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DETERMINATION OF PHYTOCHEMICAL PROFILE, ANTIOXIDANT AND ANTIBACTERIAL POTENCY OF CURCUMA LONGA EXTRACTS

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ABSTRACT

To test the antibacterial potential of prepared MeOH extracts of rhizome and leaves, well diffusion method was used as described before (Khurshid *et al*., 2019). Bacterial broth culture was spread evenly on solid agar plates along with making five wells of 6 mm diameter per In present study *Curcuma longa* (*C. longa*), a medicinal plant, reported to have antioxidant, anti-inflammatory, anticancer, antimalarial, insect repellant, antiseptic, analgesic and wound healing properties was selected. Its leaves and rhizomes were used to prepare extracts in methanol (MeOH) aiming to evaluate their antioxidant and antibacterial potential. Our results confirmed that *C. longa* extracts exhibited noticeable antioxidant and antibacterial activities. The antioxidant activity of rhizomes and leaves extracts was IC_{50} = 0.064 ± 0.01 and IC_{50} =0.28 ± 0.04mg/ml, respectively. Both extracts showed concentration dependent and bacteria dependent zones of inhibition (ZoI). The bacterial susceptibility trend at highest tested concentration (120mg/ml) was as *S. enterica>E. coli>S. pyogenes>B. cereus>S. aureus* for rhizome extract and *B. cereus=S. aureus>E. coli>S. enterica> S. pyogenes* for leaves extract. Interestingly, higher ZoI are shown by rhizomes than leaves extract. The phytochemical analysis revealed the presence of important constituents responsible for observed bacterial inhibition proposing *C. longa* rhizome as potent candidates to inhibit bacteria in future.

Keywords: *C. longa,* Rhizome, Extract, Antioxidant, Zone of Inhibition, DPPH.

INTRODUCTION

Botanical extracts are fruitful management tool to fight against pathogens. Plants, being medicinally important are used to treat many infections (Tepe *et al*., 2004). In general, many medicinal plants have antioxidant and antibacterial potential so give protection against cellular oxidation reactions and microbes (Bajpai *et al*., 2005; Mothana and Lindequist, 2005; Wojdylo *et al*., 2007). *Curcuma longa* (*C. longa)* commonly known as Turmeric, is a perennial herb of ginger family that is found in south and southeast tropical Asia. The most beneficial part of this plant is rhizome that is used both for medicinal and culinary purposes (Aggarwal *et al*., 2006). Turmeric rhizomes are usually oblongate, pyriform and short branched (Eigner *et al*., 1999). This medicinal plant, *C. longa* belongs to *Zingiberaceae* family (Chattopadhyay *et al*., 2004). Curcumin is one of the most potent components, responsible for various biological activities of turmeric (Joe *et al*., 2004; Chainani-Wu, 2003). Secondary metabolites present in turmeric include antioxidants, polyphenols and flavonoids. These phytochemicals have antibiotic activities that make them important in food and food products (Chainani–Wu, 2003).

In China, India and South East Asia, turmeric is extensively used as food preservative, spice and coloring agent. A wide range of biological activities are shown by isolated curcuminoids and sesquiterpenes of turmeric roots (Tilak *et al*., 2004; Kumar *et al*., 2006). Various pharmacological activities of turmeric are reported that

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include anti-inflammatory, antidiabetic, anticancer, wound healing (Maheshwari *et al*., 2006; Gupta *et al*., 2011; Moghadamtousi *et al*., 2014), antiplatelet, cholesterol lowering, antifungal (Luthra *et al*., 2001; Martins *et al*., 2008; Sharma *et al*., 2010), antiprotozoal (Arau´jo *et al*., 2001; Rasmussen *et al*., 2000), antiretroviral (Mazumber *et al*., 1995), nematocidal (Kiuchi *et al*., 1993), burn wound healing (Kulac *et al*., 2012), anticoagulant, antifibrotic and antivenom, antiulcer (Chattopadhyay *et al*., 2004). Its use is common in Indian traditional medicines, anorexia, biliary disorders, cough, hepatic disorders, sinusitis, rheumatism, diabetic wounds, inflammation, reducing blood cholesterol (Aggarwal *et al*., 2006), common cold, jaundice, arthritis and inflammatory bowel conditions (Ammon and Wahl, 1991). A very prominent characteristic of turmeric is its strong antioxidant activity and free radical scavenging potential which facilitates colon health along with neuroprotective activity and also maintains a healthy cardiovascular system (Luthra *et al*., 2001; Nagarajan *et al*., 2010). In animals and human trials, curcuminoids showed no toxicity even at high doses therefore turmeric is proven safe ingredient for use in medicines and cosmetics (Goel *et al*., 2008).

