Proximate and Elemental Composition of Methanolic Extract of *Boswellia dalzielii* Hutch. (Frankincense Tree: Burseracea)

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Abstract: This study is aimed at investigating the proximate contents, concentrations of some micro/macronutrients and anions of the stem bark and leaves of *B. dalzielii* with the view of validating its use as forage and medicinal by man in the treatment of diarrhoea, dysentery and in wound healing. The stem bark and leaves of *B. dalzielii* were collected from Bagale-Giere Local Government Area, Adamawa State, Nigeria. Proximate analysis was conducted following methods of Association of Official Analytical Chemists and the results showed that the stem bark had the higher dry matter (90.94 %), nitrogen free extract (54.13 %), crude fibre (30.65 %), and ash (8.34 %), while crude proteins (4.63 %), fats (21.20 %), carbohydrates (57.76 %) and moisture content (12.41 %) were estimated to be higher in the leaves of this plant. The levels of 11 elements (Ca, Mg, Na, K, Fe, Cd, Cu, Ni, Zn, Cr, Pb) were determined using atomic absorption spectrophotometer, the anions (nitrates, phosphates and sulphates) were estimated using smart spectrophotometer. The results revealed higher concentrations of macro elements and microelements in the leaves sample except for Cr concentration which was higher (4.0 x 10^2 ±0.00) mg/g in the stem bark than in the leaves (3.80 x 10^2 ±0.01) mg/g. In both the samples Pb was not detected. The concentrations of nitrates, phosphates and sulphates were higher in the leaves (1.52 x 10^2, 4.0 x 10^1, 1.33 x 10^2) mg/g than the stem bark (1.40 x 10^2, 3.0 x 10^1, 4.24 x 10^0) mg/g respectively. The proximate, elemental and anions concentrations for these parts of *B. dalzielii* were mostly found within the permissible limit set by World Health Organization.

Keywords: Anions, *Boswellia dalzielii*, leaves, elements, proximate, stem-bark.

1. Introduction

In Africa and across the globe, herbal medicines represent the fastest growing segment of pharmacy trade, surely the cost of modern clinical medicines cannot be over looked, so most people consider other alternative form of medicines. Most herbal medicines are less expensive than prescription drugs (Sofowora et al., 2013). Nature has been the source of medicine for thousands of years in the maintenance of human health since ancient time (Mamza et al., 2015). Over 50 % of all modern clinical drugs are of natural product origin (Danlami et al., 2015). The chemical constituents of plants, including metal ions, are particularly responsible for medicinal and nutritional properties, as well as the toxicity. The cumulative levels of these metals in both the roots and other tissues of plant above the ground can be transferred from soils into the food chain; thus causing phytotoxicity in plants and having potential harmful effects on animals and humans (Hussain Hussain et al., 2006). WHO (1998) recommends that medicinal plants, which form the raw materials for the finished products, may be checked for the presence of heavy metals, pesticides, bacterial or fungal contamination.

*Boswellia dalzielii* is a tree that belongs to the family of Burseraceae, from the genus of *Boswellia* and species of *B. dalzielii*. It is about 13m high of the wooden savanna with a pale papery bark peeling and ragged characteristics. It is abundantly found in West Africa in countries such as Ghana, Niger, Ivory Coast, Upper Volta, Cameroun and Northern part of Nigeria, where the Hausa speaking people of Nigeria call it “Hano” or “Arrarabbi”, Margi “Mofu” and Babur-Bura “Debro” (Moses et al., 2005; Mahamat et al., 2014a). The plant is popular in the Northern part of Nigeria due to its ethnomedical importance. A bark-decoction is used as an antiseptic wash for sores in Ivory-Coast and is an ingredient of a complicated prescription for leprosy (Burkill, 1985). In northern Nigeria, the bark is boiled up in large quantity to make a wash for fever, rheumatism etc., and the fluid is taken internally for gastrointestinal troubles (Burkill, 1985, Danlami et al., 2015). The Fulani people of northern Nigeria use a cold infusion for snake bite (Burkill, 1985). The fresh bark of the root is eaten in Adamawa State, Nigeria, to cause vomiting after a few hours and thus relieves symptoms of giddiness and palpitations as well as antidotes to arrow-poison (Burkill, 1985; Danlami et al., 2015).

