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Kalpana
School of Vocational Studies and Applied Sciences, Department of Applied Chemistry, Gautam Buddha University, Greater Noida, Uttar Pradesh, India

Dr. Jaya Maitra
School of Vocational Studies and Applied Sciences, Department of Applied Chemistry, Gautam Buddha University, Greater Noida, Uttar Pradesh, India

Correspondence
Dr. Jaya Maitra
School of Vocational Studies and Applied Sciences, Department of Applied Chemistry, Gautam Buddha University, Greater Noida, Uttar Pradesh, India

Aloin as a bioenhancer for aspirin

Kalpana and Dr. Jaya Maitra

Abstract
Aloin is a natural product isolated from the Aloe Vera leaves which has significant therapeutic effects & possess many significant properties like anti-inflammatory, antifungal, antibacterial, cardiac stimulatory etc. Aspirin is a synthetic drug which having various properties like anti-inflammatory, antihypertensive, antiplatelet anticancer. The present study is about to determine the synergistic effect of Aloin & Aspirin against micro-organisms using Agar dilution method & it was observed that they showed significant synergistic antibacterial property against the tested microorganisms.

Keywords: Aloin, Aspirin, Synergistic effect, antimicrobial

Introduction
Synergism is derived from a Greek word "synergia" i.e. syn + ergon in which syn means together & ergon means work so as whole it means working together or cooperation. Basically synergism is a cooperation of two or more components or entities to produce an effect greater than the sum of their individual effect.

Aloin is an important phyto constituent of Aloe Vera which possess significant therapeutic effect. It is a yellow colour substance having molecular formula $C_{21}H_{22}O_9$ .Aloin is basically present in the latex of Aloe vera & an anthraquinone glycoside [1]. Aloin exist in two diasterioisomers forms Aloin A & Aloin B i.e. barbaloin & isobarbaloin respectively. According to biosynthesis of Aloin, formation of Aloin B is favoured [2]. Aloin is a natural product isolated from Aloe Vera which is isolated from Aloe Vera leaves. Aloe Vera contains phytoconstituents which increases the absorption of vitamin C & E and helps to cure digestion related problems. [3] Aloin is a bioactive component which have important curative properties like it is used in the treatment of electrolyte imbalance diarrhoea, inflammation, reproductive disorders, constipation [4] & stimulate the immune system [5].

Aspirin is a salicylate drug which is also known as Acetylsalicylic acid. Aspirin is a versatile synthetic drug having molecular formula $C_9H_8O_4$ having good solubility in water. It is a white coloured NSAID (non-steroidal anti-inflammatory drug) drug which is formed by using salicylic acid & acetyl chloride in the presence of pyridine. Salicylic acid is the important metabolite of aspirin & it is an integral part of animal or human metabolism. [6] i.e. it is most widely used in medications across the world. It is widely used for various diseases like in heart attacks, stokes as it used to thin the blood, anticancer, anti-inflammatory, analgesic, antiplatelet [7] etc.

At present the main issue is the development of multi antibiotic resistance in the pathogens. Plants consists of phytochemicals which are responsible for various antimicrobial properties but when we synergise the bioactive component of plant with some synthetic drug it amends the efficacy of drug by enhancing its pharmacological activity [8] hence they are also called Bioenhancers which means that the thing which enhances the biological activity of the substance. Synergism basically done for magnifying the biological properties of drugs by making them more effective against several bacteria. Aloin is a natural product isolated from Aloe Vera leaves which have various therapeutic properties like anticancer, anti-inflammatory, laxative agent, diarrhoea etc. and Aspirin is a synthetic drug which is also widely used against various diseases like in cardiovascular diseases like heart attacks, strokes etc., antiplatelet, analgesics, antipyretics and anti-inflammation. And in present study we consider the common property i.e. anti-inflammation characteristic of both Aloin & Aspirin & examine the synergistic effect of both aspirin & aloin by varying the concentration of Aloin keeping aspirin as a standard drug. FDA gives approval to this kind of drugs but specifies the level of anthraquinone below 50ppm.

