



Formulation, Evaluation and Phytochemical screening of Shoot on *Eichhornia crassipes* (Mart.) Solms Ayurvedic Medicated oil

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Abstract

Background

Eichhornia Crassipes (Mart.) Solms is a free-floating perennial aquatic plant known as “aahayathamarai”. This is used as anti-inflammatory, anti-oxidant, analgesic, skin whitening, sedative etc. Ayurvedic medicated oil (Taila) was prepared by shoots of *Eichhornia crassipes* (Mart.) Solms and studied organoleptic property, phytochemical analysis and physiochemical parameters such as acid value, saponification value, refractive index and specific gravity as per standard methods.

Results

Preliminary phytochemical analysis of fresh juice and ayurvedic medicated oil of shoots on *Eichhornia crassipes* (Mart.) solms revealed the presence of various components like carbohydrate, glycoside, flavonoid, alkaloid, saponins, tannin and steroids. All the physio-chemical parameters like acid value (1.683 mg KOH/g), saponification value (182.25 mg KOH/g), iodine value (30.37 I₂/100g.), refractiveindex (0.148), specific gravity (0.91 kg/m³) analysed were within the normal range.

Conclusion

Ayurvedic medicated oil of shoots on *Eichhornia Crassipes* (Mart) Solms have alkaloid, flavonoid and saponins possess the various pharmacological activities. Physiochemical parameters such as acid value, saponification value, refractive index, specific gravity were within normal range. All the results showed the quality of the preparation is standard

Keywords: *Eichhornia Crassipes* (Mart.) Solms, Ayurvedic medicated oil, acid value, specific gravity, saponification value.

Introduction

Eichhornia Crassipes (Mart.) Solms is a free-floating perennial aquatic plant, family is pontederiaceae. *Eichhornia crassipes* is in the centre of each mature rosette of leaves rises broad stem, 6-10 glabrous leaves are arranged in basal rosettes, shiny and dark green. They have long, spongy and bulbous stalks, erect with distinctive swollen bladder like petioles and have purple black feathery, freely hanging roots. 6 and 4 lily-like flowers in violet colour consists of 6 petals. Simple capsule fruit containing in the region of 400 to 500 seeds. The nutritionally compounds like phenolic, flavonoids, alkaloid, sterols, terpenoids, glycosides and many other metabolites isolated from different parts of *Eichhornia crassipes* (Mart.) Solms. The plant is used as sedative, CNS depressant, analgesic, anti-epileptic, anti-psychosis, anti-anxiety, wound healer, anticancer, larvicidal, anti-oxidant property.

Ayurveda is the science of life treating mankind ancient time and its aims to treat the disease from its base. Acharya Charaka had described first primary dosage forms i.e. Swarasa (juice), kalka (paste), kwatha (decoction), hima (cold infusion) and planta (hot infusion) and mentioned them as panchavidhakasaya kalpana first time. Sneha kalpana may be defined as a pharmaceutical process to prepare oleaginous medicaments from the substances like kalka and drava dravyas in specific proportion by subjecting to unique heating pattern and duration to fulfill the certain pharmaceutical parameters according to the need of pharmacotherapeutics. Sneha kalpana are superior to other dosage forms due to its advantages increased absorption and bioavailability

However till the date there have been no investigations supporting the preparation of ayurvedic medicated oil of this plant. Hence, aim of study evaluates the phytochemical screening and pharmaceutical standardization on shoots of *Eichhornia crassipes* (Mart.) Solms ayurvedic medicated oil. Plant based ayurvedic medicated oil of has been developed in recent importance in the global market.

Materials and Methods

Plant Collection and Identification

The Shoot on *Eichhornia crassipes* (Mart.) Solms was collected from their natural habitats in Bhavani River, Keelvani, Erode District and Tamilnadu in the month of January 2022. It was authenticated by Professor P. Jayaraman, Ph.D, Director, Institute of Herbal Botany Plant Anatomy Research Centre, West Tambaram, Chennai-45.

