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Research Article

In Vitro Evaluation of Anticancer Activity of Methnaolic Extract of *borassus Flabellifer* linn. Leaves on Different Human Cancer Cell Lines

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ABSTRACT

Identification of new anticancer medicinedevoidof life threatening side effects as that of synthetic anticancer drug is the need of today's cancer treatment. Ayurveda, the Indian traditional system of medicine is based on plant as a source of medicine. Medicinal plants such as *Picrorhiza Kurroa*Royle ex Benth., *Cetrus deodara*Roxband herbal formulations like Triphala ghrita, Sthauneyaka and Madhusnuhi rasayana are mentioned in the Ayurveda for the treatment of tumour. Plants of*Borrasus* family are well known fortheir medicinal and nutritional values.*Borassus flabellifer* Linn. is traditionally important medicinal plant and is widely distributed in India.The methanolic extractof *Borassus flabellifer* Linn.was screened against colon cancer (HCT15), Human lung cancer (Hop65) and Human hepatoma (HEPG2) cell lines using *Sulforhodamine*B (SRB) assay. Adriamycin was used as a standard to compare the results of methanolic leaves extract of *Borassus flabellifer* Linn.The findings revealed that extract do not inhibit growth of these cell lines with no potential anticancer effects. These results indicate that methanolic extract of *Borassus flabellifer* Linn.is not effective against these cancer cell lines. However, further studies are needed to test extract against other cancer cell lines to reveal its anticancer effect.

Key-words: Medicinal plant, *Borassus flabelliferb*Linn., Anticancer activity, Sulforhodamine B (SRB) assay

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Introduction

Many world disease studies showed that cancer ismost dangerous disease which kills large population worldwide. Indian traditional system of medicine isbased on plant and plant preparations. From ancient time plant based medicines are used to treat severalailments including cancer. Plants like *Withania somnifera*Dunal., *Picrorhiza Kurroa*Royle ex Benth., and *Cetrus deodara*Roxb. are mentioned in Indian traditional medicine system for the treatment of cancer (Gaidhani et al., 2013). *Borassus flabellifer* Linn.is a traditionally important plant which widely distributed in tropical regions of the Asian continent (Greig et al., 1980). The plant differ as male and female and it is cultivated many regions of India(Awal et al., 1995; Greig et al., 1980).Plants of *Borassus* species are economically important(Naguleswaran et al., 2010).*Borassus flabellifer*Linn.belongs to the family Palmae and the sub-family Boracidae. It contains severalPhytochemicals such as contains carbohydrate like sucrose, albuminoids, fats,gums,and steroidal glycosides(Sandhya et al., 2010).In traditional system of medicine the*Borassus flabellifer* Linn.is usedto treat several disease conditions. Plant is reported to possessmedicinal properties like diuretic, antioxidant, anti-inflammatory activity, anthelmintics, immunomodulatory and wound healing(Paschapur et al., 2010;Pramod et al., 2013; Pattanaik et al., 2008;Keerthi et al., 2007;Rios, 2010). The aim of present study is to investigate antioxidant and anticancer activity of methanolic extract of leaves of*Borassus flabellifer*Linn. using different cell lines.

Material andmethods

Collection andauthentication of plant material

The *Borassus flabellifer* Linn.leavessample was identified and authenticated by Dr. Gachande B.D., Botanist, AssociateProfessorofBotanydepartment, N. E. S.ScienceCollege, Nanded, India.

Extract preparation

The leavesof *Borassus flabellifer* Linn.were cut into small pieces. Leaves were dried underthe shadeat roomtemperature. Grinder was used to convert dried leaves in to powder form.Soxhlet extractor was employed for extraction of powder material using 1 L methanol for 8 h at 64 ^{0}C. Obtained extractwas concentrated with the help of rotary evaporator.

Preliminary phytochemical investigation

The preliminary phytochemical investigation of methanolic extract of*Borassus flabellifer*Linn.leaves wereperformed usingstandard procedures (Khandelwal, 2006).

Anticancer activity using SRB assay

Anticancer activity of methanolic leaves extract of *Borassus flabellifer* Linn.was determined by sulphorhodamine B (SRB) assay(Vichaiand Kirtikara, 2006;Skehnet al., 1990).Human colon cancer cell line (HCT15), Human lung cancer cell line (Hop65) and Human hepatoma cell line(HEPG2) wasselected for anticancer activity.The selected cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine.All thecells were inoculated into 96 well microtiter plates in 100 µL at plating densities. After cell inoculation, the microtiter plates were incubated at 37°C, 5 % CO2, 95 % air and 100 % relative humidity for 24 h prior to addition ofextract. The extract wasinitially solubilized in dimethyl sulfoxide at 100mg/ml and diluted to 1mg/ml using water and stored frozen prior to use. At the time ofextract addition, an aliquote of frozen concentrate (1mg/ml) was thawed and diluted to 100 µg/ml, 200 µg/ml, 400 µg/ml and 800 µg/ml with complete medium containing test article. Aliquots of 10 µl ofextract dilutions were added to the appropriate microtiter wells already containing 90 µl of medium, resulting in the required finalextract concentrations of 10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml. Afterextract concentrationsaddition, plates were incubated at standard conditions for 48 hours and assay was terminated by the addition of cold TCA. Cells were fixed*in situ.* by the gentle addition of 50 µl of cold 30 % (w/v) TCA (final concentration, 10 % TCA) and incubated for 60 minutes at 4°C. The supernatant was discarded; the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (50 µl) at 0.4 % (w/v) in 1 % acetic acid was added to each of the wells, and plates were incubated for 20 minutes at room temperature. After staining, unbound dye was recovered and the residual dye was removed by washing five times

with 1 % acetic acid.The plates were air dried. Bound stain was subsequently eluted with 10 mM trizma base, and the absorbance was read on plate reader at a wavelength of 540 nm with 690 nm reference wavelength. Percent growth was calculated on a plate-by-plate basis for extract wells relative to control wells.Standard anticancer drug, Adriamycin (Doxorubicin) was used as positive control.

