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Hibiscus rosasinensis: A newer analytical detection bio-enzyme method

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Abstract

Bio-enzymes are proteins which coupled with substrate molecules using lock and key method and decreases activation energy necessary for a chemical reaction. Bio-enzyme speed up reaction rate on the basis of transition state, velocity constant variables, pH and physiological conditions. Our aim of study is projected to overcome difficulty caused by allergic reaction of enzyme, contamination, decrement degradation activity and efficiency and instability in different conditions and temperature. Stability of foam was evaluated by foaming property character with 90 days implementation process bio-enzymatic method. Our aim of study was targeted about mapping concept foaming property in relation with anti-helminthics therapeutic application and create awareness among restorative ecology of *Hibiscus rosa sinensis* Linn. This ninety days implementation bio-enzymatic process could result to analyse physical and psychological complications such as perioral chapping, dehydration, odour and social stigmatization.

Keywords: Bio-enzymatic method, antihelminthics, foaming property, restorative ecology, *Hibiscus rosasinensis*, lock and key method

Introduction

Hetero-cyclic compounds ^[1] are involved in biological implications especially presented as bio-molecules such as enzyme, vitamins, natural products and biological active compounds including anti-inflammatory ^[2], antibacterial, anticonvulsant, enzyme inhibitors, anti-allergic, anti-fungal ^[3, 4, 5], anti-oxidant, herbicidal activity, anti-HIV, anti-diabetic, anti-cancer activity, insecticidal agents and antihelminthics.

Hibiscus rosasinensis ^[6, 7] (*H. rosasinensis*) is evergreen shrub or small tree growing 2.5-5 cm (8-16 ft) tall and 1.5-3 cm (5-10 ft) wide. *H. rosasinensis* leaves are identified as simple type and petiolate with alternate phyllotaxy, ovate while tip acute, serrate margin, pinnate venation, alternate v arrangement, uncostate reticulate and glossy surface.

Previous studies have revealed that the leaves of plant contain important constituents such as alkaloids, malvalic acid, stercullic acid, tannins, saponins, glycosides, flavonoids, phenols, reducing sugars and terpenoids while petals contain compounds such as quercetin, kaempferol and anthocyanins ^[8, 9, 19, 11, 12].

Bio active compounds presented in *H. rosasinensis* such as and flavonoids, steroids, and saponins possess anti-inflammatory activity by different mechanism especially inhibiting production of reactive oxygen species (ROS) ^[13, 14, 15] and suppress inflammatory mediators such as Nuclear factor -kappa B (NF-KB) and signal transduction and activators of transcription (STAT) in the signaling pathway ^[16, 17, 18, 19, 20].

Experimental procedure

H. rosasinensis leaves were collected from Nine different places in Tamil Nadu state and Taxonomical classification were verified as follows:

Name: *Hibiscus rosasinensis* Linn

Kingdom: Plantae

Sub kingdom: Tracheophytes

Division: Magnoliopsida

Subclass: Dilleniidae

Order: Malvales

Family: Malvaceae

Genus: *Hibiscus*

Species: *rosasinensis*

These types of species were identified as shape, arrangement, venation and authenticated from Captain Srinivasa Murthy, Central Ayurveda Research Institute, Chennai, Tamil Nadu. Five different formulas were designed and coded as FAAB-1 (Formula-I), FAAB-2 (Formula-II), FAAB-3 (Formula-III), FAAB-4 (Formula-IV) and FAAB-5 (Formula-V). These bio-enzyme method [21, 22, 23, 24] were formulated using *Saccharum officinarum* (Jaggery) and *Borassus flabelifer*. L (Karupati) with and without effect of yeast and developed as ratio based project (1: 3: 10: 15). Even though it was developed as a ratio-based project (1: 3: 10: 15), sample leaves were packed in 1-litre capacity container using sequence based and Non -sequence based arrangements [25, 26, 27, 28].

The reason behind to add up Gur (Jaggery) as a natural sweetener could participate in digestion process and promote digestive flora. Meanwhile it act as detoxify the liver and blood that could improve immunity as well as reduce oxidative stress on the body [29, 30, 31].

Since it has lower glycaemic index than white sugar, palm jaggery (Karupatti) would involve in digestion process and promote digestive flora. Furthermore, it helps to maintain oral hydration and electrolyte balance in high due to its cooling effects.

Due to irregular stimulation of vata, pitta and kapha doshos with psychological factors could result loss of appetite that can be rectified using jaggery as energy boosting as well as inhibiting water retention in body due to occurrence of potassium content.

Methodology

Sample preparation: Sample were prepared using following composition Leaves (180 grams), Jaggery (60 g) /Karupatti (60 grams), yeast 500 mg and water 600 mL represented in Table: 1.

Process development

Comparatively time-based preparation method like maceration, percolation, infusion and decoction method. Therapeutic efficiency was validated high in Bio-enzyme method. Method development time period was designed as 90 days in respect with rate of reaction.

From that method identify characterization like foaming property, consistency, odour as well as Bio-Active element [32, 33, 34] using leaves. Odour of pungent- Bio-enzyme preparation from FAAB-I to FAAB-IV and acrid -decay odour of bioenzyme preparation FAAB -V were confirmed using different types of matching specification fragment, woody/resinous, minty/peppermint, sweet, chemical, popcorn, lemon, fruity, pungent, decayed. Practical difficulties like container leakage and protection from ant was rectified.

Bio-chemical reaction rate was identified by using rotational frequency method in respective two minutes continuous stirring with help of stopwatch monitoring device, faster rate of bio-chemical reaction was compared and confirmed weekly among five samples from (FAAB - I to FAAB - V) represented in fig:1. Table: 2.

