Antimicrobial activity and phytochemical screening of leaves and rhizome extracts of Turmeric (*Curcuma longa.*L)

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Abstract:

The present study aimed to investigate the *in vitro* antimicrobial activity and phytochemical screening of rhizome and leaves extracts of Turmeric (*Curcuma longa.*L) plant. *Curcuma longa* rhizome and leaves extracts were prepared using sequential extraction method by petroleum ether (PE) and methanol: chloroform (M:C) (1:1) solvents. Antimicrobial activity was investigated against four standard bacteria (*Bacillussubtilus, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli*) and two fungi (*Aspergillusniger* and *Candida albicans*) using paper disc diffusion method. Phytochemical examinations were implemented for all plant extracts using different standard methods. The results revealed that, M:C extracts of *Curcuma longa* rhizome and leaves showed the presence of flavonoids, phenols, steroids and terpenoids, whereas PE extracts of rhizome and leaves showed the presence of steroids and terpenoids only. Highest antimicrobial

activity was measured against *S. aureus* by PE extract of rhizome, while PE extract of leaves showed moderate antimicrobial activity against *B.subtillus*. At the same time, M:C extract of rhizome recorded strongest antibacterial activity against *B.subtillus*, whereas the highest effect against *E.coli* was recorded by M:C extract of leaves. PE extract of rhizome at 20 and 30 mg/ml showed antibacterial activity against *S. aureus* more than that of Ampicillin/Sulbactam 20 µg/disc, Ciprofloxacin 5 µg/disc and Gintamicin10 µg /disc whereas, M:C extract of leaves at 30mg/ml displayed higher antibacterial activity than that of Gintamicin10 µg /disc against *E.coli*. In conclusion, PE and M:C extracts of *Curcuma longa* rhizome and leaves could be exploited as a good source and alternative natural antibacterial agents especially against *S. aureus, B. subtillus*and *E.coli*.

Key words: *Curcuma longa*, leaves and rhizome, antimicrobial activity, phytochemical.

1. Introduction:

Curcuma longa.L (Turmeric) is a perennial herb, belongs to the Zingiberaceae family. *Curcuma longa* is widely used around the world as a spice, a coloring material and food preservatives. Its rhizome has been reported to possess a variety of medicinal properties such as anticancer, antimicrobial, antioxidant, anti-inflammatory and anti-parasitic properties (Sarker and Narhar, 2007, Hatcher, *et al.* 2008). Furthermore, external applications of turmeric could stop swelling and pain, heal wounds rapidly, and treat many kinds of skin diseases like acne and leprosy (Biswas, 2003). Also, it was reported that there are many therapeutic properties and medicinal values for the oil of *Curcuma longa* leaves including its good

antimicrobial activity (Apisariyakulet al., 1995; Tripathi et al., 2002). The major components responsible for the biological activity of *Curcuma longa* are phenols, such as curcuminoid and essential oils (Burke, 1994 andNeghetini, 2006). Phenolic compounds can destroy bacterial cell walls and can penetrate microbial cell affecting its metabolism (Marino *et al*., 2001). Curcumin has wide-spectrum antimicrobial activity including antibacterial, antifungal, antiviral, and antimalarial activities (Moghadamtousi *et al*., 2014). Many workers studied the biological activities and phytochemical of leaves and rhizome of Turmeric (*Curcuma longa*.L), but the comparing study of the biological activities and phytochemical screening between the leaves and rhizome extract of Turmeric (*Curcuma longa*.L) are not yet available in Sudan. This study was undertaken to investigate the antimicrobial activity and phytochemical screening of leaves and rhizome extracts of *Curcuma longa.* L.

2. Materials and methods

2.1. Source of turmeric *(Curcuma longa),* microorganisms and reference drugs:

The fresh rhizomes of *Curcuma longa* were purchased from the local market of Khartoum city. *Curcuma longa* rhizomes containing buds were cultured in the botanical garden of Faculty of Science and Technology, Al-neelain University for four months to obtain the mature *Curcuma longa* leaves.

The standard microorganisms tested in this study were: *Bacillus subtilus(*NCTC *8236* G^{+Ve}*), Staphyllo coccus aureus(*ATCC *25923* G^{+v}*), Escherichia coli (*ATCC *25922* G^{-V}*), Pseudomonas aeruginosa (*ATTC

*27853 G^{-V}), Aspergillus niger(*ATCC *9763)* and *Candida albicans (*ATCC*7596).*

The tested microorganisms were obtained from the Microbiology Department, University of Khartoum, Sudan.

Reference drugs were Ampicillin/Sulbactam 20 µg/disc, Ciprofloxacin 5 µg/disc and Gintamicin 10 µg/disc from Himedia.

