Propagation of Medicinal Capers (*Capparis Spinose* L.) and Production of Some Medicinal Secondary Metabolic Compounds Using Plant Tissue Culture Technology

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Abstract
The experiments of this study were conducted for the purpose of using plant tissue culture technology to increase the production of medically effective alkaloids capparisine A (1), capparisine B (2), capparisine C (3), and the chemical components of the Caper plant (Capparis spinosa L.). The results of the study can be summarized as follows .The MS culture media prepared to induce and maintain callus at a concentration of 40 Naphthalene acetic acid (NAA) mixed with 4.5 Adenine sulphate (AdS) gave the highest rate of fresh and dry weight of callus with increasing NAA concentrations, reaching (481.22) mg for fresh weight and (26.545) mg. For dry weight .respectively. Thecate that the MS culture media prepared with a concentration of 100 (mg/l) Progesterone gave the highest average fresh and dry weight of callus, amounting to (50.61 and 7.461) mg, respectively.The results also showed that the MS culture media prepared with a concentration of 150 (mg/l) Cholesterol gave the highest rate of callus based on fresh and dry weights, amounting to (73.733 and 14.807) mg, respectively. While a concentration of 8 (mM) of Squalene gave an average fresh and dry weight in callus induction from the growing apex of (56.882 and 20.303) mg, respectively.The alkaloids capparisine A (1), capparisine B (2), and capparisine C (3) were quantitatively and qualitatively determined using an HPLC device.Its highest value was recorded for all alkaloids when the concentration of the precursors Progesterone, Cholesterol, and Squalene was increased in the secondary metabolite accumulation medium at a concentration of 150 (mg/l) of Progesterone, Cholesterol. While the concentration of 8 (mM) Squalene for alkaloids was best for production. Phytochemical analysis using LC-ESI-MS/MS of callus tissue extract showed the presence of some chemical and nutritional compounds.

Keywords: active compounds, alkaloids ،Chemical composition، *Capparis spinosa* L

INTRODUCTION

The natural products of medicinal plants in the pharmaceutical field have been known since ancient times, and more than 2,000 species from the plant kingdom are now known as a source of medicinal and pharmaceutical products and have proven medicinal effectiveness. The World Health Organization also indicated that 80% of the world's population depends on medicines from natural sources. It is a plant in medical treatment. It is mentioned that the active part of it is originally from plant material, and it also has an important contribution to improving human life as well as the financial revenues resulting from its manufacture [10]

It occupies medicinal plants, including the Capparis spinosa L., which is one of the most important species in the family (Capparidaceae), which includes about 40-50 genera and 700-900 species [9] and about 250 different species of the genus Capparis [7]. This plant has been known by many names, including *Capparis spinosa* L.

ISSN 2072-3857

Several names have been given that differ according to the country. In the Arab world, for example, it is called caper, caper, kabbar, shafallah, mountain pepper, and mountain rose [6]. The caper plant contains many pharmaceutically active compounds in various parts of the plant, such as the leaves and roots. [13] fruits, flower buds, and seeds [28] Many studies have indicated that the various parts of the Capparis spinosa plant contain active substances of great pharmaceutical benefit, and the most important of these compounds are (Steroids) and (Alkaloids). And (Flavonoids), and rutin works to reduce the aggregation of platelets in the blood vessels and improves cardiovascular indicators and strengthens the capillaries [12]and the group of flavonoids contains several medically effective elements such as (Rutin) and (Quercetin) [23] Capparis spinosa is rich in flavonoids found in the plant that reduce the risk of cardiovascular disease [14] and these compounds have a significant positive effect on health. Due to its antioxidant properties [3]. Also, flavonoids, which are hydroxylated phenolic compounds in plants.

As a result of this fluctuation and instability in the number of secondary products, especially when it comes to a plant that is exposed to extinction, researchers resort to employing modern technologies to increase the production of secondary metabolism, including the technology of plant tissue culture from different parts of the plant, which was found to be a good source of secondary products [19]

Given the importance of alkaloids and medically important chemical compounds in general and non-alkaloids in particular in medical and pharmaceutical treatment, which are found in plants of the Capparidaceae family as naturally occurring secondary metabolites, most notably the alkaloids capparisine A (1), capparisine B (2), capparisine C (3). In order to ensure and protect its production from extinction, control it for pharmaceutical purposes, limit its production governmentally, and prevent it from being exposed to extinction, and also due to the scarcity of scientific research and studies on it, this research was conducted to find out the effect of chemical agents on the growth of calluses, the accumulation of alkaloids, and some chemical compounds with medical effectiveness, and to detect quantitatively and qualitatively the compounds produced by it. Chromatographic analysis using high-performance liquid chromatography (HPLC) and Phytochemical analysis using LC-ESI-MS/MS

