



Development of Canna-Products and Their Physiochemical Analysis

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ABSTRACT

Plant '*Cannabis sativa*' exhibits a wide range of astonishing therapeutic properties. It has a great potential to cure many diseases such as cancer, and neurological disorders. Although it is a plant with enormous beneficial properties, it also has some limitations regarding to dose range of THC. According to WHO, 10 milligrams/day of THC has been recommended for therapeutic purposes. To examine medicinal aspect of *Cannabis*, three canna-products by using *Cannabis sativa* plant leaves were prepared that includes canna-cookies, canna-candies and canna-muffins. Moisture, crude fat, ash, crude fiber and protein tests were carried out for nutritional evaluation whose result showed that they have sufficient nutritional values. Phytochemical test showed the presence of alkaloids, flavonoids, resins, cannabinoids, terpenes and steroids. As these components have great pharmaceutical importance, hence indicates beneficial aspect of canna-products. These constituents also possess different therapeutic properties involves, tumor metastasis inhibition, anti-oxidant, anti-inflammatory, immunomodulatory, antiepileptic, sedating, hypnotic, ant dystonic and anti-depressive effects. pH stability test was performed for three months with a gap of fifteen days to detect the product shelf life on different temperatures. Antioxidant activity test was performed by using DPPH assay. Antioxidant activity of methanol, ethanol, chloroform and water extract of canna-products was calculated. Results showed high anti-oxidant activity in canna-products. The results revealed that canna-products can deal with various medical problems and are cheaper, which encourages the use of *Cannabis sativa* in making food items for pharmacological purpose.

INTRODUCTION

Marijuana, scientific name *Cannabis sativa* L. is a lush, dioecian, annual, wind pollinated and flowering plant. Originally the *Cannabis* was divided by biologists just as *Cannabis sativa* L. which was further classified into three subspecies due to their distinct structural and chemical modifications from which *Ruderalis* is hardly used specie while *Sativa* and *Indica* are most commonly used species.⁶

Cannabis is a chemically well studied plant, contains around 538 known chemical constituents. But still many of them were not completely labelled as biologically active component that is why *Cannabis* plant might be known as neglected pharmacological treasure trove.⁷ Group of C21 terpenophenolic compounds, known as "Cannabinoids", are unique compounds in *Cannabis*.⁸ The glandular trichome of female flower secretes high amount of Cannabinoids.^{9,10,11,12} Due to the discovery of endogenous cannabinoid receptor ligand, ajulemic acid and formation of synthetic cannabinoids, *Cannabis* constituents were given name "phytocannabinoids".^{13,14,15,16}

Diverse chemical constituents found in *Cannabis* such as, terpenoids, cannabinoids, non-cannabinoid phenols,

simple lactone, nitrogenous compounds, simple ester, flavonoids, fatty acids, simple acids, simple aldehydes, steroids, alcohol, vitamins, pigments, amino acids, hydrocarbons, simple ketones, sugars, enzymes, protein and glycoproteins. The most significant biologically active classes of compounds are cannabinoids, flavonoids, terpenoids and hemp oil or fatty acid.

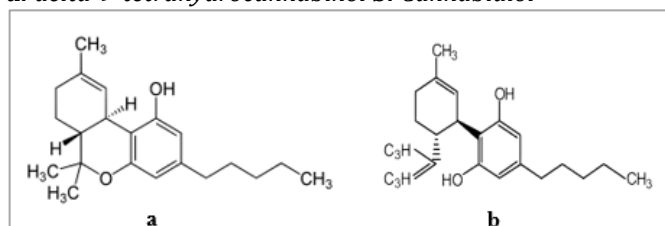
Up till now, cannabinoids have been defined as a group of terpenophenolic compounds, their analogs, carboxylic acids, and transformation products present in *Cannabis* plant. Modern definition of *Cannabis* reveals noticeable status to pharmacology and synthetic chemistry. In 1940, first cannabinoid from *Cannabis* was isolated.^{17,18} Cannabinoids are the main biologically active chemical constituent found in *Cannabis*. Among various other naturally occurring cannabinoids, some biologically active cannabinoids are THCA, THC, delta-8-THC, CBDA, CBD, THCV, CBGA, CBG, CBNA, CBN, CBCA and CBC. Among these, THC and CBD are two major important cannabinoids in *Cannabis* plants.

