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Antioxidant and bactericidal activity of wild turmeric extracts

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ABSTRACT

Naturally occurring antioxidants have considerable importance in medicine and in food processing. In this work, the antioxidant activity of dried rhizomes extract of the spice *Curcuma aromatica* (wild turmeric), a unique spice having a wide range of pharmacological and cosmetological applications is studied by the inhibition of auto oxidation of linoleic acid in aqueous alcohol system and by DPPH method along with antibacterial activity against selected organisms studied by disc diffusion method were reported.

Keywords: DPPH, Antioxidants, Curcumin, and *Curcuma aromatica*.

1. Introduction

The plant cells are highly sophisticated chemical factories where a large number of chemicals are manufactured with great precision and ease from simple raw materials at normal temperature and pressure. Besides functioning as the energy source for animals, they provide raw materials for many phytochemical based industries such as pharmaceutical, perfumery, flavour and food industries. Plant extracts are known to exert a wide range of beneficial physiological effects which is reflected in their use in traditional medicines. Even in the modern age, exploring the different pharmacological action of medicinal plants and isolation of bioactive constituents present in them constitute a major research area all over the world [1].

Zingiberaceae family constitutes a vital group of rhizomatous medicinal and aromatic plants characterized by the presence of volatile oils and oleoresins of export value [2]. *Curcuma longa* Linn. (common name: turmeric) is the most popular spice, widely used as a coloring agent and in several medicinal preparations, among the *Curcuma* genus belonging to the family *Zingiberaceae*. *Curcuma aromatica* (common name: wild turmeric) is a member of the *Curcuma* genus belonging to the family *Zingiberaceae*. Botanically close to *Curcuma australasica*, wild turmeric has been widely used as a cosmetic herbal in South Asia and nearby regions. Wild turmeric has rhizomes with a peculiar fragrance and attractive deep yellow color. The rhizomes are often used in cosmetic herbal medicines and as a culinary ingredient in limited quantities as a food flavor. Wild turmeric is recognized as a medical herb with strong antibiotic properties. It is believed to play a role in preventing and curing cancer in Chinese medicine. In an effort to remove cell accumulations such as tumors, *Curcuma aromatica* is often used. It contains aromatic volatile oils that help to remove excessive lipids from the blood, reduce aggregation of platelets (sticking of the blood cells to form masses), and reduce inflammation [3].

1.1. Antioxidants and their importance

Atmospheric oxygen is an indispensable element for life and oxidative property of oxygen plays a vital role in various biological phenomena. At the same time it has also been recognized as the principal agent responsible for the deterioration of organic materials exposed to air. The parallel role of oxygen, a molecule essential for many forms of life and as a destructive toxic agent for living tissues has been discovered much more recently [4].

Oxygen is used by the cell to generate energy and free radicals are formed as a consequence of ATP (adenosine triphosphate) production by the mitochondria. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are the byproducts resulting from the cellular redox processes [4]. These reactive species play a dual role in human as both toxic and beneficial. The delicate balance between their two opposite effects is undoubtedly a key aspect of life. At low or moderate levels, reactive species exert beneficial effects on cellular redox signaling and immune function, but at high concentrations, they produce oxidative stress, a harmful process that can damage cell function and structures [5].
Free radicals induced oxidative stress is now believed to be a fundamental mechanism underlying a number of human cardiovascular, neurologic and other disorders. It has been estimated that ~ 5% of inhaled oxygen is converted into several damaging ROS [6]. These ROS may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease.

Antioxidants are our crucial defense against free radical induced damage, and are critical for maintaining optimum health and well-being. Antioxidant compounds in food play an important role as a health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease [7]. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids and phytoestrogens have been recognized as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties.

1.2. Antibacterial agents and their importance

An antimicrobial agent is a compound that selectively destroys or inhibits the growth of micro-organisms. Antibiotics, antiseptics and disinfectants are all classified as antimicrobial agents. Based on their action, they can be broadly classified into two - the effect of an antimicrobial is whether it destroys (bactericidal) or it inhibits the growth of the micro-organism (bacteriostatic). These effects are not mutually exclusive; antimicrobials can be both cidal and static. An antimicrobial agent's spectrum of activity details its effect on various micro-organisms. The main micro-organisms concerned are usually bacteria (sometimes including bacterial spores), viruses and fungi.
The antiseptic qualities of aromatic and medicinal plants and their extracts have been recognized since antiquity, while attempts to characterize these properties in the laboratory date back to the early 1900s.The antimicrobial properties of plant volatile oils and their constituents from a wide variety of plants have been assessed and reviewed [8]. It is clear from these studies that these plant secondary metabolites have potential in medical procedures and applications in the cosmetic, food and pharmaceutical industries.

