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Titrimetric estimation of fulvic acid substances in Oriens Shilajit as a part of herbal nutraceutical standarization

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Abstract
Fulvic acids are family of organic acids and form a major part of the soil organic matter contents. In the present study, we aimed to estimate the amount of Fulvic acids in Shilajit, a well-known medicinal application in ayurveda using titration method. We obtained a fulvic acid percentage of 0.96% (w/w). Fulvic acid in Shilajit can be used to treat a variety of ailments as well protect the body against heavy metal accumulation.

Keywords: substituted Li ferrite, magnetostatic and spin waves, microstrip array antenna, X-band frequency range

Introduction
Fulvic acid is a water soluble chemical and can be extracted from humic substances (Aiken *et al*., 1985; Islam *et al*., 2005) [1, 8]. It contains several functional groups, including phenols, quinones and semiquinones which are responsible for its high antioxidant activity (Aiken *et al*., 1985; Glynn, 1995; Plaza *et al*., 2005) [1, 5, 11]. Though not much information is on the safety and toxicology of fulvic acid is available, few toxicology and mutagenicity studies have justified its safety in the food industry (Humet Product Documentation and Technical Information, 1999) [7].
High concentrations of humifulvate (upto 10 gm/kg of body weight) was not found to cause any deaths or damage to organs of Wistar rats (Antal, 1990) [2]. In ayurveda a Fulvic acid rich component namely Shilaji has been used for medical application toward the control of cognitive disorders associated with aging, and cognitive stimulation. Fulvic acid is the key active ingredient of Shilaji, which has been found to be an alternative for treating Alzheimer's. Thus, the importance of fulvic acid in human health is well demonstrated (Carlos *et al*., 2012) [3]. Shilajit has been found to contain more than 85 minerals in ionic form and humic substances (mainly fulvic and humic acid) (Mirza *et al*., 2010) [10]. The primary organic substance in aqueous extracts of Shilajit humus is fulvic acid (FA), and it has been suggested that FA may account for sevaral biological and medicinal properties of Shilajit (Ghosal *et al*., 1988; Schepetkin *et al*., 2002) [4, 13]. Oriens® Shilajit is a combination of shilajit and piperine. In the present study we aimed to estimate the the fulvic acid content in Oriens® Shilajit using titration method.

Materials and Methods
Titration method to estimate fulvic acid content
Chemicals and apparatus required
Distilled water, Potassium permanganate solution, 0.1M Hydrochloric acid, Indigosulphonic acid. Glass funnel, 1000 ml Erlenmeyer flasks, measuring cylinders (100ml, 500ml), Graduated burette, Pipette

Samples
Capsules of Shilajit (VNL-016-055) were obtained from Oriens Global Marketing (P) Ltd and its contents were carefully emptied to be used as samples for titration.

Procedure
To 0.1 gm sample add 50 ml of water, and 1ml 0.1M Hydrochloric acid. Sonicate the mixture for 15 minutes, and filter through filter paper. Completely wash the filter paper using 25 ml distilled water and collect all filtrate. Make to solution up to 750 ml using distilled water. Twenty file milliliters of Indigosulphonic acid is added to the solution as an indicator.
Titrate with constant stirring against N/10 potassium permanganate solution until a colour change of blue to golden-yellow occurs. One ml of N/10 pottasium permanganate is equivalent to 0.001701 gm of Fulvic compounds, calculated as a Galic acid. Run a blank test by titrating 25 ml of Indigosulphonic acid in 750 ml of water.

Calculation
Percentage of Fulvic acid (w/w) is calculated as:

$$\text{Assay} = \frac{(A - B)\ X\ 0.001701\ X\ 100\ x\ N}{W\ X\ 0.1}$$

Where,
A = Volume of 0.1N KMnO4 consumed in titration (Test)
B = Volume of 0.1N KMnO4 consumed in titration (Blank)
W = Weight of material taken in gm
N = Normality of Potassium permanganate

Amount of fulvic acid in per capsule of Oriens® Shilajit
The raw material procured to make Oriens® Shilajit was certified to contain 2.5% of active fulvic acid. Each capsule of Oriens® Shilajit contained 247 mg of Shilajit and 3 mg of Piperine. According to our titration data active fulvic acid per capsule can be calculated using the formula

$$\text{Active fulvic acid capsule}^{-1} = \frac{\text{Amount of fulvic acid (mg) X Percentage of Fulvic acid}}{100}$$

Results and Discussion

Humic substances belong to a faction of naturally occurring high molecular weight organic substances (Aiken *et al*., 1985) [1]. Humic and fulvic acid intermediates are produced by the microbial or chemical degradation of lignin and other carbohydrates from plant sources (Wersahw, 1989; Gramss *et al*., 1999) [12, 6]. We aimed to study the Fulvic acid content in Shilajit capsules from Oriens Global Marketing (P) Ltd using the titration method. The results obtained are tabulated in Table 1 below.

Table 1: Fulvic acid content in Shilajit

Sample	Chemical analyzed	Results	Method used
Shilajit (VNL-18-014)	Fulvic acid	0.96%	Volumetric titration

In addition to its wide applications in ayurveda, fulvic acid has been reported reduce the uptake of cadmium in intestinal segments of rats (Glynn, 1995) [5]. Sanmanee and Areekijseree (2010) [12] also showed its nature to reduce toxic metal accumulation in tissues. Fulvic acid feeding in broiler chicken during the grower period has been reported to be the most beneficial effect in terms of growth and feed conversion (Kocabagh *et al*., 2002) [9]. Calculation of the amount of active fulvic acid in per capsule of Oriens® Shilajit showed that each capsule contained 2.37% of active fulvic acid. This is in compliance with the supplier information for fulvic acid used to make Oriens® Shilajit capsules.

Conclusions

The present study shows that there is presence Fulvic acid in the ayurvedic preparation Shilajit. Fulvic acid can be understood to have several properties including improving the immune status, absorption of rare earth minerals, improve muscular growth and protect from harmful metals such as chromium and lead. Therefore, presence of high amounts of fulvic acid in Shilajit can aid nutrition and sustain health in humans.

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