Efficacy of *Embelia tsjeriam-cottam* (Roem. & Schult.) A. DC. in Calcium Chelation

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**ABSTRACT**

*Embelia tsjeriam-cottam* A. DC., commonly known as Vaividang, belongs to the family Myrsinaceae. Embelin, obtained from *E. tsjeriam-cottam* (an alternative source to *Embelia ribes*), is having diverse biological activities and used as anti-inflammatory, anti-diabetic, antimicrobial, anticancer and antioxidant. The present study aims to investigate the efficacy of *Embelia tsjeriam-cottam* fruit extract to chelate calcium under in vitro conditions. The findings of the study point to the possibility of using *Embelia tsjeriam-cottam* fruit extract as herbal calcium chelator, a substitute to EDTA. *Embelia tsjeriam-cottam* fresh fruits were shade dried and powdered. Fruit extract was prepared in 90 per cent ethanol by cold extraction method. Approximately 0.5 M CaCl2.2H2O solution was combined with 50 ml of an appropriately diluted fruit extract. The mixture was kept undisturbed for 24 hours under ambient temperature. After the incubation period, the mixture was centrifuged at ambient temperature at 4500 rpm for 15 min. The centrifugate was used for estimating leftover calcium ions. Calcium ions present in centrifugates was determined using complexometric titration with standardised EDTA solution as the titrant. Excess EDTA is challenging for the kidneys to eliminate and can even lead to kidney failure in extreme cases. It is evident that *Embelia tsjeriam-cottam* has strong Ca2+ chelating properties, can be used as an alternative to EDTA in chelation therapy treatments.

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pneumonia—the infusion of stem bark and leaves as a gargle for tonsillitis. The pulp of the bark is given to treat burns (Quattrocchi, 2012).

Figure 1: Embelia tsjeriam-cottam: twig with fruits

Figure 2: Ca^{2+} chelated by E.tsjeriam-cottam

Figure 3: Structure of Embelin

Calcium is the most abundant metal in the human body and is essential for the normal functioning of muscles, bones, blood vessels and nerves. Calcium is vital for muscle contraction, oocyte activation, the development of strong bones and teeth, blood clotting, nerve impulse, transmission, heart-beat control and fluid balance within cells. During the growth period, such as infancy, throughout pregnancy, when breastfeeding, the calcium requirements are highest. Long-term calcium deficiency can result in osteoporosis, in which the bone deteriorates and fractures increase. Consumption of a well-balanced diet can provide all the necessary nutrients and help avoid calcium deficiency (Pravina et al., 2013). Extensive calcification of the vascular system is a crucial feature of ageing. Although arterial calcification may be seen as a consistent response to vascular injury, it is a heterogeneous disorder with overlapping and distinct mechanisms for the initiation, progression, and clinical consequences. It is used to reduce the impact of vascular calcification as a risk factor. Novel drug targets may be suggested by recognising the pathways involved in these processes (Giallauria et al., 2013). Small amounts of calcium are present inside and outside of each cell in the body in a liquid state. As these cells are often sensitive to too much or too little calcium, the agency has intrinsic mechanisms for controlling the calcium levels.

Occasionally, calcium can build up in the arterial plaque on the wall of the vessel after an injury. The plaque usually is soft, to begin with, but eventually tends to harden and calcify. Hence, blood vessel cells themselves often turn into bone-forming osteoblasts, providing extra calcium at the spot. Around 50% of women over age 50 and 10% of younger women find calcifications in breast tissue (School, 2010).

Many diseases associated with calcification include dental pulp stones, biliary stones, salivary gland...
Table 1: Strength and amount of calcium chelated by *Embelia tsjeriam-cottam*

| Sl No. | Concentration of *E. tsjeriam-cottam* fruit Extracts (%) | Strength of Calcium Chelated (M) |
|--------|--------------------------------------------------------|---------------------------------|
| 1.     | Control                                                | 0.0000                          |
| 2.     | T1-10%                                                 | 0.0518                          |
| 3.     | T2-20%                                                 | 0.0997                          |
| 4.     | T3-30%                                                 | 0.1476                          |
| 5.     | T4-40%                                                 | 0.1799                          |
| 6.     | T5-50%                                                 | 0.2122                          |

stones and testicular microliths (Callaghan, 2012). Almost 80% of the kidney stones are calcium oxalate with variable calcium phosphate concentrations (Worcester and Coe, 2010).

Chelation is a process in which a large molecule is used to bind metals or minerals. Chelating agents may be used to treat conditions caused by the presence of excess metallic elements in a therapeutic manner. An ideal chelating agent is specific to the metal to be detoxified (Huheey et al., 2012). Citrate, for example, chelates calcium in the body, decreases its over-saturation, thereby inhibiting urinary crystal growth (Tiselius et al., 1993). *Embeilia ribes* shows a significant calcium chelation activity (Sebastian et al., 2019).

