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Research Article

Antimutagenic Activities of *Anisosciadium lanatum* Extracts Could Predict the Anticancer Potential in Different Cell Lines

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ABSTRACT

In spite of the tremendous progress in the development of anticancer drugs yet the cancer is still one of the leading causes of death worldwide in addition to the economic and social burdens it causes. The high cost of chemotherapy, the resistance development and the severe adverse effects mandate the continuous screening for novel, cheap, and safe anticancer drugs. In our efforts to expedite the screening process in botanical extracts, we analyzed the flavonoid, lycopene, β-carotene, and chlorophyll a and b in four different extracts from *Anisosciadium lanatum* with different polarities in addition to the essential oil. In addition, we have also measured the antioxidant, peroxide and superoxide scavenging activities of the extracts and oil. We have also estimated the antimutagenic activities of these extracts in *Salmonella typhimurium* using Ames test against two mutagens; sodium azide (NaN_3) and benzo(a)pyrene (B(a)P). All these features and measurements of the extracts were correlated with their anticancer activities in 5 cell lines; liver, lung, colon, breast, and prostate. The phytochemical and antioxidants studies did not precisely predict the potential anticancer activity of extracts or at least what is performed in the current study. Collectively, the antimutagenic activities of the extracts along with the reductions in mutant frequency reported correlates well with the anticancer activities. Therefore, we believe that the antimutagenic activity along with phytochemical analysis could serve as a plausible surrogate in the prediction of potential anticancer activity in the process of screening botanical extracts.

Keywords: *Anisosciadium lanatum*, antimutagenicity, phytochemistry, anticancer, scavenging activity, cell lines.

INTRODUCTION

The number of cancer cases rises annually and so do the social and economic burdens. The 13.3 million new cases of cancer diagnosed worldwide in 2010 cost 290 billion dollars. The 21.5 million new cancer cases anticipated in 2030 are projected to cost 458 billion dollars. In 2012 alone, it is estimated that 8.2 million died of the disease[1]. Chemotherapy remains one of the standard therapies used to destroy cancer cells. However, it can also harm healthy rapidly growing normal cells resulting in serious side effects[2]. Chemotherapy may cause secondary cancer or drive a cancer into greater malignancy. The cure rates are still extremely bad for many cancers and many cancer cell types develop resistance to the chemotherapy regimens in use[3]. All of the previous facts make the search for novel drugs a precedence goal for research. These novel anti-tumour drugs should cause fewer side-effects, cheaper, and have greater therapeutic efficiency.

Various plant products have been reported to possess chemopreventive properties. Approximately, 20% of cancer incidents are preventable by consuming more vegetables and fruits which may potentially prevent approximately 200,000 cancer-related deaths annually[4]. The drug discovery of novel antitumor agents usually begins with screening for plant extracts with antioxidant and antimutagenic activities. This is usually followed by some in vitro anticancer studies and finally with time consuming animal studies. The active ingredients should be at some point isolated and chemically characterized.

Anisosciadium lanatum Boiss is an edible plant belonging to family Apiaceae. There are about 3000 members of this family worldwide; mainly in the temperate areas. Some well-known vegetables and herbs belong to this family like carrot, parsnip, celery, fennel, angelica, cumin, parsley, coriander/cilantro, Dill, and caraway[5,6]. The biological activity of this plant has not been studied before and only the chemical composition of the essential oil was reported in literature[7]. Most members of this family are edible and showed biological activity justifying the selection of this plant for investigation.

Therefore, the aim of the present study, consistent with our continuous effort to identify antitumor plants and plant fractions, was to investigate the potential antioxidant, antimutagenic, and anticarcinogenic activities of extracts with different polarities from *Anisosciadium lanatum* against rat hepatic H4IIE1, human colon HT-29, human

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breast MCF-7, human lung A549, or human prostate PC3 cell lines. This study could offer a platform for future studies and help selecting the key feature(s) of an extract that could most probably identify the extract with potential cytotoxic and anticancer activities. This will help launch further animal studies and isolation and chemical characterization of the active agents from that promising extract.

