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Proximate and phytochemical composition of leaf extract of *Senna alata* (L) Roxb

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Abstract
Studies on proximate and phytochemical analyses of leaf extract of *Senna alata* (L) *Roxb* were carried out to evaluate its nutritional potentials and possible medicinal properties. Result obtained from proximate analysis showed the composition of moisture, total ash, crude protein, crude lipid, crude fibre and carbohydrate as: 15.4±0.17%, 6.00±0.13%, 17.50±0.57%, 9.80±0.21%, 16.91±0.51%, 2.00±0.61%, respectively. Phytochemical studies showed the presence of saponins, phenolic acids, flavonoids mainly kaemferol (148.568 ± 4.133) and quercetin (232.315 ± 1.821), tannins present as tannic acid (974.870 ± 2.315), alkaloids including coniine (30.508 ± 3.255) and coniceine (33.410 ± 3.480), and negligible amounts of quinones and acrylamides. The result from this study showed that while the leaf extract of *S. alata* (L) *Roxb* may be used for therapeutic purposes within its safety limit, usage as a source of food for man or animals may be lethal, due to the presence of poisonous phytochemicals like connine and coniceine.

Keywords: *Senna alata* (L) Roxb Proximate, Phytochemical, Kaemferol, Quercetin. connine, coniceine

Introduction
Senna alata (L) *Roxb* is a tropical shrub, but can also thrive in temperate climes. It belongs to the Fabaceae family. It can grow up to 16ft tall, branched. However, it can stand at 30ft tall, if straight. The large pinnate leaves are up to 30inches in length consisting of 7-14 smooth pairs of leaflets, each about 3in long and 1-4in in width. The yellow flowers, shaped like a cup, are closely-packed on a straight spike, looking like a candle stick (from where the name was obtained). The flower clusters are between 6-24in tall. The sepals are waxy and smooth to the touch. The fruit is a curvy or straight winged pod about 4 to 8 inches in length. The pods contain about 60 flat and brownish seeds. Some common names of *Senna alata* (L) *Roxb* are emperor's candlesticks, candle bush, Christmas candles, seven golden candle sticks and ringworm shrub. It is both an ornamental and medicinal plant [1]. In Nigeria, it is called 'Asunwo oyibo' in the West and 'Nelkhi' in the East [2]. *S. alata* (L) Roxb has an uncommon wide natural distribution. It is native to the tropics, which includes Southeastern Asia, Africa, tropical America and the Pacific Islands. It is an erect, tropical annual herb with yellow candle-like inflorescence.

S. alata (L) *Roxb* is a medicinal and ornamental plant. Studies have reported the therapeutic use of *S. alata* (L) *Roxb* leaves in such diseases as liver problems, abdominal pain, and constipation [3]. It has also been utilized in the treatment of dermatological diseases such as eczema, athlete's foot, inflamed skin, rashes on skin, as well as ring worm (from where the name 'ringworm shrub' was gotten) [4]; also it is used to manage diabetes and hyperglycemia [5]. Its antiviral, antibacterial and antifungal activities have also been proven[1], as well as its laxative properties [3].

Following the importance of the reported medicinal uses of *S. alata* (L) *Roxb*, it is pertinent to carry out some proximate and phytochemical studies on the plant for better insight on its biochemical composition, hence the present study.

Materials and Methods
Sample Collection and Identification
Fresh *Senna alata* (L) *Roxb* leaves were plucked between the 3rd and 6th of December, 2015 from an uncultivated piece of land, by the gate of the University of Port Harcourt, Abuja campus, Choba, Rivers State. The leaves, with voucher number UPH/V/125, were identified and authenticated by Dr. Chimezie Ekeke of the Department of Plant Science and Biotechnology, University of Port Harcourt, Rivers State.

Preparation of Sample
Fresh leaves of *S. alata* (L) Roxb were air-dried at room temperature (29±1 ºC) for 3 weeks and then ground with the aid of Marlex Excellent grinder (Mumbai, India). The ground sample was then passed through a sieve of 0.5mm pore size to obtain a fine uniform powder. The ground sample was then subjected to proximate and phytochemical analyses.

Proximate Analysis
The proximate analysis of the sample was done to ascertain its ash, moisture, lipid, crude protein, crude fibre, and carbohydrate composition [6].

Determination of Moisture composition
One gram of the sample was transferred into an empty pre-weighed silica dish and placed in the oven for 24 hours at about 105 ºC. This was later cooled in a desiccator and reweighed before it was taken back to the oven for another 24 hours. The cooling process was done repeatedly until a steady weight was achieved. The composition of moisture is then estimated.

