PHYTO-PHARMACOLOGICAL PROFILE AND POTENTIAL THERAPEUTIC USES OF *CURCUMA AROMATICA*

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Summary

Curcuma aromatica, commonly known as 'Jangli Haldi' is a widely cultivated plant in India for its rhizomes. It is commonly used as condiments and as flavouring agents. Medicinally, it has been proven to possess various pharmacological activities like antimicrobial, anti-angiogenic activity, cholerectic activity, anthelminitic activity, antitumour activity, wound healing activity, cytoprotective activity, anti-inflammatory activity and antioxidant effects. Further, studies reveal the presence of various phytochemical constituents mainly essential oils (1,8- cineole, carvone, camphor, borneol, limonene etc.) with curcumin, curcumene and xanthorrhizol. These studies reveal its beneficial therapeutic effects and encourage finding its new uses. It also emphasizes on the fact that these results are made more fruitful by conducting clinical trials.

Key Words: Curcuma aromatica, Phytochemistry, Essential oils, Pharmacological activities.

Introduction

Curcuma aromatica Salisb. (CA), commonly known as 'Jangli Haldi', belongs to genus Curcuma consists of about 70 species of rhizomatous herbs. *C. aromatica* is distributed throughout India and is widely used as a flavouring agent, condiment and a source of yellow dye. Medicinally, it possesses strong antimicrobial effect. It is a well-listed drug in Ayurveda and other indigenous systems of medicine. The rhizomes of *C. aromatica* possess a reputed property to promote health conditions by arresting ageing and have immunomodulatory effects. From ancient times, it is being used as an antibiotic against various microbial infections[1]. Historically, rhizomes are used as tonic, carminative, and externally in combinations with astringents, bitters and aromatics to brusises, in sprains and in snake-bite. They are also used for skin eruptions and infections and to improve complexion[2].

It is a wild plant, cultivated throughout India chiefly in Bengal and Kerala (Travancore). It is an erect, perennial herb. Rhizomes are large, tuberous, yellow or orange- red inside and aromatic in taste. Leaves are large, green, oblong-lanceolate/ oblong elliptic, with acuminate apex, 38-60 x 10-20 cm size, often variegated above, pubescent beneath, base deltoid with long petioles. Rootstock large, of palmately branched, sessile annulated biennial tubers. Flowering stem appears with or before the leafing stem, as thick as the forefinger and sheathed. Flowers are fragrant, shorter than the bracts, in spikes 15-30 cm long; flowering bracts 3.8-5 cm long, ovate, recurved, cymbiform, rounded at the tip, pale green, connate below forming pouches for the flower, bracts of the coma 5-7.5 cm long, more or less tinged with red or pink. Calyx 8 mm long, irregular with 3-lobed, corolla tube 2.5 cm long with upper half like funnel- shaped, lobes pale rose- coloured, the lateral lobes oblong, the dorsal longer, ovate, concave, arching over the anthers. Lip yellow, obovate, deflexed, subentire or obscurely 3- lobed. Lateral staminodes oblong, obtuse and as long as corolla- lobes[3]

Figure: *Curcuma aromatic*

PHYTOCHEMICAL STUDIES

Marked variations have been observed in *Curcuma aromatica* regarding chemical constituents and its content, chiefly, essential oils. The essential oils of *C. aromatica* revealed the presence of various mono- and sesquiterpenes. The oil contains sesquiterpenes (mainly 1-α and 1-β curcumenes) 65.5, two monocyclic tertiary sesquiterpene alcohols 22.0, d-camphor 2.5, d-camphene 0.8, p-methoxycinnamic and other acids 0.7, and unidentified residues 8.5%[4,5]. Early studies also reported the presence of curcumol in oil. Later on, considerable effort has been devoted for the quantification of curcumol in the essential oils[6,9]. The leaves, petioles and rhizomes of *C. aromatica* from Assam, India were subjected to steam distillation and analyzed by GLC and GC- MS.

