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PHYTOCHEMICAL, ANTIBACTERIAL AND ANTICANCEROUS ACTIVITY OF CURCUMA PSEUDOMONTANA PLANT EXTRACT (HILL TURMERIC)

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ABSTRACT: The present study has been designed to evaluate phytochemical, antibacterial and cytotoxic activities of *Curcuma pseudomontana* plant extract. In the phytochemical screening of Methanolic extract of *Curucuma pseudomontana*, different tests are performed to find the phytochemicals that are present so as to subject the plant for further medicinal uses. These chemical constituents are mainly responsible for various biological activities. Present work reports for the antibacterial activities of *C. pseudomontana* plant, dried samples were sequentially extracted with many solvents. Methanol, chloroform, petroleum ether and water extracts of plant showed considerable antibacterial activity against *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The *in-vitro* anticancer activity of methanolic extract was performed by MTT assay method against HeLa cell line. Effect of inhibition of cell growth showed significant cytotoxicity against methanolic extracts showed considerable inhibition (80–90%) of HeLa cell lines. The present study concluded that the methanolic extract of *Curucuma pseudomontana* possess the phytochemical analysis content of reducing and non-reducing sugars, amino acids, saponins are in good concentration in case of dry plant sample. Thus, it can be inferred that fresh plant sample are richer in their phytochemicals compared to the dried, powdered plant sample and the methanolic extract of *Curucuma pseudomontana* possess potent antibacterial activities.

Key words: Phytochemical, plant extract, antibacterial, HeLa cell line.

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INTRODUCTION

Hill Turmeric is an erect herb, growing to 75 cm tall, found on moist, shaded areas of wet forests and along sluggish grassy slopes of higher altitude. It has stout rootstock bearing small almond like sub-globose tubers at the ends of the fibrous roots. The tubers are fleshy and white inside, aromatic. Leaves 3-5, oblong-lancelike, 20-30 x 6-9 cm, base acute, tip sharp, margin entire, hairless; shiny; leaf stalk and the leaf sheath up to 20 cm long.

Flowering spikes seen in the center of the previously formed tuft of leaves, 10-25 cm long, bearing numerous compactly arranged flowers; flowering bracts conspicuous, inverted eggshaped to lancelike, 3-5 x l.5-2, cm, apex rounded to acute, hairless; green with a pink tip.

Non-flowering bracts (coma) are oblong-lancelike, conspicuous, purple below and pinkish purple above. Flowers 2-4 in each fertile bract, bright yellow, 3 cm long and 4 cm broad. Capsules spherical, splitting by 3-valves, smooth. Seeds ovoid or oblong, usually covered with arils. Flowering: June-September. Curcumin is the principal curcuminoid of the popular Indian spice turmeric, which is a member of the ginger family (Zingiberaceae).

The other two curcuminoids are desmethoxycurcumin and bis-desmethoxycurcumin. The curcuminoids are polyphenols and are responsible for the yellow color of turmeric. Curcumin can exist in at least two tautomeric forms, keto and enol. The enol form is more energetically stable in the solid phase and in solution. Cancer is the second leading cause of death in the world (Madhusudan et al., 2005). Plants play an important role as a source of effective anti-cancer agents.

Currently over 60% anti-cancer agents are derived from natural sources, including plants, marine organisms, and micro-organisms (Newman et al., 2003). Curcumin is a primary active compound of all curcuma plants, it is responsible for yellow color of curcuma (Lampe et al., 1913), older investigations shows that curcumin has antimicrobial (Allen et al., 1998, Apisariyakul et al., 1995, Rasmussen et al., 2000), anti-inflammatory (Surh et al., 2001), dyspepsia and gastric ulcer (Prucksunand et al., 2001), irritable bowel syndrome (Barbara et al., 2002, Bundy et al., 2004, Camilleri et al., 2001), pancreatitis (Gukovsky et al., 2003, Vaquero et al., 2001), rheumatoid arthritis, osteoarthritis (Kulkarni et al., 1991), irritable bowel syndrome, anti-inflammatory and anti-oxidant (Shoskes et al., 2005).