Determination of Protein composition
Three gram of Kjeldahl digestion catalyst and 20ml of 1.25% concentrated H_2SO_4 and little anti-bumping agents were added to 1.0g of the sample in a 100ml Kjeldahl digestion flask. The flask was heated until foaming stopped and then the content totally liquefied. Heating was further intensified, with occasional rotation of the flask, until the colour of the content changed from ash to blue-green/pale green. The content was cooled and moved to a 100ml volumetric flask, in which addition of distilled water filled it up to its capacity.
Twenty millilitre of the diluted digest was transferred into a 150ml distillation flask containing some anti-bumping chips. The flask was then connected to a condenser whose receiver linked to a Buchner funnel placed in a 400ml beaker that contains 10ml of 2% boric acid solution into which 2 drops of methyl red-methylene blue (double indicator) were added. Into the flask was added 20ml of 40% NaOH solution. When the colour of the boric acid in the receiver flask changed from purple to pale green and then the volume in the beaker was almost equal to the original volume, the distillation was stopped. The ammonia was then released into the boric acid solution. The boric acid-ammonia distillate was titrated with 0.1M hydrochloric acid. The appearance of a pink colouration marked the end of the titration. The nitrogen content, which is used to calculate the protein content, was then calculated using the titre value.

Determination of Ash composition
A clean petri dish was weighed and 1.0g of the sample was added to it. The petri dish and its content were then placed in a muffle furnace for 4 hours at 550 ºC. Then the content was cooled in the desiccator, and the heating and cooling process was done repeatedly until a steady weight was achieved. The weight of the petri-dish and residue was recorded and used to estimate the ash content.

Determination of Lipid composition
Two hundred and fifty millilitre extraction flasks were dried in the oven and cooled to the laboratory temperature and the weighed. Two hundred and fifty millilitre of petroleum ether was transferred to the flasks. About 0.25g of the sample was placed in the condenser of the Soxhlet extractor, in labelled porous thimbles, and then extracted for 4 hours. The thimbles were carefully removed and the petroleum ether collected for reuse. The extraction flask containing the oil was dried in the oven at 105 ^{0}C for 1hour. The flask with the dried oil was cooled and weighed and used to calculate the lipid composition.

Determination of Crude Fibre composition
Two hundred millilitre of 1.25% H_2SO_4 was added to 2g pulverized sample in a 1L conical flask and allowed to boil for 30 minutes. The contents were then filtered through a Buckner funnel and rinsed with deionized water. Two hundred millilitre of 1.25% boiling NaOH was added to the filtrate, gently boiled for another 30 minutes and then filtered again. Hot deionized water, 10% HCl, and dimethyl ether were respectively used to wash the residue, which was later oven-dried at 110 ºC for 12 hours. The residue was then cooled, weighed and later heated at 550 ºC for 90 minutes to ash. Finally, the ash was cooled and weighed, and the fibre content calculated for.

Determination of Total Carbohydrate composition
The composition of total carbohydrate was determined by summing up the percentage values of other components (moisture, crude protein, crude fibre, lipid and ash) and subtracting by 100.

Phytochemical Qualitative Analysis
Different qualitative tests were used to analyse for the phytochemicals in the solvent free extract of *Senna alata* (L) *Roxb* leaves. Individual tests were carried out for alkaloid, saponins, flavonoids, tannins, phenolic compounds, coumarins, protein, anthraquinones, quinines, steroids and cardiac glycosides. Standard procedures were followed [7].

Alkaloid determination
The aqueous extract of the crude dry powder of the leaves was dried by evaporating in a water bath. About 2M HCl was then used to dissolve the residue. The mixture was filtered and shared in three equal parts. To one part was added few drops of Mayer's Reagent. One part was treated with Wagner's Reagent and the other with Dragon Droff's Reagent. The presence of a creamy, orange and brownish precipitates respectively indicate the presence of reparative alkaloid [8].

Saponin determination
The presence of saponin was determined by frothing test. Zero point five milligram of the dry leaf powder was mixed thoroughly with ten millilitre of distilled water and made to stay for thirty minutes. The appearance of a stable froth over 1.5cm thick, after classification for saponin, implies saponin is present [9].

Flavonoid determination
About three drops of dilute sodium hydroxide were added to one ml of the aqueous leaf extract. The appearance of an intensive yellow colour, which then turns colourless when added with a few drops of dilute acid, implying that flavonoids are present [8].