MATERIALS AND METHODS

Chemicals and preparation of extracts

All reagents were purchased from Sigma (St. Louis, MO, USA) except where indicated. The aerial parts of *Anisosciadium lanatum* were collected from Al-Nayrria, Ajman Valley (Saudi Arabia), in April 2011. The plant material was identified by Prof. Mohamed Al Fredan and a voucher specimen (Anis-1-11) has been deposited in the Department of Biological Sciences, College of Science, King Faisal University, Saudi Arabia. The collected plant materials were stored in a dry and dark place at room temperature with passive ventilation for 2 weeks. Extraction of the plants was performed using continuous technique. The aerial parts (1.2 Kg) of *Anisosciadium lanatum* Boiss, were dried and milled. The powder of the plant was extracted with methyelene chloride (CH_2Cl_2) and methanol (MeOH) (1:1) at room temperature for 3 days and filtered. The solvents were evaporated under vacuum. Also, the essential oil of *Anisosciadium lanatum* was isolated through Clevenger Apparatus. The extract was concentrated using a vacuum rotary evaporator and stored at 4°C for chemical and biological studies. The dry extracts were subjected to general fractionations using different organic solvents (*n*-hexane, CH_2Cl_2, and MeOH, and their mixtures) according to the chemical nature of the components which they might contain (Table I). The extracts and fractions were subjected to the usual isolation and purification processes; partition between solvents, chromatography (Thin Layer Chromatography (TLC), Gravity Column Chromatography (CC) over Silica Gel, Diion, Sephadex LH-20 and Polyamide, analytical and Flash Chromatography).

The S9 mix used in antimutagenicity study consisted of a filter-sterilized NADPH (1.25 mM) and hepatic S9 fraction (4 mg protein/ml) prepared from male Sprague Dawley rats treated with a single dose (25 mg/kg, i.p. in corn oil) of Aroclor 1254.

Cell cultures and bacteria

The cell lines used were hepatic rat H4IIE cells, human colon HT29, breast MCF7, lung A549, and prostate PC3. All cells, media, fetal bovine serum, DMSO, and trypsin-EDTA were obtained from American Type Culture Collection (ATCC; Manassas, VA, USA). Cell lines were seeded in 75-cm^2 tissue culture flasks at 37ºC in a humidified atmosphere (5% CO_2) and the medium was renewed every two days.

Determination of total flavonoids

Total flavonoids content was determined as described elsewhere[8]. A standard curve for quercetin was established and the data are expressed as mg quercetin equivalents/g dried plant materials. Assays were performed in triplicate.

Determination of β-carotene, lycopene, and Chlorophyll a and b

β-Carotene, lycopene, and chlorophyll a and b were determined as described by Nagata and Yamashita[9]. Contents of β-carotene, lycopene, and chlorophyll a and b were determined according to the following equations:

lycopene (mg/100 ml) = $-0.0458\ A_{663} + 0.204\ A_{645} + 0.372\ A_{505} - 0.0806\ A_{453}$

β-carotene (mg/100 ml) = $0.216\ A_{663} – 1.22\ A_{645} - 0.304\ A_{505} + 0.452\ A_{453}$

Chlorophyll a (mg/100 ml) = $0.999\ A_{663} – 0.0989\ A_{645}$

Chlorophyll b (mg/100 ml) = $-0.328\ A_{663} + 1.77\ A_{645}$

The results are expressed as means ± standard error of the mean (SEM). The values are expressed as mg/g extract. Assays were performed in triplicate.

Determination of total antioxidant activity

The antioxidant capacity of *Anisosciadium* extracts was measured spectrophotometrically using a phosphomolybdenum method based on the reduction of Mo (VI) to Mo (V) and the subsequent formation of specific green phosphate / Mo (V) compounds[10]. Stock solutions of trolox were prepared in methanol just before use. The total antioxidant activity was expressed as equivalents of trolox (µg trolox/g extract). Assays were performed in triplicate.

Peroxide scavenging activity

Peroxide scavenging activity was measured as described elsewhere[11]. Peroxide radicals were generated from mixing $FeSO_4$ and H_2O_2.

The peroxide scavenging activity (%)= $[1 - (A_1 - A_2)/A_o] \times 100$

where A_0 is the absorbance of the control (without extract or trolox) and A_1 is the absorbance including the extract or trolox, and A_2 is the absorbance without sodium salicylate. Assays were performed in triplicate.

Superoxide anion scavenging activity

Superoxide anion scavenging activity of the extracts was determined as described elsewhere[12]. Superoxide radicals were generated in phenazine methosulfate (PMS)-nicotinamide adenine dinucleotide (NADH) systems and assayed by the reduction of nitroblue tetrazolium (NBT). The inhibition percentage of superoxide anion generation was calculated using the following formula:

Inhibition of superoxide anion (%) = $[(A_0 - A_1)/A_0 \times 100]$

where A_0 is the absorbance of control, and A_1 is the absorbance in presence of either extract or trolox. Assays were performed in triplicate.

Metal chelating activity

Plant extract or standard antioxidant (0-600 µg/ml) was added to 50 µl of 2 mM $FeCl_2$. The reaction was initiated by the addition of 5 mM ferrozine (0.2 ml) and the mixture was shaken vigorously at room temperature for 10 min[13]. The absorbance was measured at 562 nm. The metal chelating activity was calculated as follows:

Metal chelating effect (%) = $[(A_0 - A_1)/A_0 \times 100]$

where A_0 is the absorbance of control and A_1 is the absorbance in presence of either extract or standards (trolox or α-tocopherol). Assays were performed in triplicate.

