

Quality, Shelf-life and Sensory Analysis of Beef Meat Treated with *Cleome gynandra* and *Vigna unguiculata* Extracts

P. Dzomba*, I. Gwizangwe, P. Pedzisai, E. Togarepi

Chemistry Department, Faculty of Science, Bindura University of Science Education, P Bag 1020 Bindura, Zimbabwe

*Corresponding author: pdzomba@gmail.com, pdzomba@buse.ac.zw

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Abstract The aim of the present study was to assess quality, shelf-life and sensory properties of beef meat treated with *Cleome gynandra* and *Vigna unguiculata* extracts. Protein, fat content, ash (minerals), and pH were analyzed to determine the quality of the meat. Protein content was assayed using the Kjeldahl method while fat content was determined using ether-extraction on a Soxhlet apparatus. Ash analysis on defatted samples were performed by subjecting them to a temperature of 600°C in a muffle furnace for two hours and weight of the ash was used to estimate the mineral content. Shelf life analysis was measured using peroxide value and UV absorbance test. Microbial count tests were carried out using nutrient and potato dextrose agar. Semi trained and untrained panelist were used to assess sensory qualities; colour, smell and general acceptability. Fat composition ranged from 10.76 to 10.78% while pH values ranged from 5.8 to 6.0. There were no significant differences between the values, $p = 0.05$ showing that the treatment did not change meat pH and fat content. The additives maintained protein and mineral content of the meat samples. Protein content ranged from 49.47 to 52.01% while total mineral content ranged from 7.01 to 8.33%. Shelf life and sensory evaluation results revealed that treating beef meat with *Cleome gynandra* and *Vigna unguiculata* leaf extracts reduces peroxides formation, proliferation of microbes, delays colour loss and formation of unpleasant smells. *Cleome gynandra* offers best results for this purpose.

Keywords: quality, shelf-life, sensory analysis, *Cleome gynandra*, *Vigna unguiculata*

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1. Introduction

Traditional food and medicinal plants are important sources of natural antioxidants for delaying the onset of oxidative stress in meat [1,2,3]. They also consist of appreciable amounts of minerals that are required for the continued wellbeing of the human body [4,5,6]. *Cleome gynandra* and *Vigna unguiculata* are promising plants in this regard [7,8]. *Cleome gynandra* falls under the family Capparaceae and it grows in grasslands and crop fields [9]. Throughout the African continent, China and India, the tender leaves and flowers are boiled and consumed as a potherb or tasty relish [10]. Fresh leaves are one of the ingredients that may be used to make mashed foods [11]. Leaves can be dried, ground and added to weaning foods [12]. The vegetable is a rich source of nutrients such as vitamins A, C and minerals (Calcium and Iron) [13]. In many African societies, the plant is widely consumed in rural areas. Crushed leaves are used to make concoctions that are used to cure ailments such as scurvy and stomach ache [12]. Boiled leaves may be marinated in sour milk for a period of 2-3 days and consumed as a nutritious meal, which is believed to help in eyesight, providing energy

and curing of marasmus [9]. A meal consisting of boiled leaves is also highly recommended for pregnant and lactating mothers [13]. In folk medicine *Cleome gynandra* has been used to treat rheumatic and inflammatory conditions [14]. A decoction of the root is used to treat fevers [14]. Aqueous root extractions are used as antidotes for scorpion stings and snake bites [9]. The juice of the leaves is a remedy for pain in the ear [9]. In India it is used to manage diseases such as epilepsy, irritable bowel syndrome and protozoal and worm infections [13]. The seeds are anthelmintic and rubefacient [9]. *Vigna unguiculata* subsp. *Sesquipedalis* is a legume cultivated to be eaten. It is a good source of protein, vitamin A, C, thiamin, riboflavin, folate, iron, phosphorus, potassium, magnesium, and manganese [15,16]. In the effort of encouraging cultivation, consumption and preservation of *Cleome gynandra* and *Vigna unguiculata* plant species this study was designed to determine the possibility of using natural antioxidants from *Cleome gynandra* and *Vigna unguiculata* to delay the onset of oxidative stress in beef mince meat. Consequently the addition of *Cleome gynandra* and *Vigna unguiculata* extracts in meat may fortify its nutritional quality as the plants consist of appreciable quantities of minerals and other useful nutrients such as proteins.

