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Antioxidant, Cytotoxic and Phytochemical Assessment of Rhizomes of Black Turmeric (*Curcuma Caesia*)

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Abstract **– Rhizomes of *Curcuma caesia* were explored for antioxidant, cytotoxic and phytochemical profile. Four extracts were tested, these were hexane, chloroform, acetone and methanol. Antioxidant activity was tested using both qualitative as well as quantitative DPPH radical scavenging assay, whereas cytotoxic activity was tested using brine shrimp assay. In qualitative TLC based antioxidant assay, maximum number of antioxidant bands (8) were obtained in hexane & acetone extracts in BEA solvent. In quantitative assays acetone and methanol extracts showed better activity as compared to hexane and chloroform. Cytotoxic activity was found highest in acetone extract 90.99% at the dose 100µg/ml followed by hexane extract which showed 80% activity at the highest dose. Remaining extracts showed only mild activities against brine shrimp mortality assay. On phytochemical analysis flavonoids, sapponin and tannins were the major secondary metabolites present in most of the extracts.**

Keywords **– *Curcuma Caesia,* Antioxidant, Cytotoxic, Phytochemical, Flavonoids, Sapponin.**

I. INTRODUCTION

Curcuma caesia is commonly known as black turmeric, a wild species of the family Zingiberaceae. Rhizomes of the plant are used for sprains and bruises and are also employed in cosmetics [1]. It is also used as a carminative for the treatments of headaches rheumatic pains [2]. The rhizomes are aromatic and yield an essential oil [3]. The inner part of rhizome is bluish black in colour and emits a characteristic sweet smell due to the presence of essential oil [4]. The plant is in great demand in central India, due to indiscriminate exploitation without ensuring its regene--ration and conservation the plant has recently categorized as an endangered species [5]. Majority of the medicinal plants which are in high demand are collected from the wild, but keeping in view of the status of the species same was conserved in the medicinal germplasm garden of the Institute and was explored for its medicinal properties in order to adjudge whether the cultivated plants are medicinally potent or not.

II. MATERIALS AND METHODS

A. *Collection and Processing of Plant Materials*

Rhizomes of *Curcuma caesia* were collected from the medicinal germplasm garden of Regional Plant Resource Center (RPRC), Bhubaneswar. Rhizomes were washed with running tap water to remove dust and impurities followed by cutting in to fine chips. After complete drying, they were made to fine powder by using mechanical grinder. Dried powder was further used for solvent extraction.

B. *Solvent Extraction*

Solvent extraction was conducted by the process of maceration. In maceration process 50 gm of rhizome powder was taken in a beaker and 200 ml of hexane solvent was added to it, mixture was stirred with a glass rod at regular intervals and left overnight for percolation. Extract was filtered using Whatmann filter paper. Same process was repeated for each solvent thrice. Collected extract was concentrated under vacuum in rotavapor (Buchii make) at lower temperature of 40 - 45℃. Concentrated extracts were stored in screw cap vials till further use. Percentage yield for all the extracts namely hexane, chloroform, acetone and methanol was calculated.

C. *Phytochemical Tests:*

Phytochemical tests were done by standard method [6] with both fresh leaves and with solvent leaf extracts.

Test for Alkaloids: Alkaloid tests were done by using 3 different reagents.

Dragendroff's test - To 1ml of extract 2 ml of 1% HCl was added and then boiled for few minutes, after boiling 2-3 drops of dragendroff's reagent was added and sample was observed for reddish brown precipitate.

Wagner's test - To 1 ml of extract 1 ml of 1% H_2SO_4 was added followed by few drops of wagner's reagent. Formation of precipitate depicts the presence of alkaloids.

Mayer's test- To 1 ml of extract 2 ml of 1% HCl and mayer's reagent was added drop wise and was observed for the formation of precipitate.

Test for flavonoid: To 2.5 ml of extract 1 ml of 10% NaOH was added. From the side of the test tube, drops of conc. HCl were added. Yellow colour turns to colourless which indicates presence of flavonoids.

Test for Anthraquinone : To 1ml of extract 2 ml of 5% KOH was added and was observed for pink colouration.

Test for Saponin : To 1ml of extract 2 ml of $NaHCO_3$ was added and on shaking forms lather.

Test for Terpenoids : To 1 ml of extract 400µl of chloroform and 4-5 drops of conc. H_2SO_4 was added from the walls of the test tube. Redish brown ring depicts the presence of terpenoids.

Test for Cardiac glycoside : To 2.5 ml of extract 2 ml of glacial acetic acid, few drops of $FeCl_3$ and conc.H_2SO_4 was added from the walls of the test tube. Presence of cardiac glycoside was determined by reddish brown ring.

Test for Tannin : It was conducted by two methods.

Method A – 1 ml of extract was boiled and few drops of $FeCl_3$ were added to it. The sample was observed for blue, black, green precipitate.

Method B – To 1 ml of extract 500µl of lead acetate was added which gives yellow colour in the presence of tannins.