Materials and methods

This study was carried out in the Plant Tissue Culture Laboratory, Baghdad, Ministry of Science and Technology, Center for Food and Biotechnology, Department of Genetic Engineering.. Special laboratory analyzes were conducted to extract medical alkaloids and chemical compounds at the Dar Al-Bayda National Company and the Ministry of Science and Technology. For the period from November 2021 to December 2022, with the aim of producing some medicinal alkaloids from Caper (Capparis spinosa L.) ex vivo. The study included the following:

1. *Capparis spinosa* L. seeds were collected in June and July 2021 from two different places in Diyala Governorate, the first behind the garden of the old College of Agriculture and the second in the Saad camp area.

2. culture the seedling parts (growing apex) on MS medium prepared with different concentrations of Naphthalene acetic acid (NAA) and Adenine sulphate AdS) separately to induce callus.

ISSN 2072-3857

3. Culture of callus with different levels of chemical precursors Progesterone, Cholesterol and Squalene

4. Quantitative and qualitative estimation of alkaloids and chemical compounds using an HPLC device

Phytochemical analysis using LC-ESI-MS/MS

- MS nutritional environment

First, a stock solution of all plant growth regulators used in the study was prepared at a rate of (100) mg.l-1. Replace the solution monthly by preparing a new stock solution.

MS medium was used. 0.44 g of the medium was weighed, dissolved in an amount of distilled water, then 4 g of sucrose was added to it, and growth regulators were added to it according to the required concentrations, and the volume was increased to 100 ml. The hydrogen function number (pH) was adjusted to 5.6-5.8 using drops of sodium hydroxide (NaOH) 1 m and hydrochloric acid (HCl) 1 m, then 0.8 g of agar was added to the culture media for the emergence, then the components of the medium were mixed using a magnetic hot plate device (Hot plate magnetic stirrer). To dissolve the components of the culture media at a temperature of 90-100°C, distribute it directly into the vessels designated for culture. The flasks were covered with medical cotton and aluminum foil, then sterilized with an autoclave at a temperature of 121°C and a pressure of 1.5 bar for 15 minutes.

Preparing seedling explant for planting

After obtaining the best result of seed sterilization, the seeds of Capparis spinosa L. were planted in Universal Vials, containing 9 ml of MS medium free of growth regulators, at a rate of (5-6) seeds in each tube, incubated for 30 days in The growth room was used under controlled conditions in order to obtain sterile seedlings, as these seedlings were considered a source for obtaining growing shoots, which were used in most subsequent experiments.

- The effect of growth regulators used in inducing and maintaining callus

The growth regulators Auxinin, NAA at concentrations of 0.0, 20 and 40 µM and Cytokinin were used. Adenine sulphate (AdS) at concentrations of 0.0, 2.5, 4.5, 6.5 µM. The plants resulting from the previous stage were cut into growing apices. The growing apices were cut to a length of 1.3 cm, containing a pair of leaflets at the growing apex, and were scratched using a sharp blade on their lower side (abaxial). Which is in direct contact with the culture media. This process took place under sterile conditions that are often used in tissue culture technology programs.

. Measure the fresh and dry weight of callus.

The fresh and dry weight of the callus was measured seven weeks after planting using a sensitive balance. Pieces of fresh callus were extracted and placed on filter paper. The remaining culture media adhering to the callus was removed using a surgical blade, and the fresh weight of the callus was calculated. The fresh callus pieces were dried in an electric oven at 70°C and weighed until the weight was constant. The fresh and dry weight standard of induced callus at the germination stage was used to determine the best concentration of auxins and cytokinins.

Culture of callus tissue in culture media prepared with different levels of the initiators Progesterone, Cholesterol (mg/, and Squalene (mM)). Induction and maintenance of callus and production of medical secondary metabolic compounds.

A fixed weight of 100 mg of callus was taken and grown on MS culture media, which contains the same components as the MS medium at the stage of callus induction, which

ISSN 2072-3857

was prepared with a concentration of 40 µM of Auxins Naphthalene acetic acid 4.5 Cytokinins+ NAA Adenine sulphate AdS)) µM with the addition of different levels of Progesterone and Cholesterol. (0, 50, 100, 150, 200) (mg/l) with Squalene after it was prepared as a base solution at concentrations (0, 10, 20, 30,40) (mM). Ten replicates for each concentration. The cultures were grown in the dark at a temperature of 25°C ± 2°C. After five weeks, the fresh and dry weight of the callus was taken, and the extraction process was performed.