2. Materials and Methods

2.1. Plant Material

Plant samples were collected from maize and ground nuts fields in April, June and July 2012 from Mashonaland East in Mudzi, Zimbabwe and mixed to form a composite sample. The plants were validated by a taxonomist at Harare Botanical Garden and were deposited in the Chemistry Department (Natural Product Section) specimen No. 2012/4 of Bindura University for future reference.

2.2. Sample Preparation

Plant materials were shade-dried at room temperature for three weeks to obtain a constant mass and separated into roots, leaves and stems. Dried materials were later ground two times into powder using a hand mill, followed by sieving through a 0.75 μm laboratory king test sieve. The fine material which passed through the sieve was collected. The remaining residues were then discarded. A quantity of 10 g of the ground material was extracted continuously with cold organic solvent, 100 ml ethanol (Merck Co. Germany) for 5 h on a shaker at room temperature followed by filtration through Whatman no. 1 filter paper. The residues were re-extracted under the same treatment and the filtrates combined. The extracts were then centrifuged at $1536 \times g$ for 20 min at 20°C. The supernatants were taken into 100 ml flasks and then concentrated in a Buchi rotary evaporator at 40°C. The extracts obtained after evaporation were weighed to determine the yield and stored in a freezer.

2.3. Production of Treated Meat Samples

Fresh beef meats produced commercially were cut and minced using manual equipment. The composite sample was subdivided into six samples of 500 g, packed into polyethylene bags and each was treated differently. Subsample A was left as it is and it worked as a negative control. Sample B (positive control) was treated with Butylated hydroxytoluene (BHT) performed by dissolving 50mg in 2ml of distilled water and mixing the solution thoroughly with 500g of minced meat. Sample C and D were treated the same way but with 50 mg of *Cleome gynandra* and *Vigna unguiculata* extracts respectively. Samples E and F were treated by spraying on the surface of the meat using a solution made by dissolving 50 mg of *Cleome gynandra* and *Vigna unguiculata* extract in 2ml of distilled water. Polyethylene bags consisting of meat samples were sealed and stored in the dark in a refrigerator. The same process was repeated for root and stem extracts.

2.4. Meat Quality Determination

The following determinations were made on meat samples prepared by mixing with extracts to establish the nutritive and economic value of the products, pH, ash (minerals), protein and fat content. The pH value of the meat products were measured by direct contact between the sensitive diaphragm of the electrode and the meat tissue. To obtain accurate pH readings the pH-meter was calibrated before use and adjusted to the temperature of the tissues to be measured. The electrode was rinsed with

distilled water after each measurement. The protein content was determined at laboratory level by using the Kjeldahl method as described by the Association of Official Analytical Chemists [17]. Samples, 10g for fat analysis were semi-dried before being subjected to ether-extraction using a Soxhlet apparatus. After complete extraction, the fat was obtained by evaporating and recovering the ether. Defatted samples were used for ash analysis by subjecting it to a temperature of 600°C in a muffle furnace for two hours. The weight of the ash was used to calculate the mineral content in % using the formula (weight of ash, divided by total sample weight, multiplied by 100).

2.5. Shelf Life Evaluation

Peroxidation of lipids and lipid containing meat products is a complex process which results in a variety of oxidation products [18]. For this reason monitoring oxidation processes requires the use of different methods in order to establish detailed information on the efficiency of added antioxidants. In this study oxidative stability study was monitored using peroxide value determination and UV absorbance test. Microbiological counts were also assessed.