Antioxidant Activity

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Qualitative Analysis (TLC based Antioxidant Studies)

To detect antioxidant activity qualitatively 2, 2-Diphenyl 1-picrylhydrazyl (DPPH) assay was carried out. TLC sheet (Silica gel 60 F254, Merck company) coated with silica gel was used as stationary phase. It was sprayed with 0.2% DPPH in methanol as an indicator [7]. The presence of antioxidant compounds were detected by yellow spots against a purple background on the TLC sheet sprayed with 0.2% DPPH in methanol.

$$\underset{\text{(Purple colour)}}{DPPH + AH} \rightarrow \underset{\text{(Yellow colour)}}{DPPH - H + A^-}$$

For about 5 µl of each sample was loaded on TLC activated sheet, following solvent systems/mobile phases were used for TLC based antioxidant activity

a) Ethyl acetate : Methanol : water (40 : 4.5 : 4) [EMW] (polar neutral).
b) Chloroform : Ethyl acetate : formic acid (5 : 4 : 1) [CEF] (intermediate polarity/ acidic)
c) Benzene : Ethanol : ammonium hydroxide (90 :10 :1) [BEA] (Non polar/basic).

DPPH Radical Scavenging Assay

The radical scavenging activity of different extracts against DPPH was determined spectrophotometric ally by the method of Brand Williams [8]. DPPH reacts with an antioxidant compound that can donate hydrogen and it gets reduced. The change in colour (from deep violet to light yellow) was measured. DPPH is a stable free radical and accepts an electron, or hydrogen radical to become a stable diamagnetic molecule. The intensity of the yellow colour depends on the amount and nature of radical scavenger present. A reaction mixture containing 500µl of 1 mM DPPH, various concentrations of plant extracts (7.81, 15.62, 31.25, 62.5, 125, 250 and 500 µg/ml) were prepared in methanol. A test tube containing only methanol and 500 µl of DPPH solution was taken as control. Then the tubes were incubated in dark for 30 min at room temperature. The formed yellow colour chromophore was measured at 517nm. Ascorbic acid was used as standard. The percentage scavenging of DPPH free radical was calculated by following formula.

% scavenged DPPH radical = A control – A sample/A control X 100; Where A control is absorbance of control and A sample is absorbance of sample.

Cytotoxic Activity (Brine Shrimp Lethality Test):

Cytotoxic activity study was carried out by brine shrimp lethality assay [9]. Brine shrimp eggs were incubated for 48 hrs. (3.6 gm of black salt in 200 ml distilled water) to get the desired hatching of the larvae for biological evaluation. Stock solution of different extract were prepared at a concentration of 10 mg/ml, cytotoxic assay was carried out at three doses 50, 100 & 200 µg/ml. For each dose level 3 replicates were used. Motility readings were taken every hour up to 4 hrs. Motility was graded as below:

4+ = high motility, 3+ = motile, 2+ = sluggish, 1+ = slow and Nil = no activity at all After 24 hrs. live larvae in the control and experimental tubes were counted and percentage inhibition was calculated using the following f--ormula.

$$\text{Percentage inhibition} = \frac{\text{No of Live larvae in experimental}}{\text{No of live larvae in controls}} \times 100$$

III. Results and Discussions

The rhizomes oil of the plant *Curcuma caesia* possesses antibacterial and antifungal properties [10] and are discovered to be useful treating piles, leprosy, bronchitis, asthma, cancer, epilepsy, fever, wounds, impotency and migrains etc. As the rhizome is rich in oil so as expected yield of hexane extract was highest among the solvent extract followed by chloroform, methanol and acetone (Table 1). Thus, it can be said that even polar molecules are also in abundance as methanol extract's yield was also good.

Table 1. Percentage of yield.

Solvent Extracts	Percentage of Yield
HEXANE	1.287
CHLOROFORM	1.123
ACETONE	0.556
METHANOL	1.121

A. *Phytochemical Tests*

Out of 9 phytochemical screened, four were present in various solvent extracts. These were Tannin, Saponin, Flavonoid & Glycosides. (Table 2). Alkaloids, Anthra--quinone were absent in all the samples which was in contrast with other studies where alkaloids have been reported [11]. Methanol extract showed 3 numbers of secondary metabolites followed by acetone which lacked tannin. Fresh sample showed the presence of Tannin and flavonoids. Flavonoid was the predominant molecules amongst all the secondary metabolites as curcumin is reported in all the species of curcuma varying in percentage according to the species with *Curcuma longa* containing the maximum [12].

Table 2. Phytochemical analysis.

Phyto--chemical	Fresh sample	Hexane	Chloroform	Acetone	Methanol
Alkaloid	–	–	–	–	–
Tannin	+	–	–	–	+
Saponin	–	–	–	+	+
Terpenoid	–	–	–	–	–
Flavonoid	+	+	+	+	+
Antraquin-one	–	–	–	–	–
Cardiac Glycoside	–	+	–	–	–

+ indicates presence of phytochemicals, - indicates absence of phytochemicals.

