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Synthesis, Characterization and Comparative Studiesof Curcumin and Several Curcuminoids Compounds

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Abstract—*A successful collection, identification of turmeric and extraction of curcuminoid synthesis of newer curcuminoid compounds(4-Hydroxyphenyl Curcuminoid, Curcuminoid-1, 4-Chlorophenyl Curcuminoid, 3-Nitrophenyl Curcuminoid, 4-Methylphenyl Curcuminoid, 3, 4-Dimethoxyphenyl Curcuminoid, 4-Fluorophenyl Curcuminoid, 4-Methoxyphenyl Curcuminoid, DiphenylCurcuminoid and 3, 4-Dihydroxyphenyl Curcuminoid) which are separated with the help of column chromatography. Characterization of these compounds was done by various instrumental techniques like NMR, Mass and FTIR spectroscopic methods. The synthesis of many curcuminoid compounds along with curcumin itself also revealed substantially potent preliminary in vitro antioxidant activity (A.C) and selection of compounds for further in vitro antioxidant activity. The test samples exhibited effective and powerful A.C at 250ug/ml of α-Tocopherol but lower A.C than same concentration of BHA (Butylated hydroxyanisole).The reducing power of the test sample was increased with increasing concentration of the test sample. The test sample revealed higher reducing power than a-Tocopherol but reductive capability was lower than BHA. The antimicrobial activity was determined by dilution susceptibility tests based on the results of their preliminary studies of synthesized compounds and selection of compounds for further evaluation was done by their biological activity in vitro. The scavenging ability of the test sample on DPPH radical was found less than BHT. The test sample is powerful free radical scavenger, which can reduce the damage caused by free radicals in human body. The curcumin and compound 10 were the most effective anti-microbial compounds at this conc., which was below 50 ug/ml and the other compounds may be shows the activity at higher concentration. This work provides the synthesis for exploring the possibility of newer curcuminoids for use as potential curcumin like therapeutic moiety with better safety, efficacy and physiochemical feasibility.*

Keywords*:Turmeric, Curcumin, curcuminoids, antioxidant activity, antimicrobial activity.*

1. INTRODUCTION

Turmeric (curcuma longa Linn.) has a warm, bitter taste and is a primary component of curry powders. Curcumin is a constituent of the traditional medicine known as turmeric. Turmeric preparations are applied to fresh wounds and bruises and as counterirritants for insect bites. Turmeric paste is used to facilitate scabbing in chicken pox and small pox. It is used in urologic diseases, hepatobiliary diseases and as an anthelminthic. Turmeric has also been described as a cancer remedy in Indian natural medical literature(Kathryn M. Nelson et al., 2017).Curcumin is a natural, yellow coloured phenolic antioxidant and was first extracted in an impure form by Vogel et al., 1815. Curcumin is a hydrophobic phenol having the chemical name [1, 7-bis(4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-dione(Kolev et al. 2005) and empirical formula C21H20O6. Curcumin is known for its wide-ranging pharmacological applications such as antioxidant, anti-inflammatory, antimicrobial, antimalarial, anti-carcinogenic, anti-HIV agent(Sanphui et al. 2011; Agarwal and Sung 2009). Curcumin, being a di-phenolic compound extracted from the rhizome of turmeric, is a prominent candidate for treating cystic fibrosis, Alzheimer's and malarial diseases in addition to cancer (Mahesh Wari et al. 2006; Yallapu et al. 2012). Curcumin is safe even at a high dose of 12 g per day (Qureshi et al. 1992; Lao et al. 2006) proven by experiments on both animals and humans(Van Nong*et al., 2016*).Turmeric is known as golden spice of India which comes from the root of turmeric plant (Curcuma longa).Turmeric is of Zingiberaceae family, Zingiberaceae – A family of tropical monocotyledonous plants.There are various benefits and uses of turmeric powder from food to medicines. The roots, or rhizomes and bulbs, are used in medicine and food.Turmeric is widely cultivated for its rhizomes which are used as a bright yellow-orange culinary spice. In turmeric, curcumin and oleoresin are the two main components which are most important factors for the significance of turmeric. Some of the therapeutically active compounds extracted from the rhizomes of Curcuma longa are called curcuminoids. Curcuminoids are inherent compounds of the species Curcuma longa and are responsible for the antioxidant activity of the turmeric. Turmeric is generally used in various food industries as a food colour. It is mainly used in dairy products, beverages, cereal, confectionary, ice cream, bakery, and savoury products. Turmeric is added at higher levels to

