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Comparative Study of Crude and Microsuspension Forms of *Ananas comosus* Fruit Peel in Spasmolytic Activity

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Abstract:

This study investigates the spasmolytic effects of the Ethyl acetate Extract of *Ananas comosus* Fruit Peel (EAFP) and its microsuspension (EAFP MS) using an isolated chick ileum model. EAFP was prepared via Soxhlet extraction, yielding 8.1% w/w, and subjected to phytochemical analysis, revealing the presence of alkaloids, flavonoids, tannins, phenolics, and other bioactive compounds. EAFP MS was formulated to enhance bioavailability, as demonstrated by its uniform particle size and improved dissolution profile. The spasmolytic activity was evaluated by analyzing dose-response curves for acetylcholine in the presence of EAFP, EAFP MS, and atropine. The microsuspension exhibited superior spasmolytic effects, significantly reducing acetylcholine-induced contractions. These findings suggest that EAFP MS could serve as a potential therapeutic agent for managing gastrointestinal spasms.

Keywords — Spasmolytics, *Ananas comosus*, Microsuspension, Acetylcholine, Atropine.

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I. INTRODUCTION

A cramp, also known as a muscle spasm, is an abrupt, unintentional contraction of a muscle. Although usually brief, these spasms can be extremely unpleasant. Antispasmodics are widely used to treat disorders involving decreased smooth muscle relaxation and contraction disturbances [1]. They work well for respiratory problems, stomach cramps, gastric movement abnormalities, and muscular spasms. In the development of conditions like irritable bowel syndrome (IBS), smooth muscle contractility—especially in the gastrointestinal tract—is a crucial component. Antispasmodic medications are therefore essential to the pharmacological management of these disorders. But problems like drug tolerance and side effects have prompted researchers to look for new antispasmodics that can act as gastrointestinal smooth muscle relaxants or bronchodilators [2].

Non-nutrient bioactive compounds found in plants help defend them against infections, infestations, and attacks by microbes, pests, pathogens, or predators. Some of these compounds contribute to a plant's color, aroma, and other sensory characteristics. Plants produce these phytoconstituents through primary and secondary metabolic pathways, with many classified as either active drug components or inactive non-drug components [3].

Ananas comosus (pineapple), a tropical plant of the family Bromeliaceae, is widely cultivated in tropical and subtropical regions. Traditionally used in various cultures, its medicinal value is linked to bromelain, a group of proteolytic enzymes with diverse biological activities. Bromelain has demonstrated anti-inflammatory, fibrinolytic, and spasmolytic properties, making it a promising candidate for managing smooth muscle-related conditions. This study focuses on the spasmolytic potential of *A. comosus* and its applications in gastrointestinal and other smooth muscle disorders [4].

Fruit peel extract is formulated into the microsuspension for the better absorption and the bioavailability Micro suspension has been reported to enhance adsorption and bioavailability. Decrease in particle size leads to an increase in surface area

and consequently in the rate of dissolution as described by the Noyes- Whitney equation [5].

In this study the comparative evaluation of EAFP extract and EAFP Microsuspension on spasmolytic activity against the atropine on Chick ileum.

II. METHODOLOGY

A. Collection, Drying, and Authentication of Plant Material

The fruit of *Ananas comosus* was collected in December from the Kukatpally fruit market, Hyderabad, Telangana, India. The plant material was authenticated by Mr. Suresh, a botanist and Assistant Professor at Government Degree College, Kukatpally, Telangana, by comparing its morphological characteristics to standard references. The fruit peel was carefully separated, shade-dried for approximately three weeks, and pulverized into a coarse powder. The prepared material was stored in airtight containers for subsequent extraction processes.

A. Ananas Comosus fruit peel Extract

A total of 250 g of the dried coarse powder of *Ananas comosus* fruit peel was subjected to Soxhlet extraction using 1000 ml of ethyl acetate for eight hours. The resulting extract was filtered and concentrated using a water bath maintained at 50°C to obtain the Ethylacetate *Ananas Comosus* Fruit Peel extract (EAFP). The yield of the extract was calculated and recorded [6].