The major components in the leaf, petiole and rhizome oil were found to be as follows:

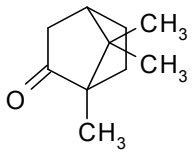
Component	Leaf (%)	Petiole (%)	Rhizome (%)
1,8- cineole	20.0	8.8	9.3
Camphor	18.0	16.8	25.6
Germacrone	11.8	0.2	10.6
Isoborneol	6.4	6.8	8.2
Camphene	9.4	1.2	7.4

Additional notable constituents included limonene (8.6%) in the leaf oil, caryophyllene oxide (8.7%), patchouli alcohol (8.4%) and elsholtzia ketone (6.0%) in the petiole oils and curzerenone (10.9%) in the rhizome oil (10). The major constituents of the leaf oil from Northeast India were found to be camphor (28.5%), ar-turmerone (13.2%), curzerenone (6.2%), 1,8-cineole (6.05), and α–turmerone (2.55) while the rhizome oil consisted mainly of camphor (32.3%), curzerenone (11.0%), α-turmerone (6.7%), ar-turmerone (6.3%) and 1,8-cineole (5.5%)[11].

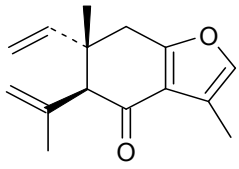
The rhizome oil from an Indian variety contained 34% β-curcumene, 15% ar-curcumene and 11% zingiberene as major constituents[12]. The oils of wild *C. aromatica* from India were also reported to contain 65% curcumene sesquiterpenes and 12% sesquiterpenes alcohols[13]. Three new sesquiterpenes; isozedoarandiol, methylzedoarondiol and neocurdione, alongwith curdione, germacrone, (4S, 5S)-germacrone-4,5-epoxide, dehydrocurdione, procurcumenol, zedoarondiol and curcumenone were isolated from Japanese *C. aromatica*[14]. Twenty-one sesquiterpenes were isolated from fresh extract of Japanese *C. aromatica* rhizomes, including (4S, 5S)-germacrone-4,5-epoxide and dehydrocurdione[15]. The rhizomes oil of *C. aromatica* from Japanese samples were found to have curdione, germacrone, 1,8-cineole, (4S, 5S)-germacrone- 4,5-epoxide, β-elemene, and linalool, whereas those in the oil from Indian samples contained β-curcumene, ar-curcumene, xanthorrhizol, germacrone, camphor, and curzerenone[16]. The essential oils of *C. aromatica* from Indonesia and India contained about 19% ar-curcumene, 26% β-curcumene and 26% xanthorrhizol[17]. The *C. aromatica* oils from Argentine were examined and found to contain mainly germacrone D, curzerene, germacrone, curzerenone, xanthorrhizol, curcuphenol and hydroxyisogermafurneolide[18]. α- and β-pinene, camphene, 1,8-cineole, isofuranogermacrene, borneol, isoborneol, camphor, germacrone, and tetramethyl pyrazine were found to be the major components of Chinese *C. aromatica* rhizomes oil[19]. Limonene, 1, 8-cineole, curcumene, zingiberene, bisabolene, β-phellandrene, arturmerone and turmerone were also identified in the oil[20]. In another study, the plant sample (100 gm) on extraction three times with 500, 300 and 200 ml alkaline water (pH 9) followed by precipitation at pH 3-4 resulted in the isolation of 82.5 % curcumin[21]. (-)-Curdione, the antipode of the antitumour principle of *C. aromatica* was prepared from (-)-carvone via Oxy-Cope rearrangement. The result also confirmed the absolute configuration of natural (+)-curdione[22]. Curcuma lactone has been analyzed and found to be an artifact formed by conversion of curdione during steam distillation of *C. aromatica*[23]. Study on the sesquiterpenes of the rhizomes of the plant by mean of repeated precise silica gel column chromatography and high performance liquid chromatography (HPLC) resulted in the isolation of eleven minor sesquiterpenes; namely epiprocurcumenol, isoprocurcumenol, neoprocurcumenol, (4S)-13-acetoxydehydrocurdione (I), (4S)-13-

hydroxydehydrocurdione (II), (4S, 5S) 13- hydroxylgermacrone-4,5-epoxide (III), (4S, 5S)-13-acetoxygermacrone-4,5-epoxide (IV), (4S, 5S)-12-acetoxygermacrone-4,5-epoxide (V), acetoxyneocurdione, curcumadione and isocurcumadione[24]. The petroleum ether and ethyl acetate extracts of the rhizomes of *C. aromatica* growing in Northern Vietnam were investigated and the study showed that the extracts consisted exclusively of sesquiterpenoids. Sesquiterpenoids with germacrene skeleton were the major constituents in both the extracts[25].

