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Screening of Antioxidant, Anticancer Activity and Phytochemicals in Methanolic Extract of *Hibiscusrosa-Sinensis* Leaf Extract.

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ABSTRACT

Leaves and flowers of selected Hibiscus species, used in traditional medicine, were evaluated for antioxidant and antibacterial activities. Information on these species is meagre and this study would contribute new and additional knowledge on the bioactivities of the genus. Antioxidant properties (AOP) of six species assessed were total phenolic content (TPC). Leaves of species with high TPC. Leaves of H.rosa-sinensis had the strong antioxidant activity. Leaves of H.rosa-sinensis developed into functional food and skin care products.
Keywords: Hibiscus leaves, antioxidant, anticancer, adenocarcinoma cells.

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INTRODUCTION

The genus Hibiscus (Malvaceae) comprises about 275 species in the tropics and sub-tropics (Dasuki, 2001). Within the Malesian region, 43 species are found. Most Hibiscus species have a remarkable colour pattern with the base of corolla forming a deep-coloured heart (Lowry, 1976). Another feature is flower colour change among species of which the most spectacular is in flowers of Hibiscus mutabilis L. Leaves of Hibiscus are simple, lobed, alternate or spiral and have paired stipules (Ng, 2006). Flowers are radially symmetrical with cup-shaped calyx, five petals joined at the base, style bearing many stamens and stigma with five hairy lobes.

With attractive and colourful flowers, plants of Hibiscus are widely planted as ornamentals and are used in traditional medicine. Of the species studied, leaves and flowers of H. mutabilis, believed to have emollient and cooling effect, are used to relieve swellings and skin infections (Dasuki, 2001). Leaves and flowers of Hibiscus rosa-sinensis leaves are used as an antiseptic for boils and ulcers. Leaves and flowers of selected Hibiscus species are used in traditional medicine. Information on their antioxidant and antibacterial activities is meagre. This study would contribute new and additional knowledge on the bioactivities of the genus.

MATERIALS AND METHODS

Plant material and preparation

The plant leaf collected from Kannur, Kerala.The fresh plant material was harvested, rinsed under tap water and air dried under shade for several days and grinded. The leaves of Hibiscus rosa-sinensis used in the form of crude 90% methanolic extract. One kilogram of shade and powderd leaf was extracted with 90% methanol in cold for 72 hours. The extract was filtered and distilled and distilled on water bath, the syrupy mass obtained was dried at low temperature under reduced pressure in a rotary evaporator and a crude residue was obtained (Akerele et al., 2008).

Phytochemical analysis

Chemical tests are conducted on the methanolic extract and also of the powderd form of the plant samples using standered methods (Edeoga et al., 2005).

Qualitative analysis on phytochemical constituents

Tests for flavonoids

A few drops of formic acid, glacial acetic acid and water added to the methanolic extract of plant sample in a test tube. A yellow coloration is observed if flavonoid compounds are present.

Tests for tannins

A few drops of toluene, ethyl acetate and formic acid are added to the methanolic extract of plant sample in a test tube. A brownish green or blue black colouration, which shows the presence of tannins.

Tests for terpenoids

n-hexane and ethyl acetate are added to the methanolic extract of plant sample in a test tube. A reddish brown coloration is formed if terpenoids constituent is present.

Tests for saponins

Chloroform, acetic acid, methanol, water are added to the methanolic extract of plant sample in a test tube and shaken vigorously to obtain a olive oil and observed for the formation of emulsion, which indicates the presence of saponins.

Antioxidant testing assays

DPPH (1, 1-Diphenyl -2-picryl- hydrazyl) activity (Shimda, 1992)

To 1ml of various concentrations of test compound, 1.0ml of 0.5mM DPPH was added (McCune and John 2002). The test tubes were incubated at 37^0C for 30 minutes. The absorbance was read at 517nm (Miliauskas et al., 2004).

$$\% \text{ of Scavenging} = \frac{\text{Absorbance control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

FRAP (Ferric Reducing Ability of plant) Assay (Pulido**,** 2000**)**

The FRAP reagent was prepared by adding 200ml of acetate buffer,20ml TPTZ; 20ml $FeCl_3$; and 24ml of distilled water. To 1ml of various concentrations of test compound added 1ml of FRAP reagent, mixed the contents thoroughly and incubated at 73^0C for 5 minutes. Read the absorbance at 593nm.