MATERIALS AND METHODS

Collection and processing of plant materials

The whole plant of *Curcuma pseudomontana* were collected on November, 2015 from local areas of IBT, Patwadangar. The collected plant material was washed with tap water thrice and then with distilled water for 2-3 times. The plant was shade dried for few days and then kept in incubator at 37°C for 2-3 days. The dried plant material was then crushed in mechanical grinder in order to make fine powder which was stored at room temperature.

Preparation of Plant Extract

The powdered material was weighed 10gm and is subjected to soxhlet extraction using methanol, chloroform, petroleum ether, water as solvents in successive mode (A. K. Mishra et al., 2009, G. K. Jayaprakasha et al., 2008).

Phytochemical analysis

These are the qualitative tests performed to analyse the presence or absence of various phytochemicals such as Reducing sugar, Non-reducing sugar, Polysaccharides (starch), Proteins, Amino-acids, Alkaloid, Steroids, Cardiac glycosides, Anthraquinone glycosides, Saponins, Tannins and phenolic compounds, Flavonoids (Harborne et al., 1978).

Microorganisms and Growth Conditions

For the Antibacterial activity against *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Staphylococcus aureus* culture was present in the laboratory. The bacterial culture was maintained at 4^0C on nutrient agar slants. Antibacterial activity of plant extracts was determined using Kirby-Bauer disc diffusion and well diffusion method (A. W. Bauer et al., 1966).

Cytotoxic activity

Chemicals

3-(4,5–dimethylthiazol–2–yl)–5–diphenyl tetra- zolium bromide (MTT), Fetal Bovine Serum (FBS), Phosphate Buffered Saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM) and Trypsin were obtained from Sigma Aldrich Co, St Louis, USA. EDTA, Glucose and antibiotics from Hi-Media Laboratories Ltd., Mumbai, Dimethyl Sulfoxide (DMSO). All chemicals were available in the laboratory.

Cell lines and culture medium

HeLa cell lines were procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) in an humidified atmosphere of 5% CO_2 at 37^0C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm2 culture flasks and all experiments were carried out in 96 microtitre plates.

Preparation of Test solutions

For Cytotoxicity studies, the ethanolic extract was separately dissolved in distilled DMSO and volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out cytotoxic studies.

Determination of cell viability by MTT Assay

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/ml using DMEM containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, the monolayer was washed once with medium and 100 µl of different test concentrations of extracts were added on to the partial monolayer in microtitre plates.

The plates were then incubated at 37^{o}C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, the sample solutions in the wells were discarded and 50 µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37^o C in 5% CO_2 atmosphere. The supernatant was removed and propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and concentration of test sample needed to inhibit cell growth by 50% (CC50) is generated from the dose-response curves for each cell line (Ali et al., 2013).

% Growth Inhibition = 100 – {(Mean OD of individual test group / Mean OD of control group) × 100}

RESULTS AND DISCUSSION

Phytochemistry of the plant extracts

According to the following phytochemical analysis, the fresh plant sample was found to contain reducing sugar and steroids in methanolic extract and cardiac glycosides in aqueous extract at high concentration while amino acids, steroids, cardiac glycosides, anthraquinone glycosides, tannins and saponins at good concentration [Table 1-2].

Table 1: Phytochemical analysis of fresh plant sample

S. No.	Aqueous Extract (37°C)	Methanol (37°C)
1. REDUCING SUGAR	+	++
2. NON-REDUCING SUGAR	-	-
3. NON-REDUCING POLYSACCHARIDES(STARCH)	-	-
4. PROTEINS	-	-
5. AMINO-ACIDS	+	++
6. STEROIDS	-	+
7. CARDIAC GLYCOSIDES	++	+
8. ANTHRAQUINONE GLYCOSIDES	-	+
9. SAPONINS	+	
10. TANNINS & PHENOLIC COMPOUNDS	-	-
11. FLAVONOIDS	-	-
12. ALKALOIDS	+	+

(-) indicates absence, (+) indicates presence at good concentration, (++) indicates presence at high concentration.

