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"PHARMCOGNOSTIC STUDY AND EVALUATION OF ANTIMITOTIC ACTIVITY OF *Curcuma longa* BY USING *Allium cepa* BIOASSAY"

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Abstract:

Curcuma longa linn commonly known as haldi reported to have various traditional application, attributed by every part, for the treatment of various ailments. Although various research studies suggested the valuable importance of haldi and still under exploration: Still much is waiting to hidden out Some of this areas include the determination of pharmacological potential of various extracts of Curcuma longa as a direction for Heading towards antimitotic activity any variation occurred with that of earlier studies along with some controversies reported by the previous researchers as an important objective. This study was investigated step wise with pharmacognostic evaluation physical evaluation followed by phytochemical screening ethanol, acetone, and water extracts of curcuma longa for the presence of various phytoconstituents groups for the determination of antimitotic activity of Curcuma longa extracts the Allium cepa root method was used respectively. On analysis of the antimitotic activity alcohol turmeric extracts showed inhibition of mitotic activity with low MI value (17.24 %), compare acetone turmeric extract (20%), water turmeric extract (20%), distilled water (control) (23.25 %) and (13.33%) for vinblastine sulphate standard.

Keywords:- Mitotic activity, pharmacognostic evaluation, allium cepa root method, phytoconstituents.

INTRODUCTION

Turmeric

Turmeric has also been used for centuries in ayurvedic medicine, which integrates the medicinal properties of herbs with food the extraordinary herbs has found it into the spotlight in the west and rest of globe, because of its wide range of medicinal benefits. uses of turmeric dates back nearly 4000 years to the vedic culture in India it is extensively. (Arawande, 2018) Ayurveda Unani and siddha medicine used turmeric as home remedy for various diseases. turmeric derived from rhizomes *curcuma longa* (family zingiberaceae) is a perennial plant having short stem with large oblong shaped leaves, and bears ovate, pyriform or oblog` rhizomes, which are oftenbranched and brownish- yellow in colouring. turmeric a native of south-east asia, is used as a food additive (spice), preservative and colouring agent in asian countries including china, Bangladesh, Burma, Nigeria, Australia, West indies, Peru, Jamaica and some other Caribbeanand Latin American Countries. . (Murugananthi D, 2008)

The naturally occurring phenolic compound known as curcumin (diferuloylmethane) is obtained from the dried and ground rhizome of the East Indian plant *Curcuma longa* Linn, which is used as a spice. (Cikricki S and Yýlmaz H , 2008 and Awasthi PK,).

Fig 1.1. Turmeric rhizomes

Curcumin has gained attention recently in scientific literature as a dietary component that effectively reduces oxidative stress in biological tissues. Indeed, studies showing growth inhibition of cancerous cells treated with pharmacological doses of curcumin have recently emerged, providing insights into potential curcumin toxicity (Paliwal P, 2011., *Sawa*nt, R. S. and Godghate, A. G. 2013. Saxena J, 2012. And Panhi Y., 2014 Joshi B et al. 2018 and Niamsa N , 2009)

. In a number of cancer cell models, curcumin induces G2/M cell cycle arrest and apoptosis- like death through an antiproliferative mechanism that is now strongly supported by data. Additionally, curcumin causes microtubule assembly disruption and mitotic cell cycle arrest in cervical and breast cancer cells. Interestingly, a phase II clinical trial for pancreatic cancer has used curcumin with encouraging results. Curcumin exhibits an exceptional degree of tolerance in people despite strong evidence of anti-mitotic activity; this could be because the processes involved in the functional translation of its anti-tubulin action are altered in cancer cells as opposed to normal cells (Parmar, Mayur P 2021).

The *Allium cepa* root growth rate is resembles to cancerous cells as we selected allium cepa bio assay method to highlight the antimitotic activity of *(Curcuma longa*) (Fiskesjo G. 1998, Shivasharanappa K et al. 2014)

MATERIALS AND METHODS

Chemicals and Reagents

For this study turmeric extract (Acetone, ethanol, distilled water) *curcuma Longa* procured from local market of durg city. For determining the antimitotic activity of the turmeric extracts All the solvents (Acetone, ethanol, distilled water) and chemicals/reagents (analytical grade) purchased from CDH Fine Chemicals, India.