B. Phytochemicals analysis

The Ethyl Acetate Fruit Peel extract (EAFP) underwent qualitative phytochemical screening to detect the presence of various bioactive compounds using standard methods. Alkaloids were confirmed using Dragendorff's test, which produced a reddish-brown precipitate. Carbohydrates were identified through the Molisch test, indicated by the formation of a violet ring. Proteins were detected using Millon's test, which resulted in the appearance of a white precipitate. Glycosides were verified by the Keller-Killiani test, showing a blue color development. Flavonoids were identified using alkaline reagent, lead acetate, and ferric chloride tests, each producing characteristic yellow or green precipitates or coloration. Phenols were confirmed by the iodine test, indicated by a transient red color. Tannins were detected using Braymer's test with ferric chloride, resulting in blue-green coloration. Saponins were identified by the sodium bicarbonate test, which produced stable honeycomb-like froth. Lastly, triterpenoids and steroids were confirmed using Salkowski's test, evidenced by the development of a golden-yellow colour and GC-MS studies [7].

Quantitative analysis i.e., total phenolic and flavonoid content of the extract determined by the FC reagent colorimetric method and aluminum chloride method respectively [8].

C. Formulation of EAFP Microsuspension

A modified precipitation method was used to prepare EAFP micro-suspensions. EAFP (2 g) was dissolved in ethanol via sonication and added at 1 mL/min to water with 1.5% PVA under stirring. The suspension was diluted with 0.2% PVA and stirred for 6 hours to prevent aggregation [9].

D. Characterization of Microsuspension

The characterization of the microsuspension included particle size determination, SEM analysis, entrapment efficiency assessment, and dissolution studies. The polydispersity index (PDI) was measured using Microtrac to evaluate size-based heterogeneity caused by aggregation. Particle size and morphology were analyzed using a Zeiss SEM at Hyderabad University, Telangana. Entrapment efficiency was determined by preparing a 100 µg/mL EAFP stock solution, measuring absorbance at λmax (200–400 nm) using Shimadzu UV-800, and creating a calibration curve for working solutions (20–100 µg/mL). This method was applied to MS to calculate unknown concentrations. Dissolution studies were conducted with 5 mL each of EAFP extract and MS using a USP type II apparatus at 100 rpm in 900 mL

dissolution medium at 37°C. Samples were taken at intervals up to 120 minutes, filtered, and analyzed for absorbance at 323 nm to calculate dissolution percentages [5].

E. Spasmolytic activity

Chick ileum was obtained from a slaughterhouse and placed in Tyrode solution, cut into 20 mm segments, and cleaned with Tyrode solution. The tissue was mounted in a 20 mL Tyrode solution-filled organ bath using a thread, with one end connected to an oxygen tube and the other to a lever. After stabilizing the tissue for 30 minutes, a dose-response curve (DRC) for acetylcholine (100 µg/mL) was recorded, maintaining a 60-second contact time and 30-second baseline. The bathing solution was replaced after each dose, with a kymograph drum speed of 0.25 rpm and a 3-minute interval between doses. Subsequently, DRCs were obtained for atropine, EAFP, and the micro-suspension in the presence of atropine. Response heights, percentage inhibition by atropine, EAFP, and the micro-suspension were calculated against acetylcholine, and response percentage were determined by plotting the data [9].

