Phytochemical analysis and comparative study of antibacterial effect of turmeric extracts using different solvent.

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Abstract:
Curcumin, the principal curcuminoid found in turmeric, is generally considered its most active constituent. Curcumin, besides its anti-inflammatory property, has been known to possess *in vitro* anti-microbial potential against a wide range of microorganisms including fungi as well as several Gram-positive and Gram-negative bacteria. Curcumin possesses a synergistic effect with important antibiotics also. The mechanism of antibacterial activity of curcumin seems to differ depending on the strain being studied. Coating of conventional wound dressing materials with curcumin composite can enhance the effectiveness of the material. Studies have shown that fabrication of silver nano-composite films impregnated with curcumin showed the stronger antibacterial activity against E. coli. This research would aim to claim the best solvent used in extraction process of curcumin and demonstrate the antimicrobial effect of curcumin in order to develop a curcumin-nano-composite coated material for wound dressing with increased efficiency.

1. Introduction

Turmeric belongs to the ginger family, Zingiberaceae. It has been traditionally used as a spice and medicine from ancient times and its potential in other fields has been harnessed in recent years. Phytochemical components of turmeric include sugars, proteins, resins, traces of volatile oils and a compound called curcuminoids which includes curcumin (diferuloylmethane), demethoxycurcumin, and bisdemethoxycurcumin. Major reported pharmaceutical activites of turmeric are antioxidant;antibacterial, anti-inflammatory, anti-tumor and anti-cancer activities and these are due to the presence of curcumin in it, hence claiming it to be the most bioactive component in turmeric. Curcumin is generally regarded as safe (GRAS) by the United States Food and Drug Administration (FDA) due to its low toxicity even when ingested at relatively high levels. Curcumin is a phenolic constituent and is hydrophobic in nature [1]. So it can be extracted efficiently by many organic solvents. Curcumin, on an average, occupies 3.14% w/w of powdered turmeric, and this percentage varies with the species of *Curcuma longa.* E.coli and S. aureus are the two bacterias mostly found in different types of wounds and play a role in delaying the wound healing process resulting wound infections [2]. In a study, the predominant bacteria isolated from the infected wounds were Staphylococcus aureus 47 (32.4%) followed by Escherichia coli 29 (20%), Proteus species 23 (16%), Coagulase negative Staphylococci 21 (14.5%), Klebsiellapneumoniae 14 (10%) and Pseudomonas aeruginosa 11 (8%) [3]. Turmeric extracts showed a prominent zone of inhibition against E. coli and S.aureusmaking it an excellent wound healing agent due to its non-toxic nature. The synergistic activity of curcumin with other antibiotics like ampicillin oxacillin, and norfloxacinhas been exploited in recent years which showed a remarkable decrease in the minimum inhibitory concentration of the antibiotics against bacterial strains [4].

So we have extracted phytochemicals from turmeric using different solvents and then compared antibacterial effect of different extracts.

2. Materials, reagents and solvents used:

Turmeric, foiln-ciocalteau reagent (FCR), gallic acid, quercetin, nutrient broth, agar agar, streptomycin, benzene, acetone, acetonitrile, ethanol, 2-propanol, methanol, dimethyl sulfoxide, silver nitrate.Test Microorganisms: Standard strains of E.coli and S. aureus

3. Methods performed:

3.1 Preprocessing of the turmeric: Before the extraction process, raw turmeric was washed with water, peeled, cut into small pieces and was dividedinto two parts- one sundried for two weeks (part A) and the other was kept in the incubator at 37 °C for two days (part B) to reduce the moisture content in the turmeric to a negligible amount. Then it was crushed into coarse powder form using mortar and pestle.

3.2. Extraction of the bioactive components: 30 grams of powdered turmeric from part A and part B was macerated with 200 ml of methanol for 3 days allowing enough time for the bioactive components to solubilize in the solvent. This was followed by digestion. Digestion was performed by adding fresh 10 grams of powder in the solution with gentle heating to increase the concentration of the bioactive components in the solution and is left for another one day. Similar process is followed for the turmeric powder from part B with solvents namely benzene, acetone, 2-propanol, ethanol, and acetonitrile.

3.3.Sample preparation: The crude extracts were filtered using Whatman No.1filter paper and concentrated using rotary evaporator. Then the highly concentrated solutions were lyophilized to achieve the final prepared samples which were stored at -20 °C for further use. Samples (i.e. the final product after lyophilization) were named from A to F depending on the solvent used in the extraction process where A, B, C, D, E, F corresponds to extraction by benzene, acetone, 2-propanol, ethanol, acetonitrile and methanol respectively.