2. Materials and Methods

Chemicals used for synthesis were of chromatographically pure grade. DPPH used for antioxidant assay was procured from Sigma-Aldrich USA. All other chemicals used were of GR grade. For analytical purposes, AR grade chemicals were used. Commercial solvents were distilled and used for synthesis. Solvents purified by standard methods were employed for physical and physicochemical measurements.

The rhizomes of wild turmeric (*Curcuma aromatica* Salisb.) were collected from Pattambi, Palakkad district (Kerala). Peeled and washed samples were cut into small pieces and dried under shade for nearly 30 days. The dried samples were ground to fine powder and used for extraction with various solvents.

2.1 Extraction of the rhizome

The rhizomes were extracted with different organic solvents with slightly varying procedure which is detailed under results and discussion. Column chromatography is used to isolate curcumin from the extract.

2.2 Antioxidant assay

Antioxidant compounds may scavenge reactive oxygen species (ROS) and peroxide radicals, thereby preventing or treating certain pathogenic conditions.

2.2.1 Ferric thiocyanate method

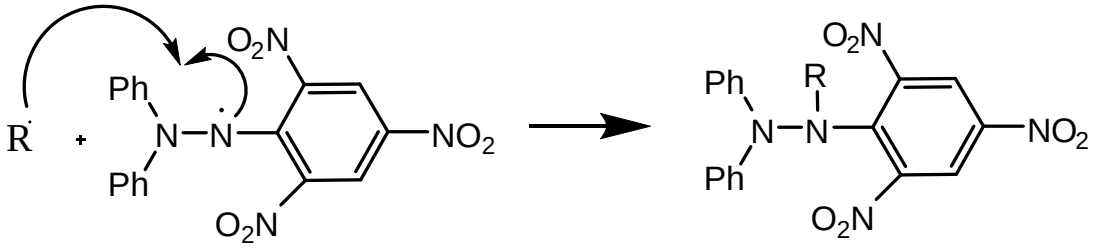
Lipid peroxidation has been extensively used as a research model for identifying natural antioxidants as well as the studies of their mechanisms of action. The antioxidant assay is usually carried out on model systems such as methyl-linoleate or linoleic acid and there are several methods to study the extent of activity [9]. In the thiocyanate method, the ability of lipid hydro peroxide (formed during the auto oxidation of lipids such as linoleic acid) to oxidize $Fe^{2+} \rightarrow Fe^{3+}$ is exploited. In this method, the sample and linoleic acid in a water-ethanol medium is incubated at 40 ^{0}C in the dark and the auto oxidation is followed at intervals by measuring the absorbance (at 500 nm) of the red colour developed after the addition of $FeCl_2$ and NH_4SCN. As the concentration of Fe^{3+} and hence absorbance depends on the extent of lipid autoxidation, the antioxidant activity can be judged qualitatively from a comparison of the absorbance of a control maintained under identical conditions.

2.2.2 DPPH Radical Scavenging Activity Assay

DPPH is a well-known radical to monitor chemical reactions involving radicals and recently it is most widely used for antioxidant assay [10]. When a solution of DPPH having a strong absorption at 517 nm is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form of DPPH (2) which can be monitored by measuring the absorbance at 517 nm. Lower absorbance at 517 nm represents higher DPPH scavenging activity. The % DPPH radical scavenging activity of sample was calculated from the decrease in absorbance at 517 nm DPPH radical's concentration.
Representing the DPPH radical by Z• and the donor molecule by AH, the primary reaction is

$$Z + AH \longrightarrow ZH + A$$

Where, ZH is the reduced form and A• is free radical produced in this first step. This latter radical will then undergo further reactions which control the overall stoichiometry, that is, the number of molecules of DPPH reduced (decolorised) by one molecule of the reductant.
The reaction [1] is therefore intended to provide the link with the reactions taking place in an oxidising system, such as the autoxidation of a lipid or other unsaturated substance; the DPPH molecule Z• is thus intended to represent the free radicals formed in the system whose activity is to be suppressed by the substance AH.



