

Antibacterial evaluation, phytochemical screening and ascorbic acid assay of turmeric (*Curcuma longa*)

Abstract

The ethanolic extract of Turmeric (*Curcuma longa*) was subjected to microbial susceptibility test using the agar well diffusion method. The extract was found to be active against the bacteria used as it inhibited all the organisms with the highest inhibition zone of 13.7mm recorded against *Shigella flexneri* and the least inhibition of 2.3mm recorded against *Staphylococcus epidermis*. Minimum Inhibitory Concentration (MIC) values range from 6.25mg/ml to 200mg/ml. *Shigella flexneri* gave MIC of 6.25mg/ml, *S. aureus* and *K. Pneumonia*, 25mg/ml, *E. coli*, 50mg/ml, *S. epidermis*, *Lactobacillus* and *P. aeruginosa*, 100mg/ml and *V. cholera* and *S. typhi* 200mg/ml respectively. Secondary metabolites were also screened using phytochemistry. Fourteen (14) secondary metabolites were screened from the ethanolic extract. The presence of these phytochemicals like tannins, alkaloids, phenols, steroids, flavonoids, phlobatannin, cardiac glycosides, terpenoids, triterpenes, saponin, etc; is indicative of the antimicrobial activity of Turmeric. The extract was found to contain 66.749mg/100ml ascorbic acid which is relatively comparable to many fruits recommended by dieticians. The value of curcumin present (Rf value 0.436) is also relatively close to that of pure curcumin. The results so far obtained are indicative of the good medicinal value of Turmeric in both pharmaceutical and pharmacological formulations.

Keywords: turmeric, phytochemicals, ascorbic acid, antibacterial sensitivity, ethanolic extract

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Abbreviations: MIC, minimum inhibitory concentration; WHO, world health organization; IZD, inhibition zone diameters; TLC, thin layer chromatography

Introduction

Herbal plant extracts used as medicine are now presently being used as a replacement for synthetic drugs. Sofowora A et al.¹ cites the definition of World Health Organization (WHO) Consultative Group concerning medicinal plants as any plant that has in one or more of its organs, metabolic substances which could be used for therapeutic purposes or which are precursors for the synthesis of useful drug. Medicinal plants are used as raw materials for herbal medicinal products and supplements due to their cheap cost, efficacy and because they have no much side effects when compared to those of synthetic formulations. The use of Turmeric has not been fully explored in the aspect of their antimicrobial properties and hence they have not been fully used as antimicrobial agents.

- a. Turmeric (*Curcuma longa*) belongs to the *zingiberaceae* family. It has a yellow rhizome. The rhizome is ovate, oblong, pyriform with short branches.²

It is an annual perennial plant with leafy and erect stem. It thrives well where there is no much rainfall under mild condition of warm and humid atmosphere. It has leaves with thin blades. It has an ovate sheath-like long petiole with entire margin.

In Nigeria, it is used to preserve and flavour food (spice.) The Urhobo people in the Niger Delta area call the Turmeric rhizome "Blu" and use it as a dye for clothes and to prevent fading of clothes. It is also used by these indigenous people of Delta State of Nigeria as

a herbal decoction with traditionally distilled gin called "ogogoro" and consumed with the claim that it cures certain ailments like diabetics, ulcers, hepatic disorders, biliary disorders, anorexia and cough. The rhizome is also used as an anti-inflammatory therapy for wounds, digestive disorders, jaundice for babies born with jaundice, dysentery/diarrhoea and for food as spice. Ahmad et al.³ reports that turmeric rhizome has antimicrobial, anti-helmintic, anticancer, anti-parasitic, antiseptic, anti-oxidative, anti-inflammatory, anti-rheumatic, antitumor and antiviral properties and that it is widely used as a stimulant and sedative in food industries.