Tannins determination
Tannins were determined by the Ferric chloride test: The extract was filtered after boiling a little amount with distilled water. To the filtrate was added two drops of five percent $Fecl_3$ solution. The appearance of a blue-black or greenish-black colour confirms the presence of tannins [8].

Determination of Phenolic compounds
To two ml of the aqueous leaf extract was added three drops of one percent ferric chloride solution. The appearance of a violet colour with ferric ion implies that phenolic compounds are present [8].

Determination of Coumarins
Three drops of one percent KOH solution was added to the aqueous leaf extract. The appearance of a yellow colour implies coumarins are present [9].

Determination of Quinones
Dilute sodium hydroxide was added to the extract in a test tube. The appearance of a red or blue-green colour implies that quinones are present [9].

Determination of Anthraquinones
Borntrager's test
About ten percent of HCl was boiled for five minutes with 5 mg of the plant extract in a water bath, filtered and then left to cool. The same volume of $CHCl_3$ was added to the filtrate and then few drops of ten percent ammonia. The mixture was subsequently heated, producing a pinkish colouration which signifies anthraquinones are present [8].

Determination of Steroids
To five milligram of the plant extract was added 2 ml of acetic acid anhydride, then few drops of concentrated sulfuric acid were slid through the side of the test tube. The appearance of a bluish-green colouration implies that steroids are present [8].

Determination of Xantho-Protein
Xantho-proteins were determined by the Xantho-Protein Test. The appearance of a yellow colouration when few drops of concentrated nitric acid were mixed with the extract indicates xantho-proteins are present [9].

Determination of Cardiac Glycosides
Keller-Killianic Test
One millilitre of $Fecl_3$ reagent (99 parts glacial acetic acid and 1 part five percent $Fecl_3$ mixture) was mixed with crude dry leaf powder of the plant. Few drops of concentrated H_2SO_4 were added to the mixture. The formation of a greenish-blue colouration in minutes implies cardiac glycosides are present [10].

Phytochemical Quantitative Analysis
The quantitative analysis was done using High Performance Liquid Chromatography (HPLC).
The leaf extract, was subjected to HPLC analysis using a μBondapak C18 column. Filtration of the extract was done using Whitman's filter paper No. 1. The suspension was allowed to settle down for about 1 hour at 4 ºC and then re-filtered with Whitman's filter paper No. 1, 0.45micron and 0.22 micron filter consecutively. The crude extract was shared in 50ml aliquots and preserved in the dark at 4 ^{0}C. The control sample used was 0.1 mg/ml Benzyl Penicillin G. A hundred millilitre of HPLC grade methanol was used to wet the C18 gravity column which was subsequently washed, using fifty millilitre Milli-Q water. Fifty millilitre aliquot of the crude extract was placed in the column for absorption to take place. Buffers A and B were 50 mM sodium acetate (P^H 4.5) and acetonitrile respectively.
The rate of flow was 1.0 ml/min, while the running conditions were: between minute 1 - 3, buffer A; minute 3 - 15, gradient buffer B, 0 to 60%; minute 15 - 18, gradient buffer B, 60 to 80%; minute 18 - 20, buffer B, 80%; minute 20 - 22, gradient buffer B, 80 - 0%; 67 and minute 22 - 25, buffer A [11,12]. The scanning of eluting peaks was done from 200 nm to 550 nm with an interval of 1nm so as to determine the absorbance maxima and minima.

Statistical Analysis
Data gathered from this study were all statistically analysed, using One-Way Analysis of Variance (ANOVA). The Statistical Package for Social Sciences (SPSS) version 20.0 (IBM Statistics, UK) was used to analyse the data gathered. All the values were reported as mean ± standard error of mean (SEM), and the results were regarded significant at $p<0.05$, that is at 95% confidence level.

