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SHORT COMMUNICATION

INVESTIGATION OF CHEMICAL AND BIOCHEMICAL PROPERTIES OF *MAERUA SUBCORDATA* PLANT EXTRACT: A LOCAL WATER CLARIFICATION AGENT

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ABSTRACT. This paper reports some findings of chemical as well as biochemical studies of *Maerua subcordata* plant juice extract. The studies include: chemical composition of the juice such as mineral contents, protein and polysaccharide contents. These tests were carried out both in the juice extracts and in the "flocs". The latter samples were analyzed to establish whether the material that settled as a result of clarification had minerals, protein and/or polysaccharides. While there were no significant amounts of minerals found in the juice or in the flocs, there were significant amounts of polysaccharides in the juice as well as in the flocs. However it was found that there were insignificant amounts of proteins in the settled flocs. This was indication that polysaccharides in the juice were precipitated along with the sediments present in water. Further biochemical experiments on the identity of polysaccharides which apparently aided flocculation indicated that they were of branched type, *amylopectin*. The paper attempts to explain the mechanism of sediments settling as aided by the plant juice components.

KEY WORDS: Turbid surface water treatment, Coagulation, Flocculation, *Maerua subcordata* plant, Amylopectin polysaccharide

INTRODUCTION

Surface water sources including rivers and dams in Kenyan rural communities have high levels of turbidity resulting from eroded soils, trampling by livestock and natural weathering processes. This is particularly true in dry areas where land cover is minimal. It is risky to drink such kind of water because most often the particulates carry a variety of water contaminants apart from looking dirty. Conventional treatment using chemicals is too expensive and often unavailable in resource poor communities. As a result, inhabitants in these communities have adapted a variety of indigenous technologies of removing water turbidity using natural plant extracts. Natural plant extracts have been demonstrated to effectively coagulate suspended solids and consequently reduce water turbidity with encouraging results. The potential use of natural coagulants has been reported by several groups including Folkard and Sutherland [1], Folkard, Sutherland and Grant, [2, 3], and Jahn [4-7]. For a long time now, research has shown that, *Moringa oleifera* seeds, among other African traditional plants could be used as an effective coagulant [7]. More recently, Chemelil *et al.* [8] have documented the effectiveness of *M. subcordata* plant extract as a natural product used for water clarification. The authors found that *M. subcordata* juice was the best water clarification agent compared with powdered *M. subcordata* or even the commonly used commercial coagulant, alum. The powder was obtained from grinding the plant tubers after drying them.

The plant is a shrub which grows widely in arid and semi arid land (ASAL) like Baringo District in Kenya. It is normally less than 1 m high but sometimes up to 2 m; the leaves are blue-green, egg shaped to rounded, stiff and leathery; the flowers are many, greenish-yellow with

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long filaments; the roots are woody and swollen, water-storing [6]. Communities with chronic water shortages in Ethiopia and Kenya have used this plant for many years to purify water.

The procedure for purification of water in these communities is explained under "Purification of turbid surface water by plants in Ethiopia" in Gottsch, [9], as follows: The swollen root which has a diameter of 3-15 cm is dug out and its bark is scraped off. The underlying tissue is of a soft consistency and has a characteristic smell. Through a number of incisions the surface area of the barked root can be enlarged. After these preparations the root is stuck on a wooden rod. A container filled with 30-40 litres of turbid water, is taken and the water is stirred for about 10 min with the rod. About half an hour later the turbid water becomes almost clear. The remaining water however, shows a slightly yellowish discoloration, but has a pleasant taste. According to Jahn [4], special polysaccharides in the root of *M. subcordata* are responsible for the flocculation of the colloidal particles. These macromolecules form bridges between mass, thus causing precipitation. The polysaccharides are also responsible for the slightly sweet taste of the root of *M. subcordata*.

Flocculation has generally been explained by Khadilar [10]. When certain chemicals are added to water containing soluble and finely suspended impurities an insoluble gelatinous substance is formed. The "floc" as the substance is known, being heavier than water it sinks to the bottom of the container. While descending it absorbs colloidal matter. This latter process is called coagulation. The most common chemical coagulant is alum (aluminium ferrous sulfate). Alum contains about 17 % Al_2O_3. Chemicals such as $Al_2(SO_4)_3$ and $FeCl_3$ when added to water combine with alkalinity (OH^-) in the water to form positive-charged sticky material. The chemical materials added neutralize the electrical charge of hydrophobic material (colloidal clay, less than 1 μm diameter, suspended particles that cause turbidity, with negative electrical charge). As a result of this electrical neutrality, the hydrophobic particles bump together. When this happens, the particles stick together and form large particles. These particles will continue to bump into one another and become large enough to settle out [11]. It has been observed that since germs are often found on the colloid surfaces, flocculation of these particles removes the germs as well [9].