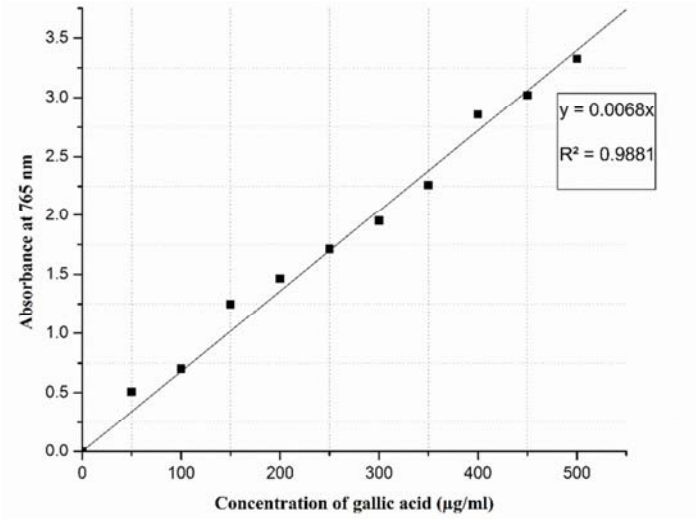

Figure 1: Linear curve of Absorbance vs Concentration of standard graph of Gallic acid

3.4. Phytochemical analysis:

3.4.1 Total phenolic content (TPC): TPC of each lyophilized samples were calculated from the calibrated standard curve using gallic acid by spectrophotometric analysis using FolinCiocalteu reagent (FCR). In this process, 0.5 ml of extracts (of concentration 1000 µg/ml) or gallic acid (of different concentrations from 0-500 µg/ml) were mixed with 3 ml of distilled water. Then 0.25 ml of FCR was added followed by 0.75 ml of saturated aqueous sodium carbonate solution. Lastly, 1 ml of distilled water was added and the mixture was incubated for 30 minutes in 37 °C. Absorbance is taken at 765 nm against a selected blank using UV-visible Spectrophotometer [5].

A standard curve of gallic acid is prepared at-first as reflected in Figure 1. Phenol content of different samples was calculated in gallic acid equivalent.

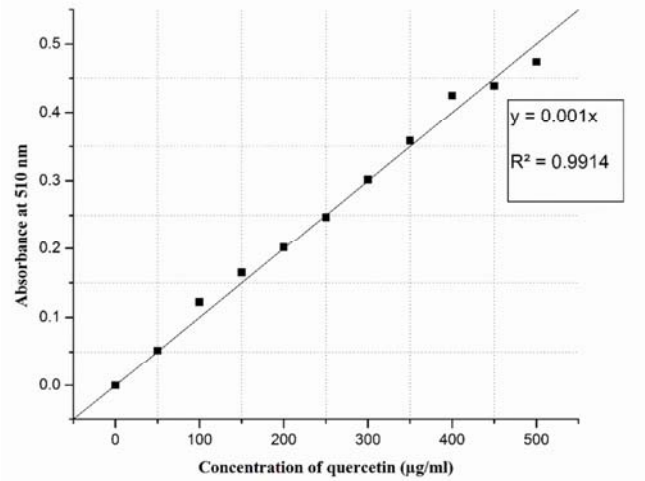

Figure 2: Linear curve of Absorbance vs Concentration of standard graph of Quercetin

3.4.2 Total flavonoid content (TFC): TFC of each extracted samples are calculated from the calibrated standard curve using quercetin by spectrophotometric analysis. In this process, 0.5 ml of extracts (of concentration1000 µg/ml) or quercetin (of different concentrations from 0-500 µg/ml) were mixed with 0.5 ml of distilled water. Then 0.3 ml of 5% sodium nitrite was added and kept at room temperature for 5 minutes after which 0.3 ml of 10 % aqueous solution of aluminum chloride was added. 5 minutes later 2 ml 1 molar sodium hydroxide solution was added and test tubes were shaken before taking the absorbance at 510 nmagainst a selected blank using UV-visible Spectrophotometer [6].

A standard curve of quercetinis prepared at-first as reflected in Figure 2. Flavonoid content of different samples was calculated in quercetinequivalent.