THC stands for delta-9-tetrahydrocannabinol (Figure 1a). THC is psychoactive in nature that is the reason it is

mostly used to measure the potency of the herb. In cancer patients, THC inhibits vomiting and nausea induced by chemotherapy. For this purpose, oral capsules are approved, which involves Dronabinol or Nabilone. Other biological activity of THC includes, increasing metabolic rate in human and animal brain.^{20,21} THC also has neuro-protective properties that include protection of the optic nerve and retina. THC is considered useful also in treating epilepsy and multiple sclerosis. THC possesses number of pharmacological effects including, inhibition of proliferation, apoptosis and inhibition of tumor metastasis and angiogenesis.²² Cannabidiol (CBD) is an important non-psychotropic cannabinoid (Figure 1b). It possesses potent anti-oxidant effects, more powerful than α -tocopherol and ascorbate. Also it has remarkable immune modulatory, reduce anxiety, anti-inflammatory, anti-psychotic, hypnotic and anti-dystonic effects.^{23,24} Studies show dominant effect of CBD in various cancer types.²⁵

Figure 1

a: delta-9-tetrahydrocannabinol **b:** Cannabidiol¹⁹



Up till now, more than 120 different terpenoids are identified in hemp plant that involves mono-terpenoids, di-terpenoid, sesquiterpenoids, and terpenoids. Two review papers have excellently summarized these compounds and their identification.²⁶ Because terpenes have antidepressive and sedative effects, it counteract towards adverse effects of THC. Limonene exhibits potent antidepressant effect and is a major constituent of hemp essential oil.²⁷ Up till Now, 23 flavonoids have been identified in *Cannabis*.²⁸ Flavonoids are often believed to minimize adverse reactions of *Cannabis* cannabinoids and gives protection against adverse UV radiation effects. Flavonoids have been isolated from the flowers,^{29,30} leaves,^{31,32} stems and pollen of the plant.³³ Some famous flavonoids include β -Stiostero, Cannaflavin B, Apigenin, Kaempferol, Luteolin, Orientin, Quercetin and Cannaflavin A. Other components found in *Cannabis* includes, nitrogenous compounds, non-cannabinoid phenols, stilbenoids, phenolic amides and Lignaamides.

Cannabinoids found in *Cannabis* interact with cannabinoid receptors in human endocannabinoid system. The cannabinoids interacting with these receptors play a beneficial role in reducing pain, appetite, sleep, inflammation and much more.³⁴ Cannabinoid receptors are spread all over the body but mostly appear as CB1 and CB2 receptors. CB1 receptors are found in intestines, bladder and heart, and mostly in brain areas related to mood, pain, memory and movement etc. While CB2 receptors found in blood, thymus gland, spleen, skin etc.

There are two medicinal products of *Cannabis* which consist of Synthetic THC. Dronabinol (Marinol) for soreness and sickness is approved by US FDA in 1985 for HIV-AIDS associated weight loss and nausea caused by chemotherapy. Nabilone (Cesamet) approved in 1985 for

nausea & neuropathic pain. Various surveys published in the medical and scientific literature suggests using approximately 75 mg to a maximum of 2-3 grams of dried *Cannabis* per day for therapeutic purposes.^{35,36,37} In this study, *Cannabis* was used to prepare canna-products which can further be used for medicinal purposes. We used the daily recommended dose of *Cannabis* in our products. Proximate and phytochemical tests were performed on these canna-products. pH stability and antioxidant activity were also determined.