*B. dalzielii* is a staple household medicine for dental problems, swellings, bronchitis and coughs (Ben-Yehoshua et al., 2012). The psychoactivity of *B. dalzielii* was recognized in ancient times in the near East and Europe. In India, the traditional Ayurvedic medical system refers to the use of the gum extracted from *Boswellia dalzielii*, which is recommended for arthritic and inflammatory conditions, gastric disorders, pulmonary diseases and skin ailments. It is also reported to have a strong action on the nervous system and reduces phlegm, asthmatic attack and stops vomiting (Ben-Yehoshua et al., 2012). *B. dalzielii* has strong antibacterial, antibiotic, antifungal and antiseptic properties, making it a valuable ingredient in natural medicine (Ben-Yehoshua et al., 2012). According to Mahamat et al. (2014a, 2014b), *B. dalzielii* has shown a potent immunological effect and immune modulatory activity both in vitro and in vivo in northern Cameroon. In the Republic of Benin, *B. dalzielii* has shown a wide spectrum of antibacterial activity (Anago et al., 2011). In Nigeria, antibacterial activities and gastrointestinal effects of *B. dalzielii* have been reported by different researchers (Oguakwa, 1980; Alemika and...
Oluwole, 1991; Ntiejumokwu and Alemika, 1991; Nwinyi et al., 2004; Moses et al., 2005; Roseline et al., 2007). Atawodi et al. (2011) reported that *B. dalzielii* has antiparasomonal activity. *B. dalzielii* has widely been reported to offer good treatment for leprosy, peptic ulcer, asthma, diarrhoea, syphilis, rashes, chickenpox, hepatitis and HIV/AIDS. In addition, it is liver protective (Burkill, 1985; Nwinyi et al., 2004; Anago et al., 2011; Ohemu et al., 2014).

It is therefore worthy to note that, whatever is taken as food could cause metabolic disturbance subject to the allowed upper and lower limits of trace metals (Prasad, 1976) the excesses of these essential nutrient and trace of toxic metals may cause serious effects on human health (Khan et al., 2008).

There is little or no report on the proximate, elemental and anions contents of *B. dalzielii*; and thus, the aim to investigate the levels in the stem bark and leaves, with the view to validating the traditional use of this plant in Adamawa State –Nigeria, as forage and remedy against diarrhoea, dysentery, for wound healing among others.

2. Materials and Methods

Collection and Identification of plant
The plant was collected from Bagale (Long. 14° 23.32’ E; Lat. 11° 01.22’ N), Gieri Local Government Area, Adamawa State, Nigeria in November, 2015. The plant material was identified and authenticated by a plant Taxonomist, Professor S. S. Sanusi, Department of Biological Sciences, University of Maiduguri and the voucher specimen number #340 was prepared and deposited at the Post Graduate Research Laboratory, Department of Chemistry, University of Maiduguri, Nigeria.

Sample Preparation
The air-dried stem bark and leaves were manually pulverized using wooden mortar and pestle and then treated onwards. Two grams of the powdered sample were processed for various parameters according to the Association of Official Analytical Chemists methods. (AOAC, 1990; AOCS, 2000).

Proximate Analysis
The proximate evaluation for the moisture, crude protein, crude fibre, ash content, crude fats, carbohydrates and nitrogen free extracts of the stem bark and leaves samples were determined using AOAC methods. The moisture and ash were determined using weight difference method. Fibre content was estimated from the loss in weight of the crucible and its content on ignition. Carbohydrate was determined when the sum of the percentages of moisture, ash, crude protein and fats were subtracted from 100. The nitrogen value, which is the precursor for protein of a substance, was determined by micro Kjeldahl method, involving digestion, distillation and finally titration of the sample (AOCS, 2000). The nitrogen value was converted to protein by multiplying with a factor of 6.25. the determination of crude lipid content of the samples was done using soxhlet type of the direct solvent extraction method. The solvent used was petroleum ether (boiling range 40 -60 °C), the nitrogen free extract (NFE) was calculated indirectly by difference as the sum of crude protein, crude fibre, ether extract and ash subtracted from 100. All the proximate values are presented in percentage (AOAC, 1990; AOCS, 2000).