Free radical scavenging activity

The free radical scavenging activity of *Anisosciadium* extracts was evaluated using 2,2-diphenyl-1-picryhydrazyl ($DPPH^{\cdot}$)[14].

$DPPH^{\cdot}$ scavenging effect (%) = $[(A_0 - A_1)/A_0 \times 100]$

where A_0 is the absorbance of control and A_1 is the absorbance in presence of either extract or α-tocopherol.

Cytotoxicity assay on Salmonella typhimurium (TA1535)

The cytotoxicity assay was performed using the bacterial growth assay on nutrient agar plates[15]. The experiment was designed with conditions that mimic those of the revertant mutagenesis/antimutagenesis assay. In a preliminary experiment, a 10^{-7} dilution of TA1535 was shown to be the appropriate dilution; therefore, it was selected to proceed with. Briefly, a 100 µl of 10^{-7} dilution of the overnight growing bacterial culture in Luria Broth (LB) medium was incubated with each plant extract (5, 10, or 20 mg/ml) and mixed with 2.5 ml of warm 0.6% top agar (NaCl/agar). The mix was then added to nutrient agar plates and the plates were incubated at 37°C for 24 hours. After the incubation period, colonies on triplicate plates were counted and compared to control plates containing no plant extracts. Concentrations investigated from hereafter were 1 and 5 mg for all extracts.

Cytotoxicity of plant extracts in combination with the selected mutagen

To rule out any possible toxic effect resulting from a combination of the mutagen used and plant extracts in the mutagenicity/antimutagenicity evaluation assays, the number of colony/plate evaluation was performed. A 100 µl of 10^{-7} dilution of overnight growing *S. typhimurium* TA1535 was incubated with each extract at 37°C for 30 minutes on a shaking incubator in the presence of 2 µg/plate of NaN_3 or 20 µM of B[a]P and S9 mix in 400 µl of phosphate buffer. The mix was then added to agar plates and incubated at 37°C and the total viable bacterial count on triplicate plates was recorded after 24 hours[16]. Control experiments were carried out simultaneously; the S9 mix alone had no effect on the bacterial viability.

Mutagenicity and Antimutagenicity Assays

The reverse bacterial mutation assay was performed for screening the mutagenic potential of the plant extracts as described elsewhere[15-18]. Spontaneous revertant colonies arise on plates containing neither mutagens nor extracts were counted. Revertant colonies seen with 20 µM B[a]P with S9 mix were performed as a positive control. All assays were performed in triplicates. Antimutagenic activity of *Anisosciadium lanatum* extracts against NaN_3 or B[a]P was determined under pre-exposure and co-exposure assays as described elsewhere[15-18]. In all assays, positive and negative controls were performed. All antimutagenesis determinations were performed in triplicates. Reveratant colonies were counted after 36-48 hours of incubation and the antimutagenic potential of the tested extracts was expressed as a percentage of reduction in mutagenicity[19], and calculated according to the following equation:

% Reduction in mutagenicity = ([Rm-Rs] - [Ra - Rs] / [Rm – Rs]) x100

Where Rm is the number of revertants /plate in the presence of mutagen

Rs is the number of spontaneous revertants /plate

Ra is the number revertants /plate in the presence of plant extract.

A 20% or less reduction means no antimutagenic activity, 20-40 % reduction means a moderate activity, and 40 % or more reduction means a strong antimutagenic activity.

The mutant frequency or mutation rate was then calculated from the mutant colonies/viable colonies for both exposure conditions for the mutagens investigated.

Determination of in vitro anticancer activity

The anticancer activity was determined as described elsewhere[16-20]. The cell lines were grown in the suitable medium. The cells were inoculated into 96 well plates at plating densities of ~ 5,000 cells/well, four wells were used for each treatment. Since the extracts are colored, control wells (extract control) were made for every extract concentration used without cells. Three independent experiments were performed. Percentage growth inhibition was calculated using the following formula:

$[(T-T0)/(C-T0)] \times 100$ for concentrations for which $T \geq T0$

$[(T-T0)/T0] \times 100$ for concentrations for which $T < T0$.