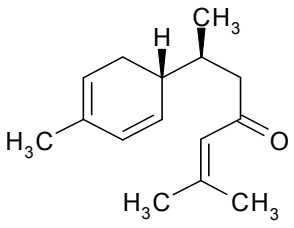
The methanolic extract of *C. aromatica* on subjection to resin D-101 silica gel column and thin layer chromatography resulted in the isolation of curdione, neocurdione, curcumol, tetramethyl pyrazine and (R)-(+)-1, 2-hexadecandiol[26].



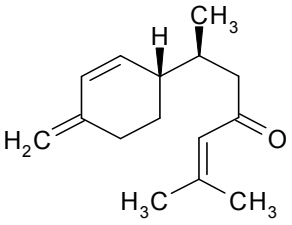
d-Camphor



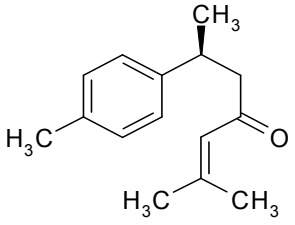
Curzerenone



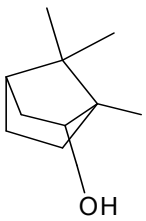
a - Turmerone



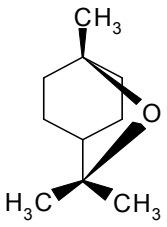
b - Turmerone



ar- Turmerone



Isoborneol



1,8 - Cineol

PHARMACOLOGICAL STUDIES

Although many pharmacological studies have been performed on the basis of chemical constituents present; a lot more are still to be exploited, explored and utilized. Important pharmacological findings are summarized below:

Anti-angiogenic activity

Demethoxycurcumin (DC), a structural analogue of curcumin, isolated from *C. aromatica* was found to have anti-angiogenic activity and its effect on genetic reprogramming in cultured human umbilical vein endothelial cells (HUVECs) using cDNA microarray analysis were studied. Of 1024 human cancerfocused genes arrayed, 187 genes were up-regulated and 72 genes were down-regulated at least 2-fold by DC. Interestingly, 9 angiogenesis-related genes were down regulated over 5-fold in response to DC, suggesting that the genetic reprogramming was crucially involved in antiangiogenesis by the compound. To verify the result obtained from cDNA micro-array analysis, matrix metalloproteinase-9 (MMP-9), the product of one of the angiogenesis-related genes down regulated over 5-fold by DC, was investigated using gelatin zymography. DC potently inhibited the expression of MMP-9, yet showed no direct effect on its activity. These data showed that gene expressional change of MMP-9 was a major mediator for angiogenesis inhibition by DC[27]. Synder and his colleagues also reported the anti-angiogenic activity of various curcumin analogues[28].

Antimicrobial activity

The essential oil from *C. aromatica* was extracted from homogenates of fresh tubers by steam distillation and was sterilized by filtration before the antimicrobial test. The antimicrobial activity was examined against four Gram-negative [non-01 *Vibrio cholerae* (NVC), *Salmonella enteritidis* (SE), entero toxigenic *E. coli* (ETEC), entero hemorrhagic *E. coli* O-157 (EHEC)] and two Gram-positive [*Staphylococcus aureus* (SA) and *Bacillus cereus* ATCC 11606 (BC)] bacteria, including food borne pathogenic bacteria. Both disc diffusion method and broth dilution methods were used for evaluating the antimicrobial activity of the essential oil. In disc diffusion method, the essential oil specimen inhibited the growth of bacteria used in the test. In broth dilution method, the essential oil specimen inhibited the growth of bacteria used in the test. In broth dilution method, the minimum inhibitory concentration (MIC) of *C. aromatica* oil against BC that has the highest sensitivity among six strains of bacteria tested proved to be 0.018 (v/v). Again, the effect of heating the essential oil on the antibacterial activity was also examined. The antibacterial activity against BC remained unaffected after heating at 121°C for 20 minutes[29]. In another study, the compound curcumin [1,7 bis (4-hydroxyl-3-methoxy- phenyl) heptan 1, 6 diene 3,5 dione] isolated from rhizomes of *C. aromatica* was subjected to antimicrobial studies against bacteria (*Staphylococcus aureus*) and fungi (*Saccharomyces cerevisiae*). The compound was found to be significantly active against both bacteria and fungi. The minimum effective concentration for antifungal activity was found to be lower than that for the antibacterial activity[30]. The volatile oil obtained from the rhizomes of *C. aromatica* showed significant antifungal activity against test organisms[31,32]. Raja and Kurucheve also showed significant antifungal activity of *C. aromatica* rhizome against *Macrophomina phaseolina*[33].

