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New HPLC method for separation and collection of Bisdemethoxycurcumin compound from Turmeric plant and study it's biological activity

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I. Abstract

New method was used to separate and collect of curcumin compound by analytical HPLC machine. The result was more economic method as the level of absorbance for the collected peak of Bisdemethoxycurcumin compound was high at low injection volume so the yield was enough to assay the biological activity of the compound. LCMS results supported the success of separation of the compound. The results showed significant inhibitory effects of Bisdemethoxycurcumin on the growth of various pathogenic bacteria, including *Pseudomonas aeruginosa* (14.81), *Staphylococcus aureus* (14.08), *E. coli* (15.15), *Enterococcus faecium* (16.50), *Staphylococcus homolyticus* (17.54), and *Staphylococcus lactis* (17.50). Additionally, Bisdemethoxycurcumin exhibited strong antifungal activity against *Candida albicans* (14.04) and *Candida rusei* (23.38). These findings align with previous studies, which demonstrated that Bisdemethoxycurcumin exerts antibacterial and antifungal properties by disrupting microbial cell structures and vital intracellular components

I. Introduction

Medicinal and aromatic plants have been traditionally used across ancient cultures for therapeutic, nutritional, and cosmetic purposes. Before the advent of synthetic pharmaceuticals, these plants served as the primary means of treating diseases (Inoue et al., 2019). Although their use declined with the rise of the pharmaceutical industry in the early twentieth century, there has been renewed interest in natural remedies due to growing global trends toward "returning to nature," as well as their availability, safety, and cost-effectiveness (Abubakar & Haque, 2020). Additionally, several medicinal plants have shown potential in minimizing the side effects associated with synthetic drugs (Mesfin et al., 2014). Notably, many modern medicines used for chronic conditions, such as cardiovascular diseases and cancer, are derived from plant-based sources (Burt, 2004).

Among these, Curcuma longa (turmeric) stands out as a widely studied and utilized medicinal herb. Belonging to the Zingiberaceae family, turmeric thrives in tropical and subtropical climates and requires moderate temperatures (20–30°C) and sufficient rainfall to grow optimally (Hanif et al., 1997; Moreira et al., 2020). Known as "Kurkum" in Arabic, "Turmeric" in English, and "Haldi" in Hindi, it has long been used in traditional medicine and as a culinary spice in South and Southeast Asia (Zahra et al., 2019).

The rhizomes of turmeric are the most valued part of the plant, containing biologically active compounds such as curcumin—a polyphenol responsible for the bright yellow pigment. Curcumin exhibits significant antioxidant, anti-inflammatory, and antimicrobial properties (Wen Sun et al, 2017; Guimarães et al., 2020). It has been widely studied for its preventive and therapeutic potential against chronic diseases including cancer and cardiovascular disorders (Xu et al., 2011; Takahashi et al., 2014).

Curcumin content varies significantly based on plant variety, geography, and post-harvest processing (Li et al., 2011), highlighting the need for further investigation. Turmeric is commercially available as dried rhizomes or powder and is widely used in both culinary and pharmaceutical applications (Gounder & Lingamallu, 2012; Li et al., 2020). Due to its high starch content, it also supports digestive function and nutrient absorption (Uzomba et al., 2019).

II. Materials and Methods

Preparation the sample of Turmeric roots

The Indian Turmeric roots were cleaned and grinded, then the powder was stored in a sterile and dried dark place. 100 mg of the powder were weighed then in 10 mL test tube, the sample was dissolved in 5 mL of acetonitrile then, to complete dissolution, the dissolved sample was vortexed, sonicated centrifuged for 3 minutes then, 4 mL of the supernatant was withdrawn into 5 mL tube as a stock solution was stored to be ready for HPLC analysis.

Analytical methods

RP−HPLC

In 1.5 mL HPLC vial, 1 mL of the stored sample was added. HPLC analysis was performed by the sykam HPLC S 600, RP−C18E, 4.6 × 250 mm, a 5 µm column (Germany), via 5 uL injection and a flow rate of 0.7 mL min^{-1}. The detector wavelength was set at 254 nm. The column oven was set at 30 °C. Unless stated otherwise, solvent A was 5% H_2O with 95% acetonitrile and add formic acid 0.1% to the solvents.

The fraction containing the purified Bisdemethoxycurcumin compound was lyophilised to remove the HPLC solvents.

The solid sample was then weighed and dissolved in dimethylsulfoxide (DMSO) to prepare a concentration of 10 mg/mL as a stock solution. 1 µL was taken from the stock solution and added to 50 µL of acetonitrile for analysis. Finally, HPLC and LC−MS analyses were performed, and the stock solution was stored at −20 °C

Bioassay of Compounds

Prepared culture media and inoculated the relevant bacteria and fungi.

Used bacterial strains resistant to antibiotics and various fungal species.

Measured the efficacy of the compounds against the bacterial and fungal samples

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Statistical AnalysisTwo-way ANOVA was conducted using the SPSS software for statistical analysis.