In-process bioactive compound formation of sample

During in-process development method, Bio active compound [35, 36, 37] were developed for all samples except sequence-based palm jaggery addition sample (FAAB-IV) until 36 days. Foaming property were identified in high at 13th day sample (FAAB-I to FAAB-V) Fig: 2. Consistency parameter was increased until 44 days Fig: 3 Table: 3. for sample

FAAB-II & FAAB IV but dramatic decrement happened from 45 days till 90 days Fig:4. Table:4. Pungent odour was confirmed for sample FAAB-II & FAAB IV at end of 7th day. Acrid odour was confirmed for sample FAAB-V at end of 6th day.

Analytical determination of anthelmintic activity [38, 39]

Phenazone test

Mix up filtered sample 10 ml with 5 ml sodium acid phosphate. Heat and cool the solution, then add up 2% phenazone reagent, precipitate was formed.

Sodium benzoate test

Mix up filtered sample 10 ml with 5 ml sodium acid phosphate. Heat and cool the solution, then add up sodium benzoate solution, precipitate was formed.

Sodium salicylate test

Mix up filtered sample 10 ml with 5 ml sodium acid phosphate. Heat and cool the solution, then add up sodium benzoate solution, precipitate was formed.

Non aqueous determination

Mix up 10 ml of filtered sample with 25 ml of ethanol, 25 ml of glacial acetic acid, 15 ml of mercuric acetate solution and 5% crystal violet 2-3 drops. Then titrate against 0.1M perchloric acid, violet colour was changed into blue colour.

Mix up 10 ml of filtered sample with 25 ml of glacial acetic acid, and 5% crystal violet 2-3 drops. Then titrate against 0.1M perchloric acid, violet colour was changed into emerald green colour.

Results and Discussion

The bio-chemical reaction rate is faster by using rotational frequency method for 2 minutes using stopwatch. During formation of biologically active compound, foaming property is reduced.

Bio enzyme was selected as the best choice when compared with maceration, percolation, infusion, and decoction method.

The reason behind that:

- Determination of foaming property:** To evaluate foaming property using 1000 value number and find out foaming height = $1000/\alpha$. From the point of view, foaming height value was calculated as less than 1cm.
- This foaming property determination using foaming height was evaluated using synthetic material (sodium lauryl sulphate and sodium sulphate) and plant material. Both samples were having foaming height less than 1cm.
- Determination of consistent parameter:** Out of 90 days examination, 50 days bio-enzyme preparation were having consistency parameter. From the obtained information, this sample had emulsifying property. By using sample, helping to prepare semisolid preparation.
- Determination of odour:** Out of 5 samples, 4 samples were tested as pungent odour, one sample was tested as acrid odour due to add up excess yeast standard preparation.
- Determination of bio-active compound:** Out of 5 samples, at week 1, Biological active compound I, III, V were formed. At end of week - VIII, all samples were tested and confirmed bio-active presence. On examination of 60 days over all samples were tested and confirmed bio-active presence till date.



Fig 1: Sampling Week-I



Fig 2: Foaming Property Formation-Week-Iii



Fig 3: Consistency Parameter Checked-Week-VI



Fig 4: Bioactive Formation Week-VIII

Table 1: Composition of bio-enzyme preparation

S. No	Formula	Leaves in grams	Jaggery in grams	Palm jiggery grams	Water	Yeast grams	Container capacity (i litre)
1.	FAAB-I	180 (NS)	60 (NS)	-	600 ml	-	Utilised
2.	FAAB-II	180 (S)	60 (S)	-	600 ml	-	Utilised
3.	FAAB-III	180 (NS)	-	60 (NS)	600 ml	-	Utilised
4.	FAAB-IV	180 (S)	-	60 (S)	600 ml	-	Utilised
5.	FAAB-V	180 (NS)	-	-	600 ml	500	Utilised

Note: All samples were prepared by ratio based project (1:3:10:15).

S- stands for sequence arrangement, NS- stands for Non-sequence arrangement.

Table 2: Property and bioactive compound identification in week: 1

Sample	Foaming Property	Consistency	Odour	BACPD
FAAB-I	Decreases	Decreases	Pungent	Formed
FAAB-II	Increases	Increases	Pungent	Not formed
FAAB-III	Decreases	Decreases	Pungent	Formed
FAAB-IV	Increases	Increases	Pungent	Not formed
FAAB-V	Decreases	Decreases	Acrid	Formed

Table 3: Property and bioactive compound identification in week: 7

Sample	Foaming Property	Consistency	Odour	BACPD
FAAB-I	Increases	Increases	Pungent	Formed
FAAB-II	Increases	decreases	Pungent	Formed
FAAB-III	Increases	Increases	Pungent	Formed
FAAB-IV	Increases	decreases	Pungent	Formed

Table 4: Property and bioactive compound identification in week:

13

Sample	Foaming Property	Consistency	Odour	BACPD
FAAB-I	Increases	Increases	Pungent	Formed
FAAB-II	decreases	Increases	Pungent	Formed
FAAB-III	Increases	Increases	Pungent	Formed
FAAB-IV	Decreases	Decreases	Pungent	Not formed

Conclusion

This method was confirmed and compared with synthetic preparation sodium lauryl sulphate 1:1.5 and 1:1. Ratio with

plant bio enzyme sample. The matching specification information belong to foaming property and mapping concepts belongs to anthelmintics therapeutic applications were clubbed. This information was evaluated as foaming height [1000/ α] less than 100 foaming index.

Total Height is 15 cm

Foaming Height is 12 cm

Foaming Height = $12/15 = 0.8$ cm

Foaming Height = $1000/12 = 83$ cm (less than 100cm foaming index).

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Conflict of interests

The authors declared no conflict of interests.

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