Percent growth = (average absorbance of the extract well/average absorbance of the control wells) X 100
The percentage growth was calculated at each of the extract concentration levelsby using the six absorbance measurements [time zero (Tz), control growth (C), and test growth in the presence of extractat the four concentration levels (Ti)].Percentage growth inhibition was calculated as [(Ti-Tz) / C-Tz)] x 100 for concentration forwhich Ti≥Tz (Ti-Tz) positive or zero and[(Ti-Tz)/Tz)] x 100 for concentration for which Ti<Tz (Ti-Tz) negative. Dose response parameters for each test article were calculated.GI50; Growth inhibition of 50%was calculated from [(Ti-Tz)/(C-Tz)] x 100= 50.The drug concentration resulting in total growth inhibition (TGI) wascalculated from Ti=Tz.The LC_{50}, concentration which inhibits 50% of protein measured from beginning was calculated from [(Ti-Tz)/Tz)] x 100= -50.GI50 value of ≤ 20 µg/ml is considered to demonstrate activity.

If the level of activity reaches then values were calculated for above three parameters. If the effect was not reached or exceeded, the values were expressed as greater or less than maximum or minimum concentration tested.

Result and discussion

Phytochemical screening

The methanol extract of leaves of *Borassus flabellifer* Linn. Was tested for the presence of major phytochemicals. Extract showed positive results for severalPhytochemicals likeflavonoids, glycosides, tannins, proteins, steroids, triterpenoids, carbohydrates, fats and fixed oils.

Anticancer activity

Human colon cancer cell line (HCT15), human lung cancer cell line (Hop65) and human hepatoma cell line (HEPG2) were employed to test anticancer efficacy ofmethanol extract of leaves of *Borassus flabellifer*Linn. at four different concentrations 10, 20, 40 and 80 (µg/ml). No human colon cancer cell line (HCT15) percentage control growth was inhibited by the extract. Minimum growth (88.9%) was observed at concentration 80 µg/ml.Adriamycin strongly inhibits percentage control growth with maximum effect (-50.3) at 80 µg/ml. LC50, TGI and GI50 for extract was >80 and forAdriamycin61.9, 21.8 and <10 respectively. Extract exerts same effects onhuman lung cancer cell line (Hop65) and human hepatoma cell line (HEPG2) with no growth inhibition of cell lines at all the concentrations.However Adriamycineffectively inhibits percentage cell growth.Similarly GI50for extract was>80 and for adriamycin <10.

Table 1:% Control growth of cell lines in presence of methanolic extract of *Borassus flabellifer* Linn.and standardadriamycin

	% Control Growth			
Concentrations (µg/ml)	10	20	40	80
Human colon cancer cell line (HCT15)				
Methanolic extract				
Experiment 1	100.0	98.8	98.8	94.4
Experiment 2	90.6	92.0	87.8	86.9
Experiment 3	94.8	94.4	88.5	85.3
Average values	95.1	95.0	91.7	88.9
Adriamycin (ADR)				
Experiment 1	-11.9	-21.6	-25.3	-39.8
Experiment 2	-28.1	-28.5	-36.1	-47.4
Experiment 3	-46.6	-60.0	-62.8	-63.7
Average values	-28.9	-36.7	-41.4	-50.3

Human lung cancer cell line (Hop65)				
Methanolic extract				
Experiment 1	100.0	100.0	100.0	86.3
Experiment 2	100.0	100.0	100.0	86.3
Experiment 3	100.0	99.7	97.2	73.6
Average values	100.0	99.9	99.1	82.0
Adriamycin (ADR)				
Experiment 1	13.6	4.9	3.5	0.6
Experiment 2	7.6	6.5	1.4	-7.0
Experiment 3	6.3	4.3	-4.1	-17.7
Average values	9.1	5.2	0.2	-8.0
Human hepatoma cell line (HEPG2)				
Methanolic extract				
Experiment 1	100.0	97.8	89.3	89.0
Experiment 2	100.0	91.5	91.1	85.0
Experiment 3	100.0	100.0	99.7	79.2
Average values	100.0	96.4	93.3	84.4
Adriamycin (ADR)				
Experiment 1	-10.8	-18.8	-43.3	-51.9
Experiment 2	-19.9	-22.1	-43.1	-53.1
Experiment 3	-20.4	-25.4	-51.8	-56.4
Average values	-17.0	-22.1	-46.1	-53.8

Table 2: lethal concentration value LC50 (µg/ml), total growth inhibition (TGI) and mediangrowth inhibition (GI50) for tested methanolic extract of *Borassus flabellifer* Linn. and adriamycin.

Cell line	Name of drug	LC50	TGI	GI50
HCT15	Extract	>80	>80	>80
	Adriamycin	61.9	21.8	**<10**
HOP62	Extract	>80	>80	>80
	Adriamycin	>80	54.5	**<10**
HEPG2	Extract	>80	>80	>80
	Adriamycin	61.2	25.4	**<10**

Conclusion

Present study revealed that though *Borassus flabellifer* Linn. contains many different chemicals, it do not exhibit any promising anticancer activity against the selected cell lines. This might be due to absence of phytochemicals of anticancer potential. Further, it can be concluded that plants of *Borassus* family do not contains anticancerous phytoconstituents and it will not be considered for cancer treatment or related complications.

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