Some secondary metabolites or phytochemicals possibly present in turmeric include anti-oxidants, poly-phenols and flavonoids. These secondary metabolites have antibiotic properties that make them to be used in food and food products.⁴ Other compounds have also been reported to be present in turmeric. Compounds like zingiberene, tumerone, ar-tumerone and curlone have been listed to be volatile components present. Curcuminoids are claimed to be the non-volatile components present. As a phenolic compound, curcumin appears to be the most essential bioactive component present in curcuminoids. It has a very good antimicrobial potency. Turmeric rhizome is yellow in colour and this colour is due to the presence of the curcumin, which is crystalline and insoluble in water⁵ but soluble in solvents like ethanol, acetone, ketone and chloroform.⁶ Turmeric has a peculiar smell which is reported to be due to the aromatic volatile oil components present. This oil component contains 25% tumerone, 11.5% curdione and 8.55% ar-tumerone.^{6,7} Aromatic tumerone extracted from Turmeric rhizome has been reported to have been used as an insect repellent while the leaf extract has been shown to have mosquitocidal activities.⁸

Turmeric has been known for its various medicinal properties. It is used as a natural antiseptic and an antibacterial agent. It is used as

a disinfectant or antiseptic and for treating burns of various degrees. It has been used topically on the body for wounds resulting from blistering (pamphigus and herpeszoster) for parasitic skin infections and diseases and for the treatment of acne (pimples.) Turmeric has also been effective and useful remedy in the treatment of common cold, liver infection/disease and urinary tract infection/disease.^{9,10} The oil extracted from turmeric has been reported to have anti-mutagenic properties.¹¹ This is especially very effective in inhibiting the formation and excretion of urinary mutagenes in people who smoke cigarette.¹² Turmeric is used orally to reduce total cholesterol, low density lipoprotein and triglycerides in obese people with recorded high level of cholesterol. It is taken orally or applied topically to reduce itching and is sometimes used as a fairly good mouth wash. However it has been reported that the use of turmeric causes some side effects; it is claimed to cause stomach upset (gastroesophageal reflux disease), nausea, dizziness and diarrhoea. It reduces blood clotting and blood sugar level in diabetic people.¹³

Materials and method

Turmeric (*Curcuma longa*) rhizome

Turmeric rhizomes were purchased from Igbudu market in Warri, in Warri South Local Government Area of Delta State, Nigeria. They were soaked in sterile distilled water and washed and then rinsed again with distilled water. The rhizomes were air dried for two hours and then grated using a sterile grater. 200g of Turmeric were macerated with 1400ml of 80% ethanol in a clean 4 litre plastic can for one week (7days.) After 7days the mixture was sifted by using clean sterile muslin. The resultant filtrate was concentrated using mild heat at 40°C in a hot air oven using sterile disposable Petri dishes. The final yield of the extract was 35g. The percentage yield was calculated to be 17.5%. The Petri dishes containing the extracts were wrapped in aluminium foil and kept in the refrigerator at 40°C prior to use.

Preparation of turmeric extract for serial dilution

A reconstitution of the plant extract was made as follows. 400mg of the extract was dissolved in 2ml 80% ethanol in a test tube as stock and twofold serial dilutions of 200mg/ml, 100mg/ml, 50mg/ml 25mg/ml, 12.5mg/ml and 6.25mg/ml were made aseptically from it. These dilutions were used for the antibacterial bioassay.

Organisms/media used

Ten (10) bacterial isolates were used for this research study (*Staphylococcus aureus*, *Staphylococcus epidermis*, *Lactobacillus spp*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella flexneri* and *Vibrio cholera*). The isolates consist of four (4) Gram-positive and six (6) Gram-negative bacteria. They were obtained from stock cultures of the Pharmaceutical microbiology laboratory of the Delta State University, Abraka. Some identification (Biochemical) tests were carried out on the selected organisms to make sure that they were contamination free. The media used for the investigation were the basic media, nutrient agar (Biotech-Himedia) and Mueller Hinton agar (Oxoid) for susceptibility test. The test organisms were inoculated into nutrient broth (Biotech-Himedia) and incubated for 24 hours at 37°C. The bottles containing the broth organisms were matched and compared with the turbidity of McFarland Standard to give approximately 1x10⁸ CFU/ml. The bioassay was then carried out using the agar well diffusion technique. Broth cultures of the organism were prepared overnight and after matching with McFarland standard they were spread unto Mueller

Hinton agar plates in triplicates with sterile cotton swab sticks. The wells were bored aseptically with a six (6mm) millimetres cork borer.