III. RESULTS AND DISCUSSION

The EAFP extract was obtained using the hot continuous Soxhlation technique, yielding 8.1% w/w. Phytochemical screening of the extract revealed the presence of carbohydrates, alkaloids, steroids, saponins, phenolics, flavonoids, tannins, and triterpenes, while proteins and cardiac glycosides were absent. Gas Chromatography-Mass Spectrometry (GC-MS) analysis identified key phytoconstituents such as stigmasterol (0.35%), cholesta-22,24-dien-5-ol (0.08%), and other derivatives of benzenedicarboxylic acid with minor peak areas. Quantitative analysis showed a total phenolic content of 71.69 mg gallic acid equivalent/g and a flavonoid content of 45.37 mg quercetin equivalent/g, determined using spectrophotometric methods.

Formulation characteristics of the EAFP extract included evaluation of particle size, morphology, entrapment efficiency, and dissolution profiles. Particle size analysis showed size-based homogeneity with minimal aggregation, while SEM analysis highlighted the microformulation's uniform morphology. Entrapment efficiency was assessed through UV spectrophotometry, creating a calibration curve for acetylcholine and determining IC50 values. Dissolution studies performed using a USP Type II apparatus revealed consistent release profiles for the EAFP extract and microformulation, indicating effective drug release characteristics. These findings suggest the formulation's potential for therapeutic applications

A. Spasmolytic activity

The kymographs of the Acetylcholine DRC of various doses and the effect of EAFP Extract, MS and Atropine against the Acetylcholine in Cumulative Method shows that EAFP MS more spasmolytic effect as Compared with EAFP (Fig. 1,2,3).