T0 or time zero represents a measurement of the cell population for each cell line at the time of extract addition, C is the control growth, and T is the test growth at different concentrations of *Anisosciadium* extracts after incubation. Three dose response parameters were calculated for each experimental agent. Growth inhibition of 50 % (GI50) was calculated from $[(T-T0)/(C-T0)] \times 100 = 50$, which is the extract concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the incubation. The extract concentration resulting in total growth inhibition (TGI) is calculated from Ti = Tz. The LC50 (concentration of extract resulting in a 50% reduction in the measured protein at the end of the extract treatment as compared to that at the beginning) indicating a net loss of cells following treatment is calculated from $[(Ti-Tz)/Tz] \times 100 = -50$.

All procedures were approved by the University of King Faisal Committee of Scientific Research Ethics.

Statistical analysis of data

Statistical analyses were performed using ANOVA, followed by Fisher's protected least significant difference multiple range test. Differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Phytochemistry, antioxidant and radical scavenging activities

Extraction of *Anisociadium* was performed using solvents with different polarities. Extract I (*n*-hexane extract) would presumably contain the highly non-polar constituents, extracts II (methylene chloride: methanol: petroleum ether) and III (water: methanol) would contain intermediate polar flavonoids and glycosides and some other polar compounds. Extract IV (water extract) would have the highly polar constituents such as glycosides, polar flavonoids and aglycones. Extract V is the essential oil.

Table 1a: Extraction Scheme of *Anisosciadium lanatum*.

Sample Code	Extraction Solvent
B1 (I)	n-Hexane 100%
B2 (II)	CH_2Cl_2:MeOH:Petrolem ether (1:1:1)
B3 (III)	H_2O:MeOH (1:1)
B4 (IV)	H_2O 100%
B5 (V)	Essential oil

Extract II has a moderate flavonoid content equivalent to ~ 25 mg quercetin / g. Extracts III and IV and essential oil have low flavonoid content (Table 1), while extract I is almost devoid of flavonoids. With the exception of extract I which is very poor in lycopene, all other extracts and essential oil have moderate lycopene content. Extract II has the highest lycopene content of ~ 41 mg/g followed by extract III which has 30 mg/g lycopene content (Table 1). The β-carotene content was higher in essential oil (31 mg/g) and polar extracts III (31 mg/g) and IV (27 mg/g) than in more non-polar extracts I and II. The chlorophyll a and chlorophyll b content varied with the extraction solvent. Extracts I, II, and III have more chlorophyll a content than extract IV and essential oil (Table 1). Extracts II and III have the highest contents of chlorophyll a and b. Extract I has the highest metal chelating activity comparable to that of tocopherol, followed by extracts III >> II >> IV. The activity of all extracts was concentration-dependent (Figure 1). The essential oil (B5) had the lowest metal chelating activity. Extracts II and III had the highest free radical scavenging activity followed by extract IV and essential oil. Extract I had the lowest activity (Figure 2). Only extract III (water: methanol) with the high β-carotene, chlorophyll a and b content resulted in a significant total antioxidant activity equivalent to ~ 101 µg trolox/g extract. It also had significant peroxide and superoxide scavenging activities (Table 2). Extract IV rich only in β-carotene resulted in a significant superoxide scavenging activity as compared to trolox. All plant extracts showed a peroxide scavenging activity higher than that of trolox and the highest activity of ~ 99% was shown by the essential oil compared to 32% shown by trolox (Table 2). Extracts III and IV have the highest superoxide scavenging activity (Table 2). From our previous study on *Conyza triloba*, we found that high chlorophyll content was a determining factor and had a strong correlation with the cytotoxic activity of extracts against tumor cells[16]. With an exception of the effect of extract I in the present study, extracts II and III rich in chlorophyll, flavonoids, lycopene, and β-carotene showed the best antioxidant and scavenging activities against peroxide and superoxide radicals. These extracts also showed the best anticancer activities. No doubt the oxidative stress is a key event during the pathogenesis of many diseases and during the initiation and progression of cancer[21]. However, extract I (hexan extract) poor in all the previous constituents was as potent as extracts II and III in antimutagenic and anticancer studies. Therefore, the phytochemistry performed in the current study alone is not a valid tool to build a platform for identification of plant extracts with potential anticancer activities. The only antioxidant/scavenging property that characterized this highly non-polar extract I was its unique metal chelating activity. Li et al found that the hexane extract of *Gynostemma pentaphyllum* arrested the cell cycle, inhibited the cancer cells and induced apoptosis in breast cell lines[22]. Although most studies focus on polar extracts, but non-polar extracts are also promising and should not be neglected. Alkaloids, esters and terpenes could be found in hexane extract and many of these were reported to have anticancer activity[23-25].