Experimental
Materials & Methods Used for Isolation of Aloin from Aloe Vera Leaves Extraction of Aloin from Aloe Vera was done using Soxhlet apparatus:

Materials Required
Aloe Vera leaves, methanol
i) Soxhlet Extraction
Aloe Vera leaves were collected & washed & rinds were removed, inner gel were scrapped out & cut into pieces & solar dried for 10days at 30-45 ^{0}Celsius & dry gel particles were collected. Powdered the dry gel particles & 2.5g of Aloe Vera powder was taken in soxhlet with 100ml methanol. The extraction was carried out for 20-24hrs.Sample were collected at the end which was free from dry gel & was stored in a freezer & analyzed [9].

ii) Characterization of Aloin
Aloin is yellow colored odourless substance having molecular formula $C_{21}H_{22}O_9$ which is characterized by using TLC (Thin Layer Chromatography) & UV visible Spectrophotometer respectively. Rf value obtained for Aloin was 0.47. T the λ_{max} of isolated compound Aloin was 279nm having absorbance 1.179 [4] by UV visible Spectrophotometer analysis.

iii) Prepation of Drug
Aspirin was made by using salicylic acid & acetyl chloride in the presence of pyridine.

Materials Required
Salicylic acid, acetyl chloride, pyridine
Procedure
6g salicylic acid & 5ml pure redistilled pyridine was taken in a conical flask & kept it on ice bath for chilling the content till 5-8 ^{0}C temperature was attained. Then 5ml Acetyl chloride was slowly added to the content drop wisely with constant stirring & vigorous shaking after that contents in conical flask were kept on water bath for 5-10minutes for heating,. Then content was cooled by using ice chips or cool water by constant stirring till semisolid residue was obtained .Then crude aspirin was filtered by Buchner funnel with suction wash with cold water & dried it by using filter paper.

iv) Antimicrobial Activity
Antibacterial & Antifungal activity was studied against gram negative bacteria *E. coli* & gram negative bacteria *Bacillus Subtilis*, *Cladosporium citri* fungus respectively.

Procedure
The in Vitro antibacterial activity of isolated Aloin was carried out by Agar dilution method

Preparation of LB Agar & LB Broth
4gm of LB Agar was measured and added to the 100ml distilled water in a conical flask & 2.5gm of LB Broth were taken and added to the 100ml of distilled water then both are autoclaved, LB Agar & LB Broth was autoclaved for 3hrs then LB Agar was used for plating of autoclaved petri plates where Microorganism was added to the LB Broth in a shaking incubator at 37 ºC for 24hrs for microorganism growth.

v) Testing Organisms
The microorganisms used for examine the antimicrobial activities are as follows:
a) *E. coli* (bacteria)
b) *Bacillus subtilis* (bacteria)
c) *Cladosporium citri* (fungus)

Antimicrobial Effect Shown by Aloin & Aspirin against Various Microbes Are As Follows
i) Aloin showed complete zone of inhibition against *E. coli* having diameter 4mm & Aspirin show 0mm zone of inhibition against *E. coli.*
ii) In case of Aloin zone of inhibition of diameter 3mm is observed and there is reduction in colonies of *Bacillus subtilis* while in case of Aspirin zone of inhibition having diameter 2mm is observed against *Bacillus subtilis*.

Fig 1: Zone of Inhibition shown by Aloin & Aspirin against *E. coli & Bacillus subtilis*

Table 1: Zone of inhibition shown by Aloin & Aspirin against *E. coli & Bacillus subtilis*

Microbes	Quantity of Aloin (µl)	Zone of inhibition (mm)
E. coli	10	4
Bacillus subtilis	10	3

Table 2: Zone of inhibition shown by Aspirin agains *E. Coli & Bacillus subtilis*

Microbes	Conc of Aspirin (1mg/ml)	Zone of inhibition (mm)
E. coli	1	0
Bacillus subtilis	1	2

A) Zone of inhibition shown by aspirin against *E. coli* at Different Concentrations

Aspirin shows least zone of inhibition at conc 1mg/ml having diameter 0mm & maximum zone of inhibition at conc 10mg/ml having diameter 7mm against *E. coli.*

Fig 2: Zone of inhibition shown by aspirin Against *E. coli* at different concentrations

Table 3: Zone of inhibition shown by aspirin against *E. coli* at different concentrations

Microbe	Conc. of Aspirin (mg/ml)	Quantity of Aspirin(µl)	Zone of Inhibition(mm)
E. coli	1	10	0
E. coli	5	10	3
E. coli	10	10	7
E. coli	15	10	6

B) Zone of inhibition shown by aspirin against *Bacillus subtilis* at different concentrations

Aspirin showed least zone of inhibition at concentration 1mg/ml having diameter 2mm & maximum zone of inhibition at conc 10mg/ml having diameter 9mm against *Bacillus subtilis*

Fig 3: zone of inhibition shown by aspirin against *Bacillus subtilis* at different concentrations

Table 4: Zone of inhibition shown by Aspirin against *Bacillus subtilis* at different concentrations.