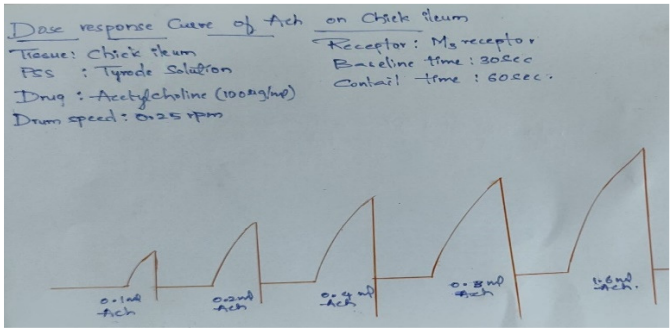

Fig.1 Dose response curve of Ach on Chick ileum

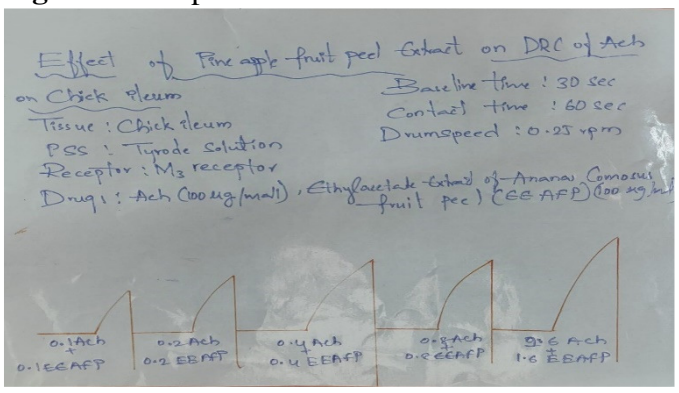

Fig.2 Effect of EAFP Extract on DRC of Ach on Chick ileum

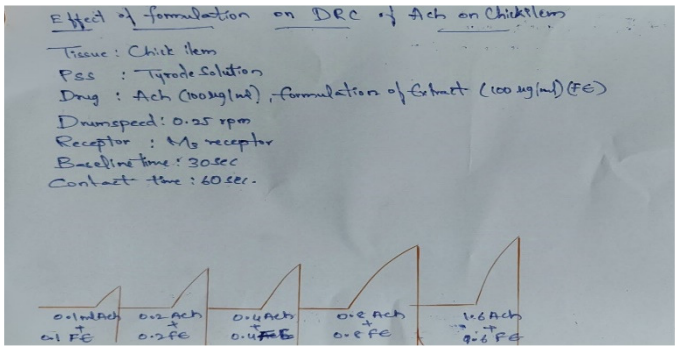

Fig.3 Effect of formulation on DRC of Ach on Chick ileum

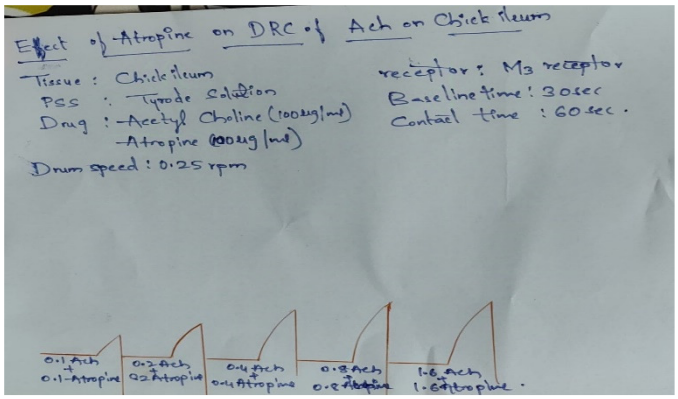

Fig. 4 Effect of Atropine against the Atropine

The spasmolytic activity of the EAFP extract and its EAFP MS was evaluated using an isolated chick ileum bioassay, focusing on their effects on the dose-response curve (DRC) of acetylcholine (Ach). Initially, acetylcholine was administered to establish a baseline DRC, showing a typical dose-dependent contraction of the chick ileum smooth muscle.

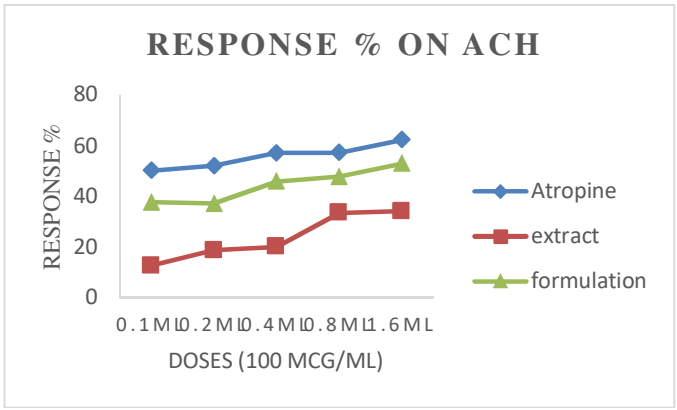

Fig. 5 Response percentage of the EAFP Extract, MS and Atropine against Ach

When the EAFP extract was added in a cumulative manner, it shifted the acetylcholine DRC to the right, indicating a reduction in acetylcholine-induced contractions. This rightward shift suggests a competitive antagonism at the muscarinic receptors, which mediate Ach-induced smooth muscle contractions. The EAFP MS formulation demonstrated an even greater rightward shift in the DRC compared to the EAFP extract, indicating that the microsuspension formulation provides more effective inhibition of acetylcholine action.

tropine, used as the standard, exhibited the strongest rightward shift of the DRC, confirming its potent antimuscarinic activity and validating the assay. The enhanced spasmolytic effect of EAFP MS may be attributed to its improved bioavailability, allowing for greater interaction with muscarinic receptors and possibly calcium channels, thus enhancing its ability to inhibit Ach-induced contractions.

These results suggest that EAFP MS has a superior antispasmodic action over the extract alone, making it a promising candidate for managing gastrointestinal spasms and related disorders.

V. CONCLUSION

The study highlights the spasmolytic potential of ethyl acetate fruit peel extract of *Ananas comosus* and its microsuspension on the chick ileum. The microsuspension demonstrated enhanced efficacy compared to the crude extract, likely due to improved bioavailability and drug delivery. The findings underscore the potential of EAFP MS as a natural alternative for managing gastrointestinal smooth muscle disorders, warranting further research to explore its mechanisms and clinical applications.

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