Antimutagenic activity

Measuring the viability of bacteria and estimating a non-toxic concentration is a pre-requisite for the validity of Ames assay results. The viability of the *Salmonella typhimurium* was not affected by either 5 or 10 mg/ml (Table 3). All extracts at 20 mg/ml significantly reduced the bacterial viability. Extracts II and V were the most toxic extracts reducing the viability by 93 and 94%, respectively (Table 3). The effect of combination of NaN_3 or B[a]P/S9 mix with plant extracts at 1 or 5 mg was investigated and was shown to have no effect on the bacterial viability (Tables 4 and 5). Extracts I-IV had no mutagenic activity when compared with B[a]P. Essential oil was mutagenic to the bacteria at 5 mg/ml resulting in colonies number comparable to those produced after B[a]P (Table 6). This essential oil was excluded from further studies. Similar properties were reported before for essential oils from mint and rosemary which should be carefully evaluated and used with great caution[26] although we usually use concentrations in the experimentation higher than those used by humans.

Extracts I, II, and III exerted a strong antimutagenic activity against NaN_3 in a dose–dependent manner (Table 7) and regardless of the type of exposure conditions. Extract IV had a strong antimutagenic activity (≥ 40%) but only under pre-exposure conditions at the high dose, where the extract was incubated with the bacteria before the addition of NaN_3 (Table 7). In a dose-independent manner, all extracts had a strong antimutagenic activity against

Table 1b: Total flavonoids, lycopene, β-carotene, and chlorophyll a and b content of different *Anisosciadium lanatum* extracts.

Extract	Total flavonoids (mg quercetin) [a]	Lycopene (mg/g) [a]	β-Carotene (mg/g) [a]	Chlorophyll a (mg/g) [a]	Chlorophyll b (mg/g) [a]
I	0.02 ± 0.01	1.0 ± 0.1	7.7 ± 0.5	58.7 ± 4.0	5.0 ± 0.4
II	24.97 ± 1.13	41.3 ± 1.1	7.0 ± 0.4	290.0 ± 9.9	94.3 ± 4.2
III	8.70 ± 0.54	30.0 ± 2.3	31.0 ± 1.2	146.0 ± 7.9	75.7 ± 6.5
IV	2.87 ± 0.31	15.7 ± 1.0	27.3 ± 2.1	35.7 ± 2.2	49.3 ± 3.4
V	0.20 ± 0.01	17.6 ± 1.4	31.3 ± 2.2	33.3 ± 2.6	55.0 ± 1.9

[a] The data are expressed means ± SEM. All assays were performed in triplicates.

Table 2: Total antioxidant, peroxide scavenging, and superoxide scavenging activities of various *Anisosciadium lanatum* extracts.

Treatment	Antioxidant activity [a,b]	Peroxide scavenging activity (%)[a]	Superoxide scavenging activity (%)[a]
I	54.3 ± 2.7	45.7 ± 5.5	19.3 ± 2.0
II	28.7 ± 1.2	45.3 ± 3.9	58.7 ± 6.3
III	100.7 ± 0.9	52.7 ± 6.1	91.7 ± 9.4
IV	64.3 ± 1.6	44.7 ± 4.2	99.7 ± 6.5
V	65.0 ± 1.7	99.0 ± 8.0	34.3 ± 2.7
Trolox	192.3 ± 3.5	32.3 ± 2.9	61.7 ± 3.7

[a] The data are expressed as means ± SEM, n = 3.
[b] The activity is expressed as equivalent of trolox (µg trolox /g extract).

Table 3: Effect of various *Anisosciadium lanatum* extracts (I-V) on *Salmonella typhimurium* TA1535 viability as assessed by colony formation on plate.

Treatment	Viability at extract concentrations (colonies/plate (% of control))		
	5 mg/ml	10 mg/ml	20 mg/ml
None	31.7 ± 4.2 (100)		
I	35.3 ± 4.5 (111)	27.0 ± 2.5 (85)	4.7 ± 1.0 (15) [a]
II	39.0 ± 2.9 (123)	28.0 ± 1.8 (88)	2.3 ± 0.7 (7) [a]
III	41.7 ± 5.1 (132)	30.3 ± 3.1 (96)	13.0 ± 2.1 (41) [a]
IV	33.3 ± 2.7 (105)	31.7 ± 2.5 (100)	16.3 ± 1.3 (51) [a]
V	29.7 ± 3.0 (94)	26.0 ± 2.2 (82)	2.0 ± 0.1 (6) [a]

[a] Significantly different ($p < 0.05$) from the number of colonies/plate (mean ± SEM) recorded in the absence of plant extracts. Assays were performed in triplicates.

Table 4: Effect of *Anisosciadium lanatum* extract and sodium azide (NaN_3) on *Salmonella typhimurium* TA1535 viability as assessed by colony formation on plate.

