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Comparative study of Phytochemical Screening and Antibacterial Activity of *Curcuma longa* (L.) and *Curcuma aromatica* (Salib.)

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Abstract

Curcuma longa (L.) and *Curcuma aromatica* (Salib.) are medicinal plants belongs to family Zingiberaceae family. *Curcuma aromatica* (Salib.) and *Curcuma longa* (L.) are rich in phytochemicals. They show the presence of alkaloids, flavonoids, tannins and terpenoids. Due to presence of these phytochemicals *Curcuma longa* (L.) and *Curcuma aromatica* (Salib.) shows antibacterial activity. The Ethanolic and Methanolic extracts of *Curcuma longa* (L.) and *Curcuma aromatica* (Salib.) were subjected to microbial susceptibility test using the agar well diffusion against *Escherichia coli*. Methanolic extract of *Curcuma aromatica* (Salib.) shows inhibition zone of 7.5mm at highest concentration of 20mg.

Keywords: *Curcuma longa* (L.), *Curcuma aromatica* (Salib.) *Escherichia coli*, Secondary Metabolites, Antibacterial properties

Introduction

Herbal extracts used as medicine are now presently being used as a replacement for synthetic drugs. Plants play the significant role in remedy and a large number of drugs which are used the derivatives from plants. Turmeric is used for many medicinal purposes in India in Ayurvedic, Unani and Siddha medicines. Turmeric is known a golden spice of India. Turmeric belongs to the Zingiberaceae or ginger family. *Curcuma longa* L. is moderately tall and perennial plant which have underground rhizomes and those rhizomes are mostly pyriform, ovate, oblong and short-branched plant. It is act as a scavenger of oxygen free radicals. It helps to protect the oxidations of haemoglobin. It will help to destroy the growth and activity of cancer cell and helps to cure prostate and breast cancer. *Curcuma aromatica* (Salib.) is commonly known as Amba haldar. It is used to relieve hiccups in infusion and the most common recipe for it is a pickle. It is used as a base for some perfumes. Despite low concentration of cucumin, volatile oil from wild turmeric exhibit anti-inflammatory property and wound healing property. It is used as cream for its healing property. It also help in quickly healing sprains, bruise and skin irritations. It has anti-bacterial action against pathogens causing infections in the body. It is highly used in curing pimples and dark spots. The present study included phytochemical screening and antibacterial activity of ethanolic and methanolic extracts of *Curcuma longa* L. and *Curcuma aromatica* (Salib.).

Materials and Methods

Collection and drying of plant material: Collecting the rhizome of *Curcuma longa* (L.) and *Curcuma aromatica* (Salib.) from Deesa dist. and air dried for 10days.

Extract preparation method: Four conical flasks were selected and weighed powder transformed into these conical flasks. Solvent methanol and ethanol were added. Cover the flask with aluminum foil. These all sets were kept on shaker for 24 hrs. After that all extracts separately filtered with help of what man filter no.1. After filtration, transfer it into Petri plates and allow it open for 24 hours for solvent evaporation. After 24 hours all the extracts were ready.

Preparation of liquid extract series for anti microbial test: Crude extract were weighed with help of weighing balance (mg) and solvent were added in appropriate proportion i.e, 5 mg

extract/ 2 ml solvent, 10 mg/2 ml solvent, 15 mg/2 ml solvent, 20 mg/2 ml solvent. Here two solvents namely Ethanol and Methanol were used for liquid extract preparation.

Qualitative analysis of secondary metabolites

A. Alkaloids

3 mg extract were dissolved individually in 3 ml ethanol and 1 N HCL was added then filtered it with whatmann filter no. 1. The filtrates were used to test the presence of Alkaloids.

Mayer's test: 1 ml filtrate was treated with 2 ml Mayer's reagent; cream colour precipitation indicates the presence of alkaloids.

Wagner's test: 1 ml filtrate was treated with Wagner's reagent; reddish brown colour indicates the presence of alkaloids

Dragendroff's test: 1ml filtrate was treated with 2 ml Dragendroff's reagent; orange red colour precipitation indicates the presence of alkaloids.

B. Flavonoids

Lead acetate test: 1 ml liquid extracted was treated with 10 % lead acetate soln; formation of yellow precipitation indicates the presence of flavonoids.

H₂SO₄ test: 1 ml extract was treated with few drops of H₂SO₄; orange colour precipitation indicate the presence of flavonoids.

Alkaline reagent test: 1 ml extract was treated with few drops of dil. NaOH and few drops of dil. HCL; yellow colour turns in to color less soln. indicate the presence of flavonoids.

