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A study on synergism between curcumin and aspirin

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Abstract
Curcumin is a natural product which is isolated from turmeric exhibit various therapeutic properties such as anti-inflammatory anti-depressant anti-bacterial anti-cancer anti-cholesterol anti-Alzheimer, wound healer, anti-diabetic anti-viral. Aspirin is synthetic product called acetyl derivative of salicylic acid used as analgesic, anti-inflammatory (in arthritis) anti-pyretic, anti-platelet anti-cancer anti-hypertensive. The present work is to examine synergistic effect of Curcumin with aspirin extracted from turmeric rind (kachchi haldi) against various micro-organism by using agar dilution test and it was observe that it impart an adequate synergistic effect against the tested micro-organisms.

Keywords: Curcumin, aspirin, synergistic effect, antimicrobial activity

Introduction
Synergism comes from Greek word "synergos" meaning working together i.e. interaction of agents, or condition such that total effect is greater than sum of their individual effects [1].
Curcumin is yellow color compound having molecular formula $C_{21}H_{20}O_6$ & molecular weight 368.385g/mol is a vital phytochemical of turmeric which is responsible for its yellow color & many biological activities [2]. Curcumin structure with several functional groups was first identified in 1910, having aromatic ring system (phenols) are connected by 2 α-β unsaturated carbonyl groups. Curcumin is easily soluble in acetone, ketone, ethanol, and chloroform but not soluble in water which indicates that it is lipophilic in nature [3]. Curcumin is most bioactive part of turmeric with numerous medicinal/pharmaceuticals/therapeutic properties such as anti-inflammatory, anti-depressant, anti-bacterial, anti-cancer, anti-cholesterol, anti-Alzheimer wound healer, anti-diabetic, anti-viral actions [4].
Aspirin is a substance called acetyl salicylic acid or acetyl derivative of salicylic acid is non-steroidal anti-inflammatory drugs (NSAIDs) with molecular formula $C_9H_8O_4$ used as a blood thinner & get rid of inflammation, pain & fever [5, 6]. It was the first drug that was available in tablet form. To study safety & efficacy of aspirin numerous studies are carried out in United States, United kingdom & Europe [7]. It is synthesized by treating salicylic acid with acetic anhydride that turn hydroxyl group of salicylic acid into ester group with the use of catalyst sulphuric acid and phosphoric acid & the process yield aspirin [8]. Aspirin exhibit various therapeutic properties such as analgesic, anti-inflammatory (in arthritis), anti-pyretic, anti-platelet, anti-cancer, anti-hypertensive [7].
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 refine the efficacy of drug by enhancing its pharmacological activity moreover encounter problems of toxicity & overdose hence they are also called Bioenhancers which means that the thing which enhances the biological activity of the substance [9, 10]. Synergism is done basically for enhancing the biological properties of drugs by making them more effective against several bacteria [1]. Curcumin is a natural product isolated from turmeric which have various therapeutic properties like anticancer, anti-inflammatory, anti-diabetic, anti-bacterial anti-platelets etc [4] and Aspirin is a synthetic drug which is also widely used against various diseases like in cardiovascular diseases like heart attacks, strokes etc, act as anti-platelets, analgesics, anti-pyretics and anti-inflammation [7]. In the present study the common property i.e. anti-inflammation characteristic of both curcumin & Aspirin is considered & examine the synergistic effect of both i.e., aspirin & curcumin is studied by varying the concentration of curcumin keeping aspirin as a standard drug [7, 4].

Materials and Methods
Extraction of Curcumin Using Soxhlet Extraction Method
Materials Required: Turmeric, acetone & soxhlet apparatus

Soxhlet Extraction: Turmeric rind (kachchi haldi) was taken dried in oven for 2 days at 50-60^0 Celsius then powdered it. 20g of powder was weighed & placed in soxhlet and 200ml of acetone was added to it & refluxed until the yellow color of extraction faded away. Final extract was collected & stored in freezer & analyzed.

Characterization of Curcumin

Curcumin bright yellow color compound with molecular formula $C_{21}H_{20}O_6$, having molecular weight 368.385 g/mol, melting point 183^o celcius was characterized by Thin Layer Chromaographyn (TLC) with R_f value 0.62 & by UV spectrophotometer with λ_{max} 423nm & absorbance 2.082.

Preparation of Drug

Materials Required: Salicylic acid, acetyl chloride, pyridine

Method: To a 6gm of salicylic acid 5ml of redistilled pyridine was added & put in a conical flask on ice bath ((5-8)^{o}c). Then 5ml of acetyl chloride was added slowly with constant stirring & was heated on water bath about 5 to 10 mins. Solution was allowed to cool & 50ml of water was added to it. Crude aspirin was filtered on Buchner funnel.

Antimicrobial Activity

Antibacterial & Antifungal activity was studied against gram negative bacteria *E. coli*, gram positive bacteria *Bacillus subtilis* & fungus *Cladosporium*.

Procedure

The in vitro antibacterial activity of isolated Curcumin and aspirin was carried out by agar dilution method.

Preparation of Agar and 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 chamber at 37 ^{o}C for 24hrs for microorganism growth.

Testing Organisms

The pathogens used for examine the antimicrobial activities are as follows:

- *E. Coli* (bacteria)
- *Bacillus subtilis* (bacteria)
- *Cladosporium* (fungus)

Determination of synergistic effect of curcumin & aspirin

Agar coated petri plates were coated by 70µl of LB Broth containing gram positive *Bacillus subtilis*, gram negative *E. Coli* & *Cladosporium* fungus respectively for determination of culture growth. Then plates having coating of LB broth containing pathogens was loaded by different concentration of isolated product Curcumin & aspirin. Finally it was placed in incubator at 37^{o}C for 24 hrs. After the incubation, zone of inhibition was measured.