Preparation of Shoot on *Eichhornia crassipes* (Mart.) Solms Ayurvedic Medicated oil (Taila) ^(1, 2)

a).Preparation of *Eichhornia crassipes* (Mart.) Solms Kalka

Fresh shoot of *Eichhornia crassipes* (Mart.) Solms were taken and washed with water. Then shoots were subjected into electrical mixer grinder with adding of sufficient quantity of water and grinded till a paste (Kalka).

b).Preparation of *Eichhornia crassipes* (Mart.) Solms Taila

500 litre of sneha dravya (*Tila Taila*) was taken in a stainless steel vessel, heated over mild flame (80°C for 5min) till complete evaporation of moisture and then added kalka dravya (*Eichhornia crassipes Kalka*). After mixing of Kalka, 2 litre of Drava dravya (*Eichhornia crassipes Swarasa*) was added and heating was continued to maintaining the temperature in between 95-100°C. The above mixture

undisturbed stage through the night and heating was given for 3 days (average 6hr 15 min). The content was stirred continuously to avoid the possibility of settling down. On 3rd day till Sneha Sidhhi Lakshana were obtained. After obtaining desired Sneha Sidhhi Lakshana, the vessel was taken out from heat and oil was filtered through two folded cotton cloth in its hot stage. After self cooling of prepared oil stored in a properly labeled air tight bottle (Table.1 and Fig.1).

Table.1: Formulation composition of Shoot on *Eichhnornia crassipes* (Mart.) Solms Ayurvedic Medicated oil (Taila)

S.no	Dravya	Name of the ingredient	Latin name	Family	Part used	Preparation part
1.	Kalka	<i>Eichhnornia crassipes</i> Kalka	<i>Eichhnornia crassipes</i> (Mart.) Solms	Pontederiaceae	Shoots	1
2.	Sneha	Tila Taila	<i>Seasumum indicum</i> L	Pedaliaceae	Seed oil	4
3.	Drava	<i>Eichhnornia crassipes</i> Swarasa	<i>Eichhnornia crassipes</i> (Mart.) Solms	Pontederiaceae	Seed oil	16

Fig.1: Preparation of *Eichhnornia crassipes* Taila

Eichhnornia crassipes Kalka



Eichhnornia crassipes Swarasa



Tila Taila



Preparation for stainless steel at 100°C



Filtration of Taila



Eichhnornia Crassipes Taila



Phytochemical Analysis ⁽³⁻⁶⁾

Extraction of phytochemical constituents from *Eichhnornia crassipes Taila* was done by mixing 5 ml of *Eichhnornia crassipes Taila* with 15 ml of 90% aqueous methanol and subjected to constant stirring by using magnetic stirrer on hot top at 60°C for 1 h and then stored in freezer to allow the solidification. The alcoholic portion was separated, filtered through Whatman filter paper no. 41 due to filtrate was used for qualitative phytochemical testing. The extraction was repeated for three times and then gets effective extraction of phytochemicals from the medicated oil.

Qualitative Analysis

1. Test for alkaloids

A small portion of the extract was stirred with few drops of dilute hydrochloric acid and then filtered due to the filtrate was tested carefully with various alkaloidal reagents such as **Mayer's reagent** (cream precipitate), **Dragendroff's reagent** (orange brown precipitate), **Hager's reagent**(yellow precipitate) and **Wagner's reagent** (reddish brown precipitate) indicated the presence of alkaloids.

2. Test for flavonoids

NaOH test: The extract was treated with aqueous NaOH and HCL due to observed the formation of yellow orange colour indicated presence of flavonoids.

H₂SO₄ test: A fraction of extract was treated with concentrated H₂SO₄ and observed for the formation of orange colour indicated presence of flavonoids.

Lead acetate test: A small amount of extract was treated with lead acetate and observed for the formation of white precipitate indicated presence of flavonoids

3. Test for tannins

Few ml of extract was treated with 10% alcoholic ferric chloride solution and then observed formation of blue or greenish colour solution indicated presence of tannins.

4. Test for Phenols

Ferric chloride test: The fraction of extract was treated with 5 % ferric chloride and observed for formation of deep blue or black colour indicated presence of phenols.

5. Test for terpenoids

Liebermann - Burchard test: The extract was treated with chloroform, acetic anhydride and then add drops of H₂SO₄ and observed for the formation of dark green colour indicated presence of terpenoids.