2.2. Preparation of plant crude extracts:

The dried *Curcuma longa* rhizome and leaves were cleaned and ground into a fine powder using a mortar and pestle. Thirty grams of the fine powder was soaked in petroleum ether 500 ml for three days at room temperature (27 ± 3 °C). The fine powder was re-extracted at the same conditions by methanol: chloroform (1:1). The obtained extracts were filtered by filter paper No.1 and evaporated at room temperature to get a dried product, and then the product was stored in dried bottles at 4°C.

2.3. Preparation of bacterial suspensions:

Bacterial suspensions were prepared according to the method described by Miles and Misra(1938).

2.4. Preparation of fungal suspensions:

The fungal cultures were maintained on agar (Sabouraud dextrose) and incubated for four days at 25 °C. The growth of fungal was harvested, then washed with sterile normal saline and finally suspended in 100ml of sterile normal saline, and the suspensions were stored in the refrigerator until used.

2.5. *In vitro* antimicrobial activity test for plant extracts:

2.5.1. Antibacterial activity test:

The paper disc diffusion method of Kil*et al.*,(2009) with minor modifications was adopted to evaluate the antibacterial activity of plant extracts. Twenty ml aliquots of the molten nutrient agar were distributed into sterile Petri dishes. About 0.1 ml of the bacterial stock suspension 10^8 –10^9 C.F.U/ ml were streaked using a sterile cotton swab on nutrient agar medium plates. Discs of sterilized filter paper (6 mm diameter) were soaked in the prepared extracts, and placed on the surface of test bacteria plates. The plates were incubated for 24 h, and the inhibition zones diameters were measured. Reference drugs and %10 DMSO were used respectively as positive and negative controls. After the incubation period, the diameters of the resultant growth inhibition zone were measured. Mean and standard error values were tabulated.

2.5.2. Antifungal activity test:

The previous method for the bacterial test was used. Instead of nutrient agar, Sabouraud dextrose agar was employed. The inoculated medium was incubated at 25 °C for two days for C. albicans and three days for A. niger.

2.6. Phytochemical screening:

Phytochemical examinations were carried out for all extracts to detect the presence of different secondary metabolites such as alkaloids, phenolic compounds, tannins, flavonoids, saponins, steroids and terpenoid by using different standard methods with some modifications. The methods described by Evans, (1997); Kokate (1999); Wolfe et al.,(2003); Peach and Tracey

(1956); Kokate (1994); Harborne (1992), respectively for the mentioned secondary metabolites.

3. Results and Discussion

3.1. Phytochemical screening:

Phytochemical screening of methanol: chloroform (1:1) and petroleum ether extracts of rhizome and leaves of *Curcuma longa* showed presence different plant secondary metabolites (Table 1).

Table 1. Screening for secondary metabolites of rhizome and leaves extracts of *C. longa.*

Key: [+] Positive test, [-] Negative test, *'+++' high; '++' moderate; '+' low*

ME:CH= methanol: chloroform extract, PE= Petroleum ether extract.

Test	Reagent	ME:CH rhizome	ME:CH leaves	PE rhizome	PE leaves	Observation
Alkaloids	Mayer's	_	_	_	_	White creamy precipitate
	Dragendorff's	_	_	_	_	yellow precipitate
Saponins	H_2O	_	_	_	_	Persistent foam
Tannins	Gelatin	_	_	_	_	White precipitate
	$Fecl_3$	_	_	_	_	bluish black colour
	NaOH	+++	+	_	_	yellow colour
Flavonoids	Lead acetate	++	+	_	_	yellow precipitate
Phenolic compounds	$FeCl_3$	+++	+	_	_	bluish black colour
Steroids and Terpenoids	Salkowski's test	++	+	++	+	reddish brown colour ring

Methanol: chloroform extract of *Curcuma longa* rhizome and leaves showed the presence of flavonoids, phenols, steroids and terpenoids. Petroleum ether extracts of rhizome and leaves showed the presence of steroids and terpenoids only. Sawant and Godghate,(2013) and Chairman*et al*.,(2015) revealed that in their results the presence of flavanoids, phenols, steroids and terpenoids in alcoholic extracts of *Curcuma longa.* These results agree with the results obtained in the present study.

The presence of plant constituents in the extract is depending on the polarity of the solvent used for extraction. The mixture of methanol: chloroform (1:1) revealed the presence of polar and non-polar compound in *Curcuma longa* rhizome and leaves. Methanol is higher polar solvent than chloroform and petroleum ether. Therefore the high polar compounds could be extracted by methanol solvent, whereas chloroform can extract non-polar or less polar components. Secondary metabolites present in medicinal plants are responsible for curing various diseases. *Curcuma longa is* rich of biologically active compounds and could be a good natural source of potent and chemotherapeutic agent.