Treatment	Viability at NaN_3 and extract concentrations (colonies/plate (% of control)) [a]	
	1 mg/ml	5 mg/ml
None	41.0 ± 2.9 (100)	
NaN_3 (2 µg/plate)	43.3 ± 3.3 (106)	
I	39.3 ± 4.0 (96)	39.0 ± 4.3 (95)
II	38.7 ± 2.5 (94)	37.3 ± 2.9 (91)
III	40.0 ± 3.7 (98)	39.7 ± 4.1 (97)
IV	44.0 ± 3.9 (107)	38.7 ± 4.5 (94)
V	39.7 ± 3.0 (97)	40.0 ± 3.0 (98)

[a] Data are expressed as mean ± SEM. Assays were performed in triplicates.

Table 5: Effect of *Anisosciadium lanatu*m extracts, benzo[a]pyrene (B[a]P), and S9 mix on *Salmonella typhimurium* TA1535 viability as assessed by colony formation on plate.

Treatment	Viability at B[a]P and plant extract concentrations (colonies/plate (% of control)) [a]	
	1 mg/ml	5 mg/ml
None	30.0 ± 2.7 (100)	
B[a]P (20 µM)	34.0 ± 3.3 (113)	
I	31.3 ± 3.0 (104)	26.7 ± 2.6 (89)
II	25.0 ± 2.2 (83)	23.7 ± 0.7 (79)
III	29.7 ± 2.7 (99)	24.0 ± 1.7 (80)
IV	36.3 ± 3.5 (121)	25.7 ± 2.7 (86)
V	28.7 ± 1.8 (96)	24.3 ± 2.0 (81)

[a] Data are expressed as mean ± SEM. Assays were performed in triplicates.

B[a]P under both exposure regimens investigated (Table 8). Extracts I-III significantly reduced the NaN_3-mutant frequency by 64-94%. Extract IV resulted in a significant reduction in NaN_3-mutant frequency (22-70%) only under pre-exposure conditions (Table 9). All extracts significantly reduced the B[a]P-mutant frequency by 52-83% (Table 10).

Mutation is an essential event in the pathogenesis of cancer and tumor formation. Ames test is used worldwide with great success to detect mutagenic chemicals[15-19]. It is a very simple assay with low cost and high efficiency and accuracy compared with other assays. *S. typhimurium* TA1535 strain contains the base-pair substitution mutation *hisG46* which is known to be more responsive to sodium azide than other direct mutagens[16]. We have chosen two different mutagens. NaN_3 which is a direct intercalation agent that needs no prior metabolism and B(a)P which requires a metabolic machinery offered by NADPH/S9 mix. This could help in the understanding of the antimutagenic activities of the plant extracts. We also performed the antimutagenicity study with two types of exposure; in pre-exposure the bacteria were incubated with the extract before the addition of the mutagen and co-exposure where the bacteria, extracts and mutagens were all added at the same time. Since the pre-exposure results for B(a)P (average 96%) were a bit better than the co-

Table 6: Determination of mutagenic activity of *Anisosciadium lanatum* extracts in *Salmonella typhimurium* TA1535 in presence of S9 mix.

Treatment	Plant extracts mutagenicity (revertant colonies/plate; mean ± SEM)	
	1 mg/ml	5 mg/ml
None (spontaneous)	25.7 ± 4.0	
B[a]P (20 µM)	184.3 ± 14.6 [a]	
I	10.0 ± 1.7	8.3 ± 1.8
II	14.0 ± 1.1	13.0 ± 2.3
III	18.0 ± 1.7	13.3 ± 1.9
IV	11.0 ± 1.1	8.7 ± 1.2
V	74.7 ± 3.2 [a]	172.7 ± 11.5 [a]

[a] Significantly different ($p < 0.05$) from non-treated bacteria (spontaneous mutations). Assays were performed in triplicates.

exposure (average of 82%). We may assume that extracts could interfere with the metabolic bioactivation of B(a)P by inhibiting to some extent CYP4501A1 responsible for the activation of the mutagen. However, this could be partly true but the 82% reported in co-exposure and the indiscrimination seen in the antimuatgenic activity of extracts against NaN_3 suggests another mechanism. One such plausible mechanism could be the direct binding and protection of DNA from the electrophilic mutagens or metabolites[27]. Another mechanism could be the elevation in the antioxidants milieu of the cells thus, promoting the DNA repair systems[28].