Chemical composition of *Capparis spinosa* L.(Caper)

Chemical profiles such as total carbohydrates Methods [21] , total cholesterol Methods ([24], total fats Methods [6] , total crude fibre Methods [25] , thiamine Methods [1] , riboflavin Methods [26] , Vitamin E Methods [11] , niacin Methods [16] , Vitamin C Methods [20] , total free amino acids Methods [2] , total proteins Methods [17] were estimated. Energy value was calculated using the formula 4 x (carbohydrates) + 4 x (proteins) + 9 (fats). [18]

Phytochemical analysis using LC-ESI-MS/MS *Capparis spinosa* L.(Caper)

Methods [22] [5] Different qualitative chemical tests were performed using standard procedures to identify the presence of major constituents. The major phytochemical constituent of Caper (*Capparis spinosa* L.) extract was investigated using LC-ESI-MS/MS (Make: Bruker, Model: MicroTOF-Q II). Solution (55 µl) was injected for liquid chromatography separations in a C18 reverse phase column (120 Å, 2.1 x 150 mm, 4.0 µm, Dionex, USA) and UV detector was set arbitrarily at 325 nm. Gradient elution at a flow rate of 0.3 ml/min was performed using mobile phase (Solvent A: Acetonitrile and B: water with 1% acetic acid). The gradient started from 2% of A for 0.1 min and it was then brought to 80% A at 16th min and then reaching at 100% A at 19th min to 5% A at 21st min and was maintained at same condition till run ends at 25th min. Eluted compounds were then identified using MS and their respective MS/MS pattern. The result of molecular mass was compared with mass bank data and the major phytochemical was identified.

Extraction of medically active alkaloids from *Capparis spinosa* L. (Caper)

Use of a high-performance liquid chromatography (HPLC) device

(High Performance Liquid Chromatography)

This effective technique is used as a means to estimate the quantity and quality of the alkaloids capparisine A (1), capparisine B (2), capparisine C (3), due to the ability of this system to separate the components of the extract of plant parts or callus tissue into their components, which enables the identification of the required materials by comparison. With standard samples, if alkaloids can be identified using Methods [27]

Silicagel column chromatography, reversed phase HPLC, NMR, X-ray crystallographic analysis.

Experimental design and statistical analysis

Single- or two-factor experiments with their interventions were designed according to Complete Random Design (CRD) with a number of replications according to what was stated in the materials and methods. The results were analyzed using the statistical program Statistical Analysis System (SAS) to study the effect of different parameters on the studied traits, and the significant differences between the means were compared with the

ISSN 2072-3857

Least Significant Differences (LSD) test with a probability of 5% [20]

Results and discussion

The effect of the interaction of Naphthalene acetic acid (NAA and Adenine sulphate AdS) in the induction and maintenance of callus tissue.

It is clear from Table 1 that NAA concentrations had a significant effect (P≤0.05) on the percentage of callus induction from the growing apex parts, as the highest fresh weight was recorded at the µM 20 concentration, which amounted to 275.22 mg, which did not differ significantly from the µM 40 treatment, while it was less Fresh weight in the control treatment. Regarding the effect of adding the cytokinin Adenine sulphate AdS, it was observed that it had a significant effect on callus induction if a concentration of 2.5 µM was applied. The highest average fresh weight amounted to 322.22 mg, which did not differ significantly from the rest of the concentrations added to the medium. As for the effect of the interaction between the two regulators Growth: The highest fresh weight rate was achieved at the intervention concentrations of 40 µM and 4.5 µM of NAA and AdS, respectively. The callus was brittle and greenish yellow in colour, while the percentage decreased in some of the growth regulator interventions and reached 179.32 when the control medium and 4.5 µM of AdS

Increasing the concentration of the regulators until reaching the ideal concentration led to an increase in the fresh weight of the callus. The reason for this may be due to the fact that cytokinins have important effects in increasing cell division, especially meristematic cells, and this in turn leads to an increase in the size of the various tissues of plant organs, whether they are attached to the mother plant or separated from it and grown in sterile nutrient media [4]. The addition of both regulators Growth into the culture medium is necessary for the induction and perpetuation of callus, as cytokinin in the presence of auxin acts as a key to starting the process of cell division. Any difference in the response of the cultivated plant parts may be due to the ratios of auxin to the added cytokinin, which may be attributed to the difference in the content of these internal parts of internal hormones, and this in turn affects In achieving the ideal concentration for callus induction and perpetuation of auxin, cytokinin, or both when added to the culture media [8]