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sausages, pickles, relishes, sauces, dry mixes, and fish due to its original usage as a spice. Turmeric powder can be used for encapsulation and preparing highly beneficial turmeric health tablets. From many years awareness of turmeric and its use as medicine is continuously increasing. Turmeric's main ingredient is curcumin which exhibit a wide range of medicinal activities.Turmeric is used for heartburn (dyspepsia), stomach pain, diarrhoea, intestinal gas, stomach bloating, loss of appetite, jaundice, liver problems and gallbladder disorders. It is also used for headaches, bronchitis, colds, lung infections, fibromyalgia, leprosy, fever, menstrual problems, and cancer. Other uses include depression, Alzheimer's disease, water retention, worms and kidney problems. Turmeric oil is widely used in pharmaceutical applications for its antioxidant, anti-mutagenic, anti-carcinogenic, antibacterial and insect repellent properties.(Jayandran, Met al.2015).The dried rhizomes or tuber a year old may be used in medicine as ether a stimulant, carminative, hematic in many kinds of haemorrhages and as a remedy for certain type of jaundice and other liver problems. Externally, it's applied to minor wounds and certain skin eruptions, decoction affords relief for a burning sensation in eye diseases, it's also considered very good for irregular menstruation. It promotes circulation, dissolves blood clots and its prescribed as a remedy for paints in abdomen chest and the back. The current traditional Indian medicine claims the use of turmeric against biliary disorders, anorexics coryza, cough, diabetic wounds, hepatic disorders, rheumatism and sinusitis (Ammon and Wahl, 1991a, Park and Darrick).Anti-proliferation and apoptosis induced by curcumin in human ovarian cancer cells (Shi, et al., 2006).

Curcumin, exhibits strong antioxidant activity, comparable to vitamins C and E (Sharma, 1976, Toda, et al., 1985).It was shown to be a potent scavenger of a variety of reactive oxygen species including superoxide anion radicals, hydroxy1 radicals (Reddy A.C. et al. 1994) and nitrogen dioxide radicals (Sreejayan and Rao, 1994, Unnikrishan and Rao, 1995).It is suggested that dietary supplementation with curcumin may be beneficial in neurodegenerative diseases such as Alzheimer's disease (Calabrese, et al., 2003, Yang, et al., 2005).It is cultivated allover India, particularly in west Bengal, Tamil Nadu and Maharashtra.

2. ACTION

Anti-inflammatory, hepato-protective, blood-purifier, antioxidant, detoxifier and regenerator of liver tissue, anti-asthmatic, anti-tumour, anti-cutaneous, anti-protozoal, stomachic, carminative, reduces high plasmacholesterol. Antiplatelet activity offers protection to heart and vessels. Also protects against DNA damage in lymphocytes.

3. KEY APPLICATION

In dyspeptic conditions (German Commission E, ESCOP, WHO.) as anti-inflammatory, stomachic. (Indian Herbal Pharmacopoeia.) The rhizomes gave curcuminoids, the mixture known as curcumin, consisting of at least four phenolic diarylheptanoids, including curcumin and mono-desmethoxycurcumin: volatile oil containing which are sesquisterpene ketones, and bitter principles, sugars, starch, resin. Curcumin related phenolic possess antioxidant, anti-inflammatory, gastro-protective and Heaton-protective activity in a variety of experimental models Used orally, curcumin prevents the release of inflammatory mediators, it depletes nerve endings of substance P, the neurotransmitter of pain receptors, curcumin's cholesterol – lowering actions include interfering with intestinal cholesterol uptake, increasing the conversion of cholesterol into bile acids and increasing the excretion of bile acids via its choleric effects. Curcuminoids prevent the increases in liver enzymes, SGOT and SGPT; this validates the use of turmeric as a hepato-protective drug in liver disorders. Curl one, obtained from the dried rhizome, is used against hepatitis, turmeric and curcumin increase the mucin content of the stomach and exerts gastro-protective effects against stress, alcohol, drug-induced ulcer formation. The ethanoic extract of the rhizome exhibited blood sugar lowering activity in alloxan-induced diabetic rats, piperine a constituent of black and long pepper enhances absorption and bioavailability of curcumin.

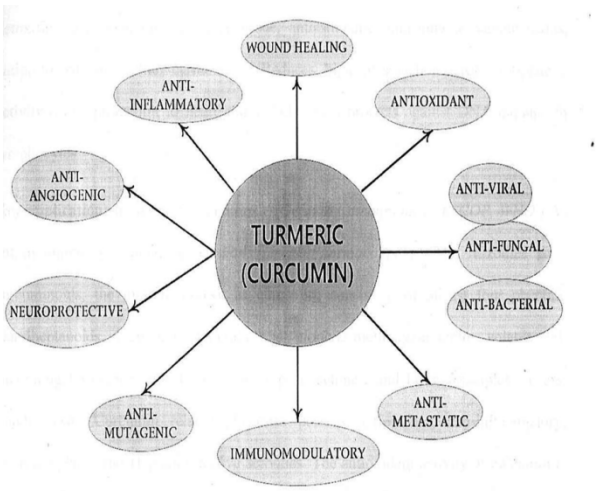

Figure 1 Biological effects of Turmeric/Curcumin Curcuma longa Linn.

4. MATERIALS AND METHODS

4.1MATERIALS AND INSTRUMENTS

Melting points were determined in open capillary tubes on an electrically heated block. IR spectra Vmax in cm-1 of the compounds were recorded on Perkin Elmer's FTIR 8201 PC spectrophotometer. H NMR spectra were recorded at 300 MHz on a Bruker DRX-300 FT spectrometer in D-chloroform solvent with TMS as internal reference (Chemical shifts on 6 ppm, J (coupling constant) in Hz.). Mass spectra were

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recorded on Joel/SX-102/DA-6000 FABMS spectrometer. Thin layer chromatography was performed on pre-coated alumina plastic plates (Aldrich). For column chromatography, Merck Silica gel 30 (120-400 mesh) was employed. Whereas the other chemicals used for the preparation of the derivatives synthesized in this work were purchased from Aldrich Chemical company. The physical and spectral data for curcumin and the synthesized compounds are in agreement with the scheme.