Superoxide dismutase activity (Robak and Gryglewski, 1988**)**

The reaction mixture contained 50mM phosphate buffer (p^H 7.6), 20mg riboflavin, 12mM EDTA, NBT 0.1mg/3ml, added in that sequence. The reaction was started by illuminating the reaction mixture with different concentrations of sample extract for 15minutes. Immediately after illumination, the absorbance was measured at 590nm.

$$\% \text{ of Scavenging} = \frac{\text{Absorbance control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

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Invitro Anticancer activity

MTT reduction assay (Methyl Trizolyl tetrazolin)

Cell viability was measured with blue formazan that was metabolized from MTT by mitochondrial dehydrogenase, which is active only in live cells. HT-29 cells were seeded in 96-well plate at a density of 1.0×10^5 cells per well, cultured overnight and pretreated with various concentrations of GBA. After incubation for 24 hr, the MTT (5mg/ml) colorimetric viability test was used to determine the viability of cells. The absorbency of each well was measured at 540 nm using an ELISA reader (BioRad, Model 680, USA), and the percentage viability was calculated (Pantazis P, 1995).

RESULTS AND DISCUSSION

Phytochemical analysis

Prliminary phytochemical analysis revealed that the plant possessedthe phytoconstituents flavonoids, terpenoids, saponins, tannins and glycosides.

Table1: Phytochemical studies

TESTS	OBSERVATION
ALKALOIDS	
1.Drogendroff's test	+
2. Wagner's test	+
3.mayer's test	+
FLAVANOIDS	+
SAPONINS	+
CARBOHYDRATES	
1. Fehlings test	_
2. Benedicts test	_
3. Mollischs test	_
PROTEINS	
1. Millions test	_
PHENOLS	
1. Ferric chloride test	+
2. Lead acetate test	+
3. Liebermanns test	+
STEROID	
1. Libermanns-Burchards test	_
2. Salkowski reaction	_
GLYCOSIDES	_
TANNINS	
1. Ferric chloride test	+
2. Lead acetate test	+
TERPENOIDS	+

(+)……. Positive **(-)……. Negative**

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Antioxidant activity

DPPH activity

The results for DPPH scavenging activity performed are presented in following table 2.

Table 2: DPPH activity

S.NO	Concentration of extract (µg)	% of DPPH scavenged
1	20	54.0
2	40	56.2
3	60	59.4
4	80	61.3
5	100	63.5

$$\text{Rate of inhibition (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

These shows increased concentrations of extract possess increased scavenging activity. The radical scavenging and antioxidant potential of the plant extracts were determined by the ability of plant extracts to scavenge the stable free radical DPPH and convert into Diphenyl picryl hydrazine. The degree of decolourization from purple to yellow colour was measured spectrometrically at 517nm. The methanolic extract of Hibiscus rosa-sinensis leaves contains high antioxidant activity.

FRAP ASSAY

The reducing power has been determined by FRAP assay and the following result was obtained. It is presented in the table 3.

Table 3: FRAP assay

Sl. No	Concentration of standard (µg)	Absorbance at 593nm
1.	20	0.04
2.	40	0.08
3.	60	0.91
4.	80	1.00
5.	100	0.91

The absorbance test is 0.75. From the table we get the concentration 95µg/ml. So the methanolic extract of Hibiscus rosa-sinensis leaves revealed the ferric reducing concentration of 95µg/ml. So the methanolic extract of Hibiscus rosa-sinensis leaves shows high ferric reducing power. The results showed that the methanolic extract of Hibiscus rosa-sinensis leaves possess ferric reducing antioxidant power.