Antitumour action

The earlier literature reported that the oil extracted from the *C. aromatica* Chinese, could inhibit the growth of the various cancer cells in vitro and in vivo[34]. The recent studies showed that *C. aromatica* oil consisted of many kinds of antitumour ingredients such as β-elemene, curcumol, curdione etc[35,39].

The antitumour effects of curcumin (Cur) in combination with adriamycin (ADM) on human tumour cell lines were studied in vitro by using MTT method. The Jin's formula was used to analyze the effect of drug combination. In simultaneous administration Cur 2.04 μmol/ L-16.29 combining with ADM 0.70 μmol/ L-5.52 μmol/ L produced a simple addition or potentiation effect. In sequential administration, the first administration of ADM followed by curcumin resulted in an antagonistic effect, while the change of the order of administration produces a simple addition effect. Simultaneous administration of curcumin and ADM produced synergistic effect but sequential administration of the drugs only produced a unidirectional synergistic effect[40].

Curcumin analogues produced from cyclohexanone and 2-hydroxyl benzaldehyde exhibited antitumour properties. The growth inhibitory concentration in the NCI anti-tumour screen was lower than cisplatin for several cell types[41]. *C. aromatica* oil (CAO) was found to possess inhibitory effect on cell proliferation of hepatoma in mice. Its inhibitory effects were evaluated by DNA image cytometry and immunohistochemical staining of proliferating cell nuclear antigen (PCNA). The tumour inhibitory rates of CAO were 52% and 51% respectively. Compared with those of the saline treated control groups, both differences were statistically significant ($P<0.01$). In the group of mice treated with CAO, the cellular nuclear DNA; OD value (249 ± 70), are as (623 um^2 ± 228 um^2) and DNA (2.38 ± 0.67) index of hepatic carcinoma were significantly lower than those of the control group (430 ± 160, 1073 um^2 ± 101 um^2 and 4.48 ± 0.71). Further, in the group of mice treated with CAO, the labeling indices of proliferating cell nuclear antigen (PCNA- LI) were 30% ± 4%, which were significantly lower than 40% ± 6% of the control group ($P<0.01$). Thus, it was concluded that the inhibition of CAO on the growth of hepatoma in mice might be associated with its depression on cellular proliferative activity[42].

Busquet et al[43], found that the systemic administration of curcumin (20 μg/ kg body weight) for 6 consecutive days to rats bearing the highly cachectic Yoshida AH-130 ascites hepatoma resulted in an important inhibition of tumour growth (31% of total cell number). Wu et al[44], found the mechanism of inhibition of curcumin on proliferative of HL-60 cells. Acute myeloid leukemic cell line HL-60 was studied by using cell culture, NBT reduction, SABC method measuring BrdU incorporation rate, FCM measuring DNA contents and TUNEL method determining apoptotic cell percentage. Curcumin inhibited the proliferation of HL-60-cells in a dose and time- dependent manner. When HL-60 cells were treated with 25 μmol/ L curcumin for 48 hours, the inhibitory rate was 0.71% and 1.22%. The study on BrdU incorporation rate and the distribution of DNA content and NBT reduction indicate that curcumin arrested the cells in G2/M phase of the cell cycle at first, and then in G0/G1 phase, the whole cell cycle progression was slowed down and DNA synthesis activities was halted. Thus, it was suggested that the curcumin was able to regulate, upto some extent, the G1/S and G2/M transmit checkpoints and disturb the HL-60 cell cycle to induce apoptosis. Curcumin induces the mitochondrial permeability transition pore mediated by membrane protein thiol oxidation. Curcumin induced the increase in rat liver mitochondrial membrane permeability, resulting in swelling, loss of membrane potential and inhibition of ATP synthesis. Curcumin pore induction involved the oxidation of membrane thiol functions and required the presence of low Ca^{2+} concentrations. These data suggested that the mitochondria might be targeted by which curcumin induces apoptosis of tumour cells[45].