Elemental Analysis of the Plant Samples
The macro and microelements were determined using Perkin-Elmer Analyst 300 single beam Atomic Absorption Spectrophotometer (AAS) and the data was obtained in parts per million (ppm) which was then converted to mg/g. Calibration curve was established using working standards for each element. Laboratory procedures for the preparation and determination of macro and microelements were used as outlined by Radojevic and Bashkin (1999) for plant samples.

Sample Digestion and Preparation for Analysis
The air-dried plant samples were pulverized manually in wooden mortar and pestle into coarse powder. 0.5 g of each sample was independently packed into an acid-washed porcelain crucible and then placed in a muffle furnace for four hours at 550 °C. The crucibles were removed from the furnace and cooled. Ten ml of 6 M HCl were added and then covered, this content was heated on a steam bath for 15 minutes. One ml of HNO₃ was later added and evaporated to dryness by continuous heating for one hour so as to dehydrate silica and completely digest organic substances. Lastly, 5 ml of 6 M HCl and 10 ml of water were added and the mixture was heated on a steam bath to complete dissolution. The mixture was cooled and filtered through a Whatman No. 1 filter paper into 100 ml volumetric flask and then made up to the mark with distilled water (Radojevic and Bashkin, 1999).

3. Anions Analysis in Plant Samples

Determination of nitrate
The concentration of nitrate in the leaves and stem bark was carried out by standard cadmium reduction method using Smart Spectrophotometer (La Motte, 2000). Plant samples solutions were prepared by chopping each sample into smaller sizes, About 0.5 g of the samples was transferred into 100 ml volumetric flask and soaked with 50 ml distilled water. The flask was corked and shaken for 30 minutes, then filtered into another 100 ml volumetric flask and the volume made to the mark with distilled water (Radojevic and Bashkin, 1999).

Determination of phosphate
The air-dried and powdered samples of the stem and leaves of *Boswellia dalzielii* were used. About 0.5 g was weighed into crucibles; this was then underlayed with 5 ml of (20 %) w/v magnesium acetate and thereafter evaporated to dryness. The content of the crucible was then transferred into the muffle furnace and heated to 550 °C. Furthermore, the crucible contents were ashed at 550 °C for 4 hours and then cooled in desiccators. Ten ml of 6 M HCl were then added to each off the crucible and covered and then heated on a water bath for 15 minutes. The contents of the crucible were completely transferred into different evaporating basins and 1 ml of concentrated HNO₃ was added. The heating was continued for 1 hour to dehydrate silica. One ml of 6 M HCl further added, swirled and then followed by the addition of 10 ml distilled water and again heated on the water bath for...
complete dissolution. The contents of the evaporating basins were cooled and then filtered through a Whatman No. 1 filter paper into 100 ml volumetric flasks and the volumes made up to the marks with distilled water (Radojevic and Bashkin, 1999). Phosphate was determined using batch direct reading 2000 spectrophotometer.

**Determination of Sulphate**
Sulphate was determined using smart spectro spectrophotometer (La Motte, 2000). The samples were prepared as follows: 5 ml of magnesium nitrate solutions were added to each of the ground samples in the crucibles which were then heated to 180 °C on a hot plate. The heating process was allowed to continue until the colour of the samples changed from brown to yellow (AOAC, 1990). The samples were then transferred to the furnace at a temperature of 500 °C for four hours. Magnesium nitrate was added to prevent loss of Sulphur. The contents of each crucible were carefully transferred to different evaporating basins; 10 ml of concentrated HCl were added to each and covered with watch glass. The content of each crucible above was boiled on a steam bath for 3 minutes. On cooling, 10 ml of distilled water were added to each of the basins and the contents filtered into 100 ml volumetric flask and the volume made up to the mark with distilled water (Radojevic and Bashkin, 1999).