Results and Discussion
Proximate Analysis

Table 1: Proximate Composition of leaf of *Senna alata* (L) *Roxb*

Parameter	Composition (%)
Moisture	15.40 ± 0.17
Total Ash	6.00 ± 0.13
Crude Lipid	9.80 ± 0.21
Carbohydrate	32.00 ± 0.61
Crude Protein	17.50 ± 0.57
Crude Fibre	16.91 ± 0.51

Values are presented as mean ± SEM (n=3)

The proximate analysis result of the leaf of *Senna alata* (L) *Roxb*, as seen in Table 1, showed that it contains: 15.40±0.17% moisture, 6.00±0.13% total ash, 9.80±0.21% crude lipid, 32.00±0.61% carbohydrate, 17.50±0.57% crude protein, and 16.91±0.51% crude fibre. The moisture content is used to evaluate the shelf life of any food [13]. It, therefore, implies that the leaves of *Senna alata* (L) *Roxb* may be more stable than *Tridax procumbens*, whose moisture content is 88%; and *Ocimum gratissimum,* whose moisture content is 82.60%; as respectively reported [14,15]. The ash content, which is a measure of the mineral content, was low (6.00±0.13%) in the leaf of *Senna alata* (L) *Roxb*. It was lower than the 13.67% reported for the stem of *Ocimum gratissimum* [15]. The protein content is moderately high (17.50±0.57%), but low compared to the 37.44% reported for *Tridax procumbens* [14] and 39.13±0.16% reported for *Moringa oleifera*. The result showed the leaf can make a good source of protein. Proteins from plants are perhaps the cheapest and most naturally abundant proteins and can be effectively used to curb malnutrition in poor populations. The crude lipid content was 9.80±0.21% and was higher than the 3.42±0.47% reported for *Moringa oleifera* [16]. The lipid content shows that it can be a good energy source, however, excessive consumption of lipid can portend a risk for certain CVDs [17]. The leaf contains 32.00±0.61% carbohydrate, which is the highest of the components analysed. The leaf can be a good source of energy, however, the carbohydrate component is lower than those of *Tridax procumbens* (41.03%) and *Moringa oleifera* (38.21±0.31%) [14, 16]. Carbohydrates are the primary sources of energy needed to carry out many biological functions. The crude fibre component was obtained from was 16.91±0.51%. Adequate consumption of crude fibre has been reported to lower plasma cholesterol and thus minimizing the risk for certain

cardiovascular diseases, certain cancers and constipation [18]; reduce excessive dietary starch intake, thereby preventing diabetes mellitus and obesity; it also helps to soften stool, increase transit time and stool bulk. Excessive intake can, however, cause increased intestinal gas production that can lead to bloating. Consuming over 30g per day can lead to mal-absorption of important minerals, such as magnesium, calcium and phosphorus [19].

Qualitative Phytochemical Analysis

Table 2: Qualitative Phytochemical Determination of *Senna alata* (L) *Roxb* leaves

Phytochemicals	Leaf Extract
Alkaloid	+
Coumarin	-
Quinones	+
Saponins	+
Phenolic Compounds	+
Steroid	-
Flavonoid	+
Tannins	+
Cardiac Glycosides	-
Xantho-Protein	-
Anthraquinones	+

Values are presented as mean ± SEM (n=3)

The result of the qualitative analysis of the leaf indicated that alkaloids, quinones, saponins, phenolic compounds, flavonoids, tannins, and anthraquinone were present; as seen in Table 2. Saponins are amphipathic glycosides reported to be utilized as adjuvants in cancer treatment [20]. They form complex with dietary cholesterol in the intestinal walls, preventing their uptake, thus lowering the amount of circulating cholesterol. They act as surfactants, aiding the uptake of macromolecules, such as proteins; by cells through the membrane. Tanins are polyphenols present in many plants. They have been implicated in the acceleration of blood clotting, blood pressure reduction, immune-response modulation and in lowering of plasma lipid [21]. Quinones play very important roles in biological systems, as in: phylloquinone (vitamin K), which plays vital roles in blood clotting, bone formation, etc.; ubiquinone, which serves as electron acceptor in the electron transport chain and so on. Quinones have been utilized as purgatives, antimicrobial and anti-parasitic agents, anti-tumor agents, and in prevention of cardiovascular diseases [22]. Flavonoids are reportedly involved in several physiological activities, such as anti-inflammatory, anti-allergic, anti-oxidant [23], anti-microbial, anti-diarrheal and anti-cancer [24]. Alkaloids are naturally-occurring nitrogen-containing compounds that are reportedly involved in several pharmacological activities, such as antimalarial, anticancer, antiasthma [25], antiarrhythmic, vasodilatory [26], analgesic, hypoglycemic, antibacterial [27], etc. Phenolic acids play vital role in scavenging for free radicals. Due to their antioxidant activities they possibly may have anti-carcinogenic properties [28]. Naturally-occurring anthraquinones, like those found in plants, may have laxative effect and also possess anti-inflammatory [29], thus may have potential for the treatment of certain inflammatory conditions.

