very likely due to the fact that the sample was mixed with sawdust, however, it gave positive results for Δ^9-THC during analysis by GC-MS and LC-MS.

GC-MS was used to determine the cannabinoids constituents whereas LC-MS was used to determine both cannabinoids and non-cannabinoids constituents in *Cannabis sativa* samples. Table 1 shows the distribution of the cannabinoids in the samples from the 11 regions. Δ^9-THC was confirmed to be present in all samples. The findings are in consistent with the preliminary testing for the presence of Δ^9-THC; even the Mtwara sample, which formed a yellowish solution in the Duquenois–Levine test, tested positive for Δ^9-THC by GC-MS.

Another interesting feature in Table 1 is that the Dar es Salaam sample contained all of the cannabinoids except one – cannabicoumaronone. In this study, seized samples from Dar es Salaam were considered as market samples. This is because, Dar es salaam is a business hub of Tanzania, with a relatively small area of 538 square miles (1393 square km), most of which is an urban area. The other studied regions have significantly larger areas, varying between 27,268 square miles (70,624 km^2) for Morogoro and 5120 square miles (13,250 km^2) for Kilimanjaro, and also characterized by thick forest vegetation. Moreover, out of 24 chemical constituents identified, Dar es Salaam was found to contain 22 of them. Being considered as a market rather than a cultivation region, the appearance of many chemical constituents suggests the possibility of blending for business purpose.

Because the samples appeared to contain the majority of the cannabinoids tested, the seized samples from Dar es Salaam were most likely a mix of samples from different regions. The Mtwara sample came in second with 9 cannabinoids, while the Tanga sample had the fewest, with only three.

When the results from Dar es Salaam and Mtwara are compared, it is possible that the majority of the cannabis seized in Dar es Salaam comes from the Mtwara region. However, this is not conclusive because cannabicoumaronone, which was detected in the Mtwara sample, was not detected in the Dar es Salaam sample. Furthermore, among the cannabinoids, cannabidivarol was discovered in only two samples, from Mtwara and Dar es Salaam. Because Dar es Salaam is considered a market for the product, one could argue that Mtwara is the possible source of *Cannabis sativa* found in Dar es Salaam. However, the argument cannot be conclusive until a full scan of the chemical constituents in all regions is conducted. In this study, 11 out of 26 regions were considered. Similarly, Cannabidiol was discovered in only two samples, the Dar es Salaam sample and the Morogoro sample, This also supports the argument that Dar es

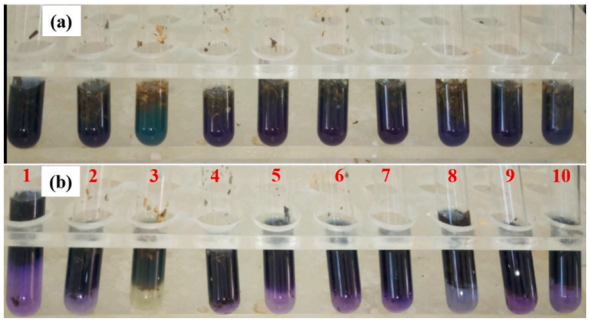

Fig. 2. Test for Δ^9- THC, indicating the appearance after the addition of Duquenois reagent and conc. HCl (a), and after addition of chloroform (b). ***Key:*** *1 = Arusha, 2 = Iringa, 3 = Mtwara, 4 = Morogoro, 5 = Singida, 6 = Coast, 7 = Dar es Salaam, 8 = Kilimanjaro, 9 = Tanga, 10 = Njombe, 11 = Ruvuma. (Sample 11 was camouflaged by test tube 1).*

Table 1
Distribution of cannabinoids among *Cannabis sativa* samples from the 11 regions from both GC-MS and LC-MS (***Key:*** *1 = Arusha, 2 = Iringa, 3 = Mtwara, 4 = Morogoro, 5 = Singida, 6 = Coast, 7 = Dar es Salaam, 8 = Kilimanjaro, 9 = Tanga, 10 = Njombe, 11 = Ruvuma).*

Cannabinoids	Regions										
	1	2	3	4	5	6	7	8	9	10	11
Δ^9-THC	+	+	+	+	+	+	+	+	+	+	+
Cannabinol	+	–	–	+	+	–	+	–	–	–	–
Δ^8-THC	–	–	–	–	–	–	+	+	–	–	+
11-nor-9-carboxy-THC	+	+	+	+	+	+	+	+	+	+	+
11-hydro-delta-THC	+	–	+	–	+	–	+	–	–	–	–
Cannabichromene	–	+	+	–	+	+	+	–	–	+	+
Cannabidivarol	–	–	+	–	–	–	+	–	–	–	–
Cannabidiol	+	+	+	+	+	+	+	+	+	+	+
Δ^9-Tetrahydrocanabivarin	+	+	+	+	–	+	+	–	–	+	+
Cannabicoumaronone	–	–	+	+	+	+	–	–	–	+	–
Tetrahydrocanabivarin	+	+	+	+	+	+	+	–	–	+	+
Total	**7**	**6**	**9**	**7**	**8**	**7**	**10**	**4**	**3**	**7**	**7**