Susceptibility test

Antibacterial sensitivity test

Different concentrations of the ethanolic extracts were made by two fold serial dilutions, 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml and 6.5mg/l respectively. Mueller Hinton agar was prepared by dissolving 3.8g of the medium in 100ml distilled water and then sterilized using autoclave at 121°C for 15 minutes. It was allowed to cool and then poured into sterile plastic Petri dishes to a depth of 4mm and left to set under aseptic conditions. Each bacterial inoculum was prepared from an overnight broth culture and matched with 0.5 McFarland Standard. This was then spread on the dried surface of the Mueller Hinton agar. A 6mm cork borer was used to bore holes in the agar and labelled according to the different concentrations. The different concentrations were filled into each well made in the culture plate using a sterile Pasteur pipette under aseptic condition. The plates were left for about 30 minutes on the work bench to allow proper diffusion before putting them in the incubator to observe growth for between 18-24 hours at 37°C. At the end of the incubation period, inhibition zone diameters (IZD) were measured using a transparent meter rule. Ciprofloxacin was used as a standard antibiotic for positive control. Analyses of the result was done using mean standard deviation and the subjected to the one way analysis of variance (ANOVA) and significance between mean was found using Turkey-Kramer test with significant level, P<0.05. ANOVA was performed using Graph pad in Stat statistical software.

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) is the highest dilution or the least concentration of an antimicrobial agent that will inhibit the growth or kill the microorganism. Various concentrations were prepared for the MIC-400mg/ml, 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.125mg/ml respectively. One (1ml) millilitre of each concentration was dispensed in sterile Petri dishes and 19ml of Mueller Hinton agar was poured. The mixture was swirled thoroughly to mix well and allowed to solidify. Each plate was divided into segments of ten (10) according to the organisms and properly labelled. The overnight broth organisms having been matched with 0.5 McFarland Standard by visual comparison were used to streak each segment aseptically, using a sterile nichrome wire loop. The plates were incubated for 24 hours at 37°C and observed. The least concentration for each organism that did not show growth was recorded as the MIC for the organism.

Phytochemistry

It is reported¹⁴ that Turmeric contains many phytochemicals (secondary metabolites) like alkaloids, tannins, steroids, saponin, flavonoids and many others. Phytochemicals are the non-nutritive chemical constituents of plants which help to protect the plant for defence and prevent them from being infected by diseases. Apart from the defensive property these phytochemicals have on the plants, they also protect humans against variety of diseases. The extraction of these secondary metabolites from plants have been found to act against growth of microbes-bacteria, fungi and even viruses.¹⁵ Turmeric (*Curcuma longa*) also contains nutritive components. Proximate studies on Turmeric reveal that it contains fats, protein, carbohydrate, minerals and moisture. Essential oils present in Turmeric include

sabinine, borneol, α -phellandrene, cineol, sesquiterpenes, zingiberene and curcumin (diferuloylmethane).¹⁴ Phytochemical screening on Turmeric extract was carried out using standard procedures with slight modifications in some aspects.^{1,16–19} The following phytochemicals were tested as described:

Alkaloids

This was tested by using Dragendorff's reagent and confirmed by using Wagner's reagent. 3ml of the crude ethanolic extract was put in a test-tube and 1ml concentrated HCl acid was added gently. The mixture was warmed for about 20 minutes, allowed to cool and then filtered using Whatman No 1 filter paper (125mm diameter.) The filtrate was then subjected to the Dragendorff's test and then Wagner's test.

- i. **Dragendorff's test:** Two drops of Dragendorff's reagent were added to 1ml of the filtrate. A creamy precipitate was observed indicating the presence of alkaloids. This was confirmed by using the Wagner's test.
- ii. **Wagner's test:** 1ml of the filtrate was mixed with Wagner's reagent. A brownish precipitate was formed revealing the presence of alkaloids.

Saponin

5ml of the ethanolic plant extract was mixed with 20ml of sterile distilled water and then vigorously agitated in a test-tube (boiling-tube) and left to stand. Frothing was observed which lasted for about 15 minutes. This shows the presence of saponin.

Tannin

3ml of the plant extract was mixed with equal proportion of freshly prepared FeCl_3 . A green colouration appeared indicating the presence of tannin.

Coumarin

1.5ml of 0.25M (10%) NaOH was mixed with 2ml portion of Turmeric extract. The formation of yellow colour showed indication of the presence of Coumarin.

Flavonoid

The Turmeric ethanolic extract was treated with 0.25M (10%) NaOH Solution. The formation of a bright yellow colour indicated the presence of flavonoids.

Diterpenes

This test was performed by using copper acetate test. 1ml of Turmeric extract was mixed with an equal proportion of water and 10 drops of copper acetate solution were added using a dropper. A deep green colouration was observed showing that diterpenes is present.