**4. Results and Discussion**

**Proximate Analysis**
The result of proximate analysis shows variant proportions of nutrients and their contents. The data for the proximate contents of the stem bark and leaves are presented on Figure 1. The result revealed that the ash content of the stem bark (8.34 %) is higher than that of the leaves (4.00 %), therefore the stem bark contains more inorganic constituents than the leaves. The result also showed that the crude fats contents (21.20 %) and the crude protein (4.63 %) content of the leaves was higher than that of the stem bark (3.56 % and 3.32 %) respectively which indicated that the leaves contain more calories constituents than the stem bark. The crude fibre content of the stem bark (30.65 %) was higher than that of the leaves (28.4 %), therefore it may provide protection against gastrointestinal disease than the leaves (Danlami et al., 2015). The moisture content was obtained as 9.06 % and 12.41 % in the stem bark and leaves respectively which was in agreement with the results reported by Danlami et al., 2015 that moisture contents for stem bark and leaves were calculated as 8.51 % and 12.24 % respectively. Our data for carbohydrate content in the stem bark and leaves were found as 45.07 % and 57.76 % respectively. Looking also at the result, it showed that carbohydrate content in the leaves which is an important source of energy is higher than that of the stem bark. The nitrogen free extract was more in the stem bark (54.13 %) than that found in the leaves (41.77 %). The highest proximate contents were presented by percentage dry matter in both parts; the stem bark had 90.94 % and the leaves had 87.59 %. The percentage dry matter observed in this study had showed a similar results reported by Danlami et al., 2015 on this plant and Usman et al., 2011 on B. rufescens Lan. From these results, the increasing order of these nutrients among the plant parts is dry matter > nitrogen free extract > carbohydrates > crude fibre > moisture content > ash content > fats content > crude protein.

**Elemental Analysis**
The results of macro- and microelements concentrations reported in mg/g are presented in Table 1.

**Macro elements**
Metals play a vital role as structural and functional components of proteins and enzymes in cells. Each mineral plays a number of different functions in the body. The most important pathway of metals to transport into human is from soil to plant and from plant to human (Maghrabi, 2014).

The macro elements analysed were potassium, calcium, magnesium, and sodium. The K, Ca, Mg and Na are available in very low to moderate concentrations respectively. This is very significant, as K, Ca, Mg and Na are known to enhance the qualities of blood, bone, teeth and blood formation and also for cardiac function; these elements likewise also play a predominant role in enzyme activation, oxygen and electron transport. There are lots of disease conditions that result due to the deficiency or excess of these essential elements in the body. For instance, a reduced extracellular blood calcium increases the irritability of nerve tissue and very low levels may cause spontaneous discharge of nerve impulse, leading to tetany and convulsion (Soetan et al., 2010). In children, calcium deficiency causes rickets due to insufficient calcification by calcium phosphate of the bones in growing children. The bones therefore remain soft and deformed by the body weight (Vohora, 1987). In adults, Ca deficiency causes osteomalacia, a generalized demineralization of bones. Toxicity symptoms occur with excess absorption due to hyperparathyroidism or idiopathic hypercalcemia. Excess calcium depresses cardiac activity and leads to respiratory and cardiac failure; it may cause the heart to stop in systole, although, normally, calcium ions increase the strength and duration of cardiac muscle contraction (Soetan et al., 2010; Maghrabi, 2013).

Sodium deficiency in young chicks cause growth retardation. Egg production and hatchability in laying chickens are depressed. Increased level of sodium in the serum is called hypernatraemia and this occurs in Cushion’s disease. Low level of sodium in the serum is hyponatraemia and this occurs in acute Addison’s disease; vomiting, diarrhoea, nephrosis-severe (non-inflammatory degeneration of kidneys) burns and intestinal obstruction. The toxicity which occur as a result of high accumulation of sodium in the body fluid may cause hypertension in susceptible individuals (Soetan et al., 2010). The health status of the digestive system and the kidneys significantly influence magnesium status. Magnesium is an active component of several enzyme systems in which thymine pyrophosphate is a cofactor. Chronic or excessive vomiting and diarrhoea may result in magnesium depletion. Deficiency diseases or symptoms is secondary to malabsorption or diarrhoea. Acute magnesium deficiency results in vasodilation, with erythema and hyperaemia. Toxicity disease or symptoms of magnesium deficiency in humans include depressed deep tendon reflexes and respiration (Murray et al 2000; Soetan et al., 2010).
Potassium is the principal cation in intracellular fluid and functions in acid-base balance, regulation of osmotic pressure, conduction of nerve impulse, muscle contraction particularly the cardiac muscle, cell membrane function and Na⁺/K⁺-ATPase (Soetan et al., 2010). Excessive amount of potassium in blood (hyperkalaemia) usually causes advanced chronic renal failure, shock and dehydration while other symptoms of accumulated potassium include dilatation of the heart, cardiac arrest, small bowel ulcers. Low level of serum potassium (hypokalaemia) causes diarrhoea, metabolic alkalosis and familial periodic paralysis. Potassium deficiency affects the collecting tubules of the kidney, resulting in the inability to concentrate urine and also causes alterations of gastric secretions and intestinal motility. Deficiency diseases or symptoms occur secondary to illness, functional and structural abnormalities including impaired neuromuscular functions, skeletal, smooth and cardiac muscle, muscular weakness, paralysis, mental confusion (Soetan et al., 2010; Sarpong et al., 2012; Maghrabi, 2014).