Zinc hydrochloride reduction test: 1 ml extract was treated with zinc dust and conc. HCL; formation of red color indicate the presence of flavonoids

Pew test: 1 ml of extract was treated with pieces of metallic magnesium and 2-3 drops conc. HCl were added; formation of brownish colour indicate the presence of flavonoids.

C. Phenols

Ferric chloride test: 1 ml extract was treated with few drops of 5% ferric chloride solution; formation of bluish black colour indicates the presence of phenols.

Lead acetate test: 1 ml extract was treated with 2-4 ml 10 % acetic acid; formation of yellow colour precipitation indicate the presence of phenols.

D. Saponins

Frothing test: About 0.5 mg of extract was shaken with 5 ml of distilled water; formation of froth (appearance of creamy small bubbles) show the presence of saponins.

E. Tannins

Lead acetate test: 1 ml of extract was treated with 1 ml 10%

lead acetate solution; white colour precipitation indicates the presence of tannins.

Ferric chloride test : Small quantity of extract was mixed with water and heated in water bath, the mixture was filtered and 0.1% ferric chloride soln. was added to filtrates; dark green colour indicates the presence of tannins.

F. Terpenoids

Salkowski's test: Few mg of extract mixed with 2 ml of chloroform and 3 ml of conc. H₂SO₄ was carefully added to form a layer; an appearance of reddish brown colour ring indicate the presence of terpenoids.

Copper acetate test: extract was dissolved in water and treated it with 5% copper acetate solution; formation of emerald green precipitation indicate the presence of terpenoids.

G. Glycosides

Bromine H₂O test: 1 ml of test soln. was dissolved in bromine H₂O; formation of yellow colour precipitation indicate the presence of glucosides.

Antibacterial Activity

Agar well Diffusion method for antibacterial activity was selected for study. 25-30 ml of nutrient agar media was poured in sterilized petri-plates and allowed it to solidify at room temperature. 24 hours broth culture of test bacteria was used as inoculums under sterile conditions. Using cork borer several wells of 6 mm in diameter were punched. 100 µl of extract was poured in each well. The plates were incubated for optimum growth conditions at 35⁰ C and 1 day. Inhibition zone was measured with zone scale of 1 mm or more was considered positive inhibition.

Results and Discussion

Results for phytochemicals Screening

Air dried plant material was extracted using Ethanol and Methanol. The prepared extracts were qualitatively analyzed for the presence and absence of secondary metabolites. The Ethanolic extract of *Curcuma longa* (L.) rhizome showed presence of Alkaloids, Flavonoids, Tannins, Terpenoids, Glycoside and absence of Phenol and Saponin. The Methanolic extract of *Curcuma longa* (L.) rhizome showed presence of Flavonoids, Tannins, Terpenoids and Glycoside and absence of Phenol and Saponin. Similarly for Ethanolic extract of *Curcuma aromatica* (Salib.) rhizome showed Alkaloids, Flavonoids and Terpenoids are present and absence of Tannins, Glycoside, Phenols and Saponin. The Methanolic extract of *Curcuma aromatica* (Salib.) rhizome showed Alkaloids, Flavonoids, Terpenoids are present and absence of Tannins, Glycoside, Phenols, Saponin. (Refer Table:1)

Table 1: Shows results for phytochemical screening of *Curcuma sp.* (+ = present, - =absent)

Phytochemical	Test	<i>Curcuma longa</i> (L.)		<i>Curcuma aromatica</i> (Salib.)	
		Ethanol	Methanol	Ethanol	Methanol
Alkaloids	Dragendroff test	+	+	+	+
	Mayer's test	+	+	+	+
	Wagner's test	+	-	+	+
Flavonoids	Lead acetate test	+	+	+	-
	H ₂ SO ₄ test	-	+	+	+
	Alkaline reagent test	-	-	+	+
	Zinc hydrochloride test	-	-	-	-
	Pew test	-	-	-	-
Phenol	Ferric chloride test	-	-	-	-
Saponins	Frothing test	-	-	-	-
Tannins	Lead acetate test	+	+	-	-

	Ferric chloride test	+	-	-	-
Terpenoids	Salkowski test	+	+	+	+
	Copper acetate test	+	+	-	-
Glycoside	Bromine H ₂ O test	+	+	-	-

Result for Antibacterial Activity

Antibacterial activity of *Curcuma longa* (L.) in Ethnolic and Methanolic extract: Upto the concentration of 20 mg we got negative result for antibacterial activity. (Figure No: 1 and 2.).

Antibacterial activity of Ethanolic extract: The result obtained shows that 5 mg concentration of extract result in 3.0 mm zone of inhibition, 10 mg concentration of extract result in 3.5 mm zone of inhibition, 15 mg concentration of extract

result in 4.0 mm zone of inhibition and 20 mg concentration of extract result in 4.5 mm zone of inhibition. (Figure No: 3).