*3.5. Nanocomposite synthesis:*As sample F has highest phenol and flavonoid content we choose it for further study. 100 mg of sample F was dissolved in 20 ml of dimethyl sulfoxide and added to an aqueous solution of silver nitrate (0.1 % w/v) dropwise under constant stirring condition. Stirring was continued for 6 hours resulting in the formation of a clear brown colored solution. Solution was centrifuged at 6000 rpm for 20 minutes. Supernatant was discarded and the pellet so formed was washed with distilled water before collecting it in a petri plate for freeze drying. The

nanocomposite(sample G) synthesized using the turmeric extract was characterized by FTIR and FESEM [7].

3.6.Antibacterialassay: To check the potency of turmeric extracts against selected bacterial strains, 50 mg of each of the lyophilized samples and 20 mg of the synthesized nanocompositewere dissolved in 1 ml of DMSO, DMSO being the standard solvent for each samples. Both well-diffusion and disc-diffusion methodswere used to determine the antibacterial activity against E.coli and S. aureus. DMSOwas used as the control and streptomycin as the standard. Plates are incubated for 24 hours and the zone of inhibition was measured. Then, the mean value of two different methods was taken into account. There was not much significant difference between two values.

4. Results and discussions:

On the first trial of extraction with methanol, both the samples designated as part A and part B showed almost same results of phytochemical analysis. So turmeric powder from part B was chosenhenceforth for the extraction process by different solvents due to its shorter preparation time.

Table 1: Total phenol content expressed in gallic acid equivalent (mg GAE /g of extract)

Sample	Absorbance at 765 nm	TPC (mg GAE / g)
A	0.846	248.79
B	0.913	268.64
C	0.929	273.12
D	1.085	319.18
E	1.132	332.91
F	1.356	398.68

Table 2: Total flavonoid content expressed in quercetin equivalent (mg QE / g of extract)

Sample	Absorbance at 510 nm	TFC (mg QE / g)
A	0.082	164.00
B	0.151	302.00
C	0.087	174.00
D	0.105	210.00
E	0.062	124.00
F	0.148	296.00

4.1. Total phenol content:

From Table 1 it is observed that methanolic extract showed the highest phenol content while benzene extract has the lowest value among others. Total phenol content varied significantly with the solvent used during the extraction process.

4.2. Total flavonoid content:

From Table 2 it is observed that Acetone extract has the highest flavonoid content followed by methanol whereas acetonitrile extract witnessed the least value. Again, the solvent used in the extraction greatly influenced the flavonoid content.

4.3. FTIR analysis of turmeric extract and its nanocomposite:

FTIR result of turmeric extract (sample F) and its comparison with the synthesized nanocomposite predicts the presence of curcumin in both the samples. The peak at 3338.78 $cm^{-1}$of turmeric extract corresponds to the alcohol/phenol O-H bond stretching and it gets shifted to 3523.95 in nanocomposite. Similar instances have been found where the peak values (in cm^{-1}) 2929.87, 1412.54, 1276.88, 1207.44, 1101.35, and 987.55 gets shifted to 2926.01, 1436.97, 1278.81, 1128.36 and 952.84 respectively. These peaks correspond to C-H stretch, aromatic C=C, enol C=O, phenol C=O, enol C-O-C and benzoate trans C-H vibration respectively. Shifting indicates the formation of co-ordination bond between silver atoms and electron rich groups present in the extract and thus claims a probability of their involvement in nanocomposite synthesis.