Cholerectic and Cholagougic activity

In the study by Beynen[46], found that the Temoe Lawak Singer (RVG 08637), a mixture of an extract of *C. aromatic*a rhizomes and whole roots of *C. amara* rhizomes and *Rhamni purshianae* cortex has cholerectic and cholagougic activity. The addition of Temoe Lawak Singer to a high cholesterol diet (but not to a cholesterol freed diet), was found to lower both serum and liver cholesterol in rats. The lowest dose induced a 20% decrease in the liver cholesterol and higher dose did not cause further reduction. The fecal excretion of bile acid was decreased by 1.0% of Temoe Lawak Singer in diet. Thus, it can be concluded Temoe Lawak Singer taken at normal doses might lower the serum cholesterol in man.

Anthelmintic activity

Extracts of *C. aromatica* showed a marked nematicidal and nematode-hatching inhibitory activity against root-knot nematode, *Meloidogyne incognita*. The butanolic extract of *C. aromatica* exhibited maximum inhibition for hatching of *M. incognita* eggs after 120 hours exposure at 1000-ppm concentration[47]. Again, the alcoholic extract of rhizomes of *C. aromatica* showed moderate in vitro anthelmintic action against *Ascaris lumbricoids*[48]. Zederone, a sesquiterpenoid isolated from the rhizomes of *C. aromatica* showed moderate antifeedant activity against 4th instar larvae of *Spilarctia oblique*[49]. Neocurdione, isoprocurcumenol, and a new sesquiterpene, 9-oxoneoprocurcumenol, were isolated from fresh rhizomes of *C. aromatica* as attachment inhibitors against the blue mussel, *Mytilus edulis galloprovincialis*[50].

Wound Healing Activity

An ointment of white soft paraffin containing 1% of powder *C. aromatica* rhizomes was applied to wounds on laboratory rabbits. The wound contracted and healed (epithelization completed) in 9 and 11 days respectively with *C. aromatica* and the corresponding times for paraffin-treated controls were 1 and 13 days, respectively[51].

Cytoprotective Activity

Turmeric and/or its main colouring component curcumin, inhibited benzo [a] pyrene [B(a)P]-induced forestomach papilomas in mice. To study the mechanism of turmeric mediated chemoprevention, the authors investigated the effects of turmeric feeding on the activities of isoenzymes of cytochrome P450 (CYP 450)-namely, ethoxyresorufin Odeethylase (EROD, CYP1A1) and methoxyresorufin (MROD, CYP 1A2)- which were predominately involved in the metabolism of B(a)P. The results indicated the activities of both EROD and MROD in forestomach (target organ), liver and lung. In vitro, studies employing curcumin, demethoxycurcumin, and bisdemethoxycurcumin suggested curcumin as the inhibitor in turmeric[52].

The effect of turmeric (Curcumin C), demethoxycurcumin (dm C), bis-demethoxycurcumin (bdm C) and Ph and phenethylisothiocynates (PITC and PEITC) on the dealkylation of ethoxyresorfin (ER), methoxyresorfin (MR) and pentoxyresorfin (PR) by rat liver microsomes (in vitro) and the chemopreventive efficacy of turmeric/curcumin against benzo [a] pyrene {B(a)P} and 4-methyl-nitrosamino-1-(3-pyridyl)-1-butanone (NNK, a tobacco-specific carcinogen), were studied. These reactions were predominantly mediated by cytochrome P450 (CYP 450) isoenzymes 1A1, 1A2, and 2B1, respectively.