Table 2: Phytochemical analysis of dry plant sample

S. No.	Cold Water Extract	Warm Water Extract	Hot Water Extract	Boiling Water Extract	Methanol
1. REDUCING SUGAR	+	+	-	+	+
2. NON-REDUCING SUGAR	-	-	+	-	-
3. NON-REDUCING POLYSACCHARIDES(STARCH)	-	+	+	-	-
4. PROTEINS	-	-	-	-	-
5. AMINO-ACIDS	+	+	-	-	+
6. STEROIDS	-	-	-	-	-
7. CARDIAC GLYCOSIDES	+	+	++	++	+
8. ANTHRAQUINONE GLYCOSIDES	-	-	-	-	-
9. SAPONINS	++	+++	+++	-	+
10. TANNINS & PHENOLIC COMPOUNDS	-	-	-	-	-
11. FLAVONOIDS	-	-	-	-	-
12. ALKALOIDS	-	-	-	-	-

(-) indicates absence, (+) indicates presence at good concentration, (++) indicates presence at high concentration

The results in the above table show that the content of reducing and non-reducing sugars, amino acids, saponins are in good concentration in case of dry plant sample.

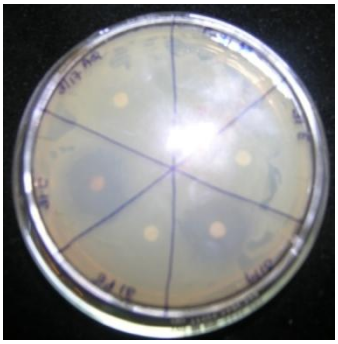
Antibacterial activity

The antibacterial activities of the extracts derived from plant of *Curcuma pseudomontana* were evaluated against *Klebsiella pneumonia, Escherichia coli, S. aureus* and *P. aeruginosa* bacterial strains. The antibacterial activity profiles of *Curcuma pseudomontana* extracts are given in Table 3. *Curcuma pseudomontana* extracts prepared in Methanol, chloroform, petroleum ether and water exhibited bacterial growth inhibition potential at 2 mg/disc concentration against *E. coli., Klebsiella pneumonia, S. aureus* and *P. aeruginosa* bacterial strains. Methanol extract of plant showed moderate activity (ZOI 15 mm) against *E.coli*., (ZOI 14 mm) *klebsiella pneumonia* and also (ZOI 14 mm) against *P. aeruginosa* . No zone of inhibition found in *S. aureus* (Fig. 1.).

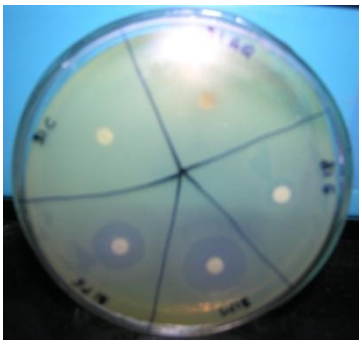
Table 3: Antibacterial activity of plant extracts along with antibiotic in terms of zone of inhibition

S.No.	Bacteria	Aqueous extracts (0.50g/50ml)				Organic solvent extracts (0.50g/140ml)		
		S-1 (mm)	S-2 (mm)	S-3 (mm)	S-4 (mm)	M (mm)	P.E. (mm)	Chl. (mm)
1.	*E. coli*	7	7	10	7	15	7	14
2.	*P. aeruginosa*	8	8	9	8	14	8	12
3.	*S. aureus*	-	-	-	-	-	-	-
4.	*K. pneumonia*	8	8	8	7	14	7	10

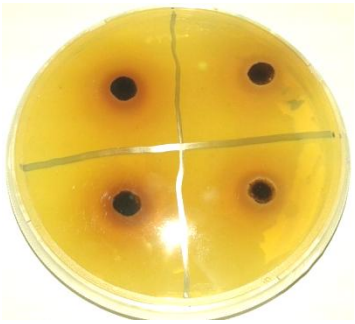
(-) indicates absence of antibacterial activity, (+) indicates presence of antibacterial activity, S-1 indicates cold water extract (10-15°C), S-2 indicates warm water extract (35-45°C), S-3 indicates hot water extract (65-75°C), S-4 indicates boiling water extract (100°C), (M) indicates methanolic extract, (P.E.) indicates petroleum ether extract, (Chl.) indicates chloroform extract



