Research Article

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Three Selected African Medicinal Plants Induce Anticancer and Phytochemical Properties in Prostate Cancer Cell Line

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Abstract

Modern pharmacology, however, relies on refined chemicals - either obtained from plants, or synthesized. This work investigated the anticancer, antioxidant and Phytochemical properties of three Sudanese Medicinal Plants commonly used as anti-inflammatory and anti-tumor. L. inermis leaves, M. piperita leaves, G. barbadense leaves. All the plant parts were extracted using 80% methanol, the anticancer activity was examined by using MTT assay against PC3 (prostate cancer) cell lines and determine their antioxidant by testing metal chelating activities, cytotoxicity using - (4, 5-Dimethyl thiazole-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT), filter and kept in dark, prepared freshly. The extract L. inermis leaves has shown very high activity against PC3, the extract M. piperita leaves has shown good activity against PC3 and G. barbadense leaves has shown none active anti- PC3 with IC50 values 48,4 >64,6 and >100 µg/ml respectively. Phytochemical screening of L.inermis leaves indicated presence of cumarin, saponins in trace amount, flavonoids, triterpenes in moderate amount, and sterols, tannins in high amount, alkaloids and anthraquenones were not detected. Phytochemical, screening of the M. piperita leaves indicated presence of cumarin, saponins in trace amount, flavonoids, triterpenes in moderate amount, sterols, and tannins in high amount, alkaloids, and anthraquenones were not detected. Phytochemical screening of G. barbadense leaves indicated presence of alkaloids, flavonoids, saponins in trace amount, triterpenes in moderate amount, tannins in high amount, and cumarin, sterols, anthraquenones were not detected. All the three plants extracts were examined for their metal chelating activity at concentration 50 µg/mL.

All the examined plant methanol extrats showed different levels of inhibition % M. piperita leaves 52.46 µg/m, L. inermis leaves 26.27 µg/m, G. barbadense leaves 11.07 µg/m.

Introduction

Since most of the standard anticancer treatments are not selective and affect both tumor and normal cells, thereby causing systemic toxicity or increased risk of other cancers. Thus, there is a need for the development of safer alternatives for the treatment of cancer which are affordable, accessible, having less toxicity and minimum side effects. A change in the life style including healthy diet and exercise still remains a better preventive measure against cancer. Furthermore, in folklore system several herbal medicines or mixtures have been used to treat cancer by either boiling the plant material in water or soaking it in alcohol. These observations and claims have generated tremendous interest of the researchers to provide scientific basis of their anticancer activities. As consequence variety of molecules with diverse mechanism of action has emerged as inhibitors of cancer and there are phenomenal number of research articles for comprehensive reviews such as Dorai and Agarwa (2004), Srivastava, et al. (2005) and Kintzios (2006) [1].

The medicinal qualities of plants are of course due to chemicals. Plants synthesize many compounds called primary metabolites that are critical to their existence. These include proteins, fats, and carbohydrates that serve a variety of purposes indispensable for sustenance and reproduction, not only for the plants themselves, but also for animals that feed on them [2].

Plants also synthesize a dazzling array of additional components, called secondary metabolites, whose function has been debated. Many secondary metabolites are "antibiotic" in a broad sense, protecting the plants against fungi, bacteria, animals, and even other plants [2].

Material and Methods

Collection of Tested Plant Parts

Tested plant parts of the *M. piperita* L, *L. inermis* L. And *G. barbadense* L. were collected from the Farm of Medicinal and Aromatic Plants Research Institute, Khartoum, Sudan (MAPRI), and identified of taxonomist team of MABRI (Medicinal and Aromatic Plants Research Institute, National Center of Research) Khartoum, Sudan. And herbarium voucher deposit at herbarium medicinal plants in the MAPRI.

Preparation of Crude Plant Extract

100gram of each plant sample was art coarsely powdered using Mortar and pistil and extracted with 80% methanol extracted for 18 hours with by using shaker (Stuart scientific, flash shaker, SF 1, UK). The extract was filter and evaporated using rotary evaporator at 40°C (Buchi, 461, Switzerland).

Culture Media and Human Tumor Cell Lines

Human Cell Lines

MCF7 (breast cancer), Hela (cervical cancer) cell line were obtained frozen in liquid nitrogen (-180 °C), the tumor cell lines were maintained in the Institute of ICCB, University of Karachi Pakistan.

Culture Media

RPMI -1640 medium was used for culturing and maintenance of the human tumor cell lines. The medium was supplied in a soluble form. Before using the medium it was warm at 37 °C in a water bath and supplemented with penicillin/streptomycin and Fetal bovine serum (FBS) with 10% concentration. The cells were maintained at 37°C in a humidified atmosphere with 5% CO_2 and were sub cultured twice a week.