4.2 METHODS

4.2.1 COLLECTION AND INDENTIFICATION OF TURMERIC

Curcuma longa (Zingiberaceae) was collected form the agricultural fields around Dehradun, Uttarakhand, India. Identification of plant was carried out by Dr. Suresh Kumar. HOD, Department of Botany, Abhilashi college of life Sciences, Mandi, H.P. it was cleaned; thoroughly washed with de-ionized water and was kept for drying in shade at room temperature for 20 days. The thoroughly air dried material was grinded to about 40-60 mesh size.

4.2.2 EXTRACTION OF CURCUMIN

Ethy1 alcohol 150 ml was taken in a 250 ml round-bottom flask attached to a soxhlet extractor. Soxhlet extractor was filled with 50 gm of turmeric powder. Extraction was started by maintaining the temperature at 50 to 60°C.Extraction continued for 3 to 4 hours until the solvent which fills the extraction unit was almost colourless. The soxhlet was removed and concentrated the extract ensuring the not all ethy1 alcohol evaporated off the extract. Then added 50 ml of hexane to the extract and stirred the solution using a magnetic stirrer. Water was added slowly to the solution and precipitating out curcumin. Curcumin was filtered out using suction filtration and recrystallize from ethanol. Checked the melting point for purity as pure curcumin melts at 179 to 180 °C.

4.2.3 GENERAL PROCEDURE FOR THE SYNTHESIS OF COMPOUNDS

2, 4-pentanedione (1.0 gm., 0.01mol) and boric anhydride (0.49 gm., 0.007mol) were dissolved in ethy1 acetate 10 ml and stirred for 0.5 hr. at 40. C. The corresponding substituted benzaldehyde 0.02mol and tributy1 borate 4.6 0.02mol were added, and the reaction mixture was stirred for 0.5 hr. after the drop wise addition of n-butyl amine 1.1 gm. 0.01mol in ethy1 acetate 10 ml over a period of 15 min., the mixture was stirred for a further 24 hrs. at 40° C. The mixture was hydrolysed by the addition of 10% HCL 10 ml and heating at 60 °C for 1 hr. The organic layer was separated, and the aqueous layer was extracted three times with ethy1 acetate. The combined organic layers were washed with water and dried over Na_2SO_4. Evaporation of the solvent left a yellow colour which was purified by column chromatography

4.2.4 COLUMN CHROMATOGRAPHY

The column was packed with silica gel 60-120 mesh for column chromatography in hexane. The eluting solvent first was hexane it was then changed to chloroform: hexane (25 ml: 75ml), (1:3). Elution of the desired product was still slow so that the concentration was again changed to chloroform: hexane (50:50) this did not work well enough and it was adjusted to a ratio of 3:1 chloroform: hexane (75 ml: 25 m). elution was still slow and at last pure chloroform 100% was used which actually did not do much improvement and so to raise the polarity the concentration of eluting solvent was adjusted to chloroform: methanol 1% and elution of the desired product was still slow, finally chloroform: methanol 2% gave good elution whereby a series of fractions were obtained and checked through TLC. Fractions which did not show any traces of impurities so they were taken and concentrated as the product. Spectroscopic analysis was done on the recrystallise product. Re-crystallization was done with methanol.

4.2.5. PRELIMINARY ANTIOXIDANT SCREENING IN VITRO

4.2.5.1 Total antioxidant activity

The total antioxidant activity of the test sample was determined according to the thiocyanate method (Mitsuda, et al., 1996).10mgsof test sample was dissolved in 10 ml water. Different concentration of test sample (50-250 $\mu g/mL$) or standard samples in 2.5ml of potassium phosphate buffer (0.04 M, pH 7.0) was added to linoleic acid emulsion 2.5 ml in potassium phosphate buffer (0.04 M, pH 7.0). 5ml linoleic acid emulsion consists of 17.5 g tween -20 , 15.5μl linoleic acid, and 0.04 M potassium phosphate buffer (pH 7.0). on the other hand, 5.0 ml control consists of 2.5 ml linoleic acid emulsion and 2.5 ml potassium phosphate buffer (0.04 M, pH 7.0). the mixed solution was incubated at 37 c in a glass flask and in the dark. After the mixture was stirred for 3 min, the peroxide value was determined by reading the absorbance at 500 nm in a spectrophotometer (UV-1601 Shimadzu, Japan), after reaction with $FeCl_2$ and thiocyanate at intervals during incubation. During the linoleic acid oxidation, peroxides formed. These compounds oxidize Fe^{2+}-Fe^{3+}. The latter Fe^{3+} inons form complex with SCN-, which had maximum absorbance at 500nm.Therefore, high absorbance at 500 nm. Therefore, high absorbance indicates high linoleic acid oxidation. The solutions without test sample or standards were used as blank samples. All data about total antioxidant activity are the average of triplicate analyses. The inhibition of lipid peroxidation in percentage was calculated by following equation:

$$\text{Inhibition (\%)} = (A_0 - A_t/A_0) \times 100$$

Where A_0value was the absorbance of the control reaction and A_t was the absorbance in the presence of the sample. All the tests were performed in triplicate and graph was plotted with

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the mean ± SD values. α-tocopherol were used as standard antioxidant compound.

