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Analysis of antimitotic activities from phytonutrients

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ABSTRACT:

Ayurveda, an ancient form of Indian medicine, speaks of several plant parts that cure different ailments when consumed. Here, we analyze different regularly consumed plant products to study their effect on mitosis. Several extracts were able to suppress mitosis, Areca nut was picked as a known mutagen, and turmeric and mango for their antimitotic activity upon eliminating other plant extracts. Preliminary analysis of a mixture of the mitogenic and antimitotic extracts showed that the antimitotic extracts were able to suppress the mitogenic activity of areca nut, indicating that the activity of areca nut is one of the factors that may be dependent on those targeted by mango and turmeric. Extracts prepared in absolute ethanol showed that the active ingredient is either inactive or absent in the ethanol extract. Two approaches were taken to identify the active ingredient. Firstly, the extracts were tested for the presence of known active ingredients, such as tannins, that are known to have beneficial health effects. Secondly, solutes miscible in aqueous and different organic solvents from the extracts have been tested for their antimitotic activity. The samples will be separated by HPLC, followed by mass spectroscopy to identify the active ingredients. These can be further tested on cell lines as a potential anticancer drug.

I. Introduction

Herbal plants are commonly used in the treatment of various ailments and are also consumed regularly as a part of the daily diet. Despite their widespread use, however, no scientific assessment for anticancer effect has been conducted in most cases. Much research has been geared towards the evaluation of plant extracts as prophylactic agents, which offer great potential to inhibit the carcinogenic process. Conventional treatment of cancer includes interventions such as psychosocial support, surgery, radiotherapy and chemotherapy._Most of these treatments are invasive, expensive and have severe side effects.

II. Materials and methods

Amitotic index (for plant cells):

Mitosis was performed using 0.2 cm of the root tip of green gram seeds. The stain used was Aceto orcein. The tip of the hypocotyl was initially incubated in 0.1N HCl for 5 to 10 minutes. This was followed by heat fixation. The tip was then stained and allowed to stand for a period of 30 minutes. The stained tip was observed under the microscope (40x) in order to count the number of dividing cells.

B. Vital staining (for animal cells)

The liver of a healthy goat was taken. Pre weighed tissue was subjected to sodium citrate for a period of 10 minutes, followed by mechanical homogenization, with the necessary volume of HBSS, the homogenate was subjected to centrifugation at 8000rpm for 8 minutes. The cell pellet was collected and the supernatant was discarded. The pellet was re suspended in necessary volumes of HBSS. A primary culture, followed by a secondary culture was performed.
To the secondary culture, the plant extracts were added, and the cell number was counted, by performing a vital staining (trypan blue was used as a stain)

C .Biochemical analysis

PREPERATION OF THE PLANT EXTRACT- An ethanol extract of the leaves of Mangnifera indica and Rhizome of Curcuma longa was prepared. The plant samples were collected, air dried and grinded. The powdered material was weighed and transferred into a conical flask, and equal volume of ethanol was added (1:1). This mixture was kept on a magnetic stirrer was 24hrs.the extracts were concentrated to dryness

and the residue was obtained as a gummy solid. The residue was then transferred into a pre weighed sample container and were stored and later used for further analysis.

- **Screening for alkaloids:** one gram of extract war stirred with ethanol containing 3% tartaric acid. The filtrate was tested for alkaloids. Mayer's reagent was added to the filtrate. Precipitation indicates the presence of alkaloids.
- **Screening for saponin:** About 0.5 g of the plant extract was shaken with water in a test tube. Frothing, which persist on warming was taking as a preliminary evidence for the presence of saponin. Few drops of olive oil was added to 0.5 g of the extract and vigorously shaken. Formation of soluble emulsion in the extract indicates the presence of Saponin (Odebiyi and Sofowora, 1978; Ngbede *et al*., 2008).