Procedure

Maintenance of the Human Cancer Cell Lines in the Laboratory

A cry tube containing frozen cells was taken out of the liquid nitrogen container and then thawed in a water bath at 37 °C. The cry tube was opened under strict aseptic conditions and its content were supplied by 5 ml complete media (RPMI- 1640 in 10% fetal bovine serum) drop by drop in a 50 ml disposable sterile falcon tubes and were centrifuged at 1200 rpm for 10 min to discard the preserving solution. The supernatant was discarded and the cell pellet was seeded in 5 ml complete media in T25 Nunclon sterile tissue culture flasks. The cell suspension was incubated at 37 °C in a humidified atmosphere with 5% CO_2 and followed up daily with changing the supplemented medium every 2-3 days. Incubation was continued until a confluent growth was achieved and the cells were freshly sub cultured before each experiment.

Collection of Cells by Trypsinization

The media was discarded. The cell monolayer was washed twice with 5 ml phosphate buffered saline and all the adherent cells were dispersed from their monolayer by the addition of 1 ml trypsin solution (0.025 % trypsin w/v) for 2 minutes. The flask was left in the incubator till complete detachment of all the cells and checked with the inverted microscope (Olympus). Trypsin was inactivated by the addition of 5 ml of the complete media. The trypsin content was discarded by centrifugation at 1200 rpm for 10 minutes. The supernatant was discarded and the cells were separated into single cell suspension by gentle dispersion several times, then suspended and seeded in 5 ml complete media in T25 Nunclon sterile tissue culture flasks.

Determination and Counting of Viable Cells

50 µl of fresh culture media was added to 50 µl of the single cell suspension. The cells were examined under the inverted microscope using the haemocytometer. Viable cells were counted and the following equation was used to calculate the cell count /ml of cell suspension.

$$\text{Viable cells /ml} = \frac{\text{number of cells in 4 quarters} \times 2\ (\text{dilution factor}) \times 10^4}{4}$$

The cells were then diluted to give the concentration of single cell suspension required for each experiment. The cell count was adjusted to 1 x 10^4 -10^5 cells/ml using medium containing 10% fetal bovine serum.

Cryopreservation of Cells

To avoid the loss of the cell line, excess cells were preserved in liquid nitrogen as follows: Equal parts of the cell suspension and freezing medium (10 % DMSO in complete media) were dispersed to cry tubes. The cry tubes were racked in appropriately labeled polystyrene boxes gradually cooled till reaching -80 °C. Then the cry tubes were transferred to a liquid nitrogen (-196 °C).

Extraction and Phytochemical Screening

General phytochemical screening to detect the chemical groups was carried out for all extracts using the methods described by with few modifications [3-7].

Identification of Tannins

0.2 g of each extract was dissolved in 10 ml of hot saline solution and divided in two test tubes. In the first tube 2-3 drops of ferric chloride were added and 2-3 drops of gelatin salt reagent were added to the other tube. The occurrence of a blackish blue colour in the first test tube and turbidity in the second one indicates the presence of tannins.

Test of Sterols and Triterpenes

0.2 g of each extract was dissolved in 10 ml of chloroform, to 50ml of the solution 0.5 ml acetic anhydride was added and then 3 drops of conc. Sulphuric acid at the bottom of the test tube. At the contact zone of the two liquids a gradual appearance of green, blue pink to purple color is indicates the presence of sterols (green to blue) and or triterpenses (pink to purple) in the sample.

Test for Alkaloids

0.5 g of each extract was dissolved in 2 ml of 2N HCl in water bath and stirred while heating for 10 minutes, then cooled filtered and divided into two test tubes. To the first test tube a few drops of Mayer's reagent were added while to the other tube few drops of Valser's reagent were added. A slight turbidity or heavy precipitate in either of the two test tubes indicates the presence of alkaloids.

Tests for Flavonoids

0.5 g of each extract was dissolved in 30 ml of 80% ethanol and filtered.

The filtrate was used for following tests:

- A/ to 3ml of the filtrate in a test tube 1ml of 1% aluminum chloride solution was in methanol was added. - Formation of a yellow color indicated the presence of Flavonoids. Flavones and or chalcone.
- B/ to 3ml of the filtrate in a test tube 1ml of 1% potassium hydroxide solution was added. A dark yellow color indicated the presence of Flavonoids compounds (flavones or flavonones) chalcone and/or flavonols.
- C/ to 2ml of the filtrate 0.5ml of magnesium turnings were added. Production of a faint pink or red color was taken as presumptive evidence that flavonones are present in the plant sample.