Throughout the experimental process, double distilled water (purchased in the laboratory) was used.

Macroscopic Evaluation

For the macroscopic evaluation of *Curcuma longa*, firstly the collected rhizomes wereclean with the water and surface was dried gently by using tissue paper further their colour, odour, taste, size, shape and texture were examined.

Microscopic Evaluation

Preparation and observation of transverse section (TS)

For the microscopic examination, TS of rhizomes clean and were taken using sharp razor blade via sections. The said TS were treated with absolute alcohol for 15-20 minutes to remove pigments. Further, the sections were treated with Pholoroglucinol:Conc. HCl (1:1) solution as a staining agent, observed under the microscope with various magnifications and pictures were recorded using HD camera.

Physical Evaluation

Determination of ash values

The accurately weighed powdered drug was incinerated using incinerator with increasing temperature up to 650 °C until the carbon free ash obtained as ‘total ash’. The resulted total ash was further used for the determination of ‘water soluble’ and

'acid insoluble' ash using dilute hydrochloric acid. The process was repeated thrice and average value is calculated.

Determination of extractive values

Macerate about 5gm accurately weighed coarsely powered air-dried turmeric` with 100ml of alcohol (90%) in a stoppered flask for 24 hrs, shaking frequently first 6 hours. Filter rapidly through filter paper taking precaution excessive loss of alcohol. evaporate 25ml of alcoholic extract to dryness in a tared flat bottomed shallow dish. Dry at 105c temp. & weigh. Calculate percentage w\w of alcohol 90% soluble extractive with reference to the air-dried drug.

Determination of foaming index

Weight accurately about 1gm of coarsely turmeric powdered drug and transferred to 500ml conical flask containing 100ml of boiling water maintain a moderate boiling at 80-90ºC temperature for about 30 min. then make it cold, filter in to volumetric flask. Clean the 10 test tube and marked with 1-10… take the successive portions of 1,2ml up to10ml drug in separate tubes and adjust remaining volume with the liquid up to 10 ml ineach test tube. After closing the tubes with stoppers, shake them for 15 sec. and allowed to stand for 15mins. Then measure the height. If the height of foam in every test tube is less than 1cm then foaming index become less than 100 .it is more than 1cm in everytest tube then it will be over 1000. if the height of the foam in any 1 test tube become 1cm then following formula was used for determination of foaming index.

$$\textbf{Foaming index} = \frac{\mathbf{1000}}{\mathbf{a}}$$

a- Volume of plant material's decoction (ml) in the test tube showing 1 cm height

Determination of loss on drying (LOD)

Loss on drying is determined by heating the sample below its melting point in an oven

and it include all volatile matter including water contain and solvent. Loss on drying is a technique removingall the volatile impurities alcohol and water.

Preparation of Plant Extracts

For the preparation of extracts, the Soxhlet extraction method was used. Firstly, the collected dried rhizomes of *Curcuma longa*. Further it was subjected to pulverization to coarse powder and successively extracted with solvents acetone, ethanol and water referenced to their polarity index as below –

Table No – 5.1: Polarity Index of some solvents

S. No.	Solvent	Polarity index units
1	Water	10.2
2	Ethanol	5.2
3	Methanol	5.1
4	Acetone	5.1
5	Chloroform	4.1
6	Diethyl ether	2.8
7	toluene	2.4
8	cyclohexane	0.2
9	hexane	0.1
10	Petroleum ether	0.1

After extractions, the liquid extracts were filtered through Whatman filter paper no.10. The extracts were further concentrated and dried using water bath and subjected to phytochemicalevaluation and *in-vitro* studies.

Phytochemical Evaluation of crude drug turmeric

Preliminary phytochemical screening was carried out for all the extracts by various standard procedures to determine the presence of Carbohydrates, alkaloids, tannins..