4.2.5.2. Reducing power

The reducing power of test sample was determined according to the method described previously (Oyaizu, 1986). The different concentrations of test sample (50-250$\mu g/mL$) in 1 ml of distilled water mixed with phosphate buffer (2 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50 C for 20 min. A portion 2.5 ml of trichloro acetic acid 10% was added to the mixture, which was then centrifuged for 10 min at 3000 rpm. The upper layer of solution 2.5 ml was mixed with distilled water 2.5 ml was mixed with distilled water 2.5 ml and $FeCl_3$ 0.5 ml, 0.1%, and the absorbance was measured at 700 nm in a spectrophotometer (UV-1601 Shimadzu, Japan). Higher absorbance of the reaction mixture indicated greater reducing power. α-tocopherol was used as standard antioxidant compound.

4.2.6. IN VITRO ANTIOXIDANT ACTIVITY OF THE SELECTED COMPOUNDS

4.2.6.1 Determination of DPPH (1-1 dipheny1 -2-picry1 hydrazy1) radical scavenging activity

The free radical scavenging activity of test sample was measured by DPPH using the method described previously (Shimada, et al., 1992). A 0.1mm solution of DPPH in ethanol was prepared and 1 ml of this solution was added to 3 ml of test sample solution in water at different concentrations (50-250$\mu g/mL$). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. then the absorbance was measured at 517 nm in a spectrophotometer (Uv – 1601 Shimazu, Japan). Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The percent DPPH scavenging effect was calculated using the following equation:

DPPH scavenging effect (%)=[$(A_0 - A_t/A_0) \times 100$]

Where A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of the standard sample or test sample. All the tests were performed in triplicate and graph was plotted with the mean ± SD values. BHA was used as standard antioxidant compound.

4.2.6.2 ABTS (2, 2-azinobis–3–ethy1benzothiazoline – 6-sulfonic acid)radical de-colourization assay

ABTS was dissolved in water to make a concentration of 7mM. ABTS was produced by reacting the ABTS stock solution with 2.45 mm potassium persulfate (final concentration) and allowing the mixture to stand in darkat room temperature for 12-16 h before use. For the test of diluted ABTS to 20 μL of sample, the absorbance reading was taken 5 min after the initial mixing (Re, et al., 1998). This activity is considered as percent ABTS – scavenging which is calculated as follows:

% ABTS – scavenging activity = [Control absorbance – Sample absorbance] / [Control absorbance] × 100

4.2.6.3Assay of nitric oxide scavenging activity

The procedure is based on Greys reaction Kumaran and Joel Karunakaran, 2006, Sreejayan and Rao. 1997. Sodium nitroprusside spontaneously generates nitric oxide at physiological pH in aqueous solution, which interacts with oxygen to produce nitrite ions that can be estimated using Greys reagent. Scavengers of nitric oxide complete with oxygen leading to reduced production of nitrite ions. Sodium nitroprusside 10 mm in phosphate buffered saline was mixed with different concentrations of test sample of the dissolved in methanol and then incubated at room temperature for 150 minutes. In the same way, a reaction mixture was prepared without the test sample but with equivalent amount of methanol was added. This served as control. After the incubation period, 0.5ml of Greys reagent (1% Sulphanilamide, 2 % H_3PO_4 and 0.1 % N-(1-naphthq) ethylenediamine dihydrochloride) was added to the mixture. The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthy1 ethylene diamine was measured at 546 nm. Standard solutions of ascorbic acid treated in the same way as test with greys reagent served as positive control. The percentage of inhibition was calculated by using the following formula:

% Inhibition = $(A_0 - A_t/A_0) \times 100$

Where, A_0 was the absorbance of the control without test sample and A_1 was the absorbance in the presence of the test sample. All the tests were performed in triplicate and graph was plotted with the mean ± SD values.

4.2.6.4 Hydrogen peroxide scavenging activity

Hydrogen peroxide (H_2O_2) scavenging ability of the test sample was measured using a method described previously (Ruch, et al., 1989). A solution of hydrogen peroxide (2mmol/L) was prepared in phosphate buffer (pH 7.4). concentration of hydrogen peroxide was determined spectrophotometrically form absorption at 230 nm with molar absorptivity 81 $(mol/L)^{-1}cm^{-1}$. The test sample (50-250 $\mu g/ml$) were added to H_2O_2 solutions 0.6 ml. absorbance of H_2O_2 at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without H_2O_2. The percentage inhibition was calculated using the following formula:

% Inhibition = $A_0 - A_t/A_0) \times 100$

Where, A_0 was the absorbance of the control (without test sample) and A_1 was the absorbance in the presence of the test sample or standard. All the tests were performed in triplicate

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and graph was plotted with the mean ± SD values. Ascorbic acid was used as standard compound.