Quantitative Phytochemical Analysis

Table 3: Quantitative Phytochemical Determination of *Senna alata* (L) *Roxb* leaves

Component	Concentration (%)	Concentration (mg/100g)
Tannic acid	55.26	974.870 ± 2.315
Quercetin	13.17	232.315 ± 1.821
Kaemferol	8.42	148.568 ± 4.133
Luteolin	1.1	19.284 ± 5.758
Catechin (+)-	1.1	19.047 ± 3.331
Spectaline	3.78	66.755 ± 0.887
Cassine	2.43	42.904 ± 0.418
Coniceine	1.89	33.410 ± 3.480
Connine	1.73	30.508 ± 3.255
Chlorogenic acid	2.56	45.201 ± 1.637
Caffeic acid	4.55	80.312 ± 4.710
4-hydroxybenzoic acid	0.92	16.161 ± 0.879
Saponine	0.52	9.213 ± 3.152
Trillin	0.34	6.024 ± 2.688
Deltonin	0.33	5.888 ± 4.517
Others	1.9	33.761 ±3.222
Total composition	100	1764.221

Values are presented as mean ± SEM (n=3)

The quantitative analysis result indicated that phytochemicals such as alkaloids, quinones, phenolic acids, tannins, saponins, flavonoids, and acrylamide were present. The most abundant of the phytochemicals was tannic acid (55.26%), a tannin, followed by quercetin (13.17%), and then kaemferol (8.42%), both of which are flavonoids. The least on the table is deltonin (0.33%), a saponin. Tannic acid reportedly possesses physiological effects such as hypolipidaemia, reduction of liver necrosis, modulation of immune-responses, and accelerates blood clotting [21]. Studies have shown that quercetin possesses some anticancer properties [30], antihistamine effect [31], antioxidant property [32], anti-stress properties [33], and also suggested to have a bronchial dilating effect [34]. Kaemferol has been implicated by several studies in many pharmacological activities, such as cardioprotective, neuro-protective, anti-osteoporotic, oestrogenic/anti-oestrogenic, anti-diabetic, anti-allergic, analgesic, anxiolytic, anti-microbial, anti-cancer, antioxidant and anti-inflammatory [35]. Caffeic acid (4.55%), a phenolic acid, is a yellow solid that inhibits the metastasis of cancerous cells by an oxidative mechanism [36]. It reportedly possesses immuno-modulatory, anti-inflammatory and antifungal properties. Certain studies have, however, shown it may be carcinogenic [37] (3.78%), an alkaloid, was reported to show anti-convulsant and anti-nociceptive abilities [38]. Chlorogenic acid (2.56%) is a phenolic acid reported to minimize blood pressure [39], and

may also have a laxative effect [40]. Cassine (2.43%) is an alkaloid that possesses both anti-inflammatory and anti-nociceptive properties [41]. Connine (1.73%) and coniceine (1.89%) are both poisonous alkaloids. High concentration of coniine causes muscular paralysis that can eventually lead to hypoxia in the brain and heart, specifically due to paralysis of the respiratory muscles. Ingestion of as little as 0.1g of coniine can be lethal to an adult human [42]. Luteolin (1.1%), a flavonoid, possesses several physiological activities, among which are anti-inflammatory, anti-allergy and anti-cancer activities. Luteolin functions biochemically as an anti-oxidant or a pro-oxidant. Its anti-cancer ability is related to its induction of apoptosis, inhibition of cell proliferation, metastasis and angiogenesis [43]. Catechin(+)- [1.1%], another flavonoid present, has been suggested to lower plasma cholesterol level and also induce vasodilation, which helps in normal blood flow [44]. It has, however, been linked to haemolytic anaemia and renal failure [45]. 4-hydroxybenzoic acid (0.92%), also known as para-hydroxybenzoic acid (PHBA), is a phenolic acid with anti-oxidant properties and reported to possess estrogenic activities [46]. Saponine (0.52%), a saponin, reportedly lowers plasma cholesterol level by binding to bile salts in the intestinal walls, preventing their return to the liver and making the liver to produce more bile from available cholesterol, thereby reducing the available cholesterol. Its other abilities are anti-fungal and anti-cancer. Trillin (0.34%) is a saponin reported to reduce gastric ulceration [47] and possesses anti-inflammatory properties [48]. Deltonin (0.33%) is a steroidal saponin reported in several studies to be a potent cancer preventive and therapeutic agent [49, 50].