MATERIALS AND METHODS

Research work was performed and carried out in Food and Biotechnology Research Center (FBRC) of Pakistan Council of Scientific and Industrial Research Lab Complex Lahore (PCSIR).

Sample Preparation

Cannabis plant was taken from Botanical Garden, Punjab University. It was allowed to dry in raw form for few days. Then converted into fine raw powder by crushing. This *Cannabis* raw powder was used in future for formation of Canna-butter and *Cannabis* infused coconut oil. The daily recommended dose for *Cannabis* was kept in mind while making these products.

Canna-butter Formation

Unsalted Butter (115g) and raw *Cannabis* Powder (2g) were used for canna-butter formation. *Cannabis* raw powder was added in melted butter, and heated for 45 minutes and stirred frequently on low heat. After that, butter was stained by pushing and smashing back with spoon against the plant matter so that every drop of available butter will squeeze out. Remaining plant matter was discarded and canna-butter was immediately allowed to frozen. After freezing, canna-butter appeared as light green in color depending on the amount of added *Cannabis*.

Canna-coconut Oil Formation

To make *Cannabis* infused coconut oil, coconut oil (130ml), raw *Cannabis* (1g) and double boiler were used. The bottom pan of a double boiler was filled with water over medium heat. The coconut oil and raw *Cannabis* was added in top pan. It was cooked for about 3 hours and stirred continuously. After 2-3 hours cooking, deep green color appeared. After cooling, liquid was poured over cheese cloth to strain out plant material in jar. The remaining oil was strained. It was then stored in a sealed container for future use.

These *Cannabis* products were further used in formation of desired products which include Canna-cookies, Canna-candies and Canna-muffins.

Canna-cookies Formation

Oven was preheated to 350°F. Baking soda, salt and flour were whisked together, set aside. With an electric mixer, melted butter and sugars were beaten at low speed for about 2 minutes. On low speed, milk, vanilla extract and the egg were added. Flour mixture was gradually added just until incorporated. Chocolate chips were folded in with a spatula. 8-10 mounds of dough were scooped onto a parchment lined cookie sheet. In preheated oven, it was

baked for 9-12 minutes. After cooling it was stored at room temperature in air tight jar.

Canna-candies Formation

Sugar, canna-butter, and honey were mixed together in a pan over medium heat. The mixture was constantly stirred until the sugar was dissolved. Then this mixture was boiled until formation of sticky foam material. It was again heated for 5-10 minutes to eliminate the stickiness of material. After this, jelly powder and gelatin were immediately added to pan while constantly stirring. The hot canna-candies mixture was poured into candy mold. After 20-30 minutes, canna-candies were wrapped in Aluminum foil and stored in refrigerator for future use.

Canna-muffins Formation

Muffin cups were lined with paper muffin cases. Oven was preheated to 400°F. Flour, granulated sugar, baking soda, baking powder and salt were stirred together in a large bowl. Milk, *Cannabis*-infused coconut oil, eggs and vanilla extract were stirred together in another bowl until blended. A well was made in the center of dry ingredients. The milk mixture was slowly added and stirred just to combine both mixtures until the smooth batter was formed. Chocolate chips were added in the batter. The prepared muffin cups were filled and baked for 5 minutes at 400°F. Then oven temperature was reduced to 375°F and baked for another 12-15 minutes. They were then allowed to cool. All the three samples were ready for different tests. Here are the tests that were performed on these products:

Proximate Analysis

The standard methods of Association of Official Analytical Chemists were used for proximate analysis of canna-products.³⁸ In moisture test, three empty petri dishes were weighed. Samples (0.5g) were deposited in the previously weighed petri dishes. The dishes were then weighed again. Dishes were placed at 100°C in the oven. Dishes were removed from the oven after 4 hours, cooled in a desiccator and weighed by using weighing balance.