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Superoxide dismutase activity

The enzymatic antioxidant activity was determined by Superoxide dismutase and the following result was obtained. It is presented in the table 4.

Table 4: Estimation of Superoxide dismutase

Sl. No	Concentration of extract in µg	Inhibition rate % of superoxide dismutase
1.	20	31.7
2.	40	32.2
3.	60	32.8
4.	80	33.1
5.	100	33.4

$$\text{Rate of inhibition (\%)} = \frac{\text{Absorbance of control-Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Superoxide radical is a highly toxic species, which is generated by numerous biological and photochemical actions. The methanolic extract of Hibiscus rosa-sinensis leaves showed 66% of superoxide dismutase antioxidant activity. In the present study showed that the methanolic extract of Hibiscus rosa-sinensis leaves possess high enzymatic antioxidant activity.

Table 4: Anticancer activity

Concentration (µg)	% Cell Inhibition
31.25	4.605678
62.5	14.19558
125	82.08202
250	100
500	100

The % cell inhibition was determined using the following formula:

% cell Inhibition = 100- Abs (sample)/Abs (control) x100.

Nonlinear regression graph was plotted between % Cell inhibition and Log_{10} concentration and IC50 was determined using Graph Pad Prism software.

RESULTS

50% of inhibition concentration =**90.79µg/ml**

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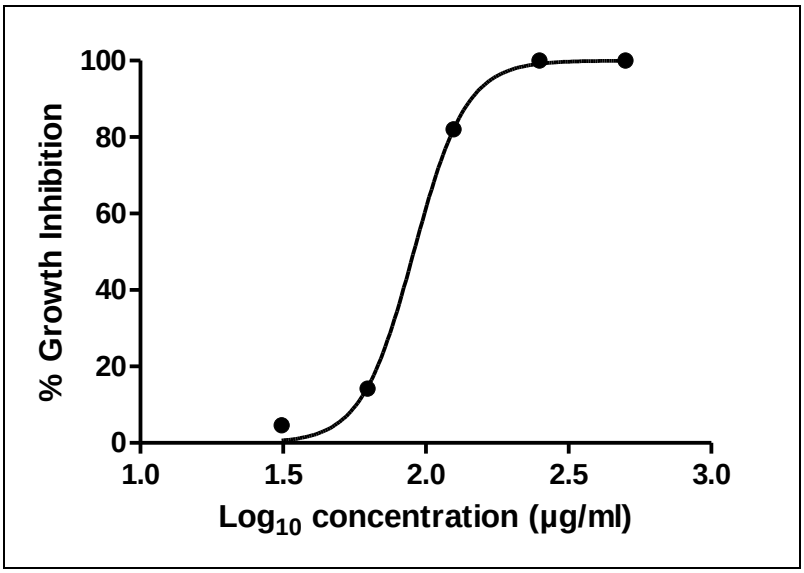
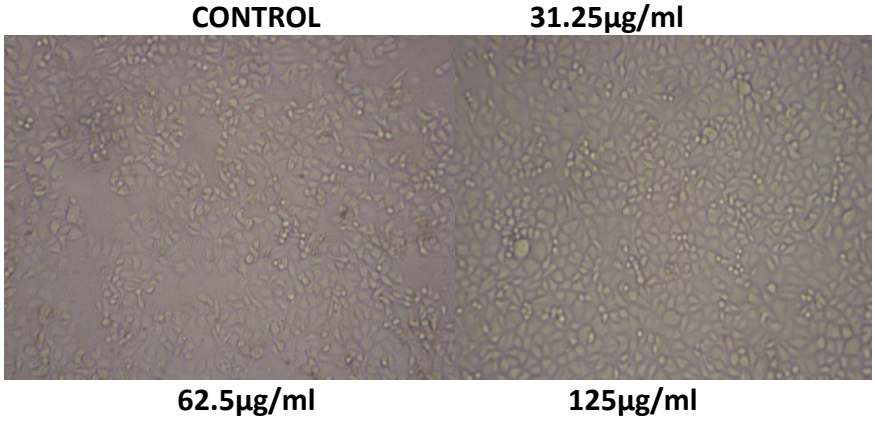


The antiproliferative effect was preceded by accumulation of cells in G2/M phase of cell cycles. The leaf extracts will be inhibit AGS cancer cell proliferation invitro mainly by accumulating cells in G2/M phase (Rhasit et al., 1997).