Table (1) The effect of the interaction of Adenine sulphate (AdS) and Naphthalene acetic acid (NAA) on the average fresh weight (mg) of callus induced from the growing shoot of Caper (Capparis spinosa L.) seedlings after five weeks of culture in MS medium.

average	Adenine sulphate AdS) (µM				concentration NAA µM
	6.5	4.5	2.5	0	
250.83	233.32	197.32	358.58	214.08	0
275.22	320.81	233.33	277.12	269.62	20
269.68	213.54	481.22	332.27	159.70	40
	255.89	303.96	322.66	214.47	average
AdS) ، 94.918 * (NAA ، * :82.210 * BAP & *2,4-D * 164.4 :*					LSD.

ISSN 2072-3857

It is noted from Table 2 that adding high concentrations of (NAA) led to a significant increase in the dry weight of callus at a concentration of 40 µM, giving the highest dry weight of (20.161) mg, except for the treatment with the control treatment, where the lowest value reached (14.531) mg. The dry weight is For callus, it decreases as the concentration of AdS increases. It was reported that the highest dry weight of callus was obtained when mixing 40 µM and 4.5 µM of NAA and AdS, respectively. The dry weight was 26.545 mg.

Table (2) The effect of the interaction of Adenine sulphate AdS) and Naphthalene acetic acid (NAA) on the average dry weight (mg) of callus induced from the growing shoot of Caper (Capparis spinosa L.) seedlings after seven or five days of culture in MS medium.

average	Adenine sulphate AdS) (µM				concentration NAA µM
	6.5	4.5	2.5	0	
14.531	19.665	9.605	22.847	5.949	0
17.758	20.081	11.926	20.034	18.990	20
20.161	18.436	26.545	21.662	14.001	40
	19.394	16.045	21.514	12.980	average
AdS) ،6.435 * (NAA ، * :5.573 * BAP & *2,4-D * 11.149 :*					LSD.

The effect of adding Progesterone to the cumulative nutritional environment

After determining the best concentration of auxins, the induced callus was transferred from the growing apex to a culture media prepared with the same components as the MS medium prepared in the induction stage, in addition to a 40 µM concentration of 4.5+ NAA Adenine Sulphate (AdS) µM, which gave the highest average callus weight. The replanting process was repeated every five weeks until the amount required for subsequent experiments was reached.

The results showed in Table (3) that moderate concentrations of Progesterone (100 mg/l) were most effective in perpetuating callus from the transplanted growing apex. The reason for this may be that high concentrations led to a reduction in the rate of callus cell division.

The highest average fresh and dry weight of callus reached 7.461 and 50.61 mg at a concentration of (100 mg/l Progesterone), and it differed significantly from the rest of the treatments, while the lowest average fresh and dry weight was achieved, amounting to 20.47 and 2.895 mg at the control treatment, which was at a concentration of (150). (mg/l). Progesterone had a significant effect on increasing the fresh and dry weight of the callus as well, while it did not differ statistically from the rest of the treatments in the average fresh and dry weight of the callus. The resulting callus was solid and golden yellow in color.

The results in the same table show that from dried callus samples grown on a culture media prepared with different concentrations of the starter Progesterone, differences in the alkaloids' averages depend on the concentrations of Progesterone added to the maintenance and accumulation medium. When calculating the concentrations of alkaloids, it was found that there were statistical differences between the averages of one compound compared to the control. The

ISSN 2072-3857

averages of the compounds increased when 150 (mg/l) of Progesterone was added, reaching 63.50 and 50.54 (µg.g-1 dry weight) for capparisine A (1) and capparisine C (3), respectively. The averages of the compounds decreased when In the control treatment, the average alkaloid capparisine B (2) also increased when 100 (mg/l) of Progesterone was added to the cumulative media and reached (µg.g-1 dry weight) 73.57. Stimulation with different concentrations of Progesterone in the cumulative media to produce active compounds led to a significant increase. In the production of capparisine A (1), capparisine B (2) and capparisine C (3) compared to the control treatment.

Table (3): The effect of adding Progesterone to the cumulative culture media on the average fresh and dry weights and the production of medically effective alkaloids for callus induced from the growing top of the Caper plant (Capparis spinosa L.) two months after planting.