Moisture (%) = $\frac{\text{Weight of fresh sample (g)} - \text{Weight of sample after drying}}{\text{Weight of sample (g)}} \times 100$

Weight of sample (g)

In Soxhlet extraction method, hexane was used as a solvent. Each sample (2g) was wrapped in filter paper and placed in Soxhlet extractor. Extraction with 75 to 100ml hexane in round bottom flask was done by setting temperature 60°C for 8 to 12 hours. After cooling, samples were weighed and crude fat was calculated.

Fat (%) = $\frac{\text{Weight of beaker with fat (g)} - \text{Weight of empty beaker (g)}}{\text{Weight of original sample (g)}} \times 100$

Weight of original sample (g)

In ash test, 0.5g of each sample was taken in clean weighed crucible and burnt at low flame until the black fumes disappeared. These crucibles were then placed in a furnace at 550°C for 24 hours till a light grey ash appeared. They were weighed after cooling and ash was calculated.

Ash (%) = $\frac{\text{Weight of sample after ashing (g)}}{\text{Weight of sample (g)}} \times 100$

Weight of sample (g)

For protein content, each sample was weighed 0.2g and transferred to digestion flask. Digestion mixture (2-3g) and concentrated H₂SO₄ (25ml) was added and

digested. After digestion, material was transferred into 100ml volumetric flask and volume was made up to 100ml with distilled water. In 5ml of each mixture, 10ml of 40% NaOH was added. Boric acid solution (2%) and phenolphthalein was used as an indicator for distillation. N/10 HCl solution was used for titration. The protein content was calculated by calculating nitrogen content.

Nitrogen (%) = $\frac{\text{Titrate} \times \text{vol.} \times \text{normality} \times 100 \times 14}{\text{Volume taken} \times \text{weight of sample} \times 1000}$

Volume taken x weight of sample x 1000

Protein (%) = Nitrogen % x Factor (6.25)

In fiber test, each weighed sample (2g) and H₂SO₄ (200ml) was transferred in separate beakers. They were allowed to reflux on a hot plate for half an hour. Filter cloth was used for filter process and washed by using distilled water. These residues were transferred into another beaker consisting of 200 ml NaOH. They were then allowed to reflux again on a hot plate for half an hour. After filtering and washing, residues were dried in an oven at 100°C for 2 hours. After cooling, they were weighed. These residues were kept in muffle furnace at 500 to 550°C and left for 24 hours for complete charring. Fiber % was then calculated.

Fiber (%) = $\frac{\text{Weight of dried sample in oven (g)} - \text{Weight of ash (g)}}{\text{Weight of sample (g)}} \times 100$

Weight of sample (g)

Carbohydrate and total energy amount in each sample was then calculated by using specific formulas.

CHO = 100 - (moisture + ash + fiber + fat + protein)

Energy = (% CHO x 4) + (% fat x 9) + (% protein x 4)

Phytochemical Analysis

Sofowora; Scott *et al.*; Butler, methods were used for phytochemical analysis.^{39,40,41} Stock solution of each sample was prepared. 5g of each sample was soaked in 200 ml of petroleum ether at 25°C for 24 hours. Then 25 ml acetone was added in them.

For resins determination, 2ml acetic anhydride was added in each 2ml extract of petroleum ether canna-samples in test tubes. Carefully 3 drops conc. tetraoxosulphate (V1) acid was added in each test tube and observed for color change to violet. For detection of tannins, 3 drops of 10% FeCl₂ was added in each 2ml extract of petroleum ether canna-samples (Ferric chloride test). Formation of reddish precipitates indicates positive result. For alkaloids detection, few drops of Dangendroff's reagents were added in 2ml extract of each petroleum ether canna-samples. Resultant mixtures were noticed for color change, mostly orange to deep orange coloration.