Conclusion

Findings from the proximate analysis of *Senna alata* (L) *Roxb* leaves showed that it can serve as a good source of energy and other nutrients in relatively moderate amount. However, it may not be suitable as food for both humans and animals, due to the presence of certain phytochemicals such as coniine and coniceine, which are poisonous. Phytochemical analysis shows that 100g of the leaf contains 30.51mg and 33.41mg of coniine and coniceine respectively. It has been reported that consuming 0.1g (100mg) of coniine can lead to death in an adult human (IPCS, 2012), due to respiratory muscle paralysis. The implication is that consuming about 330g of *Senna alata* (L) *Roxb* leaves may lead to death (due to coniine alone), to which the lethal amount could be lesser when the effect of coniceine is considered. The phytochemical screening indicated the presence of anthraquinones, flavonoids, tannins, alkaloids, phenolic acids, saponins, and quinones. The quantitative analysis showed the presence of high amount of some important phytochemicals such as tannic acid, quercetin, and kaemferol; that have been reported to possess some important pharmacological activities such as anti-cancer, hypolipidaemic, anti-oxidant, bronchial dilating, anti-microbial, and analgesic, amongst others.

References

1. Adedayo O, Anderson WA, Moo-Young M, Snieckus V, Patil PA, Kolawole DO. Phytochemistry and antibacterial activity of *Senna alata* (L) *Roxb* flower. Pharmaceutical Biology. 2001; 39(6):267-75.
2. Mutiu IK, Ganiyat AA, Anofi OTA. Effect of *Senna alata* (L) Roxb (L) Roxb (Fabaceae) Leaf Extracts on alpha-Amylase, alpha-Glucosidase and Postprandial Hyperglycemia in Rats. Tropical Journal of Pharmaceutical Research. 2015; 14(10):1843-1848.
3. Hennebelle T, Weniger B, Joseph H, Sahpaz S, Bailleul F. *Senna alata* (L) *Roxb*. Fitoterapia. 2009; 80:385-393.324
4. Ajibesin KK, Ekpo BA, Bala DN, Essien EE, Adesanya SA. Ethnobotanical survey of Akwa Ibom State of Nigeria. Journal of Ethnopharmacology. 2008; 115:387-412.
5. Abo KA, Fred-Jaiyesimi AA, Jaiyesimi AEA. Ethnobotanical studies of medicinal plants used in the management of diabetes mellitus in South Western Nigeria. Journal of Ethnopharmacology. 2008; 115:67-71.
6. Association of Official Analytical Chemists. Official Methods of Analysis of AOAC (W. Horwitiz, Ed.) 18th Ed. Washington, D.C. 2006; 1:600-792.
7. Trease GE, Evans WC. Phytochemicals in Pharmacognosy.15th ed. Saunders Publishers, London. 2002, 42-44, 221-229, 246-249, 304-306, 331-332, 391-393.
8. Santhi K, Sengottuvel R. Qualitative and quantitative phytochemical analysis of *Moringa concanensis* Nimmo. International Journal of Current Microbiology and Applied Sciences. 2016; 5(1):633-640.
9. Sheeja RR, Lawrence B. Phytochemical screening of the leaves of *Stevia rebaudiana* Bertoni. International Journal of Current Microbiology and Applied Sciences. 2015; 4(3):344-37.
10. Haliu ME, Abubakar A, Garba MK, Isah AA. Antimicrobial and preliminary phytochemical studies of methanol extract of root bark of *Crossopteryx fetrifuga* (Rubiceae). Journal of Applied Pharmaceutical Sciences. 2012; 2(12):066-070.
11. Oruc HH, Sonal S. Determination of Oxytetracycline, Penicillin G and Sulphadimidine Residues in Cow Milks. Bursa Uludag University Research Journal, Faculty of Veterinary Medicine. 2005; 24(1-2-3-4):11-13.
12. Brandao R, Roberto GJ. Determination of Beta-Lactam Residues in Milk by High Performance Liquid Chromatography. Brazilian archives of biology and technology. 2006; 49:41-46.
13. Isengard HD. Water content, one of the most important properties of food. Food Control. 2001; 12(7):395-400.
14. Ikewuchi J. Chemical Profile of *tridax procumbens* linn. Pakistan Journal of Nutrition. 2009; 8(5):548-550.
15. Idris S, Iyaka YA, Ndamitso MM, Paiko YB. Nutritional Composition of the leaves and stems of Ocimum gratissimum. Journal of Emerging Trends in Engineering and Applied Sciences. 2011; 2(5):801-805.
16. Sodamade A, Bolaji OS, Adeboye OO. Proximate analysis, mineral contents and functional properties of *Moringa oleifera* leaf protein concentrate. International Organisation of Scientific Research Journal of Applied Chemistry. 2013; 4(6):47-51.
17. Cannon C, O'Gara P. Critical Pathways in Cardiovascular Medicine (2nd ed.). Lippincott Williams & Wilkins. 2007, 243.
18. Ishida H, Suzuno H, Sugiyama N, Innami S, Todokoro T, Maekawa A. Nutritional Evaluation of Chemical Component of Leaves Stalks and Stems of Sweet Potatoes (*Ipomoea batatas Poir*). Food Chemistry. 2000; 68:359-67.
19. Abdulwaliyu L, Arekemese SO, Bala S, Ibraheem AS, Dakare AM, Sanodare R *et al.* Nutritional properties of *Senna alata* (L) *Roxb* Linn leaf and flower. *International* Journal of Modern Biology and Medicine. 2013; 4(1):1-