2.6. Peroxide Determination

Peroxide values (PV) were monitored in meat samples with added plant extracts and reference antioxidant BHT. A blank sample was run without any additives. Most extracts were hydrophilic and their homogenization in the meat was rather easy. The samples (10 g) were placed in open beakers (250 ml volume) and kept in a ventilated thermostat. PV measurements were performed and values determined according to, AOAC, [17]. Meat samples (approximately 1 g) were taken from the beakers, accurately weighed and dissolved in 25 ml of a chloroform/acetic acid mixture (3:2). Then 0.5 ml of saturated potassium iodide solution in distilled water was added, the samples were shaken for 1 min and 25 ml of distilled water was added. The liberated iodine was titrated with 0.01 M sodium thiosulphate solution using a 1 % starch solution as indicator. Peroxide values (meq/kg meat) were calculated using the formula:

$$PV = 0.01 \times N \times 1000 / m,$$

where N is the volume of sodium thiosulphate used for the titration of a sample in ml and m is the mass of meat sample in g. Measurements (in triplicate) were conducted within the period of 26 days.

2.7. UV Absorbance Test

A volume, 5ml of absolute hexane was added to 10 g of beef samples properly mixed by shaking and filtration. The absorbance of the filtrate was monitored at 232 and 268 nm in a 1 cm long quartz cell using hexane as the blank solution.

2.8. Microbial Counts

Samples of beef products, 1g stored at 20°C were taken and aseptically cultured after 2 to 26 days respectively following a method reported by [19]. Nutrient agar, for

bacterial analysis and potato dextrose agar, for fungal evaluation were prepared according to manufacturers' instructions, distributed into sterilized Petri plates and used. The meat was thoroughly agitated in 9 ml of sterile water, serially diluted and plated. After incubation (at 37°C for 24 hours and 25°C for 3 and 5 days for bacteria and fungi respectively), plate counts were carried out.

2.9. Sensory Evaluation

On day 1 and 19 meat samples were placed into white saucers and sensory attributes, colour, smell and general acceptability was tested on a five point hedonic scale, where 1 = dislike extremely, 2 = dislike moderately, 3 = neither like nor dislike, 4 = like moderately and 5 = like extremely. A total of 60 panelists were used in this study: 30 from Bindura town, 20 from city of Harare (adult, untrained) and 10 semi-trained final year undergraduate students of the department of chemical technology, Bindura University.

2.10. Statistical Analysis

Meat quality attributes data is presented as averages of three determinations and the data was subjected to one way analysis of variance (ANOVA) at $p = 0.05$. Mean separation was done through the use of Least Significant Difference (LSD) tests. Sensory analysis data includes

mean scores for each sample as tested by un-trained panelists. The results of sensory evaluation were subjected to (ANOVA) and Turkey's HSD test was used to determine the differences of the mean scores for appearance, smell, and general acceptability at $P = 0.05$ SPSS Version 16.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Meat Quality Attributes

Compositions of BHT, extract treated and untreated meats are shown in Table 1. pH values and fat composition among the meats ranged from 5.8 to 6.0 and 10.76 to 10.78 % respectively. Values did not differ significantly showing that the treatment does not have a significant effect on meat pH and fat content. Wide variability in protein and mineral content was observed among the meats. Protein content ranged from 49.47 to 52.01 % while total mineral content ranged from 7.01 to 8.33 %. The results revealed that adding *Cleome gynandra* and *Vigna unguiculata* extracts improves the quality of meat with leaves giving best results than roots and stems. *Cleome gynandra* leaves improved the protein quality of meat better than *Vigna unguiculata* leaves while the reverse is true for mineral content.

Table 1. Meat quality attributes of BHT, extract treated and untreated

% composition	Blank	BHT	C.G roots	C.G stems	C.G leaves	V.U roots	V.U stems	V.U leaves	Statistical analysis
pH	6.0	6.0	5.9	5.8	6.0	5.9	6.0	6.0	NS
Protein	49.47	49.47	51.14*	51.09*	52.01*	50.33*	50.66*	50.72*	$p > 0.05$
Fat	10.77	10.77	10.77	10.76	10.78	10.77	10.77	10.78	NS
Total mineral	7.01	7.01	7.09	7.89*	8.23*	8.14*	8.11*	8.33*	$p > 0.05$

CG = *Cleome gynandra*, VU = *Vigna unguiculata*, NS = no significant difference, * = values differ significantly $p < 0.05$, (ANOVA and LSD test at $p = 0.05$. Only values for meat samples treated by mixing thoroughly with BHT or extracts were analyzed for quality attributes.