For detection of Saponins, 100ml of distilled water was added in test tubes containing 2ml extract of each petroleum ether canna-samples. Tubes were shaken vigorously for one minute. After 30 seconds three olive oil drops were added. They were then observed for color change which mostly gives dark brown coloration. For flavonoids detection in samples, 2ml of 10% lead acetate solution was added in 2ml extract of each petroleum ether canna-samples. After 10 sec, they were observed for precipitation and color change to yellowish. For balsams identification, 2ml alcohol FeCl₃ and 2ml extract of each petroleum ether canna-samples was added in test tubes. For 5 seconds, mixtures were heated on the Bunsen flame and then observed for color change to reddish brown.

For steroids and terpenes identification, 1ml of anhydrous acetic acid was added in test tubes containing 2ml of petroleum ether canna-samples extract. Along the side of the test tubes, concentrated tetraoxosulphate acid was added carefully. Color changes into red indicate the presence of steroids and terpenes. For Cannabinoids detection, 2 ml of Duquenois reagent and a small amount of petroleum ether canna-samples extract were taken in test tubes and shaken for 60 seconds. Then 2 ml of concentrated HCl was added and again shaken for 1 minute. In end, 2 ml of chloroform was added and mixed. After 10 minutes, color change was observed for Cannabinoids detection.

pH Stability

Canna-samples stability was measured and compared by measuring pH of samples. Three temperatures 25°C, 7°C and -8°C were used. This test was performed for almost three months with a gap of fifteen days. On day 0, each sample was divided into three parts. Each part of sample was crushed into smaller pieces and then weighed. 1 gram of each sample was dissolved in 10 ml distilled water properly. After that, the nine solutions were filtered by using filter paper. Filtrates were then transferred into beakers and residues were wasted. Filtrates of three samples were then placed at three different temperatures. pH of all these three samples were measured on alternative days by using pH meter.

Antioxidant Activity

Williams *et al.*, method⁴² was used for antioxidant analysis. The extracts of four samples were prepared in methanol, ethanol, chloroform and distilled water. 0.3g of each sample was added in 10 ml of each solvent in a test tube. They were placed in test tube racks at room temperature for 2-3 days until all the samples completely dissolved in solvents. After that, a stock solution of DPPH was taken. 3ml of *Cannabis* extract was added in 25µl, 50µl and 75µl of DPPH solutions while Canna-cookies, Canna-candies and Canna-muffins extracts were added in 100µl, 200µl and 300µl of DPPH solutions respectively. DPPH mixture and extracts were placed in a glass cuvette. UV-VIS spectrophotometer was used for measuring blank reading and absorbance changes at 517nm. The percentage of DPPH consumption was calculated in each case. The higher the rate of DPPH consumption, more the antioxidant activity will be.

%age of DPPH inhibition = $\frac{\text{Blank reading} - \text{Sample reading}}{\text{Blank reading}} \times 100$

Blank reading

RESULTS

Analysis results of the proximate composition of canna-products are presented in table 1. The total energy value was 396 kcal, 395.5 kcal and 417.9 kcal for the canna-cookies, canna-candies and canna-muffins.

Table 1

Proximate analysis of canna-cookies, canna-candies and canna-muffins

Proximate Composition (%)	Canna-cookies	Canna-candies	Canna-muffins
Moisture	5.0	2.7	18.9
Fat	7.2	1.5	20.3

Ash	0.2	0.3	1.1
Crude Protein	9.1	0.0	6.4
Crude Fiber	0.5	0.0	0.9
Carbohydrate	73.7	95.5	52.4

Phytochemical tests indicated same results for all three canna-products i.e. canna-cookies, canna-candies and canna-muffins (Table 2). The incidence of light-yellow orange, violet, reddish and purple color confirmed the presence of resins, alkaloids, flavonoids, terpenes, steroids and cannabinoids.

