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In-Vitro Study of Anti-Inflammatory Activity of Polyherbal Asava Formulation

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KEYWORDS

Fermentation, anti-inflammatory, polyherbals, alcohol, sugar

ABSTRACT:

Introduction: The current research was conducted to assess the anti-inflammatory activity of a polyherbal Asava preparation consisting of ginger rhizomes (Zingiber officinale), fenugreek seeds (Trigonella foenum-graecum), and amla fruits (Emblica officinalis). Asava is a natural fermented liquid Ayurvedic formulation that increases the extraction and bioavailability of phytoconstituents.

Methods: The Asava was made by fermenting the herbal blend for 30 days with flowers of *Woodfordia fruticosa* as a natural source of wild yeast and jaggery as a fermentable sugar. The fermentation resulted in self-production of alcohol, which served as a preservative as well as a solvent to enhance the extraction of phytoconstituents. The yeasts naturally occurring on the flowers of *Woodfordia fruticosa* played a vital role in initiating and maintaining the fermentation.

Results: The resultant polyherbal Asava was rich in bioactive constituents. Synergistic interactions between the component herbs were assumed to augment its therapeutic activity, especially in terms of anti-inflammatory activity. Fermentation helped in enhanced bioavailability of the phytoconstituents.

Conclusions: This fermented polyherbal Asava extract shows promising therapeutic potential as an alternative natural preparation for the treatment of inflammatory ailments. Its boosted bioavailability as well as the synergistic outcome underscores the advantage of fermented plant products in pharmacotherapy. This traditional remedy justifies further study to confirm its efficacy and guide its development towards a standardized pharmaceutical herbal product.

1. Introduction

Recently, more people are using natural remedies because they're worried about the side effects of synthetic drugs [1]. Among these remedies, mixes of different herbs, called polyherbal preparations. They combine different herbs that work well together and tend to cause fewer side effects [2]. Our study focuses on one of these preparations, called Asava, which includes ginger, amla, fenugreek, jaggery, and *Woodfordia fructicosa*. Inflammation is a natural defense mechanism our body uses when injured or ill. However, sometimes this process becomes dysregulated and can lead to conditions such as arthritis or inflammatory bowel diseases [3]. Traditional medicinal systems such as Ayurveda have long recognized the anti-inflammatory properties of specific herbs. The Asava preparation under investigation is formulated based on Ayurvedic principles and is believed to possess potent anti-

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inflammatory properties due to the synergistic interactions among its constituent herbs [4]. Ginger contains an important compound called gingerol that helps fight inflammation by blocking certain substances in our body that cause it [5]. Amla, also known as Indian gooseberry, is loaded with vitamin C and other good stuff that help our body fight inflammation [6]. Fenugreek contains unique compounds that contribute to its anti-inflammatory effects by modulating key chemicals involved in the process [7]. Jaggery, which comes from sugarcane, has antioxidants and minerals that add to the anti-inflammatory effects [8]. *Woodfordia fructicosa*, another plant used in Ayurveda, has compounds that help with both inflammation and pain [9]. Vascular tissues' intricate biochemical reaction to pathogens, injured cells, or irritants is inflammation. Although persistent or excessive inflammation is a protective mechanism, it is linked to several illnesses, such as cancer, cardiovascular disease, and rheumatoid arthritis [10][11]. Conventional anti-inflammatory medications, like corticosteroids and nonsteroidal anti-inflammatory medicines (NSAIDs), are frequently used to treat inflammation. However, these drugs frequently have adverse effects, such as immunosuppression, cardiovascular risks, and gastrointestinal problems [13]. As a result, there is increasing interest in finding and creating substitute natural anti-inflammatory medicines that might be effective while having fewer side effects [14][15]. Early in the drug development process, in vitro investigations are essential because they enable researchers to assess a variety of compounds' anti-inflammatory potential before moving on to in vivo testing [17]. These results highlight how crucial in vitro anti-inflammatory research is for finding and creating new therapeutic medicines [18][19]. Researchers can find molecules with promising anti-inflammatory properties by screening natural products and their derivatives, opening the door to safer and more efficient treatments for inflammatory illnesses [21].

