Effect of Solvent Evaporation Methods on Biological Activity of Crude Extract of *Feronia elephantum* ***Correa*** Leaves

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In the present study, the effect on bioactivity of *Feronia elephantum* plant leaves was studies in three different methods of concentration, in order to decide the preferred method. The bioactivity of plant extract was calculated by brine shrimp lethality test (BSLT). Methanolic extract of *Feronia elephantum Correa* plant leaves was prepared by soxhlet extraction method and extract was concentrated/evaporated by three methods viz. vacuum evaporation (Rotatory vacuum evaporator) (A), room temperature evaporation (B) and hot air evaporation (C). The extract was tested for chemical constituents such as alkaloids, glycosides, carbohydrates, protein, fixed oils or fats, tannins, flavonoids, saponins and triterpenoids with standard tests. Physical properties like color, consistency, odor and nature were not varied. Percentage yields were 13.64, 16.8 and 8% in three different evaporation methods respectively. All the extracts were found positive only for glycosides and tannins in three different methods. The larvae of brine shrimp were hatched at 25ºC for 48 h in artificial sea water. The methanolic extract at the concentration of 250, 500 and 750 µg/ml was tested in comparison to negative and positive control of quantified numbers of larvae. Mortality was noted after 24 h post treatment. The LC_{50} was least (220.99 µg/ml) in method. A followed by method B (247.57 µg/ml) and highest in method C (430.09 µg/ml). Results indicated that vacuum evaporation (22 cycles at 25°C) is most superior to maintain bioactivity.

Keywords: *Feronia elephantum*, brine shrimp lethality test, plant biological activity

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1. Introduction:

Feronia elephantum is a tree belongs to *Rutaceae* family known by various names such as Kavath or Kaith, elephant apple, monkey fruit, curd fruit and kaith bel. The wood-apple is native and common in the wild in dry plains of India and cultured along roads and occasionally in orchards. It has been claimed to be useful in the treatment of jaundice, hepatotoxicity and illness by traditional practitioners (Kurian 1996), (Kamat *et al*. 2003) and (Jadhav 1998). However, the essential oil of *Feronia elephantum* having antimicrobial activity (Garg 2001).

The growing interest in therapeutically active metabolites of plants has a drawn direct attention to methods for their extraction and preparation. Natural products are extracted by conventional methods such as soxhlet and room temperature solvent extraction (Yu *et al.* 2002), (Zygmunt and Namiesnik 2003), (Gargner *et al.* 2003) and (Melecchi *et al*. 2002) and their concentration by different methods such as room temperature drying, hot air evaporation and vacuum evaporation for removal of solvent. In the process of evaporation, some of active volatile constituents may also evaporate with the solvent, which decreases the activity of particular extract. Therefore, it is desirable to test commonly used evaporation methods in respect to variation in biological property, in order to decide better and most suitable evaporation method. The brine shrimp lethality assay is considered a useful tool for preliminary assessment of biological activity and has also been suggested for screening pharmacological activities in plant extract (Carballo *et al*. 2002). The present study was made to assess the variation in bioactivity of *Feronia elephantum* leaves extract concentrated by different methods by using brine shrimp lethality test.

2. Materials and methods:

Leaves of *Feronia elephantum* were collected from Mathura district in the month of November. The plant's leaves were authenticated with taxonomy in literature. It is also done by Dr. P.K. Mathur, Head Department of Botany, B.S.A. College, Mathura (U.P.) and specimen is also deposited in the department also.

2.1. Preparation of plant extract:

The collected plant material was shaded dried and grind to coarse powder. The coarse powder (100 g) of the shade-dried leaves of the *Feronia elephantum* was exhaustively

extracted using methanol in soxhlet extractor for a period of 22 h, as per standard methods (Kokate *et al*. 2002). Prepared liquid extract was divided into three parts. The extract was concentrated by three different methods. At the first method (A), it was concentrated by vacuum rotatory evaporator (Heidolph, Japan), in which the temperature of the water bath and Rota cool was 35°C, 4°C respectively with 147 bar vacuum pressure. In second method (B) it was concentrated at room temperature (25°C), while in third method (C); it was concentrated by hot air generated by electric hair dryer.

2.2. Qualitative analysis of active chemical constituents:

Qualitative analysis of active constituents was done by standard methods (Kokate *et al*. 2002) to find out the constituents like alkaloids, glycosides, carbohydrates, protein, fixed oils or fats, tannins, flavonoids, saponins and triterpenoids. Percentage yield was also noted in three different methods.