Methanolic extract against *E. coli.*



Methanolic extract against P. aeruginosa

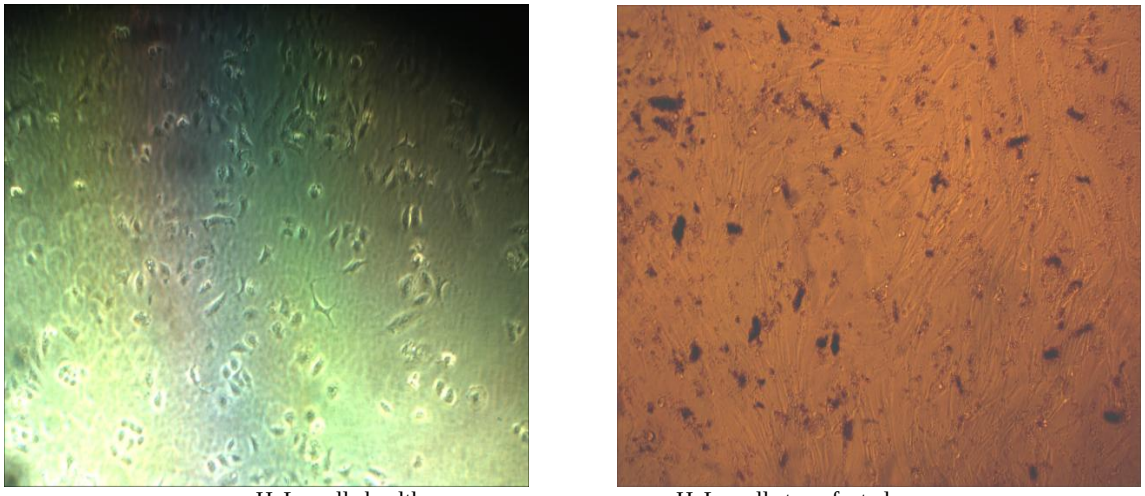


Methanolic extract against K. pneumonia

Fig. 1. Zone of inhibition of plant extract on different bacteria

Cytotoxic activity

Through the MTT method, the median cytotoxic concentration (CC50) on HeLa cell line was established for methanolic extract of whole plant *Curcuma pseudomontana*. There was gradual increase in the value of PGI (percentage of growth inhibition) as the concentration of extract was increased (30.10, 54.20, 78.50, 85.40 % for the concentrations 125, 250, 500, 1000 µg/ml, respectively) against the HeLa cell line (figure 2&3).



HeLa cells healthy HeLa cells transfected

Fig. 2. HeLa cells transfected with plant methanolic extract

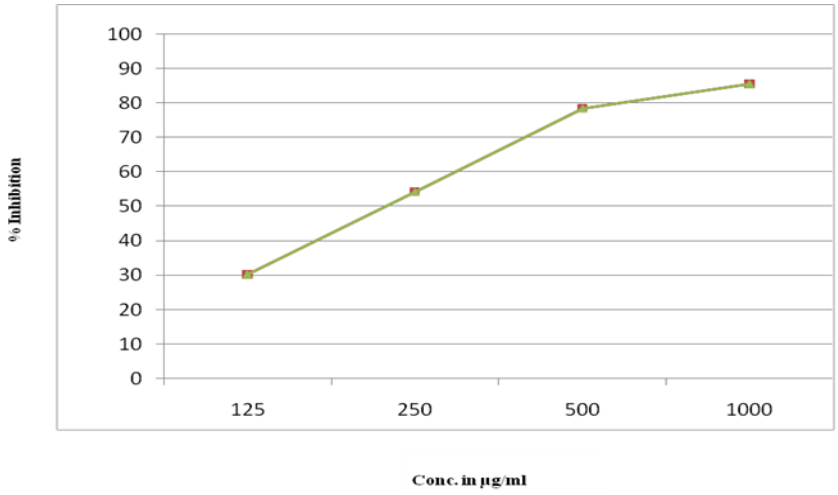


Fig. 3. Cytotoxic effect on HeLa cell line

CONCLUSION

The active phytochemical constituents present in *Curcuma pseudomontana* plant extract imparts high therapeutic properties that can prevent, various infection, flatulence, jaundice, menstrual difficulties, hematuria, hemorrhage, and colic and other ailments. The present plant *Curcuma pseudomontana* can be considered as an important source of natural products, it can be inferred that fresh plant sample are richer in their phytochemicals compared to the dried, powdered plant sample, also it have antimicrobial, anti-cancer potentials. Curcumin is main active compound of turmeric plant and many researchers prove this. Further research needed in this direction to use this safely for cancer patients.

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