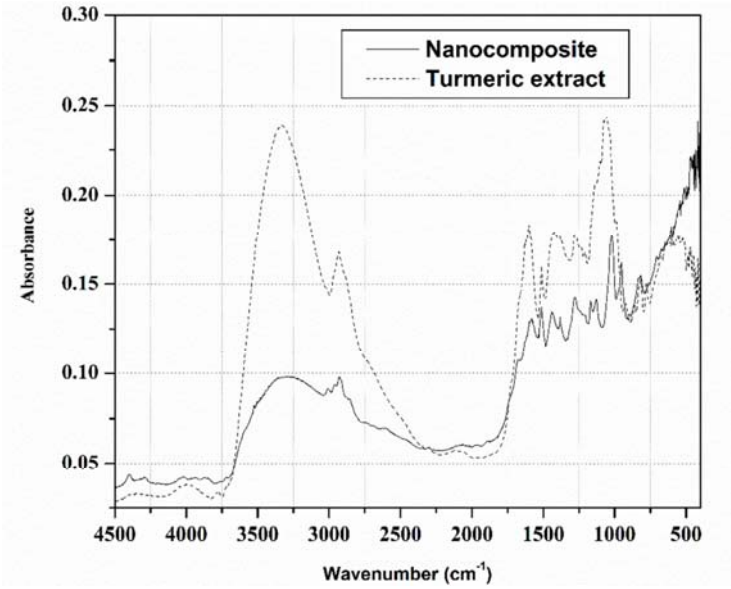

Figure 3: FTIR image of turmeric extract and its nanocomposite

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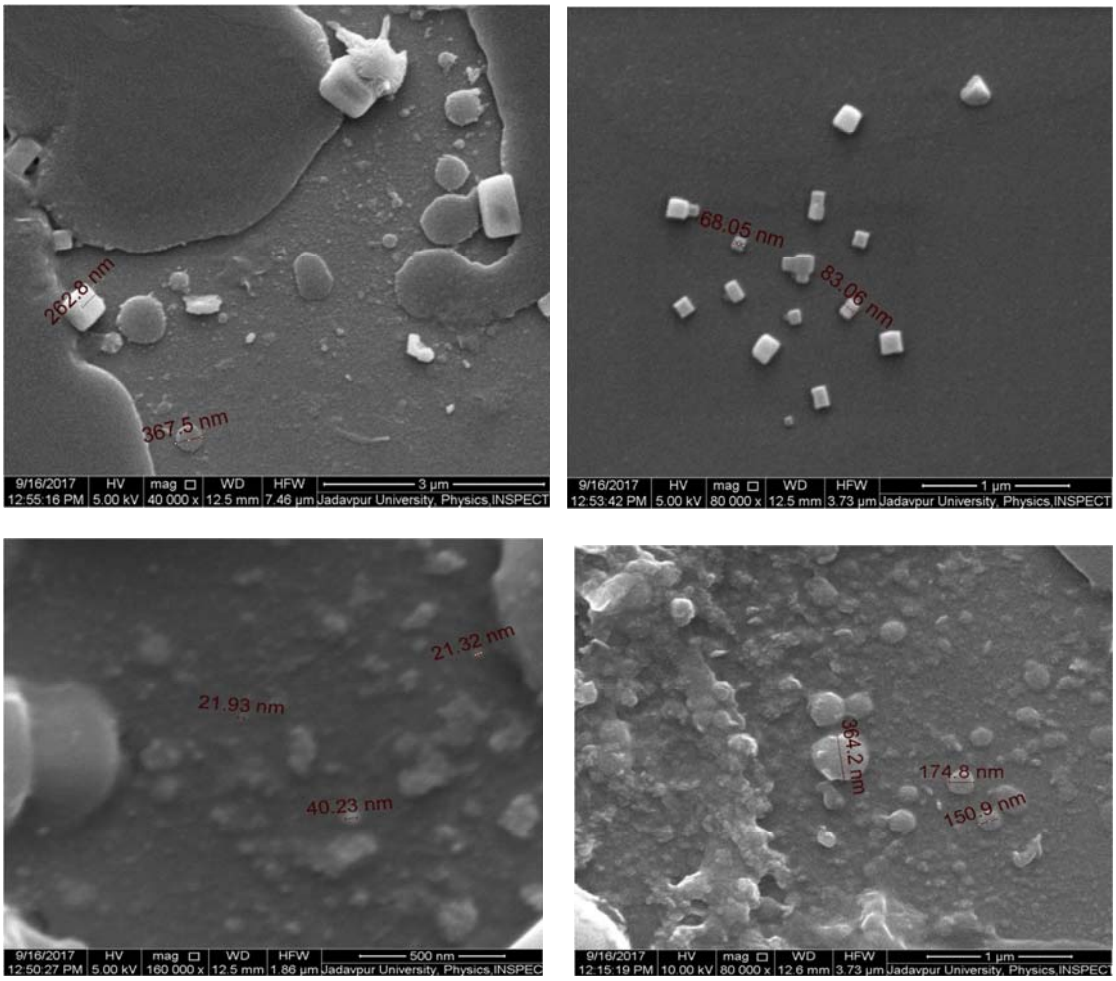

Figure 4: FESEM images of the nanocomposites

4.4. FESEM analysis of the nanocomposite:

The morphology of the nanocomposite was studied by FESEM as reflected in Figure 4. Two types of structure were found- spherical and cubical. Figure 4 shows that the nanoparticles were well dispersed and the particle size varied from 20 nm to 400 nm. Agglomeration is not visible.