Hatching and brine shrimp bioassay: The eggs of brine shrimp (*Artemia salina*) were hatched in a recommended assembly as per earlier (Michael *et al*. 1956) filled with artificial seawater, which was prepared with a commercial salt mixture in double distilled water. After 48 h, the phototrophic nauplii were collected by pipette from the lighted side. The test was conducted in multiwell plate in filtered (0.45 µm pore diameter) and sterilized seawater (final volume 5 ml). The extract was tested at 750, 500 and 250 µg/ml concentration of extract, by keeping potassium dichromate as positive control and sea water with tween-20 as negative control. Three replicate were used for each treatment and control. The entire test was performed in a temperature-controlled room at 28°C, under a continuous light regime (Meyer *et al*. 1982). The bioactivity was determined after 24 h on calculating percentage lethality and LC_{50} values by standard method (Finney 1971).

3. Results and discussion

The plant extracts were tested for chemical constituents such as alkaloids, glycosides, carbohydrates, protein, fixed oils or fats, tannins, flavonoids, saponins and triterpenoids, showed a positive reaction with glycosides and tannins only, which were similar in three different methods (Table-II). Reports are similar as earlier reports (Krishnaraju *et al*. 2005) where the chemical analysis was found unaltered in similar experiments. Physical properties like color, consistency, odor and nature, which were varied among three different methods. It was greenish black, pungent and sticky in extract concentrated by vacuum evaporation (A),

greenish black, agreeable and sticky at room temperature evaporation method (B) while, greenish black, not agreeable and sticky in extract concentrated by hot air evaporation. Percentage yield was 13.64%, 16.8% and 8% in vacuum concentrated, room temperature and hot air evaporation methods respectively (Table-I). The calculated LC_{50} was (220.99 µg/ml) in extract concentrated by vacuum evaporator method followed by 247.57 µg/ml room temperature evaporation and highest 430.09 µg/ml in hot air evaporation, which suggested that vacuum evaporation is best suitable method to maintain bioactivity in plants evaporation (Table-III). The hot air evaporation is unsuitable, because bioactivity was almost reduced to half. The bioactivity was marginally reduced in room temperature evaporation but this took more time and extracts becomes vulnerable to contamination particularly fungus. Earlier experiment has been done with essential oils but is present investigation considering, the potential use and preservation of bioactivity, vacuum evaporation is most suitable. Moreover, the LC_{50} values of the plant extract *Feronia elephantum* were shown significantly lethality to brine shrimp is an indicative of the presence of potent bioactive components, which can be utilized for further research on drug development.

In the course of our studies, the brine shrimp lethality assay has also proven a convenient method for monitoring biological activities of the plant , which has also been reported by others (Atalay 2005), (Rahuman *et al.* 2015), (Llaiyaraja *et al*. 2015) and (Rakhunde *et al.* 2014).

4. Conclusions:

The present study revealed that out of three evaporation methods viz. vacuum evaporation (Rotatory vacuum evaporator) (A), room temperature evaporation (B) and hot air evaporation (C), vacuum evaporation method is superior because it preserved the active constituents for bioactivity. Hence vacuum evaporation method should be recommended for herbal extract preparation.

Table 1. Physical characteristic of *Feronia elephantum* leaves extracts in three different evaporation methods

Properties	Concentrated by vacuum evaporation (A)	Concentrated by room temperature (B)	Concentrated by hot air evaporation (C)
Color	Greenish black	Greenish black	Greenish black
Odor	Pungent	Agreeable	Not agreeable
Nature	Sticky	Sticky	Sticky
Consistency	Semi solid	Semi solid	Semi solid

Table 2. Qualitative chemical analysis of *Feronia elephantum* leaves extract in three different evaporation methods

Constituents	Concentrated by vacuum evaporation (A)	Concentrated by room temperature evaporation (B)	Concentrated by hot air evaporation (C)
Alkaloids	-	-	-
Glycosides	+	+	+
Carbohydrates	-	-	-
Protein	-	-	-
Fixed oils and fats	-	-	-
Tannins	+	+	+
Flavonoids	-	-	-
Saponins	-	-	-
Triterpenoids	-	-	-
Percentage Yield	13.64%	16.8%	8%

- Negative, + Positive

Table 3. Bioactivity in three different solvent evaporation methods

Extract	Extract concentrated	Experiment types	LC_{50} (µg/ml)	Confidential upper limit	Lower confidential limit
Feronia elephantum	Concentrated by vacuum evaporation (A)	Test	220.99	395.73	11.23
Feronia elephantum	Concentrated by room temperature evaporation (B)	Test	247.57	406.83	24.74
Feronia elephantum	Concentrated by hot air evaporation (C)	Test	430.09	172.45	56.85
Potassium dichromate	-	Positive control	0.00	-	-
Sea water + tween- 20	-	Negative control	0.00	-	-

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