According to Osuji et al. (2013), concentrations of these elements in plants could be specifically useful as anti-infective in the treatment of diseases and disorders such as diarrhoea, dysentery, wound healing and other bacterial and fungal infections. The abundance of Ca, Mg. Na and K in the present study was also in agreements with previous studies, which indicate that these elements are the most abundant elements in many herbal drugs (Soetan et al., 2010; Sarpong et al., 2012; Maghrabi, 2014).

**Micro elements**

High levels of hazardous metals such as lead (Pb) in medicinal plants can have adverse effects on the blood, the central nervous system (CNS), the blood pressure, the kidneys, the reproductive system and vitamin D metabolism (Sarpong et al., 2012). Exposure to chromium (Cr) can cause lung cancer, irritation of the lung resulting in asthma, fever and kidney damage. When cadmium (Cd) enters the body, it severely irritates the stomach, leading to vomiting and diarrhoea. Cadmium can also cause severe lung damage leading to death. Acute toxicity of zinc may cause throat dryness, cough, general weakness, fever, nausea, anaemia and pancreas damage (Soetan et al., 2010; Sarpong et al., 2012; Kulhari et al., 2013; Maghrabi, 2014). Mercury (Hg) and its compounds are highly toxic, especially methylmercury- a potent neurotoxin. A few studies reflect that even minor increases in methylmercury exposure can cause harmful effects on the cardiovascular system, blisters in the upper gastrointestinal tract, vomiting, abdominal pain, constipation and gastritis (Mudgal et al., 2010).

According to WHO recommendation, medicinal plants which form the raw materials for the finished products may be checked for the presence of heavy metals. Further, it regulates maximum permissible limits of toxic metals like As, Cd, Hg and Pb which amounts to 1.0, 0.3, 0.5, 10.0 ppm respectively (WHO, 2007). Although, macro and micro elements are valuable for diseases and disorders control in man (Iwalewa et al., 2009) they could however produce harmful effects in excessive amounts (Hussain et al., 2006; Usman, 2012). According to the European Union (EU) Commission Regulation published by Kabata-Pendias and Mukherjee (2007), the maximum amount of Cd, Pb and Hg in foodstuffs and plants used for therapy have to be controlled. Thus, Provisional Tolerable Weekly Intake (PTWI) values for adults have been set as Cd, 7; Hg, 5 and Pb, 25 in µg/kg body weights. The Recommended Dietary Allowance (RDA) for other elements also have been estimated for safety and adequate daily intake for adults as follows: Cr, 0.6-3; Mn, 26-60; Se, 0.9; and Zn 190 in µg/kg body weights (Iwalewa et al., 2009).