(1) (2)

The extracts of rhizomes were tested for the scavenging effect on DPPH radical according to the method of Pan *et al* [11]. In this method, 0.2 mL of extract solution in ethanol (95%) at different concentrations (0.2, 0.5, 0.8 and 1.2 mg mL^{-1}) was added to 8 mL of 0.004% (w/v) stock solution of DPPH in ethanol (95%). The scavenging activity on the DPPH radical was determined by measuring the absorbance at 517 nm until the reaction reached the steady state, using a UV–VISIBLE spectrometer. All determinations were performed in triplicate. The DPPH radical scavenging activity (*S%*) was calculated using the following equation:

$$S\% = ((A_{control} - A_{sample})/A_{control}) \times 100$$

Where, $A_{control}$ is the absorbance of the blank control (containing all reagents except the extract solution) and A_{sample} is the absorbance of the test sample.

2.3. Anti-microbial assay

Determination of antimicrobial effectiveness against specific organism is essential in clinical therapy and recently several automated systems for susceptibility testing with agar or broth culture have been developed. The disc diffusion technique or Kirby-Bauer method [1, 5] is followed in the present investigation which is the most preferred to follow antimicrobial activity against rapidly growing organism. The principle behind the assay technique is fairly simple. When an antibiotic impregnated disc is placed on agar previously inoculated with the rest organism and on moistening, the antibiotic diffuses rapidly outwards through the agar producing antibiotic concentration gradient. A clear zone or ring will present if the agent inhibit microbial growth. The wider the zone surrounding the disc, the more active is the substance. The actual zone sizes have not been standardized in the method, but a comparison of zone sizes for the same chemical among organisms will provide an approximate effectiveness of the chemical.

The organism selected for studying the antibacterial activity are *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli*. The *Staphylococcus* constitute and ubiquitous group of microorganism that may be detected in air, dust and natural water. One species, *S.aureus* (a gram positive organism) is particularly associated with human beings and found on the skin and mucous membranes of nose and throat. The organism will grow in food material and secrete enterotoxins which show considerable resistance to the proteolytic digestive enzymes. The victim suffers from vomiting and sometimes diarrhea accompanied by sweating, fever, hypothermia, headache and muscular cramps. *E. coli* is a gram negative aerobic bacterium usually present in the lower portion of the intestine of warm blooded animals including human beings. Certain *E. coli* strains can cause gastroenteritis, urinary tract infections and phylogenic infections. *P. aeruginosa* is a gram negative, aerobic, coccobacillus bacterium with unipolar motility causes infections in damaged tissues or those with reduced immunity.

2.3.1 Experimental

Determination of antimicrobial effectiveness against specific organism is essential in clinical therapy and recently several automated systems for susceptibility testing with agar or broth culture have been developed. The disc diffusion technique is followed in the present investigation which is the most preferred to follow antimicrobial activity against rapidly growing organism. The principle behind the assay technique is fairly simple. When an antibiotic impregnated disc is placed on agar previously inoculated with the rest organism and on moistening, the antibiotic diffuses rapidly outwards through the agar producing antibiotic concentration gradient. A clear zone or ring will present if the agent inhibit microbial growth. The wider the zone surrounding the disc, the more active is the substance.

2.3.2 Media used and their compositions

Nutrient agar media is used for maintaining pure bacterial culture and to lawn the bacteria for detecting the antibacterial activity. It is prepared by dissolving peptone (1 g), meat extract (0.5 g), NaCl (0.5 g) and agar (2.5 g) in distilled water (100 mL) and adjusting the pH of the medium to 7.2 - 7.4 using 10% NaOH.

Nutrient broth is used to for preparing the broth culture of the test bacteria and its composition is the same as that of nutrient agar, excluding agar.

Solution of test components were prepared in DMSO and for sterilizing, all the media used were autoclaved at 121 ^{0}C for 20 minutes.

2.3.3 Detection of antimicrobial activity

Nutrient agar slants where used for maintaining the stock culture of test bacteria. A loopful of bacteria was transferred into 5mL of nutrient broth (sterilized) and was incubated at 37 ^{0}C for 6 - 8 hours. From this culture 200 µL was transferred to petriplates containing nutrient agar and spreaded evenly on the medium with the help of a glass spreader to get a uniform lawn of bacteria.