Increasing bacterial resistance to available commercial antibiotics (Chattopadhyay *et al*., 2004) and side effects in children (Khotaei *et al*., 2008) and adults (Lin *et al*., 2009) has initiated to investigate the antibacterial and antioxidant components in turmeric. To this end, MeOH extracts of *C. longa* rhizomes and leaves were prepared and evaluated their antibacterial activity against selected pathogenic bacteria and antioxidant activity by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay along with phytochemicals of turmeric rhizomes MeOH extract.

MATERIAL AND METHODS

Extract preparation: Fresh leaves and dried rhizomes of turmeric were collected from Mirpur, AJK. After essential processing both samples were pulverized separately. Powdered samples of turmeric leaf and rhizomes were soaked in 1mg:10ml methanol (MeOH) separately for ten days with daily agitation. Its filtrate was evaporated by using rotary evaporator as reported previously (Sharif *et al*., 2021). Semi solid extracts were air dried before use. These extracts were used to prepare stocks in MeOH that was further diluted with broth to get required concentrations of extracts.

Bacterial culture: Bacteria used in this study were consisted of two gram negative and three gram positive bacterial strains i.e. *Escherichia coli* (*E. coli*), ATCC 8739, *Salmonella enterica* (*S. enterica*), *Streptococcus pyogenes* (*S. pyogenes*) ATCC 12384, *Staphylococcus aureus* (*S. aureus*) ATCC 2592 and *Bacillus cereus* (*B. aureus*) ATCC 10876. Same culture conditions were used for all bacteria throughout their susceptibility testing against selected botanical extracts.

Chemicals: Analytical methanol (Sigma Aldrich), DPPH (Sigma Aldrich), ascorbic acid, Nutrient agar (OXOID CM0003, UK) and Nutrient broth (Merck, Germany) were used in this study. Then, 50 µl test extract of 120, 60, 30, 15 and 0mg/ml (control) were loaded in wells and incubated at 37°C. After 24hrs incubation, zones of inhibition (ZoI) were measured in centimeter (cm).

Antioxidant assay: For antioxidant activity, DPPH assay was done by following protocol previously described (Kanwal *et al*, 2015). Briefly, by dissolving 24 mg DPPH per 100 ml of MeOH, stock solution was prepared. For working solution, DPPH was diluted with MeOH to obtain absorbance of 0.98 ± 0.02 at 490_{nm} wavelength (BioTek Lx800). This DPPH solution was mixed with test extract and incubated for 10min in dark. Later, the sample was read at 490_{nm} wavelength to note absorbance required for IC_{50} calculation.

Phytochemical analysis: Following Priya and Chellaram (2014) phytochemical analysis for Saponins, Alkaloids, Tannins, Quinones, Glycosides, Cardiac glycosides, Terpenoids, Steriods and Flavonoids was performed.

STATISTICAL ANALYSIS

All experimentations were done twice in triplets.

RESULTS AND DISCUSSION

In this study, rhizome and leaves MeOH extracts of *C. longa* were prepared to evaluate their antibacterial activity. Later, antioxidant potential and phytochemical analysis was performed to evaluate the constituent present in most active extract.

Antibacterial activity: Rhizome MeOH extract at tested concentrations; 120, 60, 30 and 15mg/ml showed ZoI as 0.27, 0.14, 0.09, 0.07cm against *B. cereus*, 0.22, 0.11, 0.09, 0.08cm against *S. aureus*, 0.38, 0.047, 0.04, 0.036cm against *S. enterica*, 0.37, 0.13, 0.12, 0.1cm against *E. coli* and 0.3, 0.11, 0.07, 0.06cm against *S. pyogenes*, respectively after 24 hrs of incubation (Figure 1). However, ZoI by leaves MeOH extract at 120, 60, 30 and 15mg/ml concentrations were measured as 0.22, 0.09, 0.03, 0cm against *B. cereus* and 0.22, 0.14, 0.06, 0.037cm against *S. aureus* (Figure 2). In addition, ZoI by leaves MeOH extract at 120 and 60mg/ml showed 0.14 and