Table no. 5.2: phytochemical test

S.NO.	Category	Test Name	Procedure
1.	Carbohydrates	Molish test	2-3 ml sample + few drops of molish reagent shake it + conc. H2SO4 from sides of tube violet ring
		Iodine test	3ml sample + few drops of iodine solutionblue colour (Disappear on boiling)
		Benedict test	1ml sample + 5ml benedict solution shake + heat it for 3 mints (in water bath) Redor Yellow colour
		Fehling's test	Mix 1-2 ml of Fehling's solution A and B, boil for one minute.The appearance of yellow followed by brick red precipitate.
2.	Alkaloids	Draganraff's test	2-3 ml sample + few drops of dragandraff's reagent (Potassium bismuth iodide) Orange Brown ppt
		Mayer's test	3ml of sample + few drops of mayer's reagent Cream colour ppt
		Wagner's test	3ml sample + few drops of wagner's reagent Reddish brown colour ppt
3.	Tannins	Ferric chloride test	1ml sample + ferric chloride solution Dark blue\ green\ black colour
		Potassium permangnant Test (KMNO4)	1ml sample + KMNO4 colourless

Antimitotic Study

About 50 grammes of allium cepa bulbs were purchased from Durg, the state capital of

Chhattisgarh, India's spice and condiment market. Healthy onions were taken for the study, and moldy, dry onions were thrown out.

The antimitotic assay was carried out using the methodology described by Fiskesjo, with some adjustments made by us. Specifically, the onion bulbs were left to germinate in a dark room over a few beakers filled with drinkable water until roots began to grow uniformly, about 5 cm in length. Water was then regularly replaced every 24 hours. Additionally, the chosen onions (roots to be dipped) were immersed for a full day in distilled water (control), vinblastine sulphate (100 µg/ml; standard), and extracts (50 mg/ml; testing). The roots were cleaned with distilled water after a day, and tissue paper was used to remove any remaining surface content. The blade was used to cut a tiny piece of root close to the root tip, which was then placed on the slide and squeezed under the cover slip. Water was added after ethanol to further treat the crushed tissue. Methylene blue was used to stain the tissue one more time. Any excess stain was washed off with water, and the tissue was examined under a trinocular microscope at different magnifications. HD camera pictures were then captured. Counting both dividing and non-dividing cells in the tissue allowed for the calculation of the mitotic index using the following formulas

$$\textbf{Mitotic index} = \frac{\textbf{Total number of dividing cells}}{\textbf{Total number of cells}} \times \textbf{100}$$

RESULTS AND DISCUSSIONS

Macroscopic Evaluation of Turmeric:-

The plant is usually erect ranging from 0.5 to 1.0 m in height; it is differentiated into underground large ovoid tuberous rhizome often called root-stock.

Rhizomes: the rhizome is tuberous with camphoraceous sweet odor, about 1-3 cm in diameter, the shape and size are often variable. It is sessile, laterally flattened, and covered with adventitious roots, root scars, and warts; moreover, it shows longitudinal circular wrinkles on the surface giving the look of nodal and internodal zones to the rhizome. The surface (cork) of rhizome is dark brown, bluish black, or buff in colour.

Figure No. 6.1: Macroscopic study

Physical Evaluation

In the physical evaluation, the loss on drying of powdered drug was 7.2 %, as the volatile components are not reported in the rhizome; such higher moisture content can be the cause of deterioration by fungal or bacterial growth. The total ash value as 0.19 %, which explains about higher percentage of inorganic constituents present in it. The

extractive values, as a first hand representative of nature of phytoconstituents especially about its solubility in the specific solvents, were found to be- alcohol soluble (0.7 %) and water soluble (0.13 %). The foaming index of *Curcuma longa* extract was found to be 500mm which give idea about the presence of higher percentage saponins in it, somewhat quantitatively, besides preliminary phytochemical screening.