Microbes	Conc of Aspirin (mg/ml)	Quantity of Aspirin (µl)	Zone of Inhibition (mm)
Bacillus subtilis	1	10	2
Bacillus subtilis	5	10	6
Bacillus subtilis	10	10	9
Bacillus subtilis	15	10	7

C) Zone of inhibition shown by aloin against *E. coli*

Aloin show least zone of inhibition at 10 µl having diameter 13mm & maximum zone of inhibition at 40 µl having diameter 18mm against *E. coli.*

Fig 4: Zone of inhibition shown by Aloin against *E. coli* at different concentrations

Table 5: Zone of inhibition shown by Aloin against *E. coli* at different concentrations

Microbes	Quantity of Aloin (µl)	Zone Of Inhibition (mm)
E. coli	10	13
E. coli	20	15
E. coli	30	16
E. coli	40	18

D) Zone of inhibition shown by Aloin against *Bacillus subtilis* at different quantities

Aloin showed least zone of inhibition at 10 µl having diameter 13mm & there is partial loss of colonies & maximum zone of inhibition at 40 µl having diameter 19mm against *Bacillus subtilis.*

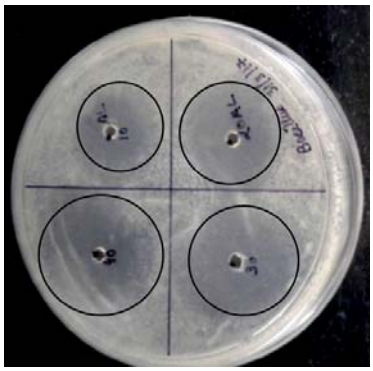

Fig 5: Zone of inhibition shown by Aloin against *Bacillus subtilis* at different concentrations

Table 6: zone of inhibition shown by Aloin against *Bacillus subtilis* at different concentrations

Microbes	Quantity of Aloin (µl)	Zone of inhibition (mm)
Bacillus subtilis	10	13
Bacillus subtilis	20	15
Bacillus subtilis	30	17
Bacillus subtilis	40	19

E) Synergistic effect shown by Aloin & Aspirin against bacteria

E. coli

On synergism of Aspirin & Aloin the least zone of inhibition shown at ratio Aloin: Aspirin ratio 10:10 having diameter 6mm & maximum zone of inhibition shown at ratio 10:40 having diameter 18mm against *E. coli.*

Fig 6: synergistic effect shown by Aloin & Aspirin against bacteria *E. coli*

Table 7: Synergistic effect shown by Aloin & Aspirin against bacteria *E. coli*

Microbe	Quantity of Aloin (µl)	Quantity of Aspirin (µl)	Ratio of Aloin: Aspirin	Zone of Inhibition (mm)
E. coli	10	10	10:10	6
E. coli	20	10	20:10	15
E. coli	30	10	30:10	17
E. coli	40	10	40:10	18

E) Synergistic effect shown by Aloin & aspirin against bacteria *Bacillus subtilis*

On synergism of Aspirin & Aloin the least zone of inhibition shown at ratio aloin: aspirin ratio 10:10 having diameter 7mm & maximum zone of inhibition shown at ratio 10:40 having diameter 14mm against *Bacillus Subtilis* but there is a partial loss of colonies not completely loss of colonies like *E. coli*.

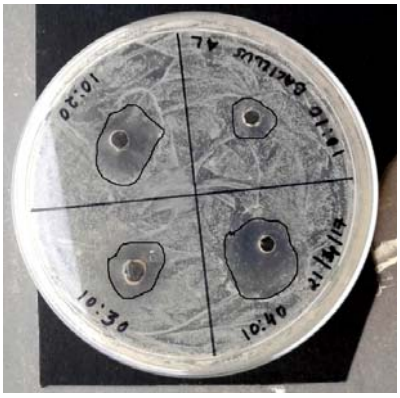

Fig 7: Synergistic effect shown by Aloin & Aspirin against bacteria *Bacillus subtilis*

Table 8: Synergistic effect shown by Aloin & Aspirin against Bacteria *Bacillus subtilis*

Microbes	Quantity of Aloin (µl)	Quantity of Aspirin (µl)	Ratio of Aloin: Aspirin	Zone of Inhibition (mm)
Bacillus subtilis	10	10	10:10	7
Bacillus subtilis	20	10	20:10	8
Bacillus subtilis	30	10	30:10	9
Bacillus subtilis	40	10	40:10	12