Test for Saponins
0.3 g of each extract was placed in a clean test tube. 10 ml of distilled water were added, the tube stoppered and vigorously shaken for about 30 seconds. The tube was then allowed to stand and observed for the formation of foam, which persisted for at least one hour, was taken as evidence for presence of saponins.

Test for Cumarins
0.2 g of each extract dissolved in 10 ml distilled water in test tube and filter paper attached to the test tube to be saturated with the vapor after a spot of 0.5N KoH put on it. Then the filter paper was inspected under UV light, the presence of coumrins was indicated if the spot was found to have adsorbed the UV light.

Test for Anthraquinone Glycosides
0.2 g of each extract was boiled with 10 ml of 0.5N KoH containing 1ml of 3% hydrogen peroxide solution. The mixture was extracted by shaking with 10 ml of benzene. 5ml of the benzene solution was shacken with 3ml of 10% ammonium hydroxide solution and the two layers were allowed to separate. The presence of anthraquinones was indicated if the alkaline layer was found to have assumed pink or red color.

Test for Cyanogenic Glycosides
0.2 g of extract fraction was placed in Erlenmeyer flask and sufficient amount of water was added to moisten the sample, followed by 1ml of chloroform (to enhance every activity). A piece of freshly prepared sodium picrate paper was carefully inserted between a split cork which was used to stopper the flask, a change in color of the sodium picrate paper from yellow to various shades of red was taken as an indication of the presence of cyanogenic glycoside.

Metal Chelating Activity Assay
The iron chelating ability was determined according to the modified method of Dinis, et al. (1994). The Fe+2 were monitored by measuring the formation of ferrous ion-ferrozine complex. The experiment was carried out in 96 micro titer plates. The plant extracts were mixed with $FeSO_4$. The reaction was initiated by adding 5mM ferrozine. The mixture was shaken and left at room temperature for 10 min. The absorbance was measured at 562 nm. EDTA was used as standard, and DMSO as control. All tests and analysis were run in triplicate.

Statistical Analysis
All data are presented as mean ±standard deviation of the mean – statistical analysis for all the assays result were done using students t-test significance was tribute to probability values $P<0.05$ or $P> 0.01$ in some cases.

Result and Discussion
The high costs of western pharmaceuticals put modern health care services out of reach of most of the world's population, which relies on traditional medicine and medicinal plants to meet their primary health care needs. Even where modern medical care is available and affordable, many people prefer more traditional practices. This is particularly true for First Nations and immigrant populations, who have tended to retain ethnic medical practices. In the last decade, there has been considerable interest in resurrecting medicinal plants in western medicine, and integrating their use into modern medical systems. *L. inermis* leaves belong to the family Lythraceae showed very high activity against HeLa cell lines (IC_{50} is 47.9µg/ml). And very high antioxidant DPPH (IC_{50}16.88µg/ml). *L.inermis* leaves were found to be not toxic on normal cell lines (IC_{50} >100µg/ml). The anticarcinogenic activity of chloroform extract of *L. inermis* leaves has been carried using microculture tetrazolium salt assay on the human breast (MCF-7), colon (Caco-2), liver (HepG2) carcinoma cell lines and normal human liver cell lines (Chang Liver). The preliminary results showed that the henna extract displayed the cytotoxic effects against HepG2 and MCF-7 and IC_{50}-value of 0.3 and 24.85 µg/ml respectively. Isoplumbagin at a concentration of 10.5-10.8 M, the compound typically produced LC_{50} – level responses against a majority of the melanoma and colon cancer cell lines as well as against several of the non-small cell lungs, colon, CNS, and renal cell lines. Isoplumbagin showed an interesting profile of cytotoxic activity [8].

Much work is done in the field of phytochemical investigation of the plant. The chemical constituents isolated from *L. inermis* are napthoquinone derivatives, phenolic compounds, terpenoids, sterols, aliphatic derivatives, xanthones, coumarin, fatty acids, amino acids and other constituents [9-12].

M. piperita leaves belong to the family Lamiaceae this plant showed good activity against PC3 cell lines (IC50 is 64.6µg/ml), and very high anti-oxidant DPPH (IC_{50} 15.4µg/ml). Also their metal chelating activity was high at 50µg/mL (inhibition persintage 52.46), and were found to be not toxic on normal cell lines (IC_{50} >100µg/ml). Phytochemical, screening of the *M. piperita* leaves indicated presence of cumarin, saponins in trace amount, flavonoids, triterpenes in moderate amount, sterols, and tannins in high amount, alkaloids, and anthraquenones were not detected. The previous study showed that *Mentha spicata* is an aromatic plant the essential oils of *Mentha spicata* are obtained by steam distillation of the fresh leaves. Malic acid is an organic acid that can reduced the number of bacteria in the solution and with decrease ACC-oxidase activity cause delay the onset of hydrolysis of structural cell components, decrease ACC-oxidase activity and sensitivity Glutamine is important as a constituent of proteins and as a central metabolite for amino acid transamination via α-ketoglutarate and glutamate, when glucose levels are low and energy demands are high, cells can metabolize amino acids for energy [13-20].