Table 1: Zone of inhibition of Curcumin against *E. Coli* & *Bacillus subtilis*

Conc of curcumin (µl)	Zone of inhibition against *E. Coli* (mm)	Zone of inhibition against *Bacillus subtilis* (mm)
10	3	4
20	5	8
30	6	10
40	11	10.2

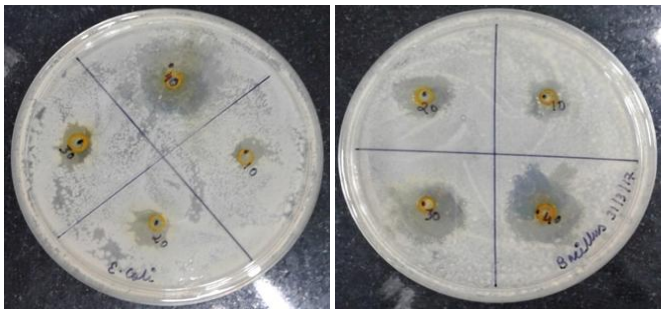

Fig 1: Zone of inhibition of Curcumin against *E. Coli* & *Bacillus subtilis*

Table 2: Zone of inhibition of aspirin against *E. Coli* & *Bacillus subtilis*

Conc of aspirin (µl)	Zone of inhibition against *E. Coli* (mm)	Zone of inhibition against *Bacillus subtilis* (mm)
1	0	0
5	3	3
10	6	5
15	5	7

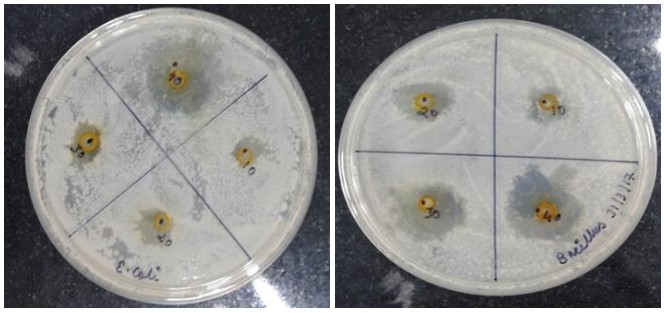

Fig 2: Zone of inhibition of aspirin against *E. Coli* & *Bacillus subtilis*

Table 3: Synergistic effect of curcumin & aspirin against *E. Coli* & *Bacillus subtilis*

Conc. Of aspirin: curcumin (µl)	Zone of inhibition against *E. Coli* (mm)	Zone of inhibition against *Bacillus subtilis* (mm)
5:10	6	7
5:20	14	15
5:30	15	16
5:40	19	17

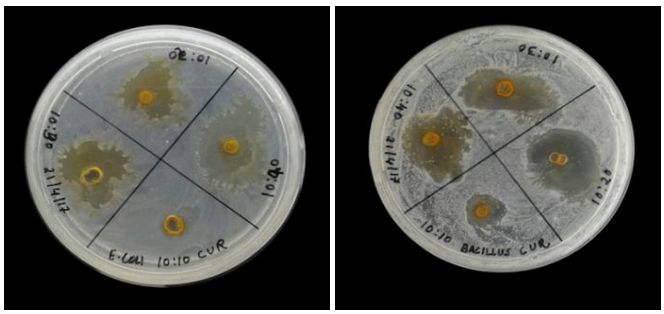

Fig 3: Zone of inhibition showing Synergistic effect of curcumin & aspirin against *E. Coli* & *Bacillus subtilis*

Table 4: Synergistic effect of curcumin & aspirin against *Cladosporium* fungus

Conc of aspirin: curcumin (µl)	Zone of inhibition against *Cladosporium* (mm)
5:10	10
5:20	12
5:30	20
5:40	26

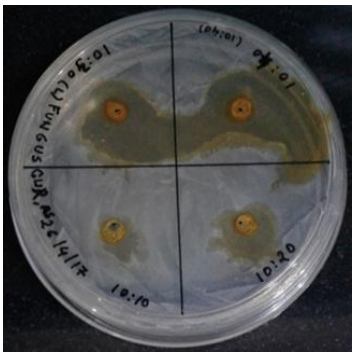

Fig 4: Zone of inhibition showing Synergistic effect of curcumin & aspirin against *Cladosporium*

Results Discussion & Conclusion

A noval and unreported work is done in which Antimicrobial activity of curcumin with different concentration was studied against gram negative bacteria *E. Coli* than in *Bacillus subtilis* (table 1, fig.1) where the zone of inhibition is (3-11) mm in case of *E. Coli* & (4-10.4) mm in *Bacillus subtilis*.

Antimicrobial activity of aspirin with different concentration 1mg/ml, 5mg/ml, 10mg/ml, 15 mg/ml showed loss of colonies against both the bacteria with maximum inhibitory effect at concentration of 15mg/ml against both bacteria *E. Coli* & *Bacillus subtilis* 5mg/ml was taken as reference to study the synergistic effect i.e. 5mg/ml & 5µl as shown in (table no.2, fig. 2).

Aspirin was synergise with varying quantity of curcumin (10µl, 20µl, 30µl, 40µl), it was observed that previously its zone of inhibition for *E. Coli* is 3mm & for bacillus it was 3mm at concentration of 5µl but it increases 19mm for *E. Coli* & 17mm for *Bacillus subtilis*. (table no. 3, fig. 3). Similarly for fungus *Cladosporium* zone of inhibition was 26mm at maximum concentration of curcumin (table no. 4, fig.4). It shows that when we synergise aspirin drug its antibacterial activity enhance & its tendency to kill pathogens increases.

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