Anticancer activity

Extracts II and III showed the most effective anticancer activity with GI50 at a concentration of as low as 80-90 ng against liver, lung, breast, and prostate cell lines used (Table 11a). Extract I was less effective with GI50 of 120-860 ng. The colon cells were the least affected and only extract IV was able to inhibit the growth of cells at very low concentration (60 ng). Extracts I-III achieved total growth inhibition (TGI) of liver, lung, and breast cells at 420-810 ng (Table 11b). Extracts II and III showed very similar pattern against prostate cells and had TGI values at 560 and 480 ng, respectively. Extract IV was very specific in affecting only the resilient colon cells, which had not been significantly affected by other extracts and had a TGI value of 380 ng. All extracts needed high concentrations to kill 50% of the cells initially present at the time of the addition of the extract (LC50). LC50 values recorded for breast, liver, prostate, colon, and lung cells were ≥100 µg. Extract III had low LC50 values of ~ 51 and 48 in liver and lung cells, respectively (Table 11c).

The NCI screened more than 50,000 plant extracts and sets up a cutoff value for potential anticancer extract at ≤ 10µg[16-20]. Since the current data showed that the plant extracts under study have their GI50 at the nanogram range, so these extracts are considered highly active and

Table 7: Determination of anti-mutagenic activity of *Anisosciadium lanatu*m extracts in *Salmonella typhimurium* TA1535 against sodium azide (NaN_3; 2 µg/plate).

Treatment	NaN_3 mutagenicity at plant extract concentrations (revertant colonies/plate; mean ± SEM (% reduction in NaN_3 mutagenicity))			
	Pre-exposure*		Co-exposure*	
	1 mg/ml	5 mg/ml	1 mg/ml	5 mg/ml
NaN_3	290.3 ± 17.1 (0)			
I	77.7 ± 6.3 (78) [a]	14.7 ± 1.2 (101) [a,b]	21.7 ± 3.6 (98) [a]	10.7 ± 2.2 (102) [a]
II	50.0 ± 5.8 (88) [a]	13.3 ± 1.5 (101) [a,b]	54.0 ± 7.1 (86) [a]	21.7 ± 5.6 (98) [a,b]
III	280.7 ± 10.8 (4)	27.7 ± 9.3 (96) [a,b]	97.7 ± 11.4 (70) [a]	17.0 ± 2.9 (100) [a,b]
IV	230.0 ± 25.8 (22) [a]	77.0 ± 9.3 (78) [a,b]	288.7 ± 20.9 (1)	273.3 ± 12.0 (6)

* Plant extracts were incubated with bacteria either 30-minutes before NaN_3 (Pre-exposure) or incubated with the bacteria and NaN_3 (co-exposure). The spontaneous revertant colonies were 17.0 ± 2.1. [a] Significant ($p < 0.05$) reduction (% of inhibition of mutagenicity indicated in parentheses) from revertant colonies seen with NaN_3. [b] Significant difference ($p < 0.05$) between plant extract concentrations.

Table 8: Determination of anti-mutagenic activity of *Anisosciadium lanatum* extracts in *Salmonella typhimurium* TA1535 against benzo[a]pyrene (B[a]P; 20 µM) in presence of S9 mix.

Treatment	B[a]P mutagenicity at plant extract concentrations (revertant colonies/plate; mean ± SEM (% reduction in B[a]P mutagenicity))			
	Pre-exposure*		Co-exposure*	
	1 mg/ml	5 mg/ml	1 mg/ml	5 mg/ml
B[a]P	324.3 ± 21.5			
I	52.0 ± 2.5 (97)[a]	53.0 ± 5.5 (97)[a]	117.7 ± 9.3 (74)[a]	121.3 ± 6.8 (73)[a]
II	64.0 ± 4.4 (93)[a]	55.0 ± 5.7 (96)[a]	86.3 ± 3.5 (85)[a]	81.0 ± 8.5 (87)[a]
III	66.0 ± 3.8 (92)[a]	51.0 ± 5.5 (98)[a]	86.0 ± 5.1 (85)[a]	85.7 ± 5.6 (85)[a]
IV	63.3 ± 5.4 (93)[a]	49.7 ± 4.4 (98)[a]	81.7 ± 10.3 (87)[a]	85.0 ± 5.1 (86)[a]

* Plant extracts were incubated with bacteria either 30-minutes before B[a]P (Pre-exposure) or incubated with the bacteria and B[a]P (co-exposure). The spontaneous revertant colonies were 45.0 ± 4.9. [a] Significant ($p < 0.05$) reduction (% of inhibition of mutagenicity indicated in parentheses) from revertant colonies seen with B[a]P.

Table 9: Effects of *Anisosciadium lanatum* extracts on sodium azide (NaN_3) mutant frequency.