3.2. Antimicrobial activity:

Table 2 revealed the antimicrobial activity of *Curcuma longa* rhizome and leaves extracts against standard microorganisms. Three concentrations (10, 20 and 30 mg/ml) of extracts were prepared to detect their effect against studied microorganisms. Highest antimicrobial activity was recorded by the highest concentration 30 mg/ml in all extracts.

Table 2 Antimicrobial activity of *Curcuma longa rhizome* and leaves extracts against standard microorganisms.

B.s= Bacillus subtillus, S. a= Staphyllococcusaureus, E. c= Escherichia coli,

Ps. a =Pseudomonas aeruginosa. As.n = Aspergillusniger, C. a = Candida albicans. (-) = Not effective

Petroleum ether extract of rhizome displayed antimicrobial activity against all microorganisms studied except *A. niger*. The highest antimicrobial activity of petroleum ether extract of rhizome was measured against *S. aureus* with a maximum zone of inhibition (24 mm) compared to other extracts (Fig.1).

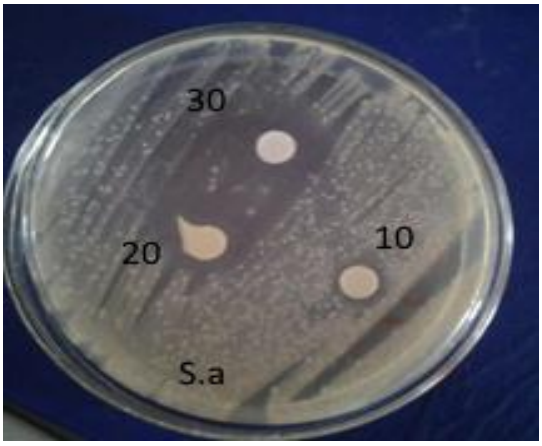

Fig.(1) * Inhibition zone (I.Z) by petroleum ether extract of *Curcuma longa* rhizome against *Staphyllococcus aureus.*

These results agree with the results of Shawket,(2013) who investigated the antibacterial effects of essential oil of rhizome of *Curcuma longa* at different concentrations(100% ,50% ,33.3% and 25%) against twenty

bacterial isolates, ten *staphylococcus aureus* and ten *staphylococcus epidermidis* by using both disc diffusion and well diffusion assays. Their results revealed that the concentrated oil (100%) had strongest inhibitory effect against the isolates among different concentrations of oil used. It could inhibit the growth of all isolates of *S. aureus* by using both methods used, while all *S. epidermidis* isolates were inhibited by well diffusion method only. Also Gupta *et al*.,(2015) who investigated the *in vitro* antimicrobial activity of different fractions prepared from rhizome of *Curcuma longa* against standard strain and clinical isolates of *Staphylococcus aureus*, they found that, the clinical isolates of *Staphylococcus aureus* were more sensitive for different fractions of *Curcuma longa* rhizome than the standard strain of *S. aureus*.

Methanol: chloroform extract of rhizome recorded strongest antibacterial activity against *Bacillus subtillus*with maximum zone of inhibition (20 mm) (Fig.2). Lawhavinit*et al*.,(2010) found that the ethanol and hexane extracts of *Curcuma long* have inhibitory effects against 13 tested bacteria, *B. subtilis*was one of them.

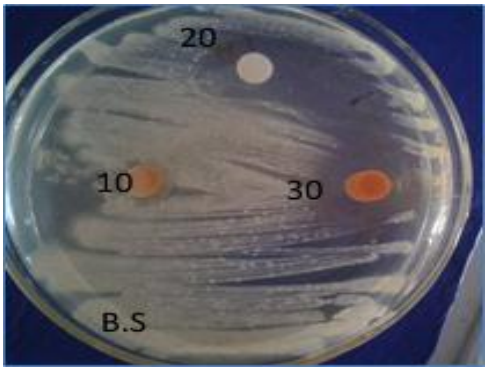

Fig. (2): * I.Z. by methanol: chloroform(1:1) extracts of Curcuma rhizome against *Bacillus subtillus.*

The effect of methanol: chloroform extract of the rhizome as an antifungal agent was only observed against *candida albicans* with a zone of inhibition (14 mm) (Fig.3).