Table 1: IC_{50} of the methanol extracts of the selected Sudanese medicinal plants for cytotoxicity against PC3 (Prostate cancer) cell line proliferation

Scientific name	Part used	IC_{50} ± SD
M. piperita L.	leaves	64.6 ± 4.6
L. inermis L.	leaves	48.4 ± 0.2
G. barbadense L.	leaves	>100

Table 2: Result of phytochemical screening the selected plants methanolic extracts

Test	Alkaloids	Sterols	Triterpenes	Flavonoids	Saponins	Cumarins	Tannins	Anthraquenones
M. *piperita*	-	+++	++	++	+	+	+++	-
L. *inermis*	-	+++	++	++	+	+	+++	-
G. *barbadense*	+	-	++	+	+	-	+++	-

References

1. Kinghorn A, SUBN, Jang D, Chang L, Lee D, et al. (2004) Natural inhibitors of carcinogensis. Planta Med 70: 691-705.
2. Cox P, Balick M (1994) The ethnobotanical approach to drug discovery. Sci Am 270: 82-87.
3. Sukhdev P, Pushpam K (2008) The Economics of Ecosystems and Biodiversity (TEEB). Wesseling, Germany, European Communities.
4. Martinez A, Valencia G, Marcha fitoquimica (2003) In Manual de prácticas de Farmacognosia y Fitoquímica: 1999. 1. St Edition. Medellin: Universidad de Antioquia; Phytochemical screening methods 59-65.
5. Sofowora A (1993) Medicinal Plants and Traditional Medicines in Africa. Chichester John, Willey & Sons New York, USA. P 256.
6. Harborne J (1984) Phytochemical methods. 2nd edition. Chapman and Hall, USA.
7. Wall M, Eddy C, Mc Clenna M, Klump M (1952) Detection and estimation of steroid and sapogenins in plant tissue. Analytical Chemistry 24: 1337-1342.
8. Ali B, Mohamed M, Osama M (2003) Relationship between genetic similarity and some productive traits in local chicken strains. African Journal of Biotechnology 2: 46-47.
9. Takeda Y, Fatope M (1988) new phenolic glucosides from Lawsonia inermis Journal of Natural Products 51: 725-729.
10. Cuong N, Nhiem N, Thao N, Nama N, Dat N, et al. (2010) Inhibitors of osteoclastogenesis from Lawsonia inermis leaves. Bioorganic & Medicinal Chemistry Letters 20: 4782-4784.
11. Chakrabartty T, Poddar G, St-Pyrek J (1982) Isolation of dihydroxy lupene and dihydroxy lupane from the bark of Lawsonia inermis, Phytochemistry 21: 1814-1816.
12. Handa G, Kapil A, Sharma S, Jagdev S (1997) Lawnermis acid a new anticomplementary tri-terpenoids from Lawsonia inermis seeds, Indian Journal of Chemistry 36: 252-256.
13. Kazemi A, Frazier T, Cave M (2010) Micronutrient-related neurologic complications following bariatric surgery, Current Gastroenterological Reports 12: 288-295.
14. Dinis T, Madeira V, Almeida M (1994) Action of phenolicderivates (acetoaminophen, salycilate and 5-aminosalycilate) asinhibitors of membrane lipid peroxidation and as peroxyl radicalscavengers, Arch Biochem Biophys 315: 161-169.
15. Kintzios S (2006) Terrestrial plant species and compounds anticancer properties. Crtitcal Reviews in Plant Science 25: 79-115.
16. Madhuri S, Govind P (2009) Some anticancer medicinal plants of foreign origin. Journal of current science 96: 779-783.
17. Matook S (1986) Antioxidant activities of water-soluble polysaccharides from Buncan (citrus grandis osbeck) fruit flavedo tissues. Pak J Biol Sci 8: 1472-1477.
18. Srivastava D, Vellend M (2005) Biodiversity-ecosystem function research: Is it relevant to conservation? Annual Review of Ecology Evolution and Systematics 36: 267-294.
19. Pietta P (1998) Flavonoids in medicinal plants In: Rice-Evans CA. Packer L. editors. Flavonoidsin health and disease. New York, USA. Dekker. pp 61-110.
20. Reza Abbas P, Nezafati S (2007) Clinical Results of Localized Alveolar Ridge Augmentation with Bone Grafts Harvested From symphysis in Comparison with Ramus. Journal of Dent Res Dent 1: 63.