Maerua subcordata juice extract seems to provide a coagulant, forming flocs through chemical or biochemical processes. This paper reports the efforts made so far in explaining the phenomenon of coagulation and subsequent flocculation of suspended matter in turbid water by the use of *M. subcordata*. In this endeavour it was necessary to determine important chemical and biochemical properties of *M. subcordata* juice that might be responsible for coagulation. Specifically it was sought to determine whether there are significant concentrations of aluminium and/or iron in the juice which would indicate that the juice works in a similar manner as alum and whether there were significant amounts of protein and/or polysaccharides in the juice. The latter two have the ability to cause coagulation of metal ions in water [12].

EXPERIMENTAL

The plant juice was extracted from peeled and chopped tubers of *M. subcordata.* Extraction was accomplished by the use of a ram press. A brownish juice obtained was filtered to remove suspended particles before it was used to treat water in a previously determined optimum dose of 0.75 mL per litre of turbid water [8]. Throughout this study, all experiments were done in triplicate.

Physical-chemical properties of Maerua subcordata juice

Physical properties of the plant juice were determined and recorded. The pH of fresh juice was determined to be 3.5. This is quite acidic. However, the pH of fresh water after adding the juice was still slightly basic (pH of 8.1). The juice had a characteristic smell, which is consequently transferred to treated water.

Metal analysis

Analyses for Al, Fe, Mg, Ca, Zn, Ni and Mn were accomplished by the use of a double beam flame atomic absorption spectrometer, model CTA 2000, using commercial standard solutions. On the other hand, K and Na were analysed using a flame photometer, model Corning-410.

The first experiment was to determine whether the plant juice works the way alum works, that is, whether the coagulation which is noticed happens due to the introduction of Al or Fe salts from the juice. It was however necessary to determine the levels of other metals normally found in water to find out whether any of them were present in unusually high concentration. Ten millilitres of the juice were digested with 1 mL of concentrated HNO_3 acid, topped up to 100 mL with distilled water. The concentration of Al and Fe were determined in the solution.

The second experiment was to determine the same two principal coagulant-metals in water that had been treated with the plant extract. Fifteen millilitres of juice was added to 20 litres of turbid water (0.75 mL per litre of water). The water was collected from Njoro River, at Egerton University after a heavy downpour the previous night. After the particulates have settled, 750 mL of the water containing the sediment was carefully sampled. Portions of 100 mL were taken, mixed with 5 mL of 1:1 HNO_3 and 1:4 HCl in a 1:1 mixture according to the standard, modified procedure [13]. The mixture was digested on a hot plate in open 125 mL flasks in triplicate. They were brought to boiling point and evaporated to about 10-20 mL. Finally the solution was cooled and filtered. The filtrate was transferred to 100 mL volumetric flask and diluted to the mark. As part of quality control, a blank consisting of distilled water was treated the same way as the sample. Because of the large absorption signal obtained, one millilitre of this digested solution was diluted 10 fold with distilled water before analysis.

Biochemical studies of Maerua subcordata

Determination of protein in the juice and in the flocs. The Biuret reagent was used. Proteins have a primary structure composed of amino acids linked by peptide bonds in a linear manner. This peptide backbone can interact with Cu(II) ions in alkaline medium to form a purple complex. Copper in the process is reduced to Cu(I). This complex is formed with any molecule containing two or more peptide bonds. Relatively high protein concentrations (1-100 mg/mL) are readily determined by the Biuret method [14]. In this study, a calibration curve was constructed using solutions made from a protein standard, BSA (Bovine Serum Albumin). The absorbance was measured at 540 nm using a UV-Vis Spectrophotometer, Model: Pharmacia Biotech Novaspec II and concentrations determined from the calibration curve. Similarly, protein concentration was obtained for real samples, that is, juice extracted from the plant tubers. Due to high concentration of protein, the juice was diluted 125 fold. About 4 mL of the juice was added to 5 litres of water (0.75 mL per litre), vigorously shaken to mix and then left to stand for 3 hours. After that, the settled sediments were obtained from the water by carefully decanting followed by filtration. Protein analysis was carried out as in the above samples.

In addition, an experiment with pure proteins' action on coagulation was carried out to determine whether it is the protein that is responsible for coagulation. Two pure proteins: *bovine albumin* and *casein* were dissolved in water and their solutions added to separate samples of turbid water.

Extraction of polysaccharides, characterization and coagulation tests. The polysaccharides from *M. subcordata* were extracted with 10 % trichloroacetic acid followed by 95 % ethanol according to standard procedure [14]. The polysaccharide isolated (white powder) was weighed and the yield recorded. The sugar content of the polysaccharide was determined by the Folic and Wu assay method [14]. Iodine test was performed on the polysaccharide where 3 drops of iodine solution was added to 2mL of the solution. The isolated polysaccharide was applied to turbid water.