Progesterone (mg/l	Fresh weight	Dry weight	Secondary compounds (µg.g^{-1} Dry weight)		
			alkaloid		
			capparisine A (1)	capparisine B (2)	capparisine C (3)
0	20.47	2.895	12.68	26.57	28.42
50	29.64	4.228	23.91	48.00	33.13
100	50.61	7.461	26.73	72.57	37.32
150	41.79	5.185	63.50	36.66	50.54
200	34.04	4.947	53.31	41.99	67.18
LSD(0.05)	20.352	1.806	31.11	32.919	21.83

Effect of adding Cholesterol to the cumulative nutritional medium

The results are shown in Table 4. It is noted that the highest average fresh and dry weight of callus reached 73.733 and 14.807 mg, respectively, at a concentration of 150 (mg/l) of Cholesterol, and it differed significantly from the rest of the treatments, while the lowest average fresh and dry weight was achieved, amounting to 21.456 and 1.787 mg, respectively, at Control treatment. The concentration of 200 (mg/l) of Cholesterol had a significant effect in increasing the average fresh and dry weights of callus as well, which did not differ statistically with the rest of the concentrations added to the continuation medium.

The results of the same table show that there are differences in the averages of medical alkaloids depending on the concentrations of Cholesterol added to the maintenance medium. And accumulation. When calculating the concentrations of alkaloids, significant differences occurred between the average concentrations of a single compound compared with the control. The averages of the compounds increased when 150 (mg/l) of Cholesterol was added, reaching 81.96 and 62.527 (µg.g-1 dry weight) for each of the compounds capparisine A (1) and capparisine C (3), respectively. Most of the compounds' averages decreased in the control treatment compared to the 100 (mg/l) Cholesterol treatment, which gave the best value recorded for the alkaloid capparisine B (2) at (58.60). (µg.g-1 Dry weight)

ISSN 2072-3857

Table (4): The effect of adding Cholesterol to the cumulative culture media on the average fresh and dry weights and the production of medically effective alkaloids for callus induced from the growing top of the Caper plant (Capparis spinosa L.) two months after planting.

Cholesterol (mg/l	Fresh weight	Dry weight	Secondary compounds (µg.g^{-1} Dry weight)		
			alkaloid		
			capparisine A (1)	capparisine B (2)	capparisine C (3)
0	21.456	1.787	25.142	23.04	34.10
50	34.026	7.925	31.351	45.81	54.65
100	33.600	6.046	34.463	58.60	30.24
150	73.733	14.807	62.527	51.93	81.96
200	40.091	10.364	45.209	56.63	49.87
LSD(0.05)	15.881	3.419	13.51	25.694	40.758

Effect of adding Squalene to the cumulative culture media

It is noted from Table 5 that adding different concentrations of Squalene led to a significant increase in the fresh and dry weight of callus when treated with 8 (mM) of Squalene, which gave the best fresh and dry weight ingested (56.882 and 16.680) mg, respectively, while it did not differ significantly from the rest. Treatments were statistically significant, except for the control treatment, which reached the lowest value (10.858 and 15.209 mg), respectively. Adding Squalene to the culture media may stimulate the growth of callus cells, as it was observed that adding different concentrations of the starter Squalene to tissue cultures of plants led to a significant increase in the accumulation of the alkaloids capparisine A (1), capparisine B (2), and capparisine C (3). Callus cells. The best concentration was 8 (mM), as the value of alkaloids reached (72.910, 71.492 and 57.493) (µg.g-1 dry weight) compared to the control under the conditions of his experiment.

Table (5): The effect of adding Squalene to the cumulative culture media on the average fresh and dry weights and the production of medically effective alkaloids for callus induced from the growing top of the Caper plant (Capparis spinosa L.) two months after planting.

Squalene (mM)	Fresh weight	Dry weight	Secondary compounds (µg.g^{-1} Dry weight)		
			alkaloid		
			capparisine A (1)	capparisine B (2)	), capparisine C (3)
0	15.209	10.858	23.442	33.568	28.092
2	36.273	14.816	28.180	50.003	36.449
4	34.331	11.280	35.555	65.956	46.487

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6	47.833	16.680	43.006	42.167	51.207
8	56.882	20.303	57.493	71.492	72.910
LSD(0.05)	12.928	9.266	18.655	18.655	19.174

Chemical composition and nutritional value of callus tissue extract from Capparis spinosa L.