Salaam is the market for samples from cultivation areas, It is possible to determine the source of the seized sample found in the market by performing a full scan of all regions. This approach could in the control of cultivation, and thus the spread of such an illegal drug.

Cannabinoids and non-cannabinoidal chemical constituents were identified using LC-MS. A total of 24 chemical constituents were identified from the 11 samples, including 4 cannabinoids, 15 non-psychoactive drugs and 5 amino acids. Despite the fact that these were just the identified chemical constituents used to study the variations across selected regions, *Cannabis sativa* has been reported to exhibit more than 500 chemical constituents.

Recall that, Δ^9-THC, the main psychoactive constituent of *Cannabis sativa,* was found in all samples from the selected regions. The percentage of occurrence of Δ^9-THC relative to other chemical constituents was determined, as shown in Fig. 3. The findings show that the *Cannabis sativa* sample from the Pwani region contains the highest percentage of Δ^9-THC (13.45%), followed by Arusha (10.92%) and Singida (10.08%). The least percentage of Δ^9-THC was observed for samples from Kilimanjaro (6.72%), Njombe (7.56%), and Tanga (7.56%). However, the observation could not be conclusive about the actual percentage of the regions. This is because the study had no control over the harvesting season and age of the plants, all of which may affect the availability and concentration of various chemical constituents.

Fig. 4(a) shows the representative LC-MS chromatogram of Δ^9-THC for a *Cannabis sativa* sample from Arusha. Its retention time is 21.51 min, corresponding to the relative intensity of 95%, indicating the large abundance of Δ^9-THC in the *Cannabis sativa* sample. At this relative intensity and RT, the corresponding M/Z ratio of Δ^9-THC is 315.2310 as shown in Fig. 4(b). A comparison was also made between the fragmentation patterns of Δ^9-THC for *Cannabis sativa* sample from Arusha.

Fig. 4 (c) shows the fragmentation pattern of Δ^9-THC in terms of intensity as a function of M/Z ratio for *Cannabis sativa* sample from Arusha, indexed with the fragmentation data from library. On the one hand, the upper fragmentation patterns in red-colored peaks represent the mass fragments of Δ^9-THC from the sample. On the other hand, the lower fragmentation patterns in blue colored peaks represent the mass fragments of Δ^9-THC from the library data. These blue peaks were used for indexing. Five fragments were formed from Δ^9-THC, with m/z of 193.12245, 93.07043, 135.11697, 123.04430, and 259.16931 for fragments 1, 2, 3, 4, and 5 respectively. By comparing with indexing fragments from the library, the two data sets matched perfectly in terms of m/z and peak intensity of each fragment. The observation confirms that the values of m/z correspond to Δ^9-THC for the *Cannabis sativa* sample from Arusha. Similar analyses were performed for the samples from all other regions, all of which were confirmed to contain Δ^9-THC.

The percentage of appearance of various chemical constituents were also analyzed. Figs. 5 and 6 show the appearance and percentage of chemical constituents for representative samples from Mtwara and Singida, plotted with chemical constituents from Dar es Salaam for comparison. The figures show that most of the chemical constituents present in the Dar es Salaam sample are also present in the Mtwara sample, while absent in the sample from Singida. Referring to Fig. 1, both Dar es Salaam and Mtwara are found in the

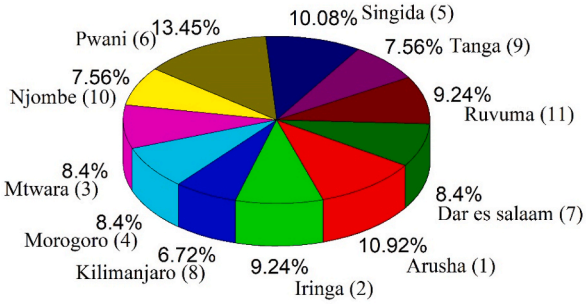

Fig. 3. The percentage of appearance of Δ^9-THC relative to other chemical constituents from the selected regions of Tanzania.