Using an agar punch, wells were made on these seeded plates and 75 µL (4000 ppm) of the test compound as a solution in DMSO were added to the wells and each well are labeled. The petriplates were prepared in duplicate and inoculated at 37 ^{0}C. Ciprofloxacin is used as the control in the solvent DMSO. The antimicrobial activity was determined by measuring the diameter of the zone inhibition.

3. Results and Discussion

The different solvents used for extraction and the mass of dried matter obtained from 5g of powdered rhizome are given in Table 1.

Table 1: The mass of crude extract using different solvents

Solvent	Mass of dry extract (in gm)
Ethanol	0.65
Hexane	0.55
Ethyl acetate	0.60
Dichloromethane	0.45
Acetone	0.50
Water	0.60

3.1 Antioxidant activity

All the extracts prepared were tested for their antioxidant activity by ferric thiocyanate method. However, only dichloromethane extract was selected for studying the DPPH scavenging activity. The absorbance data in ferric thiocyanate method is given in Tables 2-3. It is graphically represented in Figures 1-2. The results show that the ethyl acetate and dichloromethane extracts have maximum antioxidant activity probably due the solubility of phenolic compounds. The absorbance data in DPPH method is given in Table 4 and graphically represented in Figure 3.

Table 2: Absorbance data of BHT and wild turmeric extracts

Number of days	BHT	Ethanol(1)	Hexane(2)	Ethyl acetate(3)
1	0.02	0.02	0.03	0.02
2	0.04	0.08	0.08	0.05
3	0.06	0.17	0.18	0.12
4	0.09	0.33	0.29	0.25
5	0.14	0.49	0.36	0.38
6	0.21	0.71	0.49	0.51
7	0.31	0.96	0.72	0.85

Table 3: Absorbance data of BHT and wild turmeric extracts

Number of days	BHT	Dichloromethane(4)	Acetone(5)	Water(6)
1	0.02	0.02	0.02	0.02
2	0.04	0.08	0.04	0.05
3	0.06	0.18	0.09	0.12
4	0.11	0.29	0.17	0.28
5	0.16	0.42	0.22	0.39
6	0.23	0.55	0.37	0.47
7	0.31	0.78	0.54	0.62

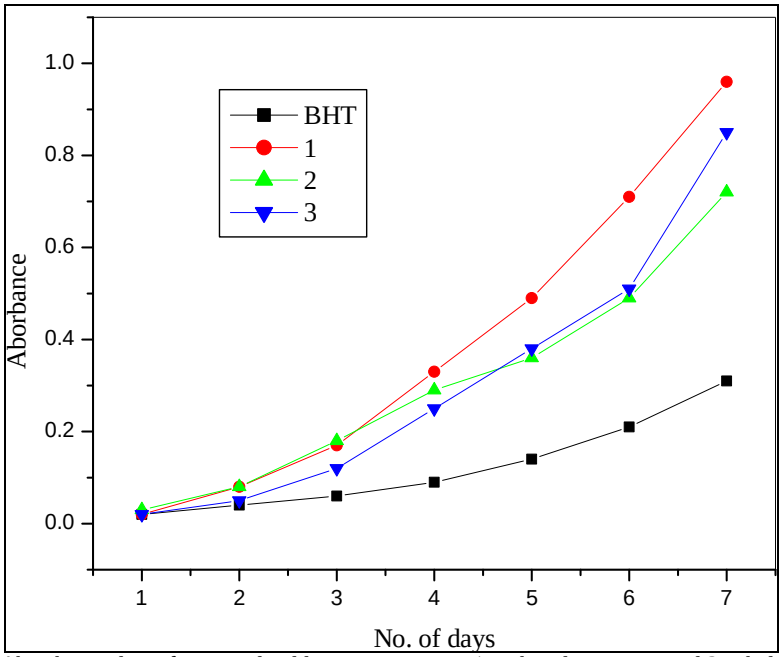


Fig 1: Absorbance data of BHT and wild turmeric extracts (1-Ethanol 2-Hexane and 3-Ethyl acetate)