4.2.6.5 Hydroxy1 radical scavenging

The reaction mixture containing 2 – deoxy – d- ribose (1mM), pheny1 hydrazine (0.2mM), (in phosphate buffer, pH 7.4) and different concentration of RG – M (50 – 250 $\mu g/ml$) were incubated for 4 h at 37 C. the reaction was stopped by the addition of 2.8% w/v trichloroacedic acid solution, followed by centrifugation at 5000 rpm for 10 min. The supernatant was mixed with aqueous 1% w/v thiobarbituric acid (TBA). The TBA reactive product thus formed was directly measured at 532 nm (Halliwell, et al., 1987).

4.7 ANTIMICROBIAL ACTIVITY

The compounds which were synthesized in this work were subjected to in vitro antimicrobial screening, for the Anti-microbial activity.

4.8. Dilution Susceptibility Tests (General Procedure)

This method can be used to determine minimum inhibitory concentration MIC and minimum lethal concentration MLC values. In the broth dilution test, a series of broth tubes containing antibiotic cone. In the range of 0.1 to 128ug/ml is prepared and inoculated with standard numbers of the test organism. The lowest conc. of the antibiotic resulting in no growth after 16 to 20 hrs of incubation is the MIC. The MLC can be ascertained if the tubes showing no growth are sub cultured into fresh medium lacking antibiotic. The lowest antibiotic concentration form which the microorganisms do not recover and grow when transferred to fresh medium is the MLC. The agar dilution test is very similar to the broth dilution test. Plates containing Mueller Hinton agar and various amounts of antibiotic are inoculated and examined for growth. Several automated systems for susceptibility testing and MIC determination with broth or agar cultures have been developed (Prescott,et at., 2005).

5. RESULTS AND DISCUSSION

5.1 PHYSICAL AND SPECTRALDATA

5.1.1 1, 7–Bis(4-hydroxy-3-methoxypheny1)-1, 6-heptadience-3, 5-dione(curcumin):47 % yield from vanillin (3.04g, 0.02mol). M.p.180-181 C (lit. M.P.182-183°C). $1_{H\ NMR}$ [300 MHz, $CDCI_3$] : δ 3.9560$(s, 6H, 2\ X_3 HCO - C6\ H4-$, 6.9262-6.9536 (d, J= 8.22 Hz, 2H, 2x - $C6H4-$), 6.4565-6.5094 (d. J_{Trans} = 15.78 Hz, 2 H, 2x- $CH_b = CH_{c-}$), 6.962-6.9536 (d, J = 8.22Hz, 2H, 2x - C_6H_{4-}), 7.1146-71475 (s, 2H, 2 x $CH_b = CH_{c-}$), 7.5709-7.6234 (d, J_{Trans} = 15.75 Hz, 2H, 2 x -$CH_c = CH_{b-}$). EIMS: m/z 369.1 $(M+1)^+$, 370.2 $(M+2)^+$, 177.0 (Base peak).IR (KBr,cm^{-1}): 3504.3, 1428.5, 1375.8, 1314.5, 1205.7, 1117.8.

5.1.2 1, 7bis (4 –hydroxypheny1) – 1, 6-heptadience – 3, 5-Dione(Compound-1):52% yield form 4 – hydro- oxy-benzaldehyde (2.4 g, 0.02mol), M.P 221-223°C (lit. M. P.224° C). H_1NMR [300 MHzCDCL$_3$]: δ 5.8176 (s, 1H, = CH_{a-}), 6.5175-6.5694 (d, J_{Trans} = 15.57 Hz, 2H, 2 x - $CH_b = CH_{c-}$), 6.9724-7.0101 (m, 4H, 4 x - $-C_6H_{4-}$), 7.3486-73942 (m, 4H, 4 x- $-C_6H_{4-}$), 7.5479-75998 (d, J_{Trans} = 15.57Hz, 2H, 2 x $CH_c = CH_{b-}$), EIMS: m/z 308.0 $(M)^+$, 309.2 $[(M+1)^+]$, 176.3 [base peak], IR (KBr, cm^{-1}) : 3213.0, 1702.1, 1441.3 1340.5, 1269.2, 1139.1.

5.1.3 1 (4 –hydroxypheny1) 7-(4-heptadience – 3- methoxy-pheny1)-1, 6 – heptadiene – 3, 5- Dione (Compound-2):18% yield form vanillin (1.52 g, 0.01mol) and 4 – hydro-ox benzaldehyde (1.2g, 0.01mol).M.P. 170-172°C (lit. M.P 224°C). H_1NMR [300 MHz, CdCL$_3$]: δ 3.8278 (3H= $1_{x_3} HCO - C_6H_{4-}$), 5.6412 (s, 1H, = CH_{A-}), 6.3271-6.3799 (d, J_{Trans} = 15.84 Hz, 2H, 2 x -C_6H_{4-}, 7.5418-75946 (d , J_{Trans} = 15.84 Hz 2H, 2 x $-CH_c = CH_{b-}$). EIMS: m/z 338.1$(M)^+$, 339.0 (M+1, base peak)$^+$, 340.1 $(M+2)^+$ IR (KBr,cm^{-1}): 3629.5, 1736.4, 1424.3, 1305.8, 1221.8, 1103.0.