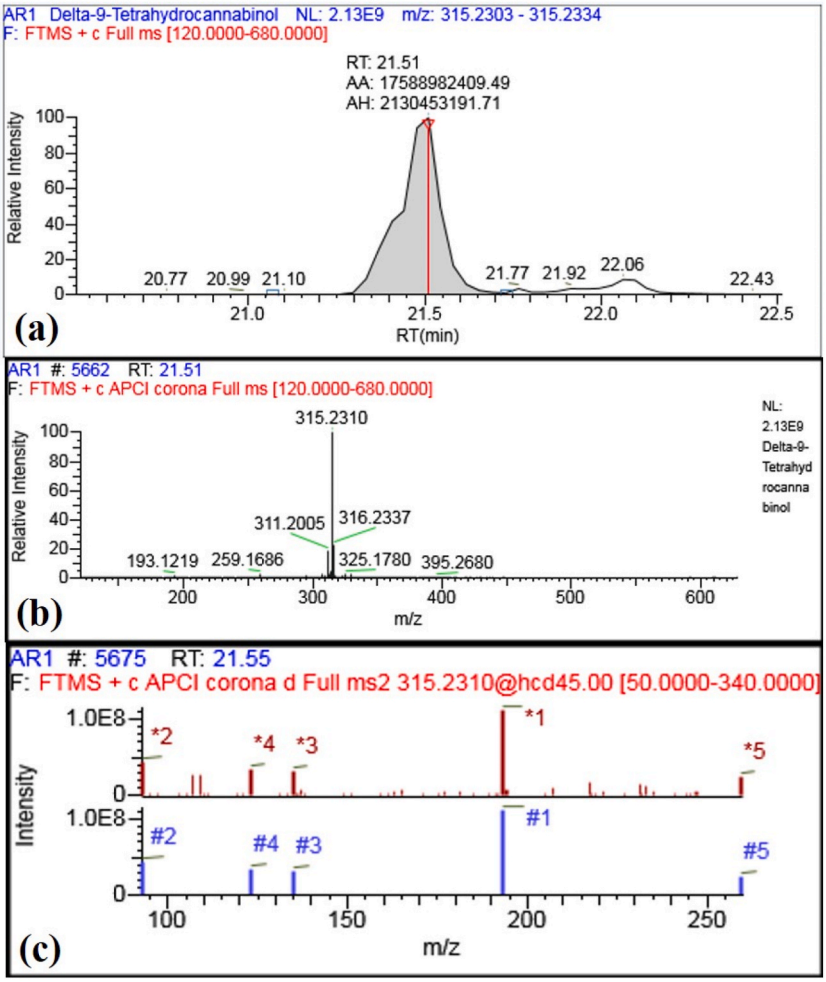

Fig. 4. The relative intensity as a function of retention time, RT **(a)**, mass-to-charge ratio, *m/z* **(b)**, and fragmentation pattern of Δ^9-THC **(c)** for a representative *Cannabis sativa* sample from Arusha.

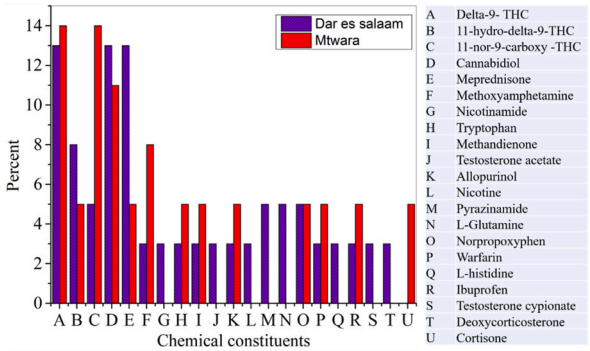

Fig. 5. Chemical constituents of *Cannabis sativa* samples from Mtwara and Dar es Salaam.

coastal regions, with Dar es Salaam found at the central part of the eastern zone while Mtwara is located at the south-eastern part of Tanzania.

Two phenomena can be used to explain the observed variations. Firstly, by both being in the coastal area, it is very likely that the geographical locations influenced the similarity of chemical composition between Mtwara and Dar es Salaam. However, that phenomenon can be supported if Dar es Salaam is considered as a cultivation area. Being a capital city that is small and densely populated,

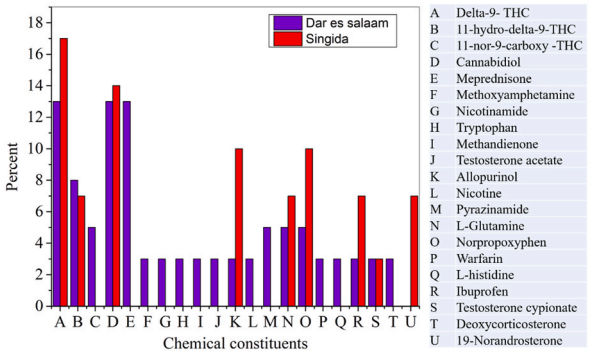

Fig. 6. Chemical constituents of *Cannabis sativa* samples of Singida and Dar es Salaam.