Phlobatannins

1ml of the plant extract was mixed with 1% HCl acid (dilute) and the mixture was slightly boiled. A reddish brown precipitate was formed, indicating the presence of phlobatannins.

Cardiac glycosides

The Legal's test was used to test for cardiac glycosides; this was performed by the addition of 1ml of pyridine to 3ml of the ethanolic Turmeric extract. 5 drops of freshly prepared 2% sodium nitroprusside

solution and 5 drops of 20% NaOH solution were then added using a dropper. A pinkish-red colouration was observed which gradually faded after standing for a while into brownish-yellow, an evidence of the presence of cardiac glycosides.

Phenol

1ml of the extract was placed in a test-tube and then mixed with 4 drops of freshly prepared alcoholic FeCl_3 solution. A bluish-black colouration was observed which shows that phenol is present in the plant extract.

Steroid

1ml of the extract was added to 10ml of chloroform in a test-tube. Concentrated H_2SO_4 acid was poured gently through the walls of the tube into the mixture without agitation. The presence of a red interface and yellow-greenish fluorescence in the H_2SO_4 acid layer showed indication of the presence of steroid in the plant extract.

Anthraquinones

This was performed by using the Borntrager's test. 0.5g of Turmeric extract was agitated with 10ml portion of benzene in a test-tube. The resulting mixture was filtered and 5ml of 10% ammonia solution (NH_4OH) was added to the filtrate. This mixture was then thoroughly agitated and left to stand. A pinkish-red colouration appeared in the lower phase (ammonia phase), which shows the presence of free hydroxy-anthraquinones.

Reducing sugars

1g of the extract was put in a test-tube and this was dissolved with 10ml of distilled water. The resulting mixture was filtered and the filtrate was subjected to Fehling's test and Benedict's test for the presence of reducing sugars. For the Fehling's test, 2ml of the filtrate was hydrolysed with dilute HCl acid and then neutralized with an alkali and then heated with Fehling's solutions A and B. A reddish precipitate was formed showing the presence of reducing sugars. This was confirmed by using the Benedict's test by mixing 2ml of the filtrate from the extract with 2ml of Benedict's reagent and then gently heating. An orange-red precipitate appeared indicating the presence of reducing sugars.

Anthocyanin

Anthocyanin was tested for by treating 1ml of the turmeric extract with an equal volume of 2M HCl acid and ammonia solution. A pinkish colour was observed which immediately changed to blue/violet colour, an indication of the presence of anthocyanin in the plant extract.

Terpenoids

This test was carried out using the Salkowski's test. 5ml of the extract was mixed with 2ml of chloroform in a test-tube and 3ml of concentrated H_2SO_4 acid was carefully added through the walls of the test-tube. A reddish-brown colour was observed at the interface indicating the presence of terpenoids.

Ascorbic acid assay

Ascorbic acid or vitamin C is a water soluble antioxidant which plays a vital role in protecting the body from infection and diseases. The human body is not known to synthesize vitamin C. It is obtained from dietary sources or food supplements, particularly fruits and vegetables.²⁰ Different methods are used for the determination of

ascorbic acid but this study used the titrimetric method using iodine solution. This method determines vitamin C concentration in a solution by redox titration using iodine. The iodine formed in the reaction is reduced to iodide as long as there is presence of ascorbic acid. When all the ascorbic acid is fully oxidised the excess iodine is free to react with the starch indicator to produce a bluish-black starch-iodine complex which is the end point of the titration. In this assay, 20ml aliquot of Turmeric ethanolic extract was pipetted into a 250ml conical flask and 150ml of distilled water was added. 1ml of starch indicator solution was added in drops and this was titrated against 0.005mol/l of iodine solution. The end point of the titration was identified as the first permanent trace of a dark bluish-black colouration due to the starch-iodine complex. The titration was carried out in triplicate to obtain concordant results (titres agreeing within 0.1ml.)

Isolation of curcumin from turmeric extract

Like all other plants, there appears to be a chemical composition variation in Turmeric rhizome because of geographical location, topography of soil, method of cultivation and handling as well as method or condition of storage and age of vegetative material.²¹ Curcumin was isolated from the turmeric extract by dissolving 0.2g of the ethanolic extract in 10ml of benzene in a separation funnel after which 0.1% w/v NaOH solution was added. Two partitions were observed. The NaOH layer was drained and then precipitated using 0.1M dilute HCl acid. The resulting yellowish-brown precipitate was filtered under vacuum, air dried for one hour in a desiccator containing concentrated H₂SO₄ acid to remove traces of moisture present. This was then used for thin layer chromatography and UV spectrophotometric analysis.