RESULTS AND DISCUSSION

Metal ions in M. subcordata and in turbid water

Results for metal analysis are given in Table 1 which indicates that there was practically no aluminium in the plant extract, and therefore the coagulation noticed when the plant juice is mixed with turbid water is not caused by aluminium salts as it happens with alum. It is also apparent that the coagulation is not caused by iron salts either because of the relatively low level of iron in the plant juice.

Table 1. Concentration of Al and Fe in *M. subcordata* juice and in turbid river water.

Sample	Al, mg/L	Fe, mg/L
Plant extract	< 0.01	46.0 ± 02
Digested water sample	214 ± 08	3,240 ± 17

The detection limit of the AAS used was 0.01mg/L. Standard deviations were calculated from 3 replicate digestions.

The acid digested water with flocs and treated with the plant juice had moderate concentration of Al and quite a high level of Fe. It appears that the metals must have originated from the minerals that are present in sediments. The high levels of metals were expected from acid digested turbid water.

The other metals were analysed in the juice to find out if any metal is present in unusually high concentration. Apart from Na (6,750 mg/L), K (3,450 mg/L) and Mg (300 mg/L), all other metals analyzed including Ni, Zn, Mn were found in trace levels. High concentrations of K and Na are expected to be found in plant material. These metals are however not known to cause coagulation of water sediments.

Protein in M. subcordata and in flocs

After taking care of the dilution factor, the concentration of protein was found to be an average of 289 mg/mL of *M. subcordata* juice (Table 2). On the other hand, results from coagulation tests indicated that both *bovine albumin* and *casein* (a conjugated phospho-protein) caused just partial clearance of water (Table 3). This may indicate that protein is not the main cause of water clarification, which happens almost immediately after adding the juice and shaking.

Table 2. Mean protein and polysaccharide composition of *M. subcordata* juice

Protein mg/L	Polysaccharide, mg/L	Hydrolysable polysac, mg/mg of polysaccharide
289 ± 12	300 ± 10	0.75 ± 0.14

Standard deviations were calculated from 3 replicate measurements using three solutions.

Protein analysis in the flocs after adding the juice was found to be only 4 mg/mL, despite the fact that the juice was found to be rich in protein. From these results, it appears that proteins do not take part substantially in causing coagulation although their structure (the presence of O and N) would suggest that they could bind with charged clay particles. This is confirmed by the result that there was just partial clearance when pure proteins were applied.

Polysaccharides in M. subcordata

The plant juice analysis indicated a very high concentration of polysaccharide, 300 mg/mL of the plant juice extract (Table 2). The amount of hydrolysed polysaccharide was found to be equivalent to 0.75 mg glucose/mg of isolated polysaccharide. This test shows that the polysaccharide polymer is made up of mostly glucose monomers, 75 % of it.

The iodine test showed a red-brown colouration when the polysaccharide was subjected to it. This indicates that the isolated polysaccharide must be a branched macromolecule (*amylopectin*), specifically a branched type of starch. It should be noted that, the absence of dark blue coloration in this test, shows that the starch was not *amylose* which is essentially an un-branched type [14].

When the isolated polysaccharide was applied to turbid water, the water was cleared almost immediately (Table 3). The polysaccharide *amylopectin* is therefore the chief agent in clearing turbid water. According to Jahn [2] special polysaccharides in the roots of *M. subcordata* are responsible for the flocculation of colloidal particles. These macromolecules form bridges between particles, increasing their mass as a consequence, thus causing precipitation. Flocculation test was also performed for pure starch from the laboratory in turbid water, causing flocculation almost immediately. However, the water looked a bit milky due to insoluble starch particulates. Starch is composed of amylopectin (70-80 %) and amylose (20-30 %) and each has their own physical and chemical properties. Amylopectin consists of large highly-branched molecules. It is soluble in water and used in food industry due to its high bonding capacity. Amylose on the other hand consists of long, chain-like molecules and generally insoluble in water [15]. Starches that are high in amylopectin are digested and absorbed more quickly than starches with high amylose content [16]. This implies that water treated with *M. Subcordata* is safe to drink. Indeed starch is the most abundant component of plant foods for humans and domesticated animals [17].

Table 3. Test results for proteins and polysaccharides in clearing water

Reagent added to turbid water	Pure proteins	Pure starch	Isolated polysaccharide	*M. subcordata* juice
Results	Partial clearance	Immediate flocculation, but with starch suspension	Immediate clearance	Immediate clearance

In conclusion, *M. subcordata* contains relatively large amounts of proteins and polysaccharides, mostly *amylopectin*. Although some proteins are known to bind and hold metal ions in solution, there is no evidence to support that in this case they are responsible for the fast coagulation and flocculation of clay particulates found in turbid, muddy water. On the other hand, in this work, the branched molecules starch has been demonstrated to cause immediate water clarification.

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