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B. *Cytotoxic Activity using Brine Shrimp Assay*

Brine shrimp assay is a good indicator of cytotoxic activity and used as primary screening model for anticancer potential [13]. All the solvent extracts were tested in three doses (25, 50, 100 µg/ml). Cytotoxic activity was found highest in acetone extracts (90.99%) at the dose 100 µg/ml followed by Hexane extract which exhibited significant (80.12%) activity at the highest dose, Methanol extract also showed more than 70% activity at the highest dose of 100microgram/ml. Remaining extracts showed only mild activities against brine shrimp motility assay (Fig.1). Another species of the genus, *Curcuma longa* has also been reported for its carcingogenic potential [14], similarly results of brine shrimp assay has provided lead for further exploration of acetone extract as anticancer agent.

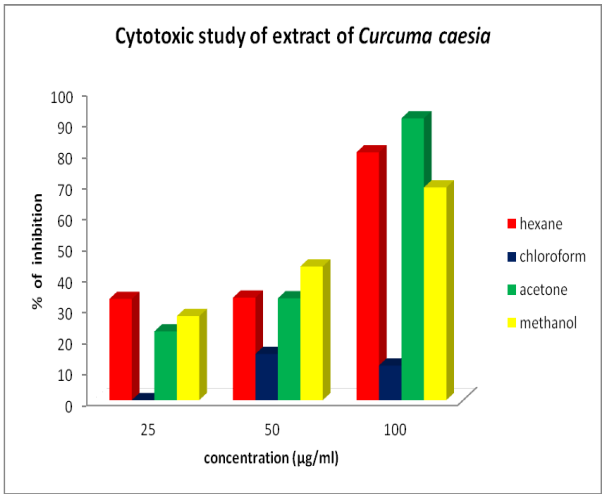

Fig. 1. Cytotoxic activity in different doses of solvent extracts of *Curcuma caesia*

C. *Antioxidant activity of Extracts of Curcuma caesia*

Antioxidant activity of *Curcuma caesia* was evaluated both by qualitative and quantitative assays. The presence of antioxidant compounds were detected by yellow spots against a purple background on the TLC sheet sprayed with 0.2% DPPH in methanol. Number of yellow bands corresponds to the molecules in the extracts capable of combining with the H^+ radical of diphenyl hydrazyl. Maximum number of antioxidant bands were obtained in hexane and acetone extracts in BEA solvent (Table 3). In quantitative assay acetone and methanol extracts showed significant antioxidant activity(>90%) at the highest dose but overall activity was less than ascorbic acid, this could be due to the fact that extracts are a combination of molecules, where as ascorbic acid is a pure molecule with antioxidant potential. Curcuma species are well-known indigenous medicine, used for the treatments of various ailments and metabolic disorders. They are mostly aromatic and medicinal in nature. They are used as spices, dyes, food, perfumes, cosmetics and tonics [15]. Regarding antioxidant potential two species *Curcuma longa* and *Curcuma amada* have been documented [16] as antioxidant and anti inflammatory. This study has exhibited antioxidant potential of *Curcuma caesia* as well.

Table 3. Qualitative antioxidant assay of solvent extracts of *Curcuma caesia* :

SAMPLES	SOLVENTS	NO. OF BANDS	R_f VALUES
Ascorbic acid	BEA	0	-
	CEF	0	-
	EMW	0	-
Hexane	BEA	8	0.03,0.16,0.24,0.29,0.34,0.41,0.84,0.93
	CEF	0	-
	EMW	0	-
Chloroform	BEA	7	0.08, 0.15, 0.19, 0.21, 0.26, 0.33, 0.91
	CEF	6	0.06, 0.52, 0.65, 0.75, 0.81, 0.86
	EMW	7	0.30, 0.40, 0.5, 0.79, 0.84, 0.86, 0.91
Acetone	BEA	8	0.07, 0.12, 0.21, 0.24, 0.28, 0.34, 0.84, 0.38
	CEF	5	0.55, 0.63, 0.76, 0.81,0.86
	EMW	5	0.48, 0.75, 0.79, 0.89, 0.92
Methanol	BEA	3	0.10, 0.12, 0.16
	CEF	5	0.12, 0.17, 0.78, 0.87, 0.92
	EMW	7	0.17, 0.24, 0.32, 0.35, 0.51, 0.82, 0.90

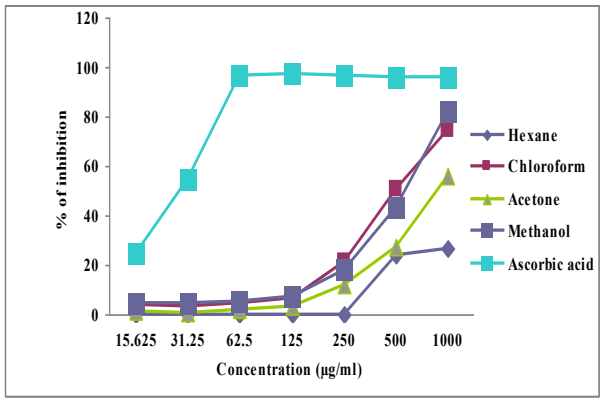

Fig. 2. DPPH radical scavenging assay of solvent extracts of *Curcuma caesia.*

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