2. Materials and Methods

The plant material of ginger, amla, and fenugreek was procured from the local market. Authentications of ginger, amla, fenugreek, and *woodfordia fruticosa* (dhataki) flowers were done at the Department of Botany, Yashvantrao Chavan Institute of Science, Satara.

To optimize the final formulation, all the ingredients were taken to identify the suitable combination of all the ingredients (Table no. 2), which will produce the formulation. In the case of the optimization of formulation ingredients, like fermenting agent and drug concentrations, they were kept as it is according to Ayurvedic procedures. Jaggery dissolves into the hot water and then cools it. After cooling the jaggery solution, add ginger, amla, and fenugreek. Dry dhataki pushpa churn will be added to the above mixture because dhataki pushpa contains mild yeast. Formulation will be kept in the sterile area. The fermentation process will be left for a month and then opened. The characterization of formulation involves pH measurement, colour, odour, specific gravity, and alcohol content [5].

In vitro anti-inflammatory activity by the Protein denaturation method [6]**.** The reaction mixture (10 mL) consisted of 0.4 mL of egg albumin (from fresh hen's egg), 5.6 mL of phosphate buffered saline (PBS, pH 6.4), and 100 µL of a different concentration sample. A similar volume of double-distilled water served as a control. Then the mixtures were incubated at (37°C ± 2) in an incubator for 15 min and then heated at 70°C for 5 min. After cooling, their absorbance was measured at 660 nm by using the vehicle as a blank. Diclofenac sodium at the concentration was used as a reference drug and treated similarly for the determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula,

% Inhibition = C –T/ C

T = absorbance of test sample, C = absorbance of control

Results and discussion

The identification of ingredients was conducted through visual observation.

For instance, the brown color of Asava might be due to the presence of dissolved jaggery, which imparts a dark brown color due to water.

The pH of the solution may affect this color change. pH variation occurs during fermentation, with a decrease observed as ethanol concentration rises. Monitoring pH is crucial for assessing fermentation completion. pH of the Asava preparation was 3.4 to 3.9

As for taste, Asava exhibited an acrid flavor. Ethanol content in Asava was determined to be 0.9% using a

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Hydrometer. Ethanol, a byproduct of anaerobic fermentation, plays a significant role in the stability and sensory attributes of the product. Saccharomyces species contribute to ethanol production during fermentation, with ethanol serving as a preservative and bioenhancer.

Asava exhibited a specific gravity of 1.01, indicating the presence of alcohol. Specific gravity exceeding 1.000 suggests alcohol production from sugar fermentation. Thus, the successful conversion of glucose to alcohol through fermentation was inferred.

***In-vitro* inflammatory evaluation of polyherbal Asava:**

Evaluating the plot of % invitro anti-inflammatory activity of this Asava concentration and standard diclofenac (Fig.1 and Fig.2). Standard showed the % Inhibition of 31.64 %, 36.70 %, 59.49 %, 67.72 %, 72.78%. Asava showed the % Inhibition 24.05 %, 27.21 %,36.70 %, 44.30 %, 44.93 % for 200, 400, 600, 800, 1000 (µg/mL) Concentration in Table 1 and Figs. 3, 4. The standard positive control Diclofenac, showed an IC50 of 490.23µg/mL calculated by graph, and the IC50 of Asava was not evaluable.