5.1.4. 1, 7-Bis (4 – cholropheny1)-1, 6-heptadiene -3, 5-dione (Compound-3): 29% yield form 4-chloro benzaldehyde (2.81 g, 0.02mol). M.P. 158-160c. 1H NMR [300 MHz, $CDCL_3$] : δ 5.8474 (s1H, = CH_{a-}), 6.5845-6.6374 (d, J_{Trans} = 153.87 Hz, 2H, 2 x $-CH_b = CH_{c-}$), 7.3978-74033 (d, J = 7.05, 4H , 4 x $-C_6H_{4-}$), 7.4986-75223 (d, J = d7.11, 4H, 4 x $-CH_bH_{4-}$), 7.6093-76647 (d, J_{Trans} = 16.62 Hz, 2H 2 x $-CH_c = CH_{b-}$). EIMS: m/z/345.1$(M)^+$, 347.1 $[(M+2)^+$, base peak]. IR (KBrcm^{-1}): 3420.9, 1636.8, 1403.5, 1090.4

5.1.5. 3.3.5 1, 7Bis (3-nitopheny1 – 1, 6- heptadiene – 3, 5-Dione (compound – 4): 19% yield from 3 – nitro – benzaldehyde (3.02 g 0.02mol). M.P. 144-146C 1H NMR [300 MHz, $CDCL_3$]: δ 5.84420 (s, 1H = CH_{a-}), 6.5952-6.6501 (d, J_{Trans} = 16.47 Hz, 2H, 2 x - $CH_b = CH_{b-0}$), 7.3637-7.3890 (m, 6H, 6 x $-C_6H_{4-}$), 7.6271-7.6820 (dJ_{Trans} = 14.47 Hz, 2H 2 x $CH_C = CH_{b-}$), 7.9415-79746 (m, 2H 2 x $-C_6H_{4-}$), EIMS: m/z 366.1 $(M)^+$, 367.2 $(M+1)^+$, 368.1 $(M+2)^+$, 190.5 (base peak). IR (KBr,cm^{-1} : 3412.9, 1627.8 1509.4 1284.5, 1141.5.

5.1.6 1, 7-Bis(4-methy1pheny1)1, 6-heptadiene-3, 5-dione (compound-5): 70% yield from 4 methy1 benzaldehyde (2.4 ml, 0.02mol). M.P. 208-210°C (lit. M.P.208-209°C). 1H NMR [300 MHz, $CDC1_3$]: δ 2.38 (s 6H, 2 $x_3 HC - C_6H_{4-}$), 5.82 (s, 1H, = CH_{a-}), 6.56-6.61 (d, J_{Trans} = 15.0 Hz, 2H, 2 x $-CH_b = CH_{c-}$), 7.19-721 (D, J = 6.0, 4H, 4 x C_6H_{4-}), 7.44-7.47 (d, J = 9.0, 4H, 4 x $-C_6H_{4-}$), 7.44-7.47 (d, J = 9.0, 4H , 4 x $-C_6H_{4-}$), 7.61-766 (d, J_{Trans} = 15.0 Hz, 2H, 2 x $-CH_c = CHb-$). EIMS: m/z 304.4 $(M)+$, 306.0 $(M+2)+$, 289.0 (base peak). IR (KBr,cm^{-1}): 3414.0, 1623.5, 1322.9, 1139.4.

5.1.7. 1, 7-Bis (3, 4-dimethoxypheny1) -1, 6-heptadiene-3, 5-dione (Compound-6):55% yield from 3, 4-dimethoxybenzaldehyde (3.3 g, 0.02mol). M.P. 130-131°C

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(lit.M.P.128—130°C). 1H NMR [300 MHz, $CDC1_3$]: δ 3.9231 (s, 12H, 4 $x_3 HCO - C_6H_{4-}$), 5.8445 (s, 1H, $= CH_{a-}$), 6.4918-65444 (d, J_{Trans} = 15.78 Hz, 2H. 2 x $-CH_b = CH_{c-}$), 6.8908-6.9185 (d, J = 8.31 Hz, 2H , 2 x $-C_6H_{4-}$), 7.1043 (s, 2H, 2 x $-C_6H_{4-}$), 7.1515-7.1791 (d, J = 8.28 Hz, 2H, 2 x $-C_6H_{4-}$), 7.6040-76565 (d, J_{Trans} = 15.78 Hz, 2H. 2 x $-CH_c = CH_{b-}$). EIMS: m/z 397.1 [$(M+1)^+$, base peak], 398.1 $(M+2)^+$, IR(KBr, cm^{-1}): 3600.2, 1725.4, 1442.9, 1263.4, 1135.9.