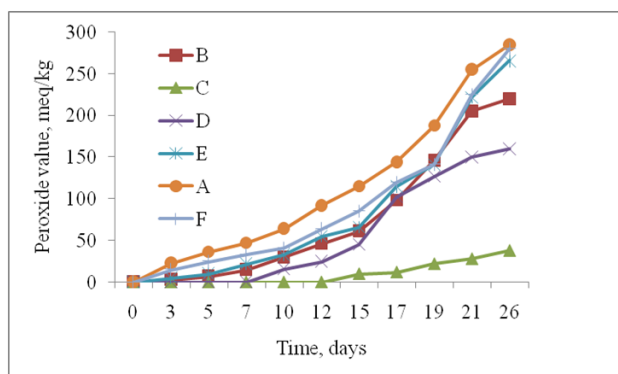


Figure 1. Peroxide value determination for samples treated with leaf extract (A = blank, B = BHT, C = 50 mg *C. gynandra* (mixed), D = 50 mg *V. unguiculata* (mixed), E = 50 mg *C. gynandra* (sprayed), F = 50 mg *V. unguiculata* (Sprayed))

3.2. Peroxide Value Determination

Oxidation of leaf, root and stem extract treated and untreated beef meat expressed as peroxide value is presented in Figure 1-Figure 3. Peroxidation in samples treated by mixing with leaf extract was observed after day 12 and 7 for *Cleome gynandra* and *Vigna unguiculata* respectively (Figure 1). Peroxidation in the other samples started almost immediately (Figure 1-Figure 3). On the basis of the presented curves peroxide values show that treating beef meat with different parts of the chosen plants

improves its oxidative stability. The profile of the untreated meat is above all other profiles. Treating beef meat by mixing with 50 mg of *Cleome gynandra* leaf extract gave more promising results with a peroxide value that was below 50 meq/Kg even after 26 days. It depicted the slowest rate of formation of peroxides as compared to BHT, and *Vigna unguiculata* treated meat. Figure 2 and Figure 3 show that antioxidant effect of stem and root extract was lower than that of leaf extract and BHT. The results also show that treating beef meat by mixing with the extract provides the best protection than spraying.

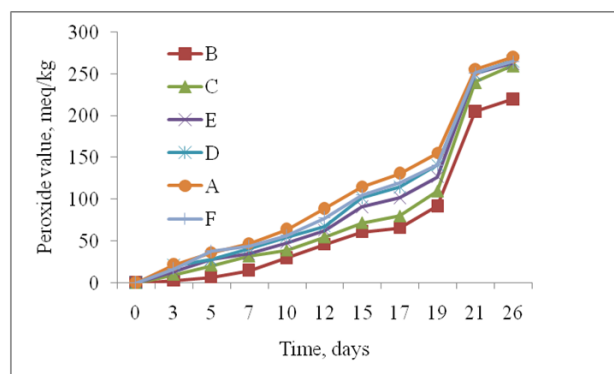


Figure 2. Peroxide value determination for samples treated with stem extract (A = blank, B = BHT, C = 50 mg *C. gynandra* (mixed), D = 50 mg *V. unguiculata* (mixed), E = 50 mg *C. gynandra* (sprayed), F = 50 mg *V. unguiculata* (Sprayed))

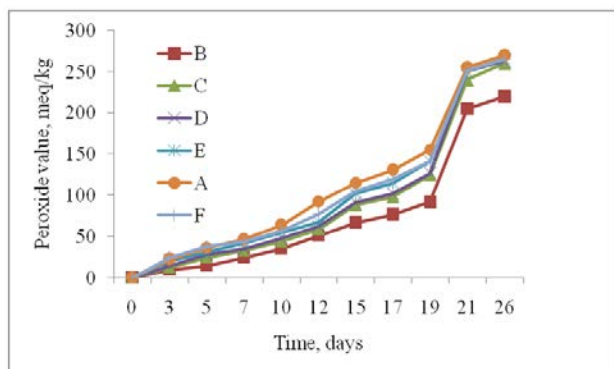


Figure 3. Peroxide value determination for samples treated with root extract (A = blank, B = BHT, C = 50 mg *C. gynandra* (mixed), D = 50 mg *V. unguiculata* (mixed), E = 50 mg *C. gynandra* (sprayed), F = 50 mg *V. unguiculata* (Sprayed))