F) Synergism shown by Aloin & Aspirin against fungus *Cladosporium citri*

On synergism of Aspirin & Aloin the least zone of inhibition shown at ratio Aloin: Aspirin ratio 10:10 having diameter 6mm & maximum zone of inhibition shown at ratio 10:40 having diameter 12mm against fungus *Cladosporium citri.*

Fig 8: Synergism shown by Aloin & ASPIRIN against fungus *Cladosporium citri*

Table 9: Synergism shown by Aloin & Aspirin against fungus *Cladosporium citri*

Microbes	Quantity of Aloin (µl)	Conc of Aspirin (µl)	Ratio of Aloin: Aspirin	Zone of Inhibition (mm)
Cadosporium citri	10	10	10:10	6
Cladosporium citri	20	10	10:20	8
Cladosporium citri	30	10	10:30	10
Cladosporium citri	40	10	10:40	14

Results

Aloin is a natural product having antibacterial property due which it is used against various disease and aspirin is a synthetic drug but its tendency to kill bacteria is less but when we synergise it with the natural product Aloin, Aaloin acts like a bio enhancer and enhances the bioactivity of the drug & its zone of inhibition increases, shown in the data (refer to Table 7, 8, 9)

Solely Aloin shows 4mm (refer to table1&2) inhibitory effect against *E. coli* & 3mm partial inhibitory effect (refer to table 1 & 2) against *Bacillus Subtilis* whereas Aspirin alone shows only partial loss of colonies against bacteria *Bacillus Subtilis* (refer to table 1).But when we make different concentration solutions of Aspirin 1mg/ml,5mg/ml,10mg/ml,15mg/ml then it was found that it shows maximum inhibitory effect at the concentration of 10mg/ml against both bacteria *E. coli & Bacillus subtilis* (refer to table 3 & 4) as a result 10 µl was taken as a reference concentration for the experiment. The concentration & quantity of Aspirin i.e. 10mg/ml & 10 µl was fixed while the concentration of drug was varied respectively to determine the synergistic effect.

During the study it was observed that formerly the zone of inhibition of aspirin at 10mg/ml concentration was 7mm. (refer to table 3) but when varying quantity of Aloin (10, 20, 30, 40) µl was added to 10 µl of 10mg/ml concentration of Aspirin the maximum inhibitory effect i.e. 18mm was shown (refer to table 7), at quantity 40 µl (Aloin) was added to aspirin against *E. coli*.

Similarly combining Aspirin drug with varying quantity of Aloin & it was found that zone of inhibition of aspirin at 10mg/ml concentration was 9mm against *Bacillus Subtilis* but there is only partial loss of colonies not clearly bacteria was killed but when we add varying quantity of Aloin (10,20,30,40) µl to 10 µl of 10mg/ml concentration of Aspirin the maximum inhibitory effect i.e. 12mm was shown(refer to table 8) against, at quantity 40 µl(Aloin) was added to aspirin against *Bacillus Subtilis*.

Combination 10 µl of 10mg/ml of Aspirin drug with varying quantity of Aloin (10, 20, 30, 40) µl also shows a good inhibitory action against fungus *Cladosporiu citri*. The zone of inhibition increases (6mm to 14mm) as the concentration of Aloin increased from 10 µl to 40 µl. From the above data it was observed that this combination effect showed the most effective antibacterial property against *E. coli*, then on fungus *Cladosporium citri*. On *bacillus subtilis* there is a partial loss of colonies when Aloin is taken alone solely but when it is synergise with drug there is formation of clear zone of inhibition.

Conclusions

Both Aloin & Aspirin have antimicrobial & anti-inflammatory, analgesic property & this was the reason for finding synergism between two. Since the use of natural product i.e. Aloin in the combination with Aspirin reduce its cost as well as toxicity associated with it.

It showed that natural products consist of phytochemicals which enhances the bioactivity of synthetic product like Aspirin. Bioenhancers constitute an innovative concept the discovery of which was based on a traditional system of medicine. They will lead to reductions in drug cost, toxicity, and other adverse effects, and have a beneficial influence on health as well as economy. Natural product is safe, effective, economical, easily procured, non-addictive, and has a widely-based effect on several classes of drug.This research is beneficial for those researcher who are interested in working on both natural product as well as on synthetic drugs.

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