Aqueous extract of callus tissue induced from the growing apex of seedlings of Caper plant (Capparis spinosa L.) and Leaves extract of a plant grown in the field are given in Table 6. The chemical composition and from the nutritional point of view, the callus tissue possesses. Caper (Capparis spinosa L.). Total proteins in callus and Leaves tissue extract (03.9 ± 0.05 and 01.9 ± 0.08) (mg/g), Total carbohydrates (23.3 ± 0.09 and 20.3 ± 0.010) (mg/g), Total free amino acids (04.5 ± 0.04 and 04.8 ± 0.05) (mg/g), Total free fatty acids (68.1 ± 0.07 and 54.3 ± 0.04) (mg/g), Total fats (68.1 ± 0.07 and 62.1 ± 0.03) (mg/g), total cholesterol (0.11 ± 0.03 and 0.08 ± 0.04) (mg/g), while total crude fiber was (22.2 ± 0.12 and 08.2 ± 0.04) (mg/g), while the level of Energy was observed value (88.4 ± 0.07 and 63.3 ± 0.06) (Kcal). An increase in the value of vitamins was observed in the plant extracts of callus and Leaves according to good rates of (0.28 ± 0.011 and 0.22 ± 0.07) (µg/g) Vitamin B1, while the value Vitamin B2 was (0.62 ± 0.013 and 0.50 ± 0.40) (µg/g), Vitamin B3 was (0.72 ± 0.16 and 0.70 ± 0.15) (µg/g), and Vitamin C was (2.58 ± 0.09 and 2.50 ± 0.07) (µg/g), Vitamin E (0.09±0.04 And 0.04 ± 0.03) (µg/g), respectively. The chemical composition values revealed are due to the presence of a good amount of carbohydrates, proteins and fats in the aerial parts of carbohydrates and vitamins. The presence of Caper (Capparis spinosa L.) proteins plays a major role in human nutrition. Deficiency or excess of nutrients, especially proteins, serves as a building block for cells, muscles, cartilage, skin, hormones, enzymes and vitamins. Eating crude fiber found in the chosen plant material can lower blood cholesterol and the risk of disease

Coronary heart disease, high blood pressure and constipation. Ascorbic acid protects low-density lipoproteins ex vivo from oxidation. The presence of large amounts of vitamin E confirms the ability to provide protection from free radicals and oxygen products. Vitamin E works together with other antioxidant nutrients found in these plant materials to quench free radicals. Vitamin E also inhibits lipoxygenase, an enzyme responsible for the formation of pro-inflammatory leukotrienes [15] Increased levels of chemicals and nutrients in callus tissue growing ex vivo and aerial parts growing in the field may be due to To the materials or growth regulators added to the growth medium of callus tissue, which played a prominent role in increasing the values of chemical compounds.

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Table (6): Detection using LC-ESI-MS/MS of the chemical composition and nutritional value of the callus tissue extract of Capparis spinosa L. seedlings grown in a medium supplemented with Adenine sulphate AdS and Naphthalene. acetic acid (NAA Leaves) of a plant growing in the field

S. No.	Parameters	Content	Content
		Callus tissue extract	Leaves extract
1	Total proteins (mg/g)	03.9 ± 0.05	01.9 ± 0.08
2	Total carbohydrates (mg/g)	23.3 ± 0.09	20.3 ± 0.010
3	Total free amino acids (mg/g)	04.5 ± 0.04	04.8 ± 0.05
4	Total free fatty acids (mg/g)	59.4 ± 0.09	54.3 ± 0.04
5	Total fats (mg/g)	68.1 ± 0.07	62.1 ± 0.03
6	Total cholesterol (mg/g)	0.11 ± 0.03	0.08 ± 0.04
7	Total crude fibre (mg/g)	22.2 ± 0.12	08.2 ± 0.04
8	Energy value (Kcal)	88.4 ± 0.07	63.3 ± 0.06
9	Vitamin B1 (µg/g)	0.28 ± 0.011	0.22 ± 0.07
10	Vitamin B2 (µg/g)	0.62 ± 0.013	0.50 ± 0.40
11	Vitamin B3 (µg/g)	0.72 ± 0.16	0.70 ± 0.15
12	Vitamin C (µg/g)	2.58 ± 0.09	2.50 ± 0.07
13	Vitamin E (µg/g)	0.09 ± 0.04	0.04 ± 0.03
(Values are mean ± S.D, n = 3).			

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ISSN 2072-3857

ISSN 2072-3857