Antibacterial activity of Methanolic extract: The results obtained shows that 5 mg concentration of extract result in 2.0 mm zone of inhibition, 10 mg concentration of extract result in 2.5 mm zone of inhibition, 15 mg concentration of extract result in 4.5 mm zone of inhibition and 20 mg concentration of extract result in 7.5 mm zone of inhibition.(Figure No: 4) (Refer Table No: 2)

***Curcuma longa* (L.)**

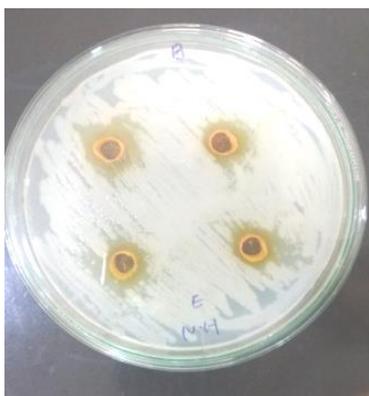


Fig 1: Ethanolic extract

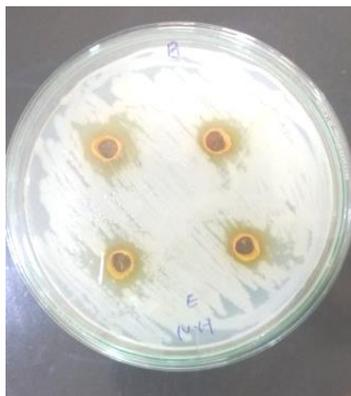


Fig 2: Methanolic extract

***Curcuma aromatica* (Salib.)**

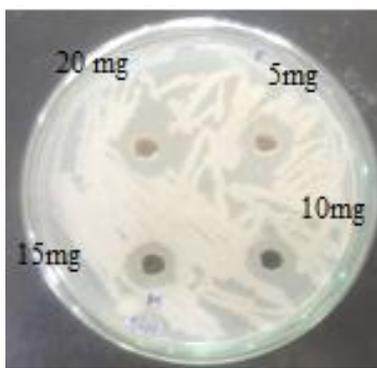


Fig 3: Methanol extract

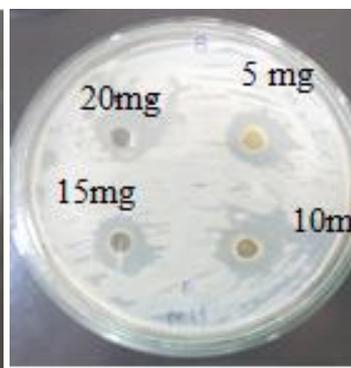


Fig 4: Ethanol extract

Table 1: Shows zone of inhibition of *Curcuma aromatica* (Salib.)

Extracts	Extract concentration (mg/2 ml)				
	Control	5mg/2ml	10mg/2ml	15mg/2ml	20mg/2ml
Ethanol	0.0mm	3.0mm	3.5 mm	4.0mm	4.5mm
Methanol	0.0mm	2.0mm	2.5mm	4.5mm	7.5mm

Discussion

Harshal Pawar *et al.*, 2014 concluded the presence of alkaloids, terpenoids, steroids and flavonoids in ethanolic extract of *Curcuma longa* (L.). Ikpeama *et al.*, 2014 concluded the presence of alkaloids, saponin, tannins, cynogenic-glycoside, phenol and flavonids in methanolic extract of *Curcuma longa* (L.). Anjusha S. *et al.*, concluded the presence of flavonoids, tannins, saponins, terpenoids and phenol in aqueous extract of *Curcuma aromatica* (Salib.) Esther *et al.*, 2009 studied the antibacterial activity in

ethanolic extract against *Escherichia coli* and *Basillus subtilis* and get 14 mm zone for *Escherichia coli* and 26 mm zone for *Basillus subtilis*. For this work they use Agar diffusion method. Nikhil Singh, 2017 find antibacterial activity against *Bacillus subtilis* by using ethanolic extract. He got inhibition zone in concentration of 25 to 300 mg. For concentration of 25mg/ml, get zone of 6.4 mm while at the concentration of 300mg/ml get zone of 7.6mm.

Conclusion

Thus the results obtained in present study indicates that *Curcuma longa* (L.) is rich in production of phytochemicals as compare to *Curcuma aromatica* (Salib.). Both *Curcuma longa* (L.) and *Curcuma aromatica* (Salib.) have potential to act as a source of useful medicines because of presence of various phytochemical components such as alkaloids, flavnoids, terpenids, glucosides. Because the presence of different phytochemicals *Curcuma sp.* shows the antibacterial activity against *Escherichia coli*. *Curcuma aromatica* (Salib.) having more antibacterial potential as compare to *Curcuma longa* L.

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