5.1.8 1, 7 Bis(4-fluoropheny1)-1, 6-heptadiene-3, 5-dione (compound-7):27% yield from 4-fluoro benzaldehyde (2.48 g, 0.02mol) M.P 151-153°C.1H NMR [300 MHz, $CDC1_3$]: δ 5.8710 (s, 1H, $= CH_{a-}$), 6.6271-6.6690 (d, J_{Trans} = 12.57 Hz, 2H 2 x $-CH_b = CH_{c-}$), 7.0583-7.1770 (d, J = 35.61, 4H, 4 x $-C_6H_{4-}$), 7.3946-7.4471 (d, J = 15.75, 4H, 4 x $-C_6H_{4-}$), 7.7104-7.7523 (d, J_{Trans} = 12.57 Hz, 2H 2 x $-CH_c = CHb-$). EIMS: m/z 312.2 [$(M)+$, base peak].313.2$(m+1)+$. IR (KBrcm^{-1}): 3417.5, 1659.5, 1356.5, 1159.0.

5.1.9 1, 7 – Bis (4-methoxyphyeny1) 1, 6-heptadiene -3, 5-dione (compound -8): 37% yield form 4-methoxybenzaldehyde (2.7 g, 0.02mol) M.P. 166-167°C (lit. M.P.164-165 °C). H_1 NMR [300 MHz, $CDC1_3$]: δ 3.8415 (s, 6H, 2 $x_3 HCO - C_6H_{4-}$), 5.7830 (s, 1H, $= CH_{a-}$), 6.4723-6.58249 (d, J_{Trans} = 15.78 Hz 2H, 2 x $-CH_b = CH_{c-}$), 5.7830 (s 1H, $= CH_{a-}$), x $-C_6H_{4-}$), 7.4952-7.5238 (D J = 8.58, 4H, 4 x C_6H_{4-}), 7.4952-7.5238 (d, J = 8.58, 4H, 4 x C_6H_{4-}), 7.5943-7.6469 (d, J_{Trans} = 15.78 Hz 2H, 2 x $-CH_c = CHb-$). EIMS: m/z 335.1 [$(M-1)+$, base peak],336.3$(M)+$. IR (KBr$cm - 1$): 3415.5, 1735.8, 1358.9, 1037.1.

5.1.10. 1, 7-Dipheny1-1, 6-heptadiene-3, 5-dione (Compound-9):21% yield from benzaldehyde (2.1 g, 0.02mol) 140-141 °C). 1 H NMR[300 MHz, $CDC1_3$] : δ 5.8413 (s, 1H, $= CH_{a-}$), 6.5941-6.6471 (d, J_{Trans} = 15.90 Hz, 2H, 2 x -$CH_b = CH_{c-}$), 7.3648-73886 (m, 6H, 6 x $-C_6H_{4-}$), 7.5324-7.5640 (m, 4H, 4 x $-C_6H_{4-}$), 7.6282-7.6811 (d, J_{Trans} = 15.90 Hz, 2H, 2 x -$CH_c = CH_{b-}$). EIMS: m/z 277.2 [$(M+1)^+$, base peak], 278.2 $(M+2)^+$IR (KBr, cm^{-1}): 3407.8, 1728.1, 1376.2, 1044.7.

5.1.11. 1, 7-Bis(3, 4-dihydroxypheny1)-1, 6-heptadiene-3, 5-dione (compound-10):40% yield form 3, 4-dihydroxybenzaldehyde (2.76 g, 0.02mol),M.P.= 304-306 °C (lit. M.P.=306-308 °C). 1 H NMR [300 MHz, $CDC1_3$]: δ, 5.8397 (s, 1H, $= CH_{a-}$), 6.2737-63267 (d, J_{Trans} = 15.9 Hz, 2H, 2 x $-CH_b = CH_{c-}$), 6.8590-6.8864 (d, J = 8.22 Hz, 2H 2 x $-C_6H_{4-}$), 7.0032-7.0306 (d J = 8.22 Hz, 2H, 2 x $-C_6H_{4-}$), 7.0710-7.1018 (s, 2H, 2 x $-C_6H_{4-}$), 7.4068-7.4598 (d, J_{Trans} = 15.9 Hz, 2H, 2 x $-CH_c = CH_{b-}$). EIMS: m/z 340 $(m)^+$, 339.2 $(m-1)^+$, 289 [base peak]. IR (KBr, cm^{-1}) : 3234.5, 1651.6, 1445.0, 1388.7, 1298.0, 1192.2, 1117.9.

6. TOTAL ANTIOXIDANT ACTIVITY DETERMINATION IN LINOLEIC ACID SYSTEM

Thiocyanate method was used to evaluate the total antioxidant activity of the test sample. The test sample exhibited effective and powerful antioxidant activity at a concentration of 250 ug/ml. The effect of 250ug/ml concentration of the test sample on peroxidation of linoleic acid emulsion is shown in figure 2. The antioxidant activity of the test sample initially was increased with an increasing time of incubation. The studied concentration of the test sample exhibited higher antioxidant activity than 250ug/ml concentration of α-Tocopherol but lower antioxidant activity than same concentration of BHA (Butylated hydroxyanisole).The percentage inhibition of peroxidation of the test sample linolenic acid system were depicted n figure 15. And percentage inhibition of 250ug/ml concentration of BHA and α-tocopherol was found 95. 13 and 32.58 % respectively.