III. Results and discussion

Results of HPLC Analysis

Figure 1 represents HPLC chromatogram of crude turmeric sample (Acetonitrile extract). There are three clear peaks with different intensities, without any overlap, between retention time 3 to 10 minutes.

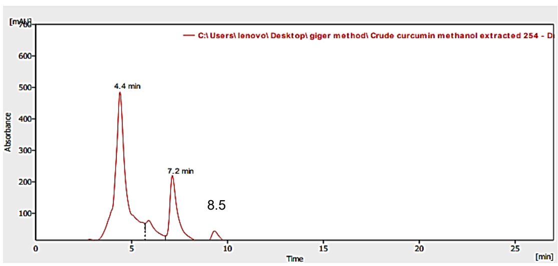

Figure 1: HPLC chromatogram of crude turmeric sample, 254 nm, inject. Vol. 10 µL, flow rate 0.7 mL/min

The first high intensity peak at the retention time 4.4 minutes was collected and checked by HPLC as in Figure 2, the single, high intensity peak at the retention time 8.5 min was consistent to the peak at the same retention time in the Figure 1, this peak could be considered to the target compound (Bisdemethoxtcurcumin) as the LC−MS analysis for the collected peak at the retention time 8.5 min in Figure 1 supported the HPLC result.

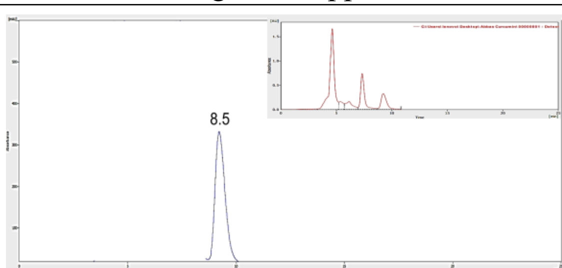

Figure 2: HPLC chromatogram of purified Bisdemethoxycurcumin compound, 254 nm, inject. Vol. 10 µL, flow rate 0.7 mL/min

Mass spectrum clearly showed a peak of 308 *m/z* as in Figure 3,this peak was consistent with the theoretically calculated value of 308 *m/z* for the compound in the -1charge state as in Figure3.

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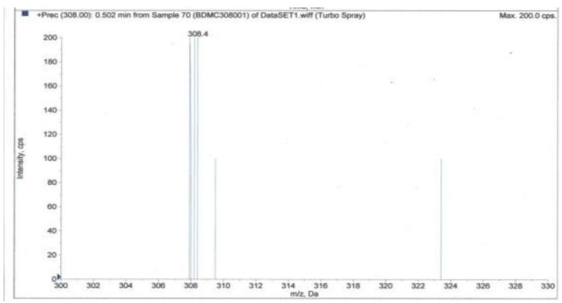

Figure 3: mass spectrum of purified Bisdemthoxycurcumin compound at retention 8.5

Total ion chromatogram (TIC) in the Figure 4 A chromatographic graph displaying the total ion response at each retention time this is illustrated in Figure 4.

Biological Activity of Compound

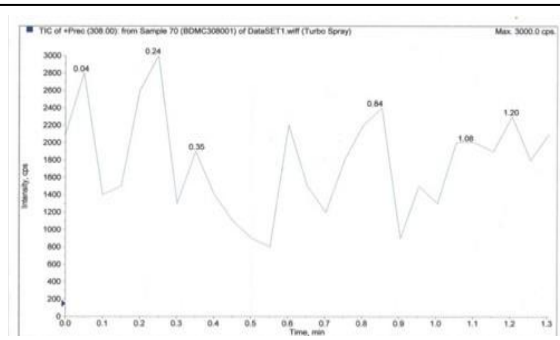

Figure 4: total ion chromatogram of purified Bisdemethoxycurcumin compound

Activity of Compound against Bacteria

The results showed that Compound had a
significant inhibitory effect on the growth of various bacteria, including:

-*Pseudomonas aeruginosa*: with an average effect of 14.81.
- *Staphylococcus aureus*: an average effect of 14.08.
- *E. coli*: significant effect with an average of 15.15.
- *Enterococcus faecium*: with an average effect of 16.50.
- *Staphylococcus homolyticus*: an average effect of 17.54.
- *Staphylococcus lactis*: with an average effect of 17.50.

These results are consistent with studies showing that Bisdemethoxycurcumin possesses effective antibacterial properties against a wide range of pathogenic bacteria[27]. BisdemethoxyCurcumin inhibits bacterial growth by interfering with cell membranes and intracellular proteins, leading to the destruction of bacterial cell structure.[28]

Activity of Compound against Fungi

- Candida albicans: with an average effect of 14.62.
- Candida rusei: with an average effect of 17.38.

Research indicates that Bisdemethoxycurcumin exhibits strong antifungal properties[29], inhibiting fungal growth by interfering with fungal cell walls and other vital components inside the fungal cell.[30]

4-4. Statistical Analysis and Graphical Representation

Statistical results demonstrated that Compound has clear biological efficacy against most tested bacteria and fungi.

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