11.
20. Sun H, Xie Yong, Ye Y. Advances in saponin-based adjuvants. Vaccine. 2009; 27(12):1787-1796.
21. Chung KT, Wong TY, Wei C, Huang Y, Lin Y. Tannins and Human Health: A Review. Critical Reviews in Food Science and Nutrition. 1998; 38(6):421-64.
22. Liu HW. Extraction and Isolation of Compounds from Herbal Medicines: In Traditional Herbal Medicine Research Methods: Identification, Analysis, Bioassay, and Pharmaceutical and Clinical Studies (W. J. H. Liu. Ed.). Hoboken, NJ: John Wiley & Sons, 2011.
23. Yamamoto Y, Gaynor RB. Therapeutic potential of inhibition of the NF-κB pathway in the treatment of inflammation and cancer. Journal of Clinical Investigation. 2001; 107(2):135-42.
24. Cazarolli LH, Zanatta L, Alberton EH, Figueiredo MS, Folador P, Damazio R *et al.* Flavonoids: Prospective Drug Candidates. Mini-Reviews in Medicinal Chemistry. 2008; 8(13):1429-1440.
25. Kittakoop P, Mahidol C, Ruchirawat S. Alkaloids as important scaffolds in therapeutic drugs for the treatments of cancer, tuberculosis, and smoking cessation. Current Topics in Medicinal Chemistry. 2014; 14(2):239-252.
26. Russo P, Frustaci A, Del Bufalo A, Fini M, Cesario A. Multitarget drugs of plants origin acting on Alzheimer's disease. Current Medicinal Chemistry. 2013; 20(13):1686-93.
27. Qiu S, Sun H, Zhang AH, Xu HY, Yan GL, Han Y *et al*. Natural alkaloids: basic aspects, biological roles, and future perspectives. Chinese Journal of Natural Medicine. 2014; 12(6):401-406.
28. Ghasemzadeh A, Ghasemzadeh N. Flavonoids and phenolic acids: Role and biochemical activity in plants and humans. Journal of Medicinal Plants Research. 2011; 5(31):6697-6703.
29. Shih-Chang C, Yueh-Chen W, Zeng-Weng C, Wen-Chin Y. Naturally Occurring Anthraquinones: Chemistry and Therapeutic Potential in Autoimmune Diabetes. Evidence-Based Complementary and Alternative Medicine. 2015; 20(15):13.
30. Long Q, Xiel Y, Huang Y, Wu Q, Zhang H, Xiong S *et al.*, Induction of apoptosis and inhibition of angiogenesis by PEGylated liposomal quercetin in both cisplatin-sensitive and cisplatin-resistant ovarian cancers. Journal of Biomedical Nanotechnology. 2013; 9(6):965-75.
31. Chirumbolo S. The role of quercetin, flavonols and flavones in modulating inflammatory cell function. Inflammation and Allergy-Drug Targets. 2010; 9(4):263-85.
32. Williams RJ, Spencer JP, Rice-Evans C. Flavonoids: antioxidants or signalling molecules? A Review. Free Radical Biology & Medicine. 2004; 36(7):838-49.
33. Cheng LC, Li LA. Flavonoids exhibit diverse effects on CYP11B1 expression and cortisol synthesis. Toxicology and Applied Pharmacology. 2012; 258(3):343-50.
34. Townsend EA, Emala CW. Quercetin acutely relaxes airway smooth muscle and potentiates-agonist induced relaxation via dual phosphodiesterase inhibition of PLC and PDE4. American Journal of Physiology- Lung Cellular Molecular Physiology. 2013; 19(6):563-75.
35. Calderon-Montaño JM, Burgos-Moron E, Perez-Guerrero C, Lopez-Lazaro M. A review on the dietary flavonoid kaempferol. Mini-Reviews in Medicinal Chemistry. 2011; 11(4):298-344.
36. Rajendra PN, Karthikeyan A, Karthikeyan S, Reddy BV. Inhibitory effect of caffeic acid on cancer cell proliferation by oxidative mechanism in human HT-1080 fibrosarcoma cell line. Molecular and Cellular Biochemistry. 2011; 349(1):11-9.