- **Screening for tannin:** Water extract of the sample was treated with 15% ferric chloride test solution. The resultant color was noted. A blue color indicated the presence of hydrolyzable tannin. (Odebiyi and Sofowora, 1978; Sofowora, 1982).
- **Screening for steroids (Salkowski's test):** About 0.1 g of the extract was dissolved in 2 mL of chloroform. Sulphuric acid was carefully added to form a lower layer. A reddish brown color at the interface is indicative of the presence of steroidal ring (Sofowora, 1982).
- **Screening for flavonoid:** About 2 g of the powder was completely detanned with acetone. The residue was extracted in warm water after evaporating the acetone in a water bath. The mixture was filtered while, still hot. The filtrate was cooled and used. Five ml of 20% NaOH was added to equal volume of the detained water extract. A yellow solution indicates the presence of flavonoid.
- **Screening for anthraquinone (Borntrager's test):** About 0.5 g of the extract was taken into a dry test tube and 5 mL of chloroform was added and shaken for 5 min. The extract was filtered and the filtrate was shaken with equal volume of 10% ammonia solution. A pink violet or red color in the ammoniacal layer (lower layer) indicates the presence of anthraquinone.

D. Solvent extraction :

- ethanol extract- ethanol extract of the leaves of Mangnifera indica and Rhizome of Curcuma longa was prepared. The plant samples were collected, air dried and grinded. The powdered material was weighed and transferred into a conical flask, and equal volume of ethanol was added (1:1). This mixture was kept on a magnetic stirrer was 24hrs.the extracts were concentrated to dryness and the residue was obtained as a gummy solid.

- Ether extract- the gummy solid obtained by the above mentioned method was dried to powdered form and was dissolved in water to which equal amounts of ether was added and the organic layer was separated from the aqueous layer using a separation funnel. the organic layer is dried and is used for further analysis.

E. SDS –PAGE

The separation of macromolecules in an electric field is called electrophoresis . a very common method for separating protein by electrophoresis uses a discontinuous polyacrylamid gel as a support medium and sodium dodecyl sulphate (SDS) to denature the proteins. This method is called sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS -PAGE) . The most commonly used system is also called the Laemmli method. SDS is an anionic detergent , meaning that when dissolved its molecules have a net negative charge with in a wide pH range. A polypeptide chain binds amounts of SDS in portion to its related molecular mass. The negative charges on SDS destroy most of the complex structure of proteins, and are strongly attracted towards anode in an electric field. An SDS – PAGE was performed to determine if there was any difference in the protein expression in the samples.

F. HPLC:

Mangifera indica
System: agilent 1200 series HPLC
Detector:UV detector, 258nm
Column temperature : 26°c

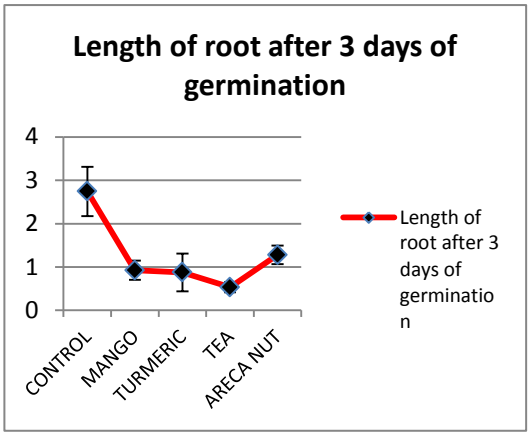
Flow rate: 1.0ml/minute isocratic

Curcuma longa
System: agilent 1200 series HPLC
Detector:UV detector, 430nm
Column temperature : 25°c
Flow rate: 1.2ml/minute isocratic

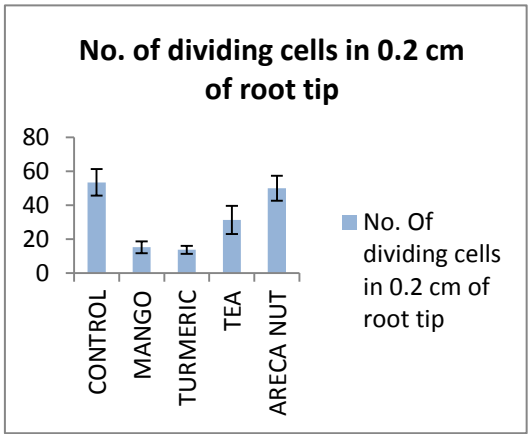
III.Results and discussion

A. Results of mitotic index

The length of the hypocotyl measured on the third day:



The length of the hypocotly was measured on the 3 rd day of germination. It was observed that control had a average length of 2.7cm where as Magnifera indica had an average length of 0.92cm, Curcuma longa with an average length of 0.87cm. At this stage tea was considered due to its average length, which was 0.53 cm. areca nut was used as a negative control due to its mitogenic properties.
Mitotic index performed on the third day of germination



The numbers of dividing cells were observed under the microscope at 40x magnification.

Sample	No of dividing cells
1.Control	53.42
2.*Mangifera indica*	12.6
3.*Curcuma longa*	13.75
4.*Camellia sinensis*	31.33
5. Areca nut	50.0

At this stage *Camellia sinensis* (tea) was eliminated because, in spite of the short hypoctyl the no of dividing cells were found much higher.

B. Results of the phytochemical analysis:

Upon performing the tests as mentioned above , the results were obtained as follows:

Test	Magnifera indica	Curcuma longa
1. Alkaloids	Positive	Positive
2. Saponin	Positive	Negative
3 tannin	Positive	Negative
4.steroids	Positive	Positive
5.flavonoids	Negative	Positive
6.anthraquinone	Negative	Negative

C.Results for solvent extraction:

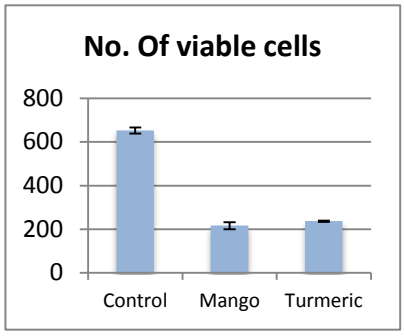
The number of dividing cells obtained from the ethanol and ether extracts of Mangifera indica &.Curcuma longa were comparable to that of the water extracts of the same.

Sample	No of dividing cells in ethanol extract	No of dividing cells in ether extract

Mangifera indica	21	27
Curcuma longa	12.5	16.5

D.Vital staining for animal cells

The cell count was determined by performing vital staining. The number of vital cells, were determined microscopically.

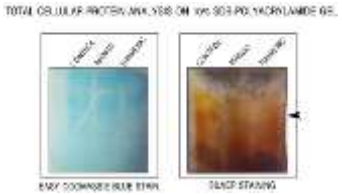


control had a cell count of 652 cells , Mangifera indica with 216 cells & Curcuma longa with 237 cells .

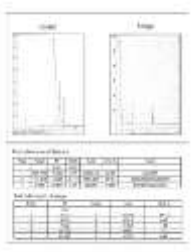
Sample	No of viable cells
Control	652
Mangifera indica	216
Curcuma longa	237

E. SDS PAGE

SDS Polyacrylamide Gel Electrophoresis was performed to study the differences in total protein content from the root tip of green gram seeds grown in mango and turmeric compared to the control. Extracts from 7 root tips of 0.5 cm in length were used for analysis. Equal quantity of protein was loaded into each well. Two staining methods were used. While no difference was observed among the samples with Coommassie Blue staining, some protein bands were differentially expressed on silver staining



F. Results for HPLC:



IV. Conclusion and on going work.

With respect to plant cells, A characteristic reduction in the length of the hypocotyl as well as the number of dividing cells were observed when exposed to turmeric and mango plant extracts But there was no characterisctic reduction in the size of the cell as a whole. With respect to animal cells, it was seen that , there was a radical reduction in the cell count. All the cells observed were vital, which indicates that the extracts inhibited the cell proliferation as a whole and did not obliterate the already proliferated cells. However the active ingredients responsible for this activity was processed for identification by HPLC, in order to identify the active component among the various other substances present. The results from HPLC indicated that the mango leaf had five fractions and the turmeric rhizome had three fractions. further analyzes are to be performed on tumor cell lines. The active component can be used as a potential drug for treating cancer.

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