Thin layer chromatography

Curcumin was separated using thin layer chromatography (TLC) with ethyl acetate and n- hexane mixture in the ratio of 3:7 and the plate was sprayed using 1% alcoholic KOH solution. The R_f values for the separated spots were compared with the R_f value of the pure curcumin.

Preparation of test sample for UV analysis

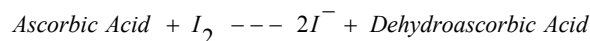
0.2g of the extract was dissolved in 50ml of 80% ethanol in a 100ml measuring cylinder. The mixture was agitated thoroughly and made up to the 100ml mark by further addition of 50ml of the 80% ethanol. (80% ethanol was used because the extract was made by using 80% ethanol.) The resultant mixture was filtered and 4ml aliquot of it was diluted with 46ml of the 80% ethanol. The percentage content of curcumin present in the sample was calculated from a calibration curve obtained by using standard solutions with concentrations ranging from 1µg/ml to 8µg/ml that is percentage concentrations from 40% to 320% of the standard concentration of 2.5µg/ml using a wavelength of 425nm. The UV analysis was carried out by using UV-Vis Spectrophotometer (Double Beam, LABTECH- Model 2806.)

Results

From the susceptibility tests carried out using the agar well diffusion method the following inhibition zone diameters were obtained for the test organisms used as shown in Table 1. The minimum inhibitory concentration (MIC) carried out on the different organisms at various concentrations are shown in Table 2. The result of the preliminary phytochemical screening of the crude ethanolic extract of Turmeric shows the presence of the compounds as enumerated in Table 3. Ascorbic acid assay carried out on the crude extract gives the result

as shown in Table 4.

Average titre is calculated as $27.00+27.00+26.90/3=29.96$



1 Mole 1 Mole

From $C_1V_1/C_2V_2=n_1/n_2$ where C_1V_1 represent molar concentration and volume of Iodine used and C_2V_2 represent molar concentration and volume of ascorbic acid used and n_1/n_2 =mole ratios from equation

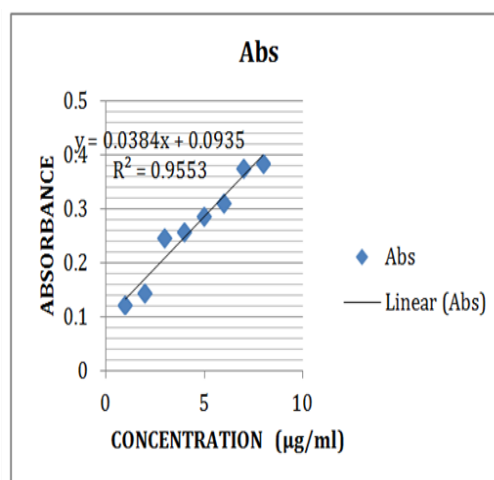
$$0.005 \times 26.96 / C_2 \times 35.5 = 1 / 1.$$

$$C_2 = 0.005 \times 26.96 / 35.5 = 0.00379 \text{ mol / l} = 667.49 \text{ mg / l}$$

Amount of ascorbic acid present in mg/100ml sample=66.749mg/100

Thin layer chromatography result

The Thin Layer Chromatography (TLC) result shows both intense yellowish-brown spot and two other dull yellowish-brown spots which could be as a result other types of curcuminoids present in the sample. The R_f values of the yellowish-brown spots are shown in table 5 as compared with the pure R_f value of curcumin (Table 5). The calibration curve from the UV spectrophotometry gives a regression equation of $y=0.0384x+0.0935$ with a correlation coefficient of 0.955 which gives a good indication of linearity. The result also shows that there is reasonable percentage of curcumin in Turmeric (Figure 1 & 2).