Table 2

Qualitative phytochemical screening of canna-cookies, canna-candies and canna-muffins

Phytochemicals	Canna-products	Color
Resins	+ve	Violet
Tannins	-ve	-
Alkaloids	+ve	Orange
Saponins	-ve	-
Flavonoids	+ve	Light yellow
Balsams	-ve	-
Terpenes and steroids	+ve	Reddish
Cannabinoids	+ve	Purple

The pH of the canna-products at different temperatures (7°C, 25°C and -8°C) with fifteen days gap is shown in table 3. The overall result of canna-cookies and canna-muffins indicates that these samples are basic in nature. While canna-candies result indicates that this sample is slightly acidic in nature.

Table 3

pH stability of canna-cookies, canna-muffins and canna-candies at different temperature

Samples	Days	7	25	-8
Canna-cookies	0	8.3	8.2	8.1
	15	8.3	8.3	8.2
	30	8.4	8.5	8.2
	45	8.6	8.5	8.3
	60	8.8	8.6	8.4
	75	8.8	8.7	8.6
	90	8.9	8.8	8.6
	0	6.2	6.1	6.3
	15	6.1	6.0	6.2
Canna-candies	30	6.0	5.8	6.2
	45	5.8	5.7	6.1
	60	5.7	5.6	6.0
	75	5.5	5.4	5.9
	90	5.6	5.4	5.8
	0	9.2	9.4	9.2
	15	9.3	9.5	9.4
	30	9.3	9.6	9.4
	45	9.5	9.4	9.5
Canna-muffins	60	9.6	9.6	9.7
	75	9.5	9.5	9.6
	90	9.5	9.6	9.7

Antioxidant activity (AOA) of different canna-extracts is present in below tables. The absorbance of blank was 0.678 observed by UV-spectrophotometer. AOA of methanol extracts of canna-samples are presented in table 4. Methanol extract of all three samples with different concentrations shows different % of radical scavenging activity.

Table 4

Antioxidant activity of canna-cookies, canna-candies and canna-muffins in methanol

	Concentration	Absorbance	Inhibition (%)
Canna-cookies	100 µl	0.345	49.11
	200 µl	0.221	67.40
	300 µl	0.106	84.36
Canna-candies	100 µl	0.204	69.91
	200 µl	0.114	83.18
	300 µl	0.041	93.95
Canna-muffins	100 µl	0.294	56.63
	200 µl	0.271	60.02
	300 µl	0.245	63.86

AOA of ethanol extracts of canna-samples are presented in table 5. Ethanol extract of all three samples with different concentrations shows different % of radical scavenging activity.

Table 5

Antioxidant activity of canna-cookies, canna-candies and canna-muffins in ethanol

Sample	Concentration	Absorbance	Inhibition (%)
Canna-cookies	100 µl	0.358	47.19
	200 µl	0.238	64.89
	300 µl	0.126	81.41
Canna-candies	100 µl	0.224	66.96
	200 µl	0.138	76.64
	300 µl	0.068	89.97
Canna-muffins	100 µl	0.305	55.01
	200 µl	0.285	67.96
	300 µl	0.258	61.94

AOA of chloroform extracts of canna-samples are presented in table 6. Chloroform extract of all three samples with different concentrations shows different % of radical scavenging activity.

Table 6

Antioxidant activity of canna-cookies, canna-candies and canna-muffins in Chloroform

Sample	Concentration	Absorbance	Inhibition (%)
Canna-cookies	100 µl	0.454	33.03
	200 µl	0.372	45.13
	300 µl	0.296	56.34
Canna-candies	100 µl	0.365	46.16
	200 µl	0.301	55.60
	300 µl	0.257	62.09
Canna-muffins	100 µl	0.420	38.05
	200 µl	0.405	40.26
	300 µl	0.391	42.33

AOA of water extracts of canna-samples are presented in table 7. Water extract of all three samples with different concentrations shows different % of radical scavenging activity.