6. Test for Anthraquinones

Borntrager's test: The extract was heated with 10% ferric chloride solution and 1ml concentrated HCl. The extract was cooled and filtered. The filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia. The pink or deep red colourations of aqueous layer indicated the presence of anthraquinone.

7. Test for anthocyanin

NaOH test: The extract was treated with 2M NaOH due to observed for the formation of blue green colour indicated presence of anthocyanin.

8. Test for Proteins

Biuret test: The extract is treated with an equal volume of 1% strong sodium hydroxide and then add few drops of copper (II) sulphate due to formation of purple colour indicated the presence of protein.

Million's test: To the extract treat with million's reagent due to white precipitate is produced, while heating it turns brick red colour indicated the presence of protein.

9. Test for sterols

Liebermann-Burchard test: The extract was treated with chloroform, acetic anhydride and added drops of H₂SO₄ due to observed for the formation of dark pink or red colour indicated presence of sterols.

10. Test for saponins

Foam test: The extract was diluted with 5ml distilled water. The suspension was shaken in graduated cylinder for 15 min. A 2cm layer foam indicated the presence of saponins

11. Test for mucilage

The extract is treated with aqueous potassium hydroxide. Swelling indicated the presence of mucilage.

12. Test for carbohydrates

Molish's test: To the extract few drops of α -naphthol solution in alcohol. The con.H₂SO₄ is added at the side of test tube due to formation of violet ring at the junction of two liquids indicated the presence of carbohydrates

Evaluation of Physicochemical Parameters of *Eichhnornia Crassipes* (Mart.) Solms Ayurvedic Medicated oil (Taila) ⁽⁷⁻¹¹⁾

The physicochemical evaluation of *Eichhnornia Crassipes* Ayurvedic Medicated Oil (Taila) was carried out with respect to acid value, saponification value, refractive index, Specific gravity as per standard methods

a).Refractive index

The refractive index is the quotient of the speed of light as it passes the through two media. It is a dimensionless number that depends on the temperature and wavelength of the beam of light. To determine refractive index by using abbe's refractometer. The refractive index is calculated by following formula,

$$\text{Index of refraction, (n)} = \frac{\text{The speed of light in a vaccum (c)}}{\text{Speed of light in the oil (v)}}$$

The Refractive index measurement can be used for qualitative and quantitative analysis as well as structural study. It is an intrinsic property of a substance. Hence it is used in determining the identity and purity of a chemical. It is also useful in analysis of commercial products and in identifying unknown substance.

b). Saponification value

Saponification value is defined as the number of milligrams of potassium hydroxide required to neutralize the fatty acids resulting from complete hydrolysis of 1 g of the sample of oil or fat. Saponification value occurs in an inverse proportion to the average molecular weights of fatty acid present in the oil.

Weigh accurately about 2g of the substance into a tared 250ml flask of borosilicate glass and fit with a reflux condenser. Add 25ml of 0.5M ethanolic potassium hydroxide and boil under reflux condenser on a water bath for 30 minutes. Cool and add 1ml of solution of phenolphthalein and titrate the excess of alkali with 0.5 M hydrochloric acid. Note the number of ml required (a). Repeat the experiment with the same quantities of the same reagents in the manner omitting substance. Note the number of ml required (b). Calculate the saponification value from the following formula,

$$\text{Saponification Value} = \frac{(b-a) \times 0.02805 \times 1000}{W}$$

Where, a - Volume of HCl required by sample, b - Volume of HCl required by blank, W - Weight in g of the substance taken.

The amount of alkali needed to saponify a given quantity of fat will depend upon the number of - COOH group present. Thus fats containing short chain fatty acids will have more - COOH groups per gram than long chain fatty acids and this will take up more alkali and hence will have higher saponification number.

c). Iodine value

Iodine value is defined as the weight of iodine absorbed by 100 parts by weight of the sample of fat or oil. Iodine value is a measure of the extent of unsaturation. Susceptibility to rancidity increases for the oil or fat having higher iodine values.