Eleven human colorectal AGS cell lines established in the lab were classified into three groups based on morphological features (Light & Electron microscopy). Modal chromosome number and ability to synthesize carcino embryonic cell lines G1 cell lines contains both dedifferentiated and differentiated cells growing in tight clusters. G2 cell lines were more dedifferentiated were hyper diploid.G3 cell line were morphologically similar to those of G1 (Albert leibovitz et al., 2002).Thus the results showed that the methanolic extract of Hibiscus rosa-sinensis leaves possess antitumor activity.

Anticancer activity of Hibiscus rosa-sinensis leaves extract



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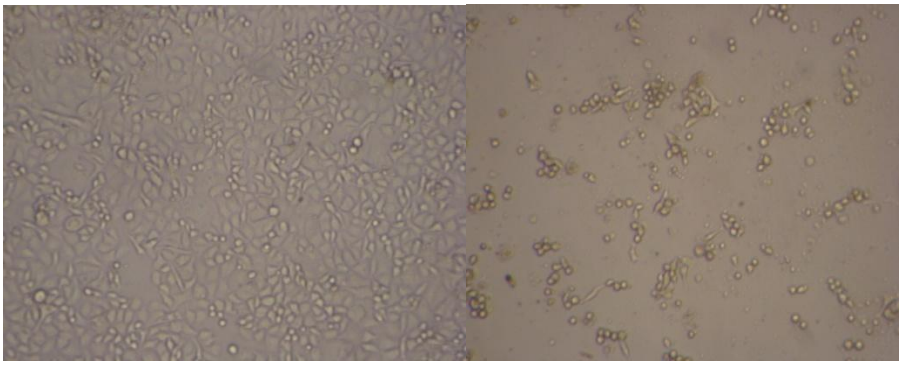
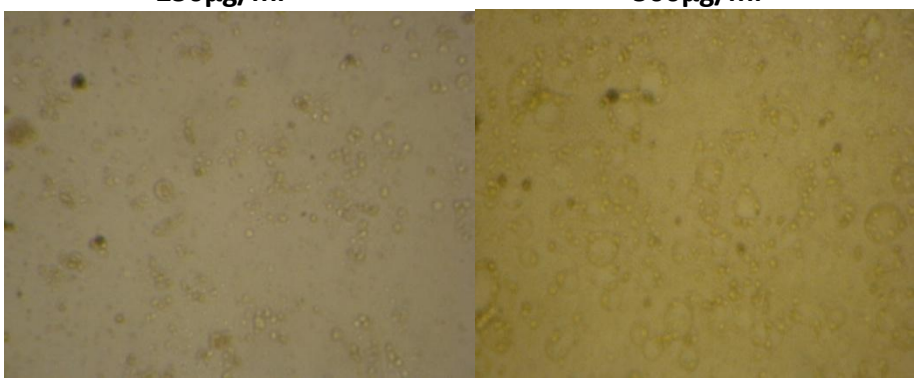


250µg/ml 500µg/ml



CONCLUSION

The result obtained from present study reveal that 90% methanolic leaf extract of Hibiscus rosa-sinensis exhibit significant antioxidant and anticancer activity due to the increased flavonoids and terpenoids level. The phytochemical analysisl of leaf extract indicate the constituents present are responsible for pharmacological effects. The investigation validate used of Hibiscus rosa-sinensis as herbal drug for anticancer and antioxidant activity.

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