Dar es Salaam is considered a market for Cannabis. Secondly, the seized Cannabis from Dar es Salaam originated from the Mtwara region which is considered a cultivation area. This, however, cannot be conclusive unless an investigation of the chemical constituents from all regions is performed, including the control of harvesting season and age of the Cannabis plant. Singida, on the other hand is a semi-arid region found in the central part of Tanzania.

The variation of chemical constituents between Singida and Dar es Salaam can also be discussed by considering the two phenomena. If Dar es salaam is a cultivation area, the large difference in chemical constituents can be associated with the difference in weather conditions. And if is indeed a market for the cultivated Cannabis from elsewhere, the seized sample from Dar es Salaam does not originate from Singida. Generally, this baseline study indicates that by analyzing samples from all regions and controlling the growth parameters, it is possible to establish the origin of a seized sample found in the market.

The variation of retention time with mass to charge ratio of the various chemical constituents were also evaluated. The corresponding data are shown in Table 2. The highest retention times were observed for pyrazinamide (23.84 min), testosterone cypionate (22.87 min), and deoxycorticosterone acetate (22.06 min), which have the mass to charge ratio of 124.0506, 413.3041, and 373.2359 respectively. The lowest retention times, on the other hand, were observed for L-glutamine (2.82 min), allopurinol (3.20 min), and nicotinamide (3.24 min) with M/Z ratios of 147.0763, 137.0457, and 123.0954 respectively. Table 2 also shows that the mass to charge ratio of most of the chemical constituents with RT between 20 and 23 min ranges from 250 to 310.

From Table 2, a scatter plot of RT versus M/Z was drawn, as shown in Fig. 7. The general trend is that the RT of chemical constituents increases with increasing M/Z. This observation implies that it takes longer for heavier compounds to be eluted from the column. However, Fig. 7 also shows that pyrazinamide, L-histidine, and acetaminophen have long RT values despite the relatively lower M/Z ratios.

It was also interesting to see that pyrazinamide with an m/z of 124.0506 exhibited the highest RT among all the chemical

Table 2
Variation of retention time (min) with a mass-to-charge ratio of various chemical constituents of *Cannabis sativa* sample, analyzed by LC-MS.

S/No	Chemical constituent	Retention Time (RT)	Precursor (m/z)
1	Delta-9- Tetrahydrocannabinol	21.51	315.231
2	11-nor-9-carboxy –THC	21.51	345.2047
3	11-hydro-delta-9-THC	20.69	313.2157
4	Cannabidiol	21.51	315.2310
5	Meprednisone	19.09	373.2001
6	Methoxyamphetamine	3.69	166.1224
7	Nicotinamide	3.24	123.0954
8	Tryptophan	9.18	205.0969
9	Methandienone	20.95	301.2160
10	Testosterone acetate	20.69	331.2260
11	Allopurinol	3.20	137.0457
12	Nicotine	3.48	163.0452
13	Pyrazinamide	23.84	124.0506
14	L-Glutamine	2.82	147.0763
15	Norpropoxyphen	20.44	326.2105
16	Warfarin	20.44	309.1112
17	L-histidine	21.92	156.0765
18	Ibuprofen	11.98	207.1378
19	Testosterone cypionate	22.87	413.3041
20	Deoxycorticosterone-acetate	22.06	373.2359
21	Tyrosine	3.48	182.0810
22	Caffeine	12.71	194.1901
23	Cortisone	18.13	361.2003
24	Acetaminophen	15.49	157.0711

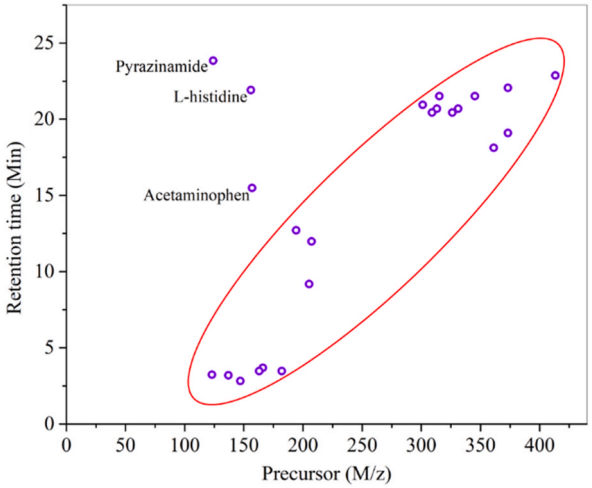

Fig. 7. A scatter plot of retention time (min) as a function of *m/z* ratio.

constituents, indicating that the high RT value is caused by a factor other than the M/Z ratio. A reverse-phase column whereby a stationery phase is nonpolar and a mobile phase is polar (consisting of water and acetonitrile), was used to perform the LC-MS analysis. The highest retention times of the compounds are attributed to the affinity of these compounds to the nonpolar stationary phase.