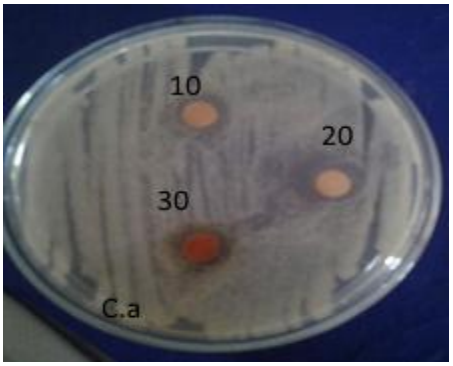

Fig.(3) * I.Z. by methanol: chloroform(1:1) extracts of Curcuma rhizome against candida *albicans.*

Petroleum ether extract of leaves showed moderate antimicrobial activity against *Bacillus subtillus*with zone of inhibition (16 mm) (Fig.4). Its effect on the other microorganisms was weak.

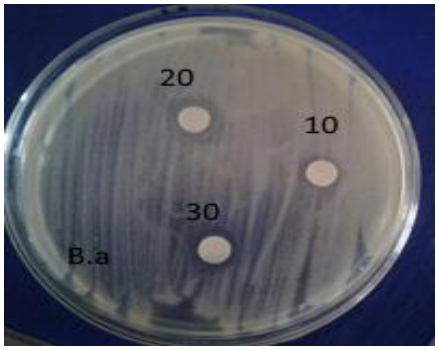

Fig. (4) * I.Z. by petroleum ether extract of Curcuma leaves Bacillus *subtillus.*

This results could be in harmony with that reported by Saifur*et al*.,(2014) who examined the antibacterial activity of *Curcuma longa* leaves against six *Bacillus species*. Their findings revealed that the ethanolic extract of

Curcuma longa leaves could be considered as potentially effective antibacterial agents against tested *Bacillus species*.

Methanol: chloroform extract of leaves was effect against all bacteria and fungi studied except *Pseudomonas aeruginosa which* was resistance for methanol: chloroform extract of leaves. Highest effect against *Escherichiacoli* was recorded by methanol: chloroform extract of leaves with a maximum zone of inhibition (19 mm) (Fig.5) when compared to another extract. Previous studies recorded the antibacterial activity of *Curcuma longa* against *E.coli* as that reported by Selvam *et al*.,(2012)**;** **(**Thakur *et al*.,2013) and (Gul and Bakht, 2015).

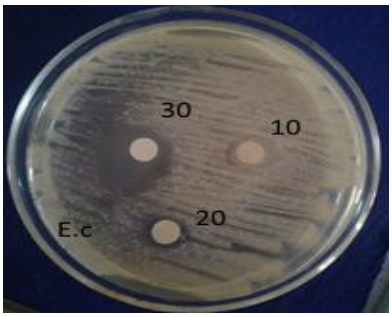

Fig.(5) * I.Z. by methanol: chloroform(1:1) extracts of Curcuma leaves against *Escherichia coli.*

Table 3 represents the antibacterial activity of reference drugs against standard bacteria examined.

Table 3 Antibacterial activity of reference drugs (antibiotics) against standard bacteria.

Drug	M. D. I. Z .(mm)**			
	B.s	*S. a*	*E. c*	*Ps. a*
Ampicillin/Sulbactam 20 µg /disc	20.0	11.0	20.0	_
Ciprofloxacin 5 µg /disc	20.0	10.0	33.0	35.0
Gintamicin 10 µg /disc	25.0	17.0	17.0	8.0
***Control**	_	_	_	_

**M. D. I .Z . (mm); Mean diameter of growth inhibition zone(mm).

* Control ;10% Dimethyl sulphoxid in D.W.

The comparison of results given in table 2&3 showed that the petroleum ether extract of *Curcuma longa* rhizome at 20 and 30 mg/ml showed activity higher than Ampicillin/Sulbactam 20 µg/disc, Ciprofloxacin 5 µg/disc and Gintamicin10 µg /disc against *S. aureus*, also all *Curcuma longa* extracts used at 20 and 30 mg/ml (except methanolic extract of leaves) showed activity more than that recorded by Gintamicin10 µg /disc against against *Pseudomonas aeruginosa*. Methanol: chloroform extract of *Curcuma longa* rhizome at 30mg/ml showed activity equal to Ampicillin/Sulbactam 20 µg/disc and Ciprofloxacin 5 µg /disc against *Bacillus subtillus*, whereas methanol: chloroform extract of *Curcuma longa* leaves at 30mg/ml showed activity higher than that of Gintamicin10 µg /disc against *Escherichia coli.*

In conclusion, the results of the present study suggested that, petroleum ether and methanol: chloroform extracts of *C. longa* rhizome and leaves could be exploited as a good source and alternative natural antibacterial agents

especially against *S. aureus, B. subtillus* and *E.coli*. This antibacterial activity is attributed to the presence of active secondary metabolites detected by phytochemical examination.

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