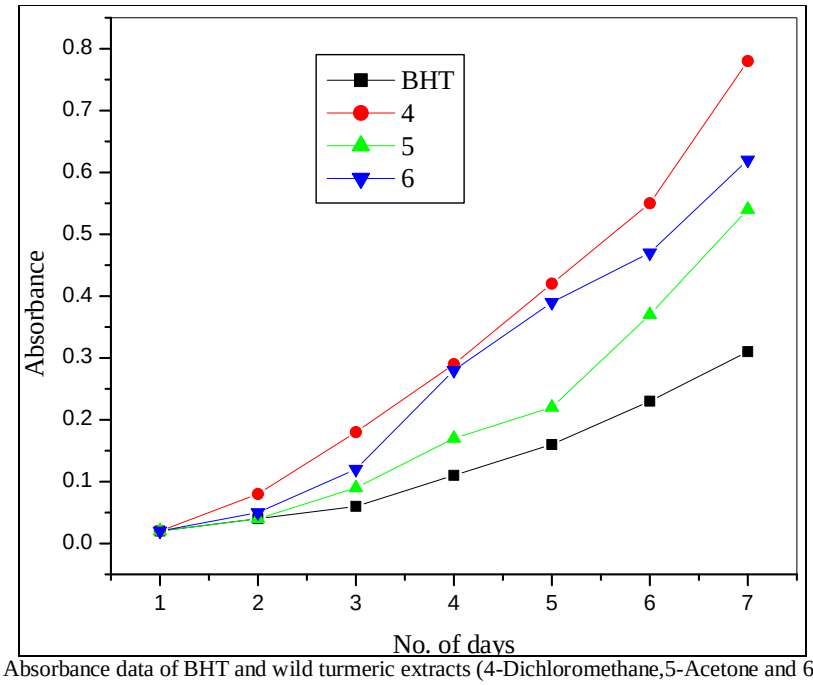


Fig 2: Absorbance data of BHT and wild turmeric extracts (4-Dichloromethane,5-Acetone and 6-Water)

Table 4: DPPH scavenging activity

Sl. No.	Concentrations (mg mL^{-1})	Absorbance of control	Absorbance of sample	S%
1	0.2	0.74	0.68	08.108
2	0.4	0.74	0.59	20.270
3	0.6	0.74	0.42	43.243
4	0.8	0.74	0.31	58.108

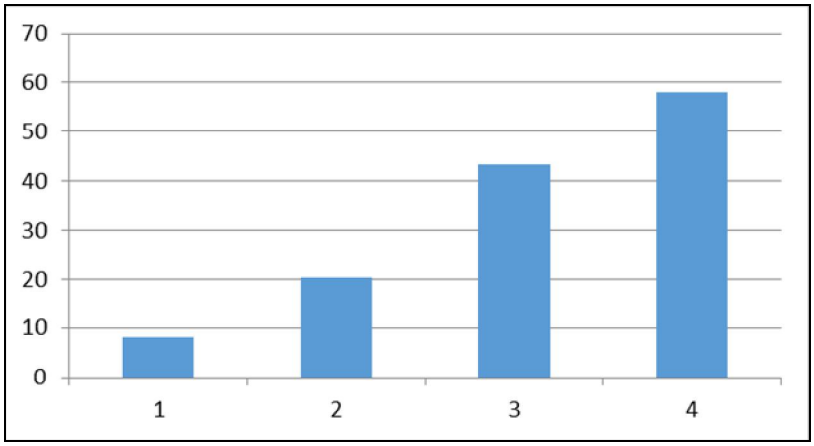


Fig 3: DPPH scavenging activity (%S against concentration)

3.2 Antibacterial assay

The organism selected for studying the antibacterial activity are *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 25922). The diameter of the zone inhibition (mm) are given in Table 6 and graphically represented in figure 6. From the results it follows that hexane, dichloromethane and ethyl acetate extracts were more effective against the tested organisms.

Table 5: Average diameter of the zone of inhibition

Extracts	Average diameter(mm) of the zone of inhibition		
	S.aureus	***P. aeruginosa***	***E.coli***
Hexane	9	10	12
Ethyl acetate	10	11	10
Dichloromethane	9	9	18
Acetone	7	7	9
control	32	31	32

4. Conclusions

Curcumin can be easily extracted from wild turmeric rhizome in relatively good yield. It give usual reactions of phenolic OH group and carbonyl group. Hence, functional modification of curcumin can be carried out. The presence of enolizable β- diketo group make it a good chelating ligand and hence it can form stable metal complexes. Besides the curcuminoids, wild turmeric extracts contains other phytochemicals which are to be explored and the biochemical studies with individual compounds shall give interesting results. Our studies reveal that ethyl acetate and dichloromethane extracts have the maximum antioxidant activity. Among the extracts screened for antimicrobial activity, hexane, dichloromethane and ethyl acetate extracts showed maximum activity against all tested organisms.

5. Acknowledgement

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