0.07cm, against *S. enterica*, 0.15 and 0.03cm against *E. coli* and 0.13 and 0.008cm against *S. pyogenes*, respectively however, no ZoI were observed at 30 and 15mg/ml concentrations (Figure 2). Thus, trend for bacterial susceptibility at highest tested concentration (120mg/ml) was as: *S. enterica>E. coli>S. pyogenes>B. cereus>S. aureus* for rhizome extract and *B. cereus=S. aureus>E. coli>S. enterica>S. pyogenes* for leaves extract, respectively (Table 1). Moreover, rhizome MeOH extract presented higher ZoI than leaves MeOH extract. These results depicted that *S. aureus* and *B. cereus* were most susceptible against turmeric leaf MeOH extract, these results are in accordance with Gul and Bakht, 2015; Sayeed *et al*., 2014; Wang *et al*., 2009 that microcapsule curcumin had more potency against *S . aureus.* Due to presence of a phenolic compound, curcuminoids; turmeric has effective antibacterial potential against *E.coli, B. subtills* and *S. aureus*. Moreover, antibacterial activity of turmeric is associated with the presence of an alkaloid, curcumins, veleric acid and turmerol. Curcumin, the coloring principle of turmeric has yellow color and is a vital constituent of turmeric plant (Ammon *et al*., 1992). Turmeric's powder is reported beneficial against diabetic wounds, inflammation, anorexia, coryza, cough, sinusitis, rheumatism and gastrointestinal diseases, especially for hepatic and biliary disorders (Ammon *et al*., 1992). Many activities of turmeric are stated in conventional literature such as antioxidant, anti-inflammatory, anticancer, antimalarial, insect repellant, antiseptic, analgesic and wound healing activities (Araujo and Leon, 2001). Bioactive compounds like antioxidants, polyphenols and flavonoids can be acquired from turmeric promptly, so it may be the substitute of antibiotics used in food and food products (Chainani, 2003).

Table 1. Bacterial susceptibility trend

Extract Type	Bacterial susceptibility trend
C. longa leaves	*B. cereus=S. aureus>E. coli>S. enterica>S. pyogenes*
C. longa rhizomes	*S. enterica>E. coli>S. pyogenes>B. cereus>S. aureus*