Table 7

Antioxidant activity of canna-cookies, canna-candies and canna-muffins in water

Sample	Concentration	Absorbance	Inhibition (%)
Canna-cookies	100 µl	0.499	26.40
	200 µl	0.436	35.69
	300 µl	0.371	45.28
Canna-candies	100 µl	0.471	30.53
	200 µl	0.431	36.43
	300 µl	0.399	41.15
Canna-muffins	100 µl	0.498	26.54
	200 µl	0.489	27.87
	300 µl	0.479	29.35

DISCUSSION

In this research, development, proximate analysis, phytochemical analysis, pH stability and antioxidant activity of canna-cookies, canna-candies and canna-muffins consisting of *Cannabis sativa* L. were examined which were not studied before.

Firstly, canna-butter and canna-oil was prepared by using accurate amount of raw *Cannabis*. According to Hazekamp and Heerdink,⁴³ Carter *et al.*,⁴⁴ Ware *et al.*,⁴⁵ dosage of *Cannabis* for medical use should be less than one gram and not more than five grams per day. So, two grams of raw *Cannabis* was added in butter and 1 gram in coconut oil. Later, canna-butter and canna-oil were used in canna-cookies, canna-candies and canna-muffins formation respectively.

In the proximate analysis, the canna-samples were first tested for moisture determination. Moisture test tells us about the quantity of water in product and plays important role in controlling shelf life and determining product quality. The result of the moisture content of canna-cookies, canna-candies and canna-muffins were 5.0%, 2.7% and 18.9%. Soxhlet extraction method was done for calculating the quantity of fat content in the food sample. The fat values of the samples were canna-cookies (7.2%), canna-candies (1.5%) and canna-muffins (20.3%). Ash test tells us about the existence of inorganic residues left after the removal of organic matter. 0.7%, 0.3% and 1.1% were the results of ash content in the canna-cookies, canna-candies and canna-muffins samples respectively. Kjeldahl method developed by Johan Kjeldahl was used for analysis of protein in the samples. Nitrogen percentage and then protein percentage was calculated. 9.1%, 0.0% and 6.4% protein contents were present in the samples of canna-cookies, canna-candies and canna-muffins respectively. Fiber test was conducted which determined that food samples canna-cookies, canna-candies and canna-muffins contained 0.5%, 0.0% and 0.9% of fiber content respectively. According to Singh *et al.*,⁴⁶ carbohydrate plays a major role in reducing the risk of heart disease, inflammatory bowel disease, colorectal cancer etc. The amount of carbohydrate in the canna-cookies, canna-candies and canna-muffins samples was 73.7%, 95.5% and 52.4% respectively which is healthier for human body.

Different tests were performed in order to analyze the presence of phytochemicals in the samples. Dillard and German,⁴⁷ demonstrated that phytochemicals present in plant species have strong anti-parasitic, anti-inflammatory, antiviral, anticancer, antibacterial properties.

Resins presence was determined by violet color appearance which is consider as positive result indicating the presence of resins in samples. No red precipitate was formed in Ferric chloride test so it was concluded that no tannins were present in the samples. Tannins absence is beneficial as tannins have toxic effects in humans like minimizing mineral uptake, digestive enzymes activities, amino acid presence, and other harmful effects indicated by Salunkheet *al.*⁴⁸ Dragendorff test was performed for the detection of alkaloids presence in all three samples. Test results in the deep orange color change. Aniszewski,⁴⁹ in his book related to alkaloids demonstrated that in human's