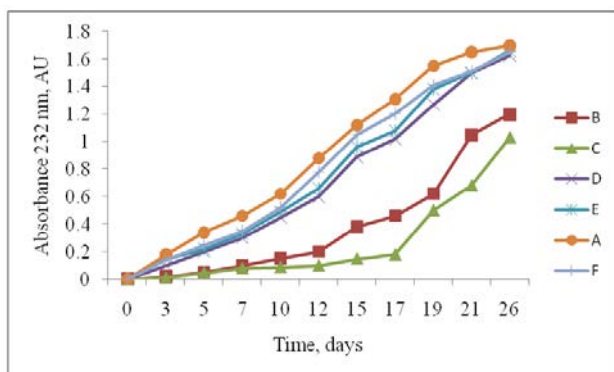


Figure 4. UV absorbance at 232 of leaf extract treated beef meat (A = blank, B = BHT, C = 50 mg *C. gynandra* (mixed), D = 50 mg *V. unguiculata* (mixed), E = 50 mg *C. gynandra* (sprayed), F = 50 mg *V. unguiculata* (Sprayed))

3.3. UV Absorbance Test

Oxidation of lipids in meat produces conjugated dienes and trienes products. Measuring the UV absorbance of these compounds at certain wavelengths affords information about the degree of oxidation and the efficiency of added natural antioxidants. The effect of extracts on the formation of dienes and trienes in beef meat expressed as UV absorbance at 232 and 268 nm is presented in Figure 4 and Figure 5. The results show that changes in peroxide value correlate well with differences

in conjugated diene and trienes in the beef meat. The lowest absorbance was realized in the sample treated with 50 mg of *Cleome gynandra* leaf extract (profile C). The absorbance was almost constant until day 17 and 12 for maximum absorbance wavelength of 232 and 268nm respectively (Figure 4 and Figure 5). Highest absorbance values were observed in the untreated beef sample (profile A).

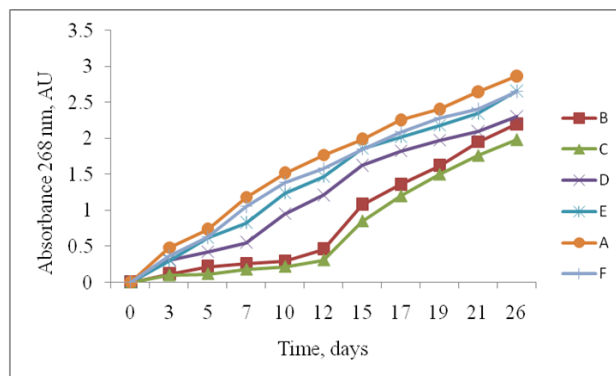


Figure 5. UV absorbance at 268 of leaf extract treated beef meat (A = blank, B = BHT, C = 50 mg *C. gynandra* (mixed), D = 50 mg *V. unguiculata* (mixed), E = 50 mg *C. gynandra* (sprayed), F = 50 mg *V. unguiculata* (Sprayed))

3.4. Microbial Counts

Total viable bacterial count and total viable fungal count in beef products treated by mixing with leaf extracts and stored for 26 days at 20°C are shown in Table 2 and Table 3 respectively. On day 7 total viable counts for the untreated sample was too many to count while for all the treated samples the highest was only 984 for sample E observed on day 26. No bacteria were observed in the sample treated by mixing with *Cleome gynandra* leaf extract. Development of bacteria was observed in samples treated by mixing with *Vigna unguiculata* on day 12 (Table 2). No fungal development was observed in the other samples except for the untreated and BHT treated samples. The results show that treating beef meat with *Cleome gynandra* leaf extract prevents the growth of micro organisms. High microbial loads lead to spoilage of foods. It was observed in this study that the untreated meet quickly developed an unpleasant smell.