Table No. 6.1: Result of Physical Evaluation

S.NO.	**Parameters**	**%Compositions**
1	Loss on drying	7.2 %
2	Total ash value	0.19 %
3	Alcohol soluble extractivevalue	0.7 %
4	Water soluble extractivevalue	0.13 %
5	Acetone soluble extractivevalue	0.6 %
6	Chloroform solubleextractive value	0.03 %
7	Foaming index	500mm
6	Swelling index	5cm

Fig. No. 6.2: Moisture content

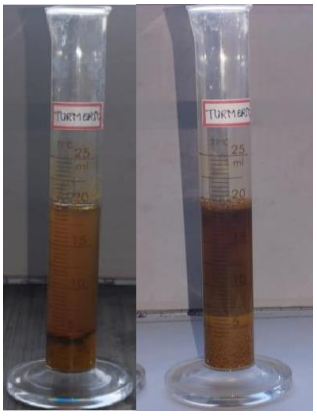
Fig. No.6.3 Ash value

Alcohol **Water**

Chloroform **Acetone**

Fig. No. 6.4: Extractive value

Fig. No. 6.5: Foaming index of turmeric



Before **After**

Fig. No. 6.6: Swelling index

Extraction of dried rhizomes of *Curcuma longa* using different solvents

For the preparation of extracts, the Soxhlet and decoction extraction method was used. Firstly, the collected dried rhizomes of *curcuma longa*. Further it was subjected to pulverization to coarse powder and successively extracted with solvents acetone, ethanol and water). The liquid extracts were filtered through Whatman filter paper no. 1. The extracts were further concentrated and dried using water bath and subjected to phytochemical evaluation, antimitoticactivity.

Fig. No. 6.7: Crude drug (turmeric) extraction by soxhlet method

Fig. NO. 6.8: Ethanol, Acetone and Aqueous extract of *curcuma longa*

Preliminary phytochemical screening

On phytochemical investigation of various extract (alcohol, acetone, water) carbohydrate is present in molisch's test, fehling solution test and Benedict test, alkaloid is present in dragondroff and Mayer's test and Wagner test, were reported tannins is absent in ferric chloride test and potassium permanganate test.

Table No. 6.2: Phytochemical analysis

1.	**Carbohydrate s**	**A. Molisch` test**	**Present**
		B. Iodine test	**Absent**
		C. Benedict test	**Present**
		D. Fehling's solution test	**Present**
2.	**Alkaloids test**	**A. Dragendroff test**	**Present**
		B. Mayer's test	**Present**
		C. Wegner test	**Present**
3.	**Tannins**	**A. Ferric chloride test**	**Absent**
		B. Potassium permanganate test	**Absent**

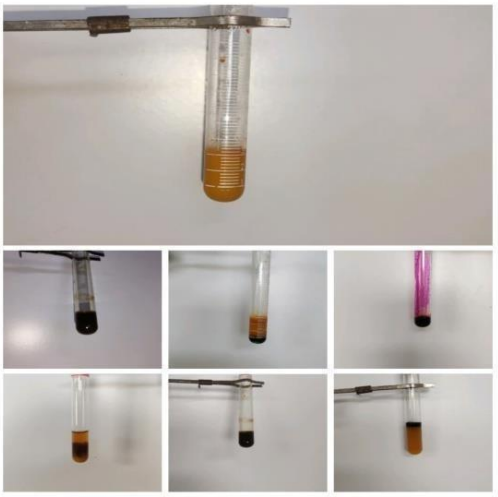


Fig. No. 6.9: Chemical test

In vitro study

Antimitotic study

The results of antimitotic assay of different *Curcuma longa* extracts using *Allium cepa* bioassay are summarized in (Table No. 6.3) It is observed that alcohol extract shown the significant antimitotic action with mitotic index 17.24 % compared acetone extract (20 %) water extract while it was 23.25 % for distilled water (control)

and 13.33 % forvinblastine sulphate (standard).