4.5. Antibacterial activity:

Methanolic extract showed the highest antibacterial activity while benzene extract had the least potency against the selected strains of bacteria. It was observed that the total content of phenols plus flavonoids directly influenced the % inhibition, i.e. more the value of summation of TPC and TFC, more was the zone of inhibition. The synthesized nanocomposite showed high antibacterial activity even at much lower concentration than the extracts, thus claiming to enhance the activity of the bioactive components present in the extracts.

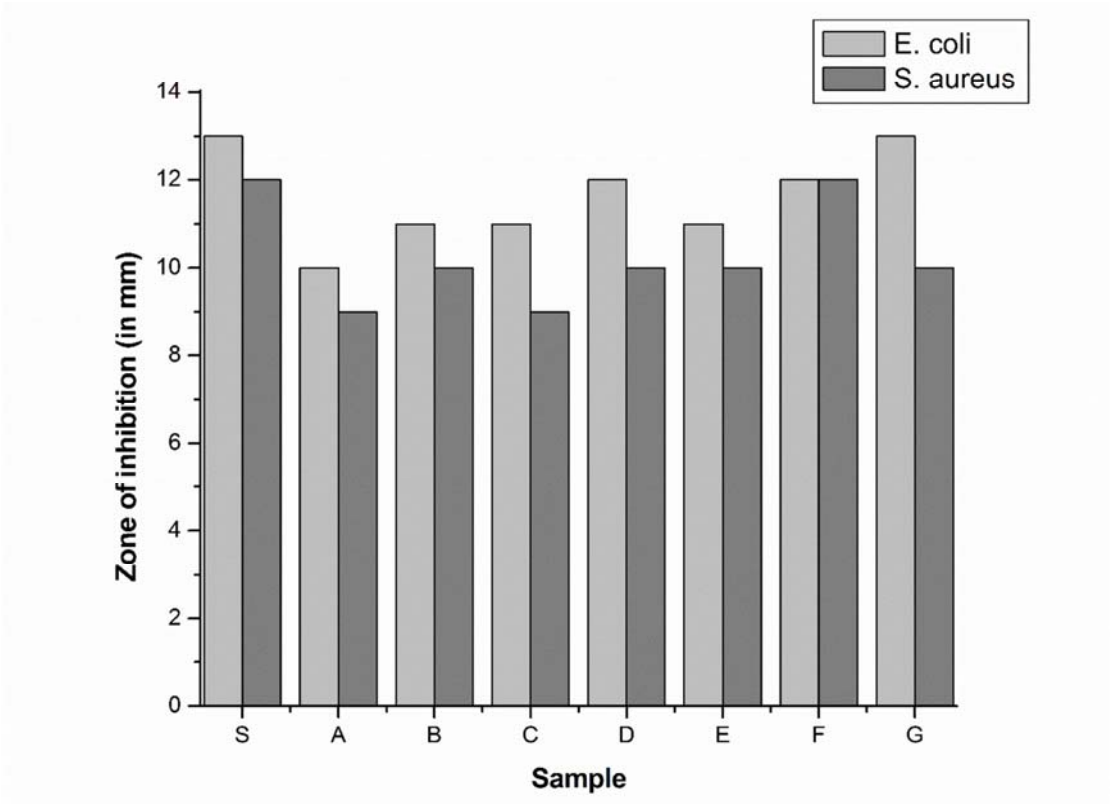

Figure 5: Zone of inhibition of different samples against different strains

5. Conclusion:

Six solvents were used in the extraction process of turmeric. TPC and TFC varied with the solvent used in extraction. The highest and the lowest TPC were observed in methanolic and benzene extract respectively. Acetone extract achieved the highest TFC while acetonitrile extract had the lowest value. Nanocomposite synthesized using turmeric extract and $AgNO_3$ was found to be existed in 2 shapes-cubical and spherical with sizes ranging from 20-400 nm. FTIR analysis confirms the interactions between $AgNO_3$ and turmeric extract in the nanocomposite. The antibacterial activity of different extracts seemed to be directly dependent on the summation of TPC and TFC. Nanocomposite showed a high potency against bacterial strains of E. coli and S. aureus at a much lower concentration than the extracts, thus enhancing the activity of the extracts.

6. References

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