Since the *Embelia tsjeriam-cottam* is related species to *Embelia ribes*, can be used as an alternative. In this context, the present study aims to investigate the efficacy of *Embelia tsjeriam-cottam* fruit extract to chelate calcium under *in vitro* conditions.

**MATERIALS AND METHODS**

**Chemicals**

CaCl$_2$.2H$_2$O, ZnSO$_4$.7H$_2$O, NH$_3$, NH$_4$Cl, Ethylenediaminetetraacetic acid (EDTA) and Eriochrome Black T (EBT) were of analytical grade and were supplied by Merck. The solutions used have been formulated in the Milli-Q water.

**Preparation of Fruit Extract**

Fruits of *Embelia tsjeriam-cottam* were selected for this study. They were collected from Wayanad District, Kerala (Figure 1). They were identified and authenticated. *Embelia tsjeriam-cottam* fresh fruits were shade dried and powdered. Fruit extract was prepared in 90 per cent ethanol by cold extraction method.

The extract thus obtained was taken as 100% and diluted using water to get dilutions of 10, 20, 30, 40 and 50%. Approximately 0.5 M CaCl$_2$.2H$_2$O solution was combined with 50 ml of an appropriately diluted fruit extract. The mixture was kept undisturbed for 24 hours under ambient temperature. A similarly prepared solution of 50 ml water and 50 ml 0.5 M CaCl$_2$.2H$_2$O was used as the control. After the incubation period, the mixture was centrifuged at ambient temperature at 4500 rpm for 15 min. The centrifugate was used for estimating leftover calcium ions.

Standard 0.25 M ZnSO$_4$ was prepared and was used to standardise EDTA, and this standardised EDTA was used to estimate the strength of CaCl$_2$ solution.

**Estimation of Calcium**

Calcium ions present in centrifugates was estimated using complexometric titration with standardised EDTA solution as the titrant. In the burette, the standardised EDTA solution was taken. To a clean conical flask, 20 ml of centrifugate was pipetted, and 2 ml of ammonia buffer (pH 10) and a pinch of EBT indicator was added. It was then titrated against EDTA taken in the burette. At the endpoint, a change in colour from vine red to blue was observed. The volume of EDTA consumed was noted. For concordant values, the titration was repeated. The strength of the Ca$^{2+}$ left in the centrifuge was calculated using the following equation

\[ M_1V_1 = M_2V_2 \]

The difference between the strength of CaCl$_2$ stock solution and the estimated power of centrifugate gave the concentration of calcium chelated by the fruit extract.

**RESULTS AND DISCUSSION**

**Standardisation of solutions**

The strength of the prepared EDTA solution was estimated as 0.5125 M using standard ZnSO$_4$ solution. Calcium chloride stock solution’s strength was found to be 0.4971 M.

**Estimation of Ca$^{2+}$ after chelation**

The amount of Ca$^{2+}$ present in the samples was estimated using standardised EDTA, and results are
given in the Table 1.

The bar diagram (Figure 2) shows the efficacy of the plant extract in different concentrations to chelate Ca\(^{2+}\). It is evident from the diagram that the amount of calcium chelated was directly proportional to the strength of the fruit extract. Embelin is one of the biologically active benzoquinone derivatives that act as the active principle compound in the fruits of *Embelia tsjeriam-cottam* and responsible for its medicinal properties (Stasiuk and Kozubek, 2011). Embelin is a member of the class of dihydroxy-1, 4-benzoquinones that is 2, 5-dihydroxy-1, 4-benzoquinone, which is replaced at position three by an undecyl group (Figure 3).

Embelin in the fruit extracts might have interacted with calcium ions in the samples, chelated it and precipitated. From embelin's structure, it is clear that the compound can form hydrogen bonds at 1,2,4 and 5 positions with Ca\(^{2+}\) ions due to the partial negative charges on the oxygen present in those positions.

The leftover Ca\(^{2+}\) forms a complex with EDTA during titration (Figure 4) (UOC, nd).

EDTA is excessively used as a chelating agent in chelation therapy. EDTA chelation therapy can reverse and eliminate damage from heavy metal intoxication (Bamonti et al., 2011). But EDTA can cause a hypocalcemic effect (Gerhardsson and Aaseth, 2016). Excess EDTA is challenging for the kidneys to eliminate and can even lead to kidney failure in extreme cases. Herbal extracts having metal chelating properties can be a viable option to replace EDTA in this context. As it is evident from this analysis that *Embelia tsjeriam-cottam* has strong Ca\(^{2+}\) chelating properties, it can be used as an alternative to EDTA in chelation therapy treatments.

**CONCLUSIONS**

EDTA, a widely used chelating agent, can cause fatal damage to kidneys in extreme cases. The outcomes of this work indicate that the *Embelia tsjeriam-cottam* fruit extract can chelate and precipitate calcium. Embelin, an important compound present in the fruit can form multiple hydrogen bonds with Ca\(^{2+}\) ions. The results of the present study point out the possibility of using *Embelia tsjeriam-cottam* fruit extract as herbal calcium chelator, a substitute to EDTA.

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**Conflict of Interest**
None.

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