FIG NO. 6.10 : Growing Onion

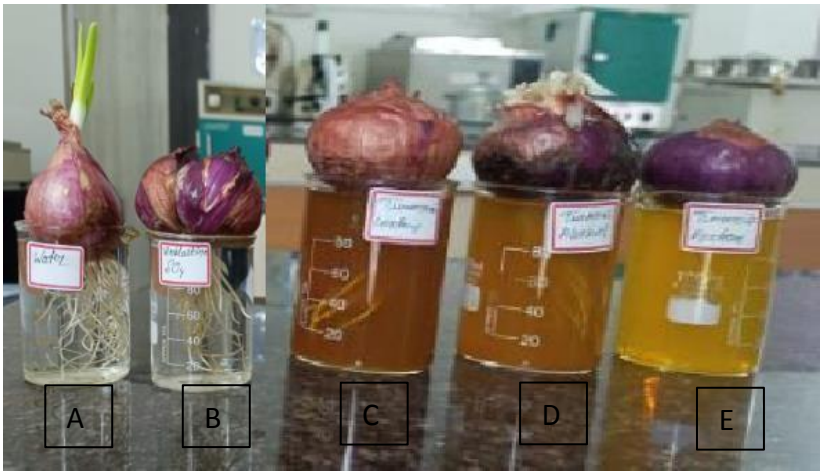


Figure No. 6.11 : Antimitotic assay using *A. cepa*. (A)- Distilled water/control; (B)- Vinblastine sulphate/standard, 100 µg/ml; (C)- Water extract, 50 mg/ml; (D)- Turmericalcohol extract, 50 mg/ml; (E)- Acetone extract, 50 mg/ml

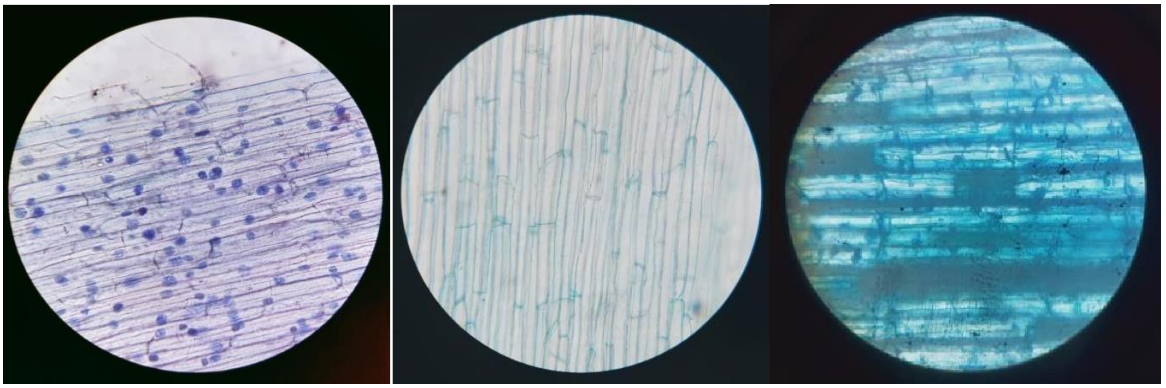


Fig.(A) Distilled Water **Fig.(B) Vinblastine Sulphate Extract** **Fig.(C) Water**

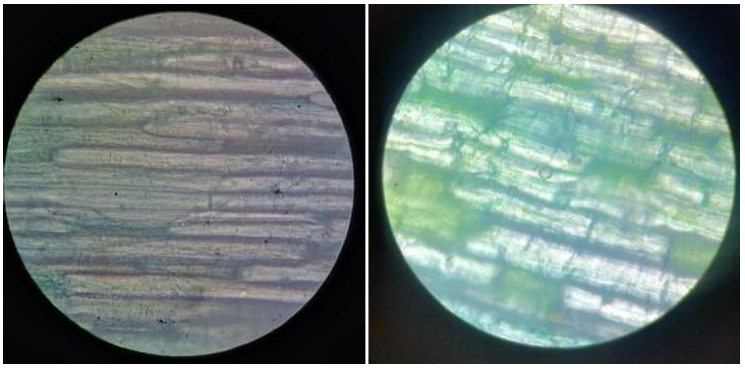


Fig.(D) Turmeric alcohol **Fig.(E) Turmeric Acetone**

Figure no. 6.12 : Microscopic observation of *A. cepa* meristematic cells treated with: (A)- Distilled water/control; (B)- Vinblastine sulphate/standard; (C)- Water extract; (D)- alcoholextract; (E)- Acetone extract;