Table 1. Anti-inflammatory assay of the test compound against PDA

Sr. no.	Sample Code	Concentration (µg/ml)	Absorbance at 660nm				% Inhibition	IC50 (µg/ml)
			Test 1	Test 2	Test 3	Mean		
1	Control	-	1.58	1.58	1.58	1.58	-	490.23
2	Standard (Diclofen ac S)	200	1.09	1.07	1.09	1.08	31.64%	
		400	1.01	1.00	0.99	1.00	36.70%	
		600	0.64	0.64	0.64	0.64	59.49%	
		800	0.51	0.51	0.51	0.51	67.72%	
		1000	0.43	0.44	0.43	0.43	72.78%	
3	Asava	200	1.20	1.20	1.20	1.20	24.05%	NE
		400	1.15	1.15	1.15	1.15	27.21%	
		600	1.01	1.00	1.01	1.00	36.70%	
		800	0.89	0.89	0.87	0.88	44.30%	
		1000	0.87	0.87	0.86	0.87	44.93%	

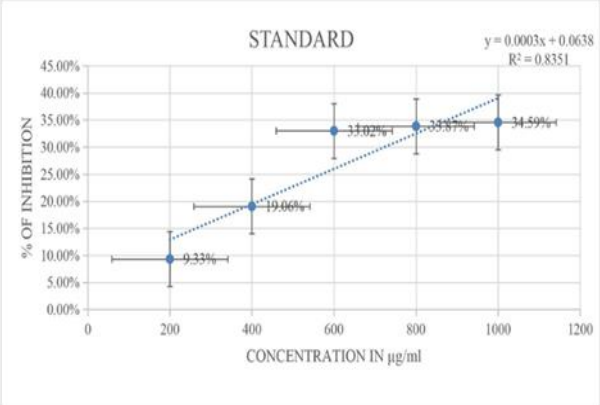

Fig. 1. Percentage of inhibition of ascorbic acid

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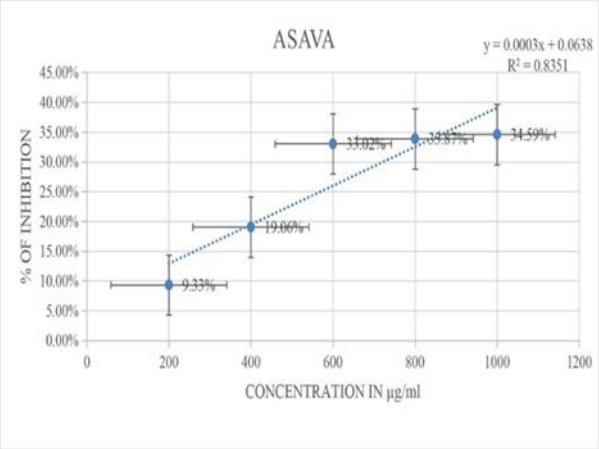

Fig. 2. Percentage of inhibition of Asava

Fig. 3. Anti-inflammatory activity of ascorbic acid (standard)

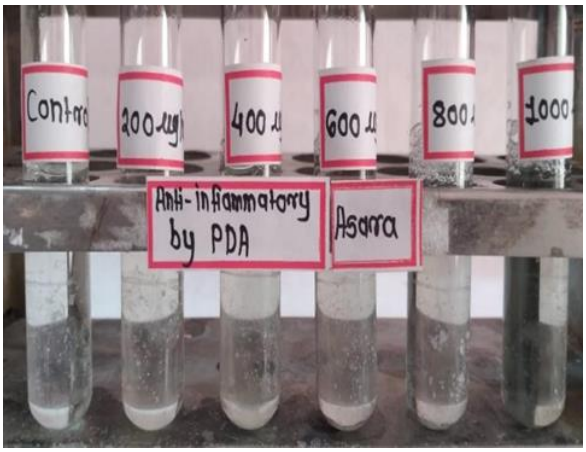

Fig. 4. Anti-inflammatory activity of Asava

3. Conclusions

The anti-inflammatory profile of compound Asava was evaluated by measuring the percent of inhibition against protein denaturation via test tube method. The compound Asava exhibited moderate anti-inflammatory activity against protein denaturation and however the concentration increases also the anti-inflammatory activity of the compound increases moderately as compared to the standard Diclofenac Sodium.

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