Conc	Abs
1	0.122
2	0.144
3	0.246
4	0.257
5	0.288
6	0.312
7	0.376
8	0.384

Figure 1 Calibration Curve from UV Spectrophotometer

Table 1 Inhibition zone diameters (IZD) in mm for *Staphylococcus aureus*, *Staphylococcus epidermis*, *Lactobacillus*, *Vibrio cholera*, *Proteus vulgaris*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Shigella flexneri*, *Escherichia coli* and *Salmonella typhi*; Cipro: (Ciprofloxacin was used as positive contro)

S/n	Test organisms	Concentration (mg/ml) and inhibition zone diameters (IZD in mm)_																		
		200			100			50			25			12.5			6.25			Cipro
		1 st .	2 nd .	3 rd .	1 st .	2 nd .	3 rd .	1 st .	2 nd .	3 rd .	1 st .	2 nd .	3 rd .	1 st .	2 nd .	3 rd .	1 st .	2 nd .	3 rd .	
1	<i>S. aureus</i>	12	12	11	9	9	10	7	8	8	5	4	7	4	4	4	4	3	3	35
2	<i>S. epidermis</i>	9	7	7	7	8	8	6	5	6	5	5	6	4	5	4	2	2	3	30
3	<i>Lactobacillus</i>	11	10	10	10	11	8	11	13	10	9	9	8	8	10	7	8	8	8	32
4	<i>V. cholera</i>	7	8	7	6	6	6	4	4	5	3	4	4	4	4	4	3	3	4	35
5	<i>P. vulgaris</i>	6	5	8	5	5	4	5	5	6	4	5	5	4	4	4	5	5	4	40
6	<i>K. pneumonia</i>	9	10	7	7	6	6	5	5	4	4	4	4	4	5	5	3	3	3	32
7	<i>P. aeruginosa</i>	9	7	9	7	7	7	6	5	6	5	7	6	6	8	7	5	4	4	45
8	<i>S. flexneri</i>	14	13	14	10	12	10	8	8	8	6	8	8	5	5	7	4	4	6	35
9	<i>E. coli</i>	12	11	11	10	10	12	6	8	7	6	6	6	7	9	9	6	5	8	30
10	<i>S. typhi</i>	7	7	9	6	5	6	6	6	8	5	4	4	5	5	5	6	8	6	35

Table 2 Minimum inhibitory concentration (MIC) in mg/ml

S/n	Test organisms	Concentrations in mg/ml							
		400	200	100	50	25	12.5	6.25	3.125
1	<i>S. aureus</i>	-	-	-	-	-	+	+	+
2	<i>S. epidermis</i>	-	-	-	+	+	+	+	+
3	<i>Lactobacillus</i>	-	-	-	+	+	+	+	+
4	<i>V. cholera</i>	-	-	+	+	+	+	+	+
5	<i>P. vulgaris</i>	-	-	+	+	+	+	+	+
6	<i>K. pneumonia</i>	-	-	-	-	-	+	+	+
7	<i>P. aeruginosa</i>	-	-	-	+	+	+	+	+
8	<i>S. flexneri</i>	-	-	-	-	-	-	-	+
9	<i>E. coli</i>	-	-	-	-	+	+	+	+
10	<i>S. typhi</i>	-	-	+	+	+	+	+	+

(-) No Growth; (+) Growth

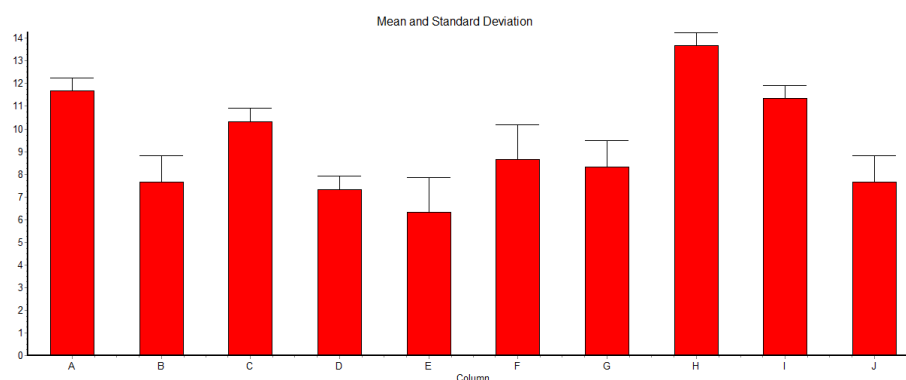
**Figure 2** Graphical representation of the mean zone of inhibition and Standard Deviation. (A: *S. aureus*; B: *S. epidermis*; C: *Lactobacillus* spp; D: *V. cholera*; E: *P. vulgaris*; F: *K. pneumonia*; G: *P. aeruginosa*; H: *S. flexneri*; I: *E. coli*; J: *S. typhi*).