regulation, stimulation and inductions are the functions which are directly related to alkaloids. Wink *et al.*,⁵⁰ and Fahey *et al.*,⁵¹ has demonstrated that alkaloids provide protection and is important to human health as it has pharmacological properties. The saponins test of canna-products resulted in negative, no brown color appeared which meant that no saponins were present in the samples. In Lead acetate test light yellow color change was observed which shows the presence of sufficient flavonoids in all three samples. According to Kinsella *et al.*,⁵² flavonoids have biological activities against allergies, platelet aggregation, tumors etc. Aherne and Brien,⁵³ also tells about anti-mutagenic and antioxidant activities of flavonoids while Duthie *et al.*,⁵⁴ demonstrate that it has an important role in prevention of cancer and heart diseases. Balsams were detected in samples by observing reddish brown color change. No color change was occurred. Terpenoids and steroids detection in the samples were done by Burchard test. Reddish brown color change in the test tube had shown their presence. Langenheim,⁵⁵ and Dudareva *et al.*,⁵⁶ indicated that terpenoids have certain properties which are beneficial for human like anti-ulcer, anti-carcinogenic etc. Terpenoids also prevent different diseases mainly pancreatic cancer, growth dysregulation, chronic damage, protect lung tissue, according to Dorow *et al.*⁵⁷ While steroids in the human body affect various tissues and play a major role in various biology features like in sexual differentiation, metabolism, reproductive physiology, indicated by Henley *et al.*⁵⁸ Duquenois-Levine test was performed for determination of cannabinoids presence in the samples which is a major component in *Cannabis* and has important therapeutic effects. The purple color change was observed in all samples for cannabinoids detection. Holdcroft *et al.*,⁵⁹ demonstrated that in a patient having chronic abdominal pain, the need of morphine was decreased by using *Cannabis* extract. According to Nahas,⁶⁰ cannabinoids are neuroprotective agents as they can play important role in curing Alzheimer and Parkinson's disease.

Bello *et al.*,⁶¹ in his review article discussed pH effect on texture and color of the product. He also discussed that

water-holding capacity of the product is affected by pH stability. pH stability also affects the product's protein denaturing property, enzymatic activities etc. Canna-cookies, canna-candies, canna-muffins stability was measured by using pH meter. Three temperatures on which these three samples were stored was at room temperature 25°C, 7°C and freezing temperature -8°C. The overall result showed that canna-cookies showed pH 8.1 to 8.9 within 90 days which indicates that this sample is basic in nature. While canna-candies showed 5.3 to 5.4 pH within observing 90 days. Results indicate that canna-candies are slightly acidic in nature. While canna-muffins showed 9.2 to 9.7 pH at different temperature for three months. The results for canna-muffins revealed that these are basic in nature.

The antioxidant activity of canna-cookies, canna-candies and canna-muffins were determined by using DPPH as it is a simple and quick process. According to McPartland and Russo,⁶² *Cannabis* has major anti-oxidant property. Different reagents methanol, ethanol, chloroform and water were used as solvents. The result indicates that samples have good antioxidant activity. The overall figures of the samples showed that all samples have higher antioxidant activity in methanol solvent as compared to other solvents which were ethanol, chloroform, and water. Formation of such canna-products will be healthy for human consumption.

CONCLUSION

Canna-products are cheaper, have no side effects (when used in recommended dose range) and are grown easily, so they are easily available. The scope of this study is to use canna-cookies as a great alternative to medicines. This study reveals that canna-products have good nutritional values and the presence of flavonoids, terpenes, and cannabinoids showed that they are rich in antioxidant, anti-cancer, anti-inflammatory and many other medicinal properties. By using these canna-products as an alternative of medicines many diseases can be treated.

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Appendix

Table 1

Canna-cookies formation

All purpose flour	90g
Baking soda	2g
Salt	0.71g
Canna-butter	58g
Brown sugar	75g
Granulated sugar	39g
Egg yolk	20g
Milk	7ml
Pure vanilla extract	2.1g

Table 2

Canna candies formation

Honey	6 oz
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Gelatin	1.5g
Canna-butter	113g
Strawberry jelly powder	84g
White sugar	10.6g oz

Table 3

Canna muffins formation

All-purpose flour	400g
<i>Cannabis</i> infused coconut oil	125g
Granulated sugar	200g
Milk	170ml
Eggs	2
Baking soda	5g
Baking powder	13g
Salt	2.5g
Vanilla extract	12ml
Chocolate chip	100g