Again, pretreatment of rats with 1% turmeric through the diet resulted in a significant decrease in induction of B(a)P-induced CYP-1A1 and 1A2 and phenobarbitone (Pb)-induced CYP 2B1 in liver, lung and stomach, although the extent of the decrease was different. These results suggested that the turmeric/curcumin as in the case of isothiocanate, PEITC, are likely to inhibit activation of carcinogens metabolized by CYP 450 isoenzymes, namely CYP 1A1, 1A2 and 2B1[53].

Sood et al[54], studied that the curcumin, an antioxidant compound extracted from the spice turmeric inhibited the cell death induced by Shiga toxin (Stx) 1 and 2 in HK-2 cells, a human proximal tubule cell line. Cells were incubated for 24-46 hours with Stx 1 or Stx 2, 0-100 ng/ ml. Exposure to Stx 1 and Stx 2, 100 ng/ ml, reduced cell viability to approximately 25% of control values after 24 hours and 20 μM curcumin restored viability to nearly 75% of control. Stx 1 caused apoptosis and necrosis in 12.2 ± 2.2 % and 12.7 ± 0.9% of HK-2 cells, respectively. Similarly, Stx 2 caused apoptosis and necrosis in 13.4 ± 2.1% and 9.0 ± 0.5% of HK-2 cells, respectively. Addition of 20 μM curcumin decreased the extent of apoptosis and necrosis to 2.9 ± 2.0% and 3.8 ± 0.2%, respectively, in the presence of Stx 1 and to 3.0 ± 2.1% and 3.9 ± 0.3%, respectively, for Stx 2 ($P<0.01$). Stx- induced apoptosis and its inhibition by curcumin were confirmed by DNA gel electrophoresis and by an assay for fragmentation. Thus, the cytoprotective effect of curcumin against Stx-induced injury in cultured human proximal tubule epithelial cells may be a consequence of increased expression of HSP 70.

Antioxidant action

Kim and Kim[55], found that the methanolic and aqueous extracts of *C. aromatica* when screened along-with other plants for antioxidant action using Fenton's reagent/ ethyl linoleate system, has potential antioxidant effect. In another study, Masuda et al[56], found the mechanism of curcumin as antioxidant on polyunsaturated lipids, which consisted of an oxidative coupling reaction at 3' position of the curcumin with the lipid and a subsequent intramolecular Diels-alder reaction.

Anti-inflammatory activity

The alcoholic and aqueous extracts of *C. aromatica* (100 mg/ Kg) showed the significant anti-inflammatory activity on the paw of mice treated with carrageenan. The effect was similar to prednisolone. The alcoholic extract was slightly more effective than the aqueous extract[57]. The volatile oil obtained from the rhizomes of *C. aromatica* showed significant anti-inflammatory activity[58].

Anti-complementary activity

Shim et al[59], showed the relatively potent anticomplementary activity of *C. aromatica*, which decreased TCH50 more than 70% in comparison with control. Again, hot aqueous extract of *C. aromatica* has partially purified and analyzed for chemical properties. These activities were resistant to digestion with promise but decreased by treatment with $NaIO_4$. These results indicated that the complement activating activity was due to polysaccharide.

Hypoglycemic activity

Ara et al[60], found that (4S, 5S)-(+)-germacrone 4,5-epoxide extracted from *C. aromatica* given as IV to male mice prior to glucose loading lowered the blood sugar level.

Miscellaneous activity

The tablets containing *C. aromatica* alongwith other Chinese medicines were found to be effective against schizophrenia, hysteria and epilepsy[61,63]. The preparation containing curcumin, demethoxycurcumin extracted from *C. aromatica* was effectively used as hair tonic. These preparations also showed the anti-dandruff and depilation preventing activities[64]. Some pharmaceutical preparations (capsules, tablets, granules) containing *C. aromatica* roots were found to be effective for the treatment of cholecystitis, biliary calculi and other biliary tract diseases[65]. The aqueous extract of *C. aromatica* antagonized the inhibitory action of purified Naja naja sianensis neurotoxin. The mechanism of antagonism between the plant extract and the neurotoxin was direct inactivation of the toxin by the plant extract[66,67]. The aqueous extract of *C. aromatica* was found to act as termiticides and wood preservatives. It can also be used for the control of cockroaches, files, mosquitoes, bed bug, lyctidae etc[68].

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