Table 3 Phytochemical compounds found in ethanolic extract of turmeric

S/n	Secondary metabolites	Ethanolic extract of turmeric
1	Alkaloids	+++
2	Saponins	+
3	Tannins	+
4	Coumarins	+++
5	Flavonoids	++
6	Diterpenes	+
7	Phlobatannins	+
8	Cardiac Glycosides	+
9	Phenol	++
10	Steroids	++
11	Anthraquinones	+
12	Reducing Sugars	+++
13	Anthocyanins	+
14	Terpenoids	++

(+++ Present in high concentration; (++) Present in moderate concentration; (+) Present

Table 4 Titre values of ascorbic acid

Volume of tumeric extract used = 35.5ml				
Trial Titre (ml)	1 st . Reading (ml)	2 nd . Reading (ml)	3 rd . Reading (ml)	Average Titre (ml)
27.2	27	27	26.9	26.96

Table 5 R_f Values of curcumin

Sample	R _f values
Pure Curcumin	Spot = 0.487 Spot 1 = 0.068
Isolated Curcumin	Spot 2 = 0.212 Spot 3 = 0.436

Discussion

Over the years, interest has diverted towards the investigation of natural plants as alternative to synthetic antimicrobial agents. Traditional herbs have been found to be very effective against microbes. Herbal extracts are now becoming useful in modern medicine as well as food supplements. The antibacterial activity of ethanolic extract of Turmeric as shown in Table 1 indicates that the plant extract is very effective against the various organisms used. The extract inhibited all the organisms with the highest average zone of inhibition (13.7mm) against *Shigella flexneri* at 200mg/ml and least average zone of inhibition (2.3mm) against *Staphylococcus epidermis* at 6.25mg/ml. The antimicrobial susceptibility test is an essential technique used in pharmacology to determine the efficacy of novel antimicrobial agents from biological extracts against microorganisms.²² The minimum inhibitory concentration (MIC) result in Table 2 shows that *Shigella flexneri* had MIC of 6.25mg/ml while *Vibrio cholera*, *Proteus vulgaris* and *Salmonella typhi* gave

MIC values of 200mg/ml each. *Staphylococcus aureus* and *Klebsiella pneumonia* gave MIC values of 25mg/ml each. The result of the MIC table also indicates that *Staphylococcus epidermis*, *Lactobacillus* and *Pseudomonas aeruginosa* had MICs of 100mg/ml while *Escherichia coli* gave an MIC of 50mg/ml. The result suggests that Turmeric is active against both Gram-positive and Gram-negative bacteria.

Work done by Odhav et al.²³ & Chandarana et al.²⁴ show that phenols and their derivatives are used in disinfection and as standards with which other disinfectants are compared. Thus the phenolic compounds that are present in Turmeric could be responsible or promote the antimicrobial efficacy of the plant extract. This may likely be attributed to the H-bonding and possibly the hydrophobic interaction of the phenolic compounds present in the plant to the membrane proteins, membrane damage, disruption of electron transport chain and possible cell wall distraction. The extract was effective against *Lactobacillus*, *Staphylococcus aureus* and *Escherichia coli*. It is believed to have broad spectrum activities. The antimicrobial properties could be attributed to the phenolic compounds and essential oils like curcumin, turmerol and veleric acid present in turmeric.

Phytochemical screening carried out on the ethanolic extract of Turmeric reveals the presence of reasonable amount of alkaloids, saponin, tannins, coumarin, flavonoids, diterpenes, phlobatannins, cardiac glycosides, phenols, steroids, anthraquinones, reducing sugars anthocyanins and terpenoids-(14 phytochemical compounds) as shown in Table 3. These antibacterial inhibitory effects could be attributed to the presence of these secondary metabolites. The results obtained from the phytochemical screening are comparable to those obtained by Rajesh et al.,²⁵ Swadhini et al.²⁶ and Saxena et al.²⁷ The presence of flavonoids and curcumin in Turmeric has been reported to be responsible for its chemopreventive and physiological effects in many tumor bioassays and the reduced increased growth of tumor cells.²⁸ Phytochemicals which constitute the non-nutritive components of plants protect the plant against pests and diseases and they also help to protect humans against variety of diseases.^{15,29} Turmeric has also been reported to be an anti-diabetic.³⁰ It is claimed that it reduces the risk of diabetic by lowering the level of glucose-6-phosphate and raises the action of liver. The level of serum hexokinase and lactate dehydrogenase in blood is said to be improved by Turmeric and this is indicated in the reduction in cellular outflow of alkaline phosphatase lactate dehydrogenase and acid phosphatase into the blood of diabetic whister rats.