Iodine Monochloride method

Place an accurately weighed quantity of the substance in a dry 500 ml iodine flask, add 10 ml of carbon tetrachloride and dissolve. Add 20 ml of iodine monochloride solution insert the stopper and allow to stand in the dark at a temperature between 15°-25°C for 30 minutes. Then add 15 ml of potassium iodide and 100 ml water in to flask and shake it. The flask contents titrate with 0.1 M sodium thiosulphate by using starch solution as indicator, which is added towards the end of the titration. Note number of ml required (a). The procedure is repeated without the sample and the number of ml required is noted as (b). Calculate the iodine value from the following formula,

$$\text{Iodine Value} = \frac{(b-a) \times 0.01269 \times 100}{W}$$

Where, a - Number of ml required, b - Without the sample and the number of ml required, W - Weight in g of the substance taken.

d). Acid value

Acid value is defined as the number of milligrams of potassium hydroxide required to neutralize the free acids present in one gram sample of fat or oil. Generally Acid value indicates acidic nature of the preparation.

Weigh accurately about 10 g of the substance into a 250 ml flask and add 50 ml of a mixture of equal volumes of ethanol (95%) and ether, which has been neutralized after the addition of 1 ml of solution of

phenolphthalein. Heat gently on a water-bath, if necessary until the substance has completely melted, titrate with 0.1 M potassium hydroxide, shaking constantly until a pink colour which persists for 30 seconds is obtained. Note the number of ml required. Calculate the acid value from the following formula,

$$\text{Acid value} = \frac{a \times 0.00561 \times 1000}{W}$$

Where, a - Number of ml of 0.1 M potassium hydroxide required, W - Weight in g of the substance taken.

e). Specific Gravity

The specific gravity of a liquid is the weight of a given volume of the liquid at 25°C (unless otherwise specified). Compared with the weight of an equal volume of water at the same temperature, all weighing being taken in air.

Specific gravity bottle is a flask made to hold a known volume of oil at a specified temperature (25°C). The bottle is weighed, filled with the oil whose specific gravity is to be found and weighed again. The difference in weights is divided by the weight of an equal volume of water to give specific gravity of oil. Calculate the Specific gravity from the following formula,

$$\text{Specific gravity} = \frac{\text{Density of the oil}}{\text{Density of the water}}$$

Results and Discussion

Organoleptic parameters

Shoots on *Eichhornia crassipes* (Mart.) Solms ayurvedic medicated oil was subjected to observed the various organoleptic parameters and results obtained are illustrated in Table.2

Table.2: Organoleptic Parameters of *Eichhornia crassipes* (Mart.) Solms Ayurvedic Medicated oil

Organoleptic characteristics	Colour	Odour	Taste	Touch	Texture
Observation	Brown	Mild smell	Tasteless	Viscous	Liquid

India, as in many less developed areas, phytotherapy is a common method of primary health care, because pharmaceutical products are expensive and the folk pharmacopoeia provides apparently effective remedies for many diseases. Normally oils give different characteristics like colour and odour relative to ingredients which were used to prepare the medicated oil. Ayurvedic medicated oil of shoots on *Eichhornia crassipes* (Mart.) Solms possess the light brown colour the characteristic odour is due to Tila Taila which was used in preparation (Table.2).

Phytochemical analysis

Qualitative Analysis

Shoots on *Eichhornia crassipes* (Mart.) Solms fresh juice and ayurvedic medicated oil was subjected to various chemical test for detection of phytoconstituents and results obtained are illustrated in Table.3.

Table.3: Phytochemical Analysis of Shoots on *Eichhornia Crassipes* (Mart.) Solms Fresh Juice and Ayurvedic Medicated oil

S.no	Phytochemical Test	Fresh Juice	Ayurvedic Medicated Oil
1.	Alkaloids	+	+
2.	Flavonoids	+	+
3.	Tannins	+	+
4.	Phenols	+	+
5.	Terpenoids	+	+
6.	Anthraquinones	+	+
7.	Anthocyanin	-	-
8.	Proteins	+	+
9.	Sterols	+	+
10.	Saponin	+	+
11.	Mucilage	+	+
12.	Carbohydrate	+	+

NOTE: (+) Present (-) Absent

Preliminary phytochemical analysis of fresh juice and ayurvedic medicated oil of shoots on *Eichhornia crassipes* (Mart.) solms. revealed the presence of various components like on carbohydrate, glycoside, flavonoid, alkaloid, tannin and steroids (Table.3). Most of the plant Derived drugs containing alkaloids and have remarkable physiological and pharmacological properties like stimulant, spasmolytic, vasodilator etc. Active phytochemical such as alkaloid, flavonoids, terpenoids and saponins are known to responsible for various pharmacological activities.