37. Hirose M, Takesada Y, Tanaka H, Tamano S, Kato T, Shirai T. Carcinogenicity of antioxidants BHA, caffeic acid, sesamol, 4-methoxyphenol and catechol at low doses, either alone or in combination, and modulation of their effects in a rat medium-term multi-organ carcinogenesis model. Carcinogenesis. 1998; 19(1):207-212.
38. Amat-Sain A, Amanah A, Zahari Z, Salim RJ, Mansor SM, Adenan MI. (+) - Spectaline, A piperidine alkaloid from *Senna spectabilis* DC: Effective in reducing the in vitro infection of leishmania major. International Journal of Pharmacology, Phytochemistry and Ethnomedicine. 2016; 3:1-8.
39. Onakpoya IJ, Spencer EA, Thompson MJ, Heneghan CJ. The effect of chlorogenic acid on blood pressure: A systematic review and meta-analysis of randomized clinical trials. Journal of Human Hypertension. 2014; 29(2):77-81.
40. Stacewicz-Sapuntzakis M, Bowen PE, Hussain EA, Damayanti-Wood BI, Farnsworth NR. Chemical composition and potential health effects of prunes: a functional food? Critical Reviews in Food Science and Nutrition. 2001; 41(4):251–86.
41. Da Silva, KA, Manjavachi MN, Paszcuk AF, Pivatto M, Viegas C Jr, Bolzani VS *et al.* Plant derived alkaloid (-)-cassine induces anti-inflammatory and anti-hyperalgesics effects in both acute and chronic inflammatory and neuropathic pain models. Neuropharmacology. 2012; 62(2):967-77.
42. International Programme on Chemical Safety [PCS] *Conium maculatum* L. In chem. 2012.
43. Lin Y, Wang X, Shen HM. Luteolin, a Flavonoid with potential for cancer therapy and prevention. Current Cancer Drug Targets. 2008; 8(7):634-46.
44. Khalesi S, Sun J, Buys N, Jamshidi A, Nikbakht-Nasrabadi E, Khosravi-Boroujeni H. Green tea catechins and blood pressure: A systematic review and meta-analysis of randomised controlled trials. European Journal of Nutrition. 2014; 53(6):1299-311.
45. Martinez SE, Davies NM, Reynolds JK. Toxicology and Safety of Flavonoids. Methods of Analysis, Preclinical and Clinical Pharmacokinetics, Safety, and Toxicology. Hoboken, NJ: John Wiley & Son. 2013, 257.
46. Sushil K, Khetan. Endocrine Disruptors in the Environment. Wiley. 2014, 109-112. ISBN 978-1-118-89115-5.
47. Tong C, Wenjiao J, Huixin Z, Xintong Y, Manling L, Wang L *et al.* Protective effect of trillin against ethanol-induced acute gastric lesions in an animal model. Royal Society of Chemistry journal. 2016; 6(24):20081-20088.
48. Huabing T, Qin H, Rugui L, Feifei L, Xu L. Trillin Reduces Liver Chronic Inflammation and Fibrosis in Carbon Tetrachloride (CCl_4) Induced Liver Injury in Mice. Immunological Investigations. 2016; 45(5):371-382.
49. Shu D, Qing Y, Tong Q, He Y, Xing Z, Zhao YL *et al.* Deltonin isolated from Dioscorea zingiberensis inhibits cancer cell growth through inducing mitochondrial apoptosis and suppressing Akt and mitogen activated protein kinase signals. Biological and Pharmaceutical Bulletin. 2011; 34(8):1231-9.

50. Qing-Yi T, Yong Q, Dan S, Yang H, Ying-Lan Z. Deltonin, a Steroidal Saponin, Inhibits Colon Cancer Cell Growth in Vitro and Tumor Growth in Vivo via Induction of Apoptosis and Antiangiogenesis. Cellular Physiology and Biochemistry. 2011; 27:233-242.