4. Discussions

Despite the fact that *Cannabis sativa* has more than 500 chemical substances, some of them may exist in amounts that are below the detection limit of the instrument. Moreover, some chemical components may be completely absent from all *Cannabis sativa* species depending on the geographical origin of the samples. 24 chemicals were successfully identified in this study. This suggests that even if the instrument's detection limit affected some substances, the constraint could not have an impact on the comparison because it would apply to all samples as a common denominator.

From the results, THC, the active ingredient in *C. sativa* was observed in all samples from the selected regions, while cannabidivarol was observed in the sample from Mtwara region only. Dar es Salaam had nine cannabinoids, the most of any region, whereas the sample from Tanga had the lowest, with only two. The variation of the composition of the cannabinoid constituents could be associated with the geographical locations and other factors associated with it, such as nature of the soil and weather.

On the other hand, the LC-MS analyses revealed both cannabinoids and non-cannabinoid chemical constituents. Apart from cannabinoids, the majority of other chemical substances, which included standard drugs, were found in the Dar es Salaam region sample. This could be due to the fact that the city is the epicenter of business and considered as the market rather than the cultivation area of cannabis. This implies that the samples were seized from petty traders and last users. The chemical compositions were not observed in other samples from most of the other regions.

The results, also revealed that the similarity between CBD and THC which were due to the fact that they are both naturally occurring with the same molecular formula and follow similar biosynthesis pathway. Both, CBD and THC were sparingly soluble in water but highly soluble in organic solvents. The existence of an extra cyclic ring in Δ^9-THC is one significant structural variation that results in the two molecules having radically distinct pharmacological effects.

5. Study limitations

According to several drug related acts of Tanzania, possessing and use of Cannabis is illegal and can lead to prison sentence. Therefore, samples for this study were obtained and analysis performed at the Government Chemist Laboratory Authority (GCLA), the legal entity which is a center for laboratory analysis of samples/exhibits related to Forensic Sciences. The samples were therefore obtained as seized samples, meaning that the researchers had less control over the sampling process at the studied regions. Another limitation of this study is that researchers had no information about when the plants were cultivated (season), and the age of the plant during its harvest, all of which could affect the chemical composition of the seized sample. Therefore, the findings hereby reported gives the preliminary information about the correlation between the chemical compositions of seized samples from different regions.

Future study may therefore involve a systematic and controlled cultivation, and the analysis takes into consideration of the season of cultivation as well as the age of the plant during harvesting with a further comparison between samples from the controlled cultivation and seized samples. In this study, five samples from each region were considered for analysis. Considering that the study relied on the seized samples, the regions in which less number of seized samples was obtained was excluded from this study.

6. Conclusions

Samples from all 11 regions tested positive for *Cannabis sativa* by the presence of Δ^9-THC. Through GC-MS analysis, 9 cannabinoids were identified, while LC-MS revealed 24 chemical constituents which included 15 drugs, 5 amino acids, and 4 cannabinoids. The variation in chemical constituents between the studied regions was evident. Out of 24 chemical constituents identified, Dar es Salaam was found to contain 22 of them. Being considered as a market rather than a cultivation region, the appearance of many chemical constituents suggests the possibility of blending for business purpose. Since the study involved seized samples with no control over the age of the plant and harvesting season, it is recommended to control these conditions in future study, because they may affect the variability of chemical constituents. A future study could also involve similar investigations in other regions, to establish a pattern of chemical constituents concerning the region or source.

Ethical considerations

The permit to undertake this research was granted by the Drug Control and Enforcement Authority (DCEA) of the United Republic of Tanzania, under the respective DCEA act of 2015, R.E 2018. All sample analyses were performed with confidentiality at the Government Chemist Laboratory Authority (GCLA), the country's leading laboratory for Forensic Science related analysis.

Author contribution statement

Husna B. Mhando: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Mtabazi G. Sahini: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

John J. Makangara: Analyzed and interpreted the data; Wrote the paper.

Data availability statement

No data was used for the research described in the article.

The authors declare no competing interests.

Declaration of competing interest

The authors declare no competing interests.

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