This study reports the concentrations of seven microelements; Cd, Cr, Pb, Cu, Fe, Ni and Zn. The results showed the highest concentration of Cu in the leaves as (4.22 x 10⁻³±0.63) mg/g than stem bark (2.56 x 10⁻³±0.04) mg/g samples as shown in Table 2. On the other hand, the concentration of chromium (Cr) in both leaves and stem bark were the lowest (3.8 x 10⁻³±0.01 and 4.00 x 10⁻⁴±0.00)mg/respectively. The levels of Cd were low in both stem bark and leaves with values of 7.80 x 10⁻²±0.01 and 8.10 x 10⁻²±0.40 mg/g respectively. The concentrations of Ni in both stem bark and leaves equally showed low values of 1.25 x 10⁻²±0.00 and 1.33 x 10⁻²±0.01 mg/g respectively. The concentration of Fe in this part of plant (stem bark and leaves) was found to be 3.00 x 10⁻⁵±0.01 and 3.80 x 10⁻³±0.33 mg/g while the levels of Zn are 3.9 x 10⁻²±0.02 and 3.82 x 10⁻²±0.01 mg/g respectively. This is in agreement with most medicinal plants studied as reported by several workers that these metals were either very low in concentration or below the detection limit of the instruments used (Khan et al., 2008; Kulhari et al., 2013; Maghrabi, 2014). Lead (Pb) was not detected in the samples. Cadmium (Cd) which is known to be practically toxic (Khan et al., 2008; Kulhari et al., 2013), was found to be below the permissible limit of 0.3 mg/kg (WHO, 2007). The concentration of Cr for both stem bark and leaves (4.00 x 10⁻⁵±0.00 and 3.80 x 10⁻³±0.01 mg/g) determined falls below the permissible region in plant as reported by Adrano (1986), that most phytoxic level of Cr in plant occurs at 10 mg/kg. The concentration of copper (Cu) was determined as 2.56 x 10⁻⁴±0.04 mg/g and as reported by Suttle and Jones (1989), copper as a trace element has as inhibitory effect on edema and inflammation as well as being universally important cofactor for many hundreds of enzymes (Suttle and Jones, 1989; Tilson, 1982). It is therefore pertinent to say that there is reason for the use of this part of this plant in the management of diarrhoea and dysentery. The effect of copper on resistance to bacterial and viral infections in ruminants has been highlighted (Suttle and Jones, 1989). A Cu deficiency can result in a decrease in the tissel strength of arterial walls, leading to aneurysm formation and skeletal maldevelopment (Tilson, 1982).

Ni concentration was lower in the sample studied (1.25 x 10⁻³±0.00 mg/g) and these values fall within the safety margin, since phytotoxic concentration of Ni ranges between 40 to 246 mg/l (Usman, 2012) and also lower than the Ni concentration of 0.05-5 mg/kg for most of plant foods (FAO/WHO, 1993). The concentration of Zn was less than 0.15 mg which falls within the normal range of 25-150 mg/kg (Jones, 1972) and was found to be far below the concentration which affects human health that ranges between 100 to 500 mg/l (Macnicol and Beckett, 1985). This
element being essential is utilized in the development of brain, bone formation and wound healing (Khan et al., 2008). These observations could be the possible reason for the use of this plant in wound healing in the traditional system of medicine. Therefore, it may properly be said that the anti-infective effects of this plant may be related to these elements.

According to WHO recommendation, medicinal plants which form the raw materials for the finished products may be checked for the presence of heavy metals, further it regulates maximum permissible limits of toxic metals like arsenic (As), cadmium (Cd) and lead (Pb), which amount to 1.0, 0.3 and 10 ppm, respectively (WHO, 1989, 1998). Although, macro- and microelements are valuable for disease and disorders control in man (Iwalewa et al., 2009), they could however produce harmful effects in excessive amounts (Hussain et al., 2006).

**Anions Analysis**

The levels of anions in the leaves and stem of *B. dalzielii* are presented in Figure 2. The results revealed that the concentrations (mg/g) of nitrates were highest in stem bark (1.52 x 10^{-2}) mg/g compared to those in the leaves (1.40 x 10^{-2}) mg/g while the phosphate content in both stem and leaves were lowest (4.0 x 10^{-4} and 3.0 x 10^{-4}) mg/g respectively. The levels of sulphates in the *B. dalzielii* parts (stem bark and leaves) were 1.33 x 10^{-2} and 1.24 x 10^{-2} mg/g respectively. The levels of nitrates and sulphates are below WHO/FAO standard permissible limits while phosphates concentration were higher than the WHO/FAO limits.

![Figure 1: Proximate levels of the stem bark and leaves of *B. dalzielii*](chart.png)

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<th>Table 1: Macro- and microelements contents of the stem bark and leaves of <em>B. dalzielii</em></th>
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Key: ND = no detected
5. Conclusion

In conclusion, both the proximate, elemental and anions concentrations for these parts of *B. dalzielii* were mostly found within or below the permissible limits set by WHO/FAO. This further corroborates the use of this plant in some parts of Adamawa State, Nigeria, in the treatment of diarrhoea, dysentery and wound healing among others.

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