Pharmaceutical Standardization of *Eichhornia Crassipes* (Mart.) Solms Ayurvedic Medicated oil

The common parameters mentioned in Ayurvedic Pharmacopeia of India and CCRAS guidelines i.e. Refractive index, Specific gravity, Acid value, Iodine value, Saponification value were taken. The physiochemical parameters of Shoots on *Eichhornia Crassipes* (Mart.) Solms Ayurvedic Medicated oil results obtained are illustrated in Table.4.

Table.4: Evaluation of Physiochemical Parameters of Shoots on *Eichhornia Crassipes* (Mart.) Solms Ayurvedic Medicated oil

S.no	Physiochemical Parameters	Results
1.	Specific gravity	0.91 kg/m ³
2.	Refractive index	0.148
3.	Saponification Value	182.25 mg KOH/g
4.	Acid value	1.683 mg KOH/g
5.	Iodine value	30.37 g I ₂ /100g
6.	Rancidity	Nil

Evaluation of physico-chemical parameters helps to assess the quality and identify the presence of specific ingredients in a formulation and also to assess the purity by comparing with the standard ones. Refractive index indicates the density of sample compared to air and liquid media the value (0.148) of *Eichhornia crassipes* medicated taila was within the limit. Specific gravity indicates the presence of solute

content in the solvent, the value (0.91kg/m^3) for the same was appropriate for this Taila. The amount of alkali needed to saponify a given quantity of oil will depend upon the number of - COOH group present in it. The saponification value also indicates the average molecular weight /chain length of all fattyacids present in a sample. Fatty acids with longer chains have low saponification value and the shorter chain fatty acids have high saponification value. Shorter chain fatty acids (high saponification value) have faster rate of absorption than longer chain fatty acids. Saponification value of *Eichhornia crassipes* medicated Taila was found to be 182.25 mg KOH/g. It is more than normal saponification value of Tila taila 169.5 mg KOH/g. The Acid number is a measure of the amount of carboxylic acid groups in a chemical compound such as fatty acids or in a mixture of compounds. The acid number is used to quantify the amount of acid present. The Acid value indicates the presence of free fatty acid in the Taila which is responsible for rancidity of compounds. Higher the free fatty acid more is the rancidity, this helps to decide the shelf life of the Taila. Acid value for *Eichhornia crassipes* Taila was found to be 1.683mg KOH/g thus indicating the longer shelf life of Taila. Iodine value indicates the degree of unsaturation of Taila, greater the degree of unsaturation will be the possibility of absorption and atmospheric oxidation leading to rancidity. The more iodine number, the more unsaturated fatty acid bonds are present. Unsaturated fatty acid better absorbed than saturated fatty acids. The iodine value of Taila was found to be fair enough which indicates the less rancidity of this formulation. The determination of iodine number is useful to the chemist in determining the quality of oil or its freedom from adulteration. The iodine value of *Eichhornia crassipes* Taila was found to be 30.37g I₂/100g. Iodine value of *Eichhornia crassipes* Taila was found to fair enough which indicates the less rancidity of this formulation (Table. 4).

Conclusion

The preliminary phytochemical analysis of *Eichhornia crassipes* (Mart.) solms ayurvedic medicated oil indicated the presence of alkaloids, flavonoids, proteins, saponins, terpenoids, phytosterols and carbohydrates. The Physio-chemical parameters helps to assess the quality and identify the presence of specific ingredients in a formulation and also to assess the purity by comparing with the standard ones. All the physio-chemical parameters like acid value (1.683 mg KOH/g), saponification value (182.25 mg KOH/g), iodine value (30.37 g I₂/100g) and refractive index (0.148), specific gravity (0.91 kg /m^3) analysed were within the normal range. All the results showed the quality of the preparation is standard. Further studies can be carried out various *in-vitro* and *in-vivo* various pharmacological activities and elucidate their mechanism of action of *Eichhnornia Crassipes* (Mart.) Solms Ayurvedic Medicated oil (Taila) which may be followed and clinical studies to establish its efficacy in human.

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