Alkaloids have been known to be the largest groups of secondary metabolites in plants. They are claimed to have powerful effects on humans and could be used as pain killers.³¹ Stray F et al.³² reports that alkaloids are very efficient therapeutically among the plant phytochemicals. The pure isolated alkaloids and their synthetic derivatives are used medicinally because of their analgesic antispasmodic and bacterial activities. The high concentration of alkaloids present in the ethanolic extract suggests its usefulness as a pain killer. Tannin is present in low concentration in the Turmeric extract (Table 3). However, the presence of tannin could be responsible for its use in the treatment of intestinal disorders like diarrhoea and dysentery as also shown in the high inhibition zone diameter for *Shigella flexneri*. Most herbs that contain tannin as a major constituent are claimed to be astringent in nature and find use in the treatment of intestinal disorders.³¹ Arora et al.³³ suggest that the anti-inflammatory properties responsible for Turmeric effect against arthritis may be due the decrease in the histamine levels and increase production of cortisone by adrenal glands. This could be responsible for the use of Turmeric in the treatment of rheumatoid arthritis,

arthritis, osteoarthritis, trauma and stiffness of the body parts. The coumarin present in Turmeric gives the rhizome (extract) its vanilla like flavour. Coumarin is an oxygen heterocyclic compound (C_9H_6O) and occurs in free state or in a combined state with sugar glucose (coumarin glycoside.) It is claimed to have a blood-thinning, anti-fungal and anti-tumor properties and it is believed to increase the flow of blood in the veins and cause reduction in capillary permeability.³⁴

Turmeric has been reported³⁵ to be a potent plant in the treatment of rheumatic arthritis, arthritis, osteoarthritis, trauma and stiffness of body parts, with great immune modulating properties. It is said to decrease triglyceride and cholesterol level and thus reduces the risk of cardiovascular accident. Table 4 gives the result of the ascorbic acid assay. The result shows that Turmeric has fairly good Vitamin C (66.749mg/100ml) content. This value is comparable to fruits such as papaya (62mg/100ml), oranges (59mg/100ml), strawberries (59mg/100ml), pineapple (56mg/100ml), and etcetera. Vitamin C is a water soluble antioxidant which plays a vital role in protecting the human body from infection and diseases. The human body does not synthesize Vitamin C so it must be obtained from dietary sources or food supplements such as fruits and vegetables. Deficiency of Vitamin C results in pain in the joints, fatigue, muscle weakness, bleeding of gum, leg rashes, anaemia, haemorrhage, muscle ache, defective skeletal calcification which results in scurvy when prolonged. Turmeric could be a good source of Vitamin C as shown in the analysis.

The presence of curcumin in Turmeric could be responsible for its use as a potent antioxidant, antiplatelet, antimicrobial properties and its use to reduce cholesterol level and inhibit cancer growth.³⁶ Curcumin is known to inhibit *Helicobacter pylori*, a bacterium which is responsible for gastric ulcers which has also been linked to gastric cancers. It is one of the three curcuminoids of Turmeric, the other two being Demethoxycurcumin and Bisdemethoxycurcumin. But curcumin is reported among them to be protective against heart and is also antiviral. However, climatic conditions and topography could have significant impact on the content of curcumin in Turmeric.²¹ Curcumin is also a lipophilic phenolic compound³⁷ which gives Turmeric some anticancer properties. The TLC result shows that the Turmeric extract is comparable to the pure curcumin sample since the R_f value of the extract (0.436) is relatively close to the R_f value of the pure sample (0.486). From the results obtained in this study it is found that Turmeric is a plant that could be cultivated because of its usefulness as an antibacterial agent and a good source of ascorbic acid. It is recommended therefore that further research should be done on it for its use as a plant medicinal especially in this period that herbal drugs are becoming significant as antimicrobials.

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Conflict of interest

The authors declare no conflict of interest.

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