Table 2. Total viable bacterial count (cfu/g) in beef samples treated with leaf extract

Sample	0	3	5	7	10	12	15	17	26
A	188	1343	4800	TNC	TNC	TNC	TNC	TNC	TNC
B	14	23	33	39	47	80	84	188	315
C	ND	ND	ND	ND	ND	ND	ND	ND	ND
D	ND	ND	ND	ND	ND	260	390	770	980
E	ND	ND	ND	388	460	565	690	780	984
F	ND	ND	ND	370	455	565	685	773	982

TNC = Too numerous to count, ND = Not detected

A = blank, B = BHT, C = 50 mg *C. gynandra* (mixed), D = 50 mg *V. unguiculata* (mixed), E = 50 mg *C. gynandra* (sprayed), F = 50 mg *V. unguiculata* (Sprayed)

Table 3. Total Viable Fungal Count (CFU/g) in beef samples treated with leaf extract

Sample	0	3	5	7	10	12	15	17	26
A	188	1330	3260	4850	5560	TNC	TNC	TNC	TNC
B	15	23	35	38	47	80	86	188	314
C	ND	ND	ND	ND	ND	ND	ND	ND	ND
D	ND	ND	ND	ND	ND	ND	ND	ND	ND
E	ND	ND	ND	ND	ND	ND	ND	ND	ND
F	ND	ND	ND	ND	ND	ND	ND	ND	ND

TNC = Too numerous to count, ND = Not detected

A = blank, B = BHT, C = 50 mg *C. gynandra* (mixed), D = 50 mg *V. unguiculata* (mixed), E = 50 mg *C. gynandra* (sprayed), F = 50 mg *V. unguiculata* (Sprayed)

3.5. Sensory Evaluation

Sensory evaluation results of beef meat treated differently are shown in Tables 4 and 5. Results for the untreated and treated samples for day 19 (Table 5); colour, smell and overall acceptability were significantly different ($p > 0.05$). Differences in colour scores for BHT treated sample (B) and *Cleome gynandra* leaf extract (C) were not significant however differences in smell and acceptability were significant. Results shown in Table 4 generally show that at the beginning of the study meat samples were the same in terms of appearance and smell as there was no significance differences between the scores. The overall acceptability and flavor did not differ between treatments, indicating that all the products were acceptable to the panelists. Samples treated with BHT and *Cleome gynandra* leaf extract was rated higher than all other samples. Results of trained (results not shown) and untrained respondents (Table 4 and Table 5) did not differ significantly and it was concluded that prior training was not necessary for this study.

Table 4. Mean scores for sensory Evaluation of beef samples on day 1 treated with leaf extract on a 7-point hedonic scale

Sample	Colour	Smell	Acceptability
A	4.98 ^a	5.98 ^a	5.15 ^a
B	5.04 ^a	6.00 ^a	5.15 ^a
C	5.00 ^a	6.08 ^a	5.10 ^a
D	4.98 ^a	6.25 ^b	5.05 ^a
E	5.02 ^a	6.02 ^a	5.15 ^a
F	5.00 ^a	6.02 ^a	5.03 ^a

Means in a column followed by different letters are significantly different ($p < 0.05$).

A = blank, B = BHT, C = 50 mg *C. gynandra* (mixed), D = 50 mg *V. unguiculata* (mixed), E = 50 mg *C. gynandra* (sprayed), F = 50 mg *V. unguiculata* (Sprayed)

Table 5. Mean scores for sensory Evaluation of beef samples on day 19 treated with leaf extract on a 7-point hedonic scale

Sample	Colour	Smell	Acceptability
A	4.78 ^a	3.98 ^a	3.15 ^a
B	6.04 ^b	5.00 ^b	5.65 ^b
C	6.00 ^b	5.30 ^c	5.50 ^c
D	5.94 ^c	5.25 ^c	5.95 ^b
E	5.02 ^b	4.20 ^c	5.15 ^c
F	4.80 ^a	4.02 ^a	3.03 ^a

Means in a column followed by different letters are significantly different ($p < 0.05$).