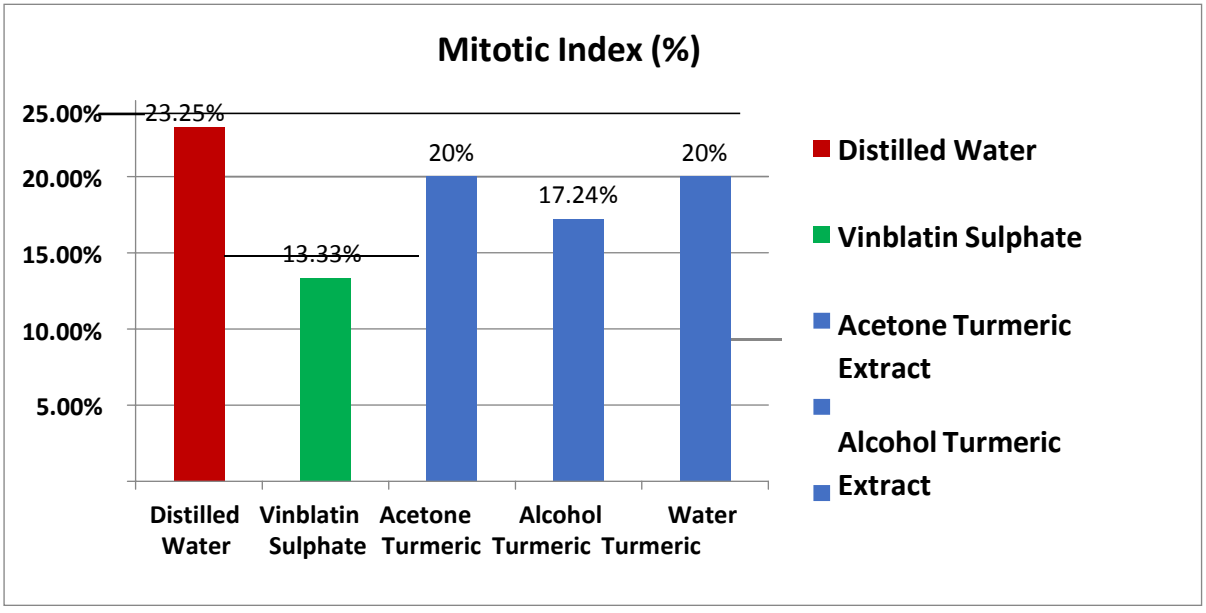


Figure no. 6.13: Antimitotic assay (Graphical representation of mitotic index)

CONCLUSION

Concluding the study, "Pharmacognostic Study of *Curcuma longa linn*", was mainly divided into two parts-

1) Pharmacognostic and phytochemical investigation of *curcuma longa linn.* rhizome.
2) Investigation of antimitotic potential of *curcuma longa linn.* rhizome extracts using *Allium cepa* bioassay.

The first part of pharmacognostic and phytochemical investigation of *curcuma longa linn.* rhizome was carried out under different steps-macroscopic evaluation, microscopic evaluation, physical evaluation followed by phytochemical screening of acetone, ethanol and aqueous extracts for the presence of various phytoconstituents groups.

In the second part, the antimitotic bioassay revealed that, the alcohol extract of rhizome showed higher antimitotic activity with mitotic index 17.24 % followed by, acetone and water extracts. The reason for such arrest of cells division probably because of presence of saponin carbohydrates, alkaloids, and tannins compounds in the extract as revealed by the phytochemical screening. Although the concentration of extracts as compared to standard drugis higher but as already known fact that, the extract is a mixture of large number of constituentsand if we could process further especially, the fractional separation assisted isolation of particular components of said alcohol extract, the breakthrough molecule for treatment of cancer can open the door of future surely.

According to the study's findings, pharmacognostic analyses of *Curcuma longa linn.* from different geographical regions can reveal more details about the variation in phytoconstituents, histological traits and physical parameters. Further studies into the rhizome extract may lead to the development of novel anticancer medication.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABRIVIATIONS

SD: Standard deviation; **TS:** Transverse section.

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