7. EFFECT ON REDUCING POWER ASSAY

The reducing power of the test sample compared to BHA and a-Tocopherol are shown in figure 16. In the reductive ability measurement $Fe^{3+} - Fe^{2+}$ transformation the presence of test sample was investigated The reducing power of the test sample was increased with increasing concentration of the test sample. At all the studied concentrations, the test sample revealed higher reducing power than α-tocopherol but reductive capability was lower than BHA. Reducing power of the test sample and standard compounds followed the order: BHA> Test sample (except compound-10) > α-Tocopherol.

8. EFFECT ON DPPH RADICAL SCAVENGINE ACTIVITY

Evaluation of antioxidant activities by measuring scavenging capability of stable DPPH radical is a widely used method which requires short time compared to other methods. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. Antioxidants induced decrease in absorbance at 517 nm by scavenging of DPPH radicals. Hence, DPPH is usually as a substrate to evaluate antioxidant activity of different antioxidants. In this present study, BHA was used as a standard radical scavenging capability of the test sample and standard compound (BHT) at different studied concentration (50-250ug/ml). The scavenging ability of the test sample on DPPH radical was found to be less than BHT. The percent DPPH scavenging effect of the test sample and the standard were depicted in figure 17. The results indicated the compounds as strong scavenge of DPPH radical comparable to standard BHT. The IC_{50} values of the test sample and BHT were calculated using the equation obtained from linear regression analysis. The calculatedIC_{50} values of the test sample and the standard compound (BHT) were tabulated in table 5. When free radical formation exceeds the body's ability to protect itself, oxidative stress occurs and

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forms the biological basis of chronic condition (Janie and Devi, 2005). Data form this present study indicate that the test sample is powerful free radical scavenger, which can reduce or reverse the damage caused by free radicals in the human body.

8.1 ABTS (2, 2 – AZINOBIS – 3 ETHYLABENZOTHIAZOLINE-6- SULFONIC ACID) RADICAL DECOLORIZATION ASSAY

The synthesized and selected compounds produced a concentration dependent scavenging of ABTS radical. The IC_{50} values of the selected compoiunds, quercetin, curcumin and selected compounds were calculated.

9. EFFECT ON NITRIC OXIDE SECAVENGING ACTIVITY

Nitic oxide or reactive nitrogen species (RNS) are very reactive compounds which can change pathologically the structural and functional behaviour of many cellular components (Ashok Kumar, et a., 2008). In this present experimental design, scavenging of nitric oxide by the test sample was evaluated by observing the reduction of linear time-dependent nitrite production in the sodium nitroprusside – PBS system which shows the concentration dependent scavenging of nitric oxide by the test sample and standard compound (Ascorbic acid). It is observed that all the concentrations of the test sample (50-250ug/ml) are likely to have the nitric oxide scavenging activity. The nitric oxide scavenging activity of the test sample was found to be lower than ascorbic acid but nitric oxide scavenging effect of curcumin was found to be higher. The percentage inhibitions of nitric oxide by the test sample and standard were calculated. The IC_{50} values were calculated form the equations obtained from linear regression analysis of the data, and given in the table 1.Hydrogen peroxide gives rise to hydroxy1 radicals. Removing bydroxy1 radicals (OH) is very essential for the protection of living system as they react with most biomolecules and other cellular components to cause tissue damage leading to cell death (Dhulia, et al., 1993, Reddy, et al., 2010). In this present study, ability of test sample at different concentrations (50-250ug/ml) to scavenge H_2O_2 scavenging effect by different concentrations of the test sample and standardcompound (ascorbic acid). Figure 20 shows hydrogen peroxide scavenging activity of the test sample and standard antioxidant compound at concentration of 50ug/ml to scavengeH_2O_2 was evaluated. Figure 7shows the percentage H_2O_2 scavenging effect by different concentrations of the test sample and standard compound (ascorbic acid). Figure 20 shows hydrogen peroxide scavenging activity of the test sample and standard antioxidant compound at concentration of 50ug/ml. The test sample showed good H_2O_2 scavenging ability when compared to standard compound. The pervrnyshr H_2O_2scavenging effect by the same concentration (50ug/ml) of the test samples and ascorbic acid were depicted in figure 20. But 250ug/ml concentration of the test sample showed higher percentage scavenging effect than the same concentration of ascorbic acid.

10. ANTIMICROBIAL ACTIVITY

From the data it was clear that the curcumin and compound 10 were the most effective anti-microbial compounds at this conc. Which was below 50ug/ml and the other compounds may be shows the activity at higher concentration.

11. SUMMARY AND CONCLUSION

The present study demonstrated a successful synthesis and identification of curcumin and several curcuminoid compounds. Curcumin is a successful and potential investigational molecule with various biological activities. The study demonstrated successful synthesis of several curcuminoid compounds along with curcumin itself and also revealed substantially potent antioxidant and antimicrobial activity in vitro. Further the compounds, which were selected based upon the preliminary in vitro. Studies, were found to be potentially antioxidant in vitro. The present study paved a way for a line of synthesis for exploring the possibility of synthesis of newer curcuminoids for use a potential curcumin like therapeutic moiety with better safety, efficacy and physiochemical feasibility.

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