A = blank, B = BHT, C = 50 mg *C. gynandra* (mixed), D = 50 mg *V. unguiculata* (mixed), E = 50 mg *C. gynandra* (sprayed), F = 50 mg *V. unguiculata* (Sprayed)

4. Discussion

Food plants that are used as food in traditional settings have been tested as food-based solutions for combating nutrient deficiencies [20,21]. Food-based approaches for dealing with nutrient deficiencies are a potentially sustainable, affordable, effective, and feasible approach to improving the quality of a food product. Nestel et al [22] reports that adding plant extracts to meat products improves mineral quality and absorption of iron by the body. The present results revealed that addition of *Cleome gynandra* and *Vigna unguiculata* improves the quality of the meat in terms of nutrient content, shelf life and sensory attributes. *Cleome gynandra* was found to be of great value to this respect. Results of the present study are

in line with the results reported by Muchuweti et al. [23]; Mishra and Dash, [13]. Muchuweti et al. [23] reported that *Vigna unguiculata*, and *Cleome gynandra* extracts contained vanillin, caffeic acid, p-coumaric acid and ferulic acid while protocatechuic acid and catechin were detected only in *Cleome gynandra*. Vanillin, caffeic acid, p-coumaric acid, ferulic acid, protocatechuic acid and catechin are known natural antioxidants that have antiradical [24] and antibacterial activity. *Cleome gynandra* was found to consist of larger amounts of phenolic contents ($1327.333 \pm 1.658 \text{ mg g}^{-1}$) than *Vigna unguiculata* ($1136.603 \pm 3.869 \text{ mg g}^{-1}$). Vegetable extracts of both the plants showed a time dependent DPPH antiradical activity which was attributed to hydrogen-donating capability. *Cleome gynandra* was also found to exhibit the highest inhibitory effect on rat brain peroxidation than *Vigna unguiculata*. Presence of flavonoids, protocatechuic acid and catechin in *Cleome gynandra* may account for antioxidant and antimicrobial activity observed in the beef meat samples. Flavonoids consist of more hydroxyl groups. Ismail et al. [25] reported that orthosubstitution flavonoids with electron-donating alkyl or methoxy groups increases the stability of the antioxidant radical and hence its antioxidant capacity.

Meat color is an important factor when consumers choose meat freshness. Meat color deterioration is due to oxidation of red oxymyoglobin to metmyoglobin, and these renders the meat an unattractive brown color [26]. Different reports demonstrated that natural antioxidants may retard color loss in meat by delaying formation of metmyoglobin [27,28,29,30]. Example of dietary natural antioxidants reported in literature include, antioxidants from oregano and rosemary, ascorbic acid, α -tocopherol and vitamin C. However Carpenter et al. [31]; Chouliara et al. [32] obtained different results. Carpenter et al. [31] reported that the color of raw pork meat was not affected by adding grape seed and bearberry extracts. Similar results were obtained for fresh chicken breast meat by Chouliara et al. [32]. Mishra and Dash, [13] reported that crude ethanolic extract and aqueous extracts of *Cleome gynandra* are a potential source of natural antioxidants like flavonoids. It is important to note that addition of *Cleome gynandra* and *Vigna unguiculata* extracts in meat products will increase the likelihood of consumption of useful nutrients from these plants by many people. More often people favour meat products as compared to vegetables especially traditional vegetables. Traditional vegetables are usually believed to be inferior and are usually are considered as weeds in fields. Inclusion of polyphenolic compounds from *Cleome gynandra*, in the diet of many people can have a substantial protective effect against carcinogenesis, cardiovascular and renal disorders, memory and cognitive function, age-related neurological dysfunction such as Alzheimer's disease, diabetes and ulcers [33,34,35,36]. Chouliara et al. [32]; Djenane et al. [28]; Skandamis et al. [37] reported that plant extracts exhibiting antimicrobial activities may be used to increase shelf life of refrigerated meat.

5. Conclusion

Cleome gynandra and *Vigna unguiculata* leaf extract incorporation into beef meat has beneficial effects. It

improves nutritional content and shelf life. Beef meat fortified with *Cleome gynandra* leaf additive had the most beneficial effects on nutritional, sensory quality and shelf life. The plants delay proliferation of micro-organisms in the meat thereby helping maintain nutritional value and prevention of food borne diseases. The plants also offer some economic advantage since they can be grown easily. Both can be cultivated from seed.

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