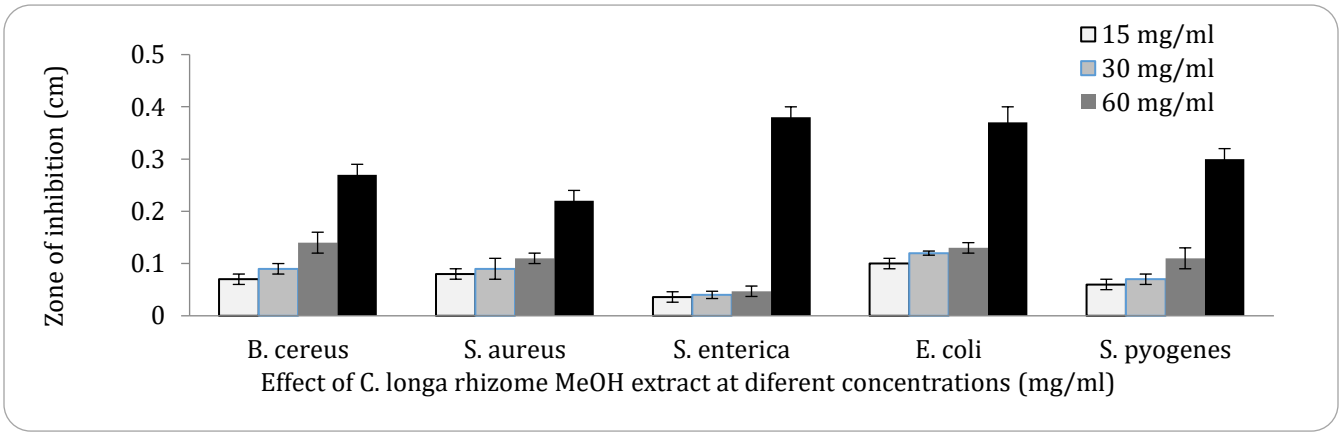

Figure 1. Bacterial susceptibility against MeOH rhizome extract of *C. longa.*

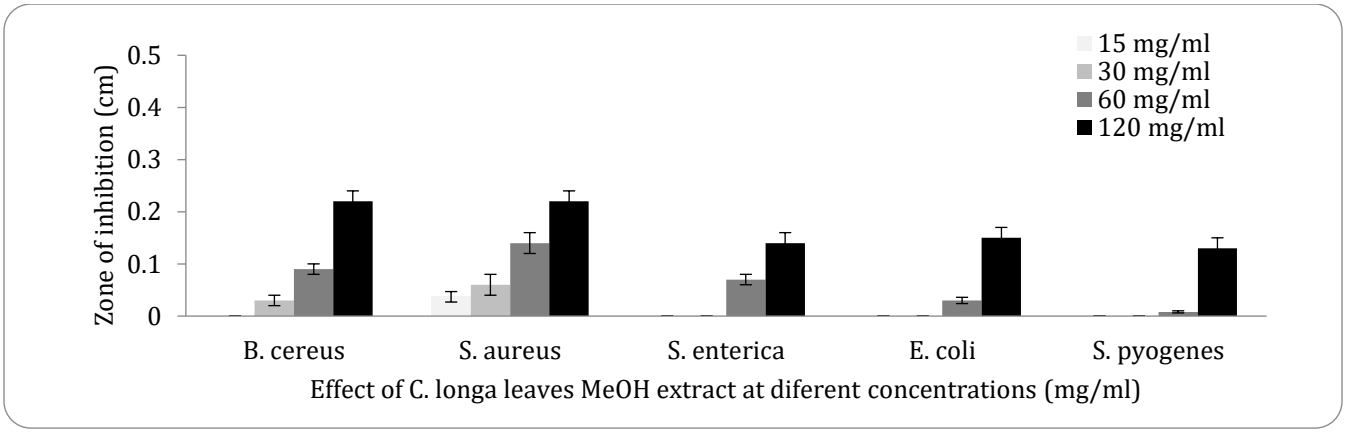

Figure 2. Bacterial susceptibility against MeOH leaves extract of *C. longa.*

Antioxidant potential of test extracts: Turmeric antioxidant activity was evaluated by performing DPPH assay. The IC_{50} values recorded for *C. longa* rhizome and leaves were 0.064 ± 0.01 and 0.28 ± 0.04mg/ml, respectively (Table 2). The antioxidant potential of rhizome extract is higher than leaves. According to Nagarajan *et al.,* the most important property of turmeric is its free radical scavenging potential and antioxidant activity (Nagarajan *et al.,* 2010).

Table 2. Antioxidant activity of *C. longa* rhizome and leaves MeOH extracts.

C. longa extract type	DPPH inhibition IC_{50} ± SEM (mg/ml)
Rhizome	0.064 ± 0.01
Leaves	0.28 ± 0.04
Ascorbic acid	0.003±0.0001

Phytochemical analysis: Phytochemical analysis of rhizome extract (most potent extract) showed the presence of flavonoids, tannins, alkaloids, terpenoids, cardiac glycosides, quinone and steroids (Table 3).

Table 3. Qualitative phytochemical analysis of *C. longa* rhizome MeOH extract

Tested phytochemicals	Status
Saponins	-
Alkaloid	+
Tannins	+
Quinone	+
Glycosides	-
Cardiac glycosides	+
Terpenoids	+
Steroids	+
Flavonoids	+

+ (Present), - (Absent)

CONCLUSION

In current study, *C. longa* leaves and rhizome MeOH extracts were prepared. Both extracts presented noticeable antioxidant and antibacterial activities. Bacterial inhibitory trend was as: *S. enterica, E. coli, S. pyogenes, B. cereus, S. aureus* for rhizome extract and *B. cereus, S. aureus, E. coli, S. enterica, S. pyogenes* for leaf extract. Additionally, rhizome MeOH extract showed higher ZoI than leaf. Interestingly, antioxidant activity of rhizome was also higher than leaves. Phytochemical analysis revealed the presence of important constituents responsible for observed bacterial inhibition. Thus, results of the present investigation support the use of *C. longa* extracts against a wide range of pathogenic bacteria.

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CONFLICT OF INTEREST

No conflict of interest is expressed.

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Muhammad Rafiq	:	Data Analysis and Proofread
Zahid Hassan Tarar	:	Research Designed , Sampling and Proofread