Treatment	Mutant frequency and (% of NaN_3)[#]			
	Pre-exposure[*]		Co-exposure[*]	
	1 mg/ml	5 mg/ml	1 mg/ml	5 mg/ml
NaN_3	6.70 (100)			
I	1.98 (30) [a]	0.38 (6) [a]	0.55 (8) [a]	0.27 (4) [a]
II	1.29 (19) [a]	0.36 (5) [a]	1.40 (21) [a]	0.58 (9) [a]
III	7.02 (105)	0.70 (10) [a]	2.44 (36) [a]	0.43 (6) [a]
IV	5.23 (78) [a]	1.99 (30) [a]	6.56 (98)	7.06 (105)

[#] Calculated from mutant colonies (table 8)/ viable colonies (table 5). [*] Plant extracts were incubated with bacteria either 30-minutes before NaN_3 (Pre-exposure) or incubated with the bacteria and NaN_3 (co-exposure). [a] Significant difference ($p < 0.05$) from NaN_3.

Table 10: Effects of *Anisosciadium lanatum* extracts on benzo[a]pyrene (B[a]P) mutant frequency.

Treatment	Mutant frequency and (% of B[a]P)[#]			
	Pre-exposure[*]		Co-exposure[*]	
	1 mg/ml	5 mg/ml	1 mg/ml	5 mg/ml
B[a]P	9.54 (100)			
I	1.66 (17) [a]	1.99 (21) [a]	3.76 (39) [a]	4.54 (48) [a]
II	2.56 (27) [a]	2.32 (24) [a]	3.45 (36) [a]	3.42 (36) [a]
III	2.22 (23) [a]	2.13 (22) [a]	2.90 (30) [a]	3.57 (37) [a]
IV	1.74 (18) [a]	1.93 (20) [a]	2.25 (24) [a]	3.31 (35) [a]

[#] Calculated from mutant colonies (table 9)/ viable colonies (table 6). [*] Plant extracts were incubated with bacteria either 30-minutes before B[a]P (Pre-exposure) or incubated with the bacteria and B[a]P (co-exposure). [a] Significant difference ($p < 0.05$) from B[a]P.

Table 11a: The 50% growth inhibition (GI50) data of different *Anisosciadium lanatum* extracts in cell lines.

Extract	Potency of extracts (µg) in cell lines (mean ± SEM), n = 5				
	H4IIE1	A549	HT29	MCF7	PC3
I	0.16 ± 0.01	0.36 ± 0.05	0.60 ± 0.07	0.12 ± 0.01	0.86 ± 0.11
II	0.09 ± 0.01	0.09 ± 0.01	>> 100	0.20 ± 0.01	0.08 ± 0.01
III	0.08 ± 0.01	0.08 ± 0.01	0.74 ± 0.03	0.08 ± 0.01	0.08 ± 0.01
IV	1.28 ± 0.20	0.28 ± 0.01	0.06 ± 0.01	30.32 ± 2.95	0.36 ± 0.04

Table 11b: The total growth inhibition (TGI) data of different *Anisosciadium lanatum* extracts in cell lines.

Extract	Potency of extracts (µg) in cell lines (mean ± SEM), n = 5				
	H4IIE1	A549	HT29	MCF7	PC3
I	0.70 ± 0.05	0.81 ± 0.09	6.84 ± 0.91	0.54 ± 0.06	8.40 ± 1.02
II	0.58 ± 0.04	0.56 ± 0.03	>>100	0.68 ± 0.04	0.56 ± 0.06
III	0.42 ± 0.05	0.42 ± 0.03	27.42 ± 2.44	0.50 ± 0.02	0.48 ± 0.06
IV	8.56 ± 0.91	0.72 ± 0.08	0.38 ± 0.02	> 100	1.14 ± 0.15

Table 11c: The 50% lethality (LC50) data of different *Anisosciadium lanatum* extracts in cell lines.

Extract	Potency of extracts (µg) in cell lines (mean ± SEM), n = 5				
	H4IIE1	A549	HT29	MCF7	PC3
I	94.20 ± 10.37	> 100	> 100	> 100	> 100
II	100.01 ± 9.54	> 100	>> 100	> 100	99.60 ± 10.01
III	50.66 ± 4.50	47.74 ± 6.04	> 100	> 100	94.22 ± 7.07
IV	> 100	> 100	97.8 ± 8.94	>> 100	> 100

[a] extrapolated from dose-response curve. H4IIE1 (rat liver), A549 (human lung), HT29 (human colon), MCF7 (human breast), PC3 (human prostate). GI50 is the concentration of an extract (µg) that causes 50% growth inhibition. TGI is the concentration of an extract (µg) that yields no net growth over the course of the assay. LC50 is the concentration of an extract (µg) that kills 50% of the cells that were present at the time of the addition of the extract.

worthy of further investigations. One other unique feature that makes these extracts promising is their safety which is evident from LC50 data. Having LC50 at much higher concentrations away from those needed for GI50 means these extracts are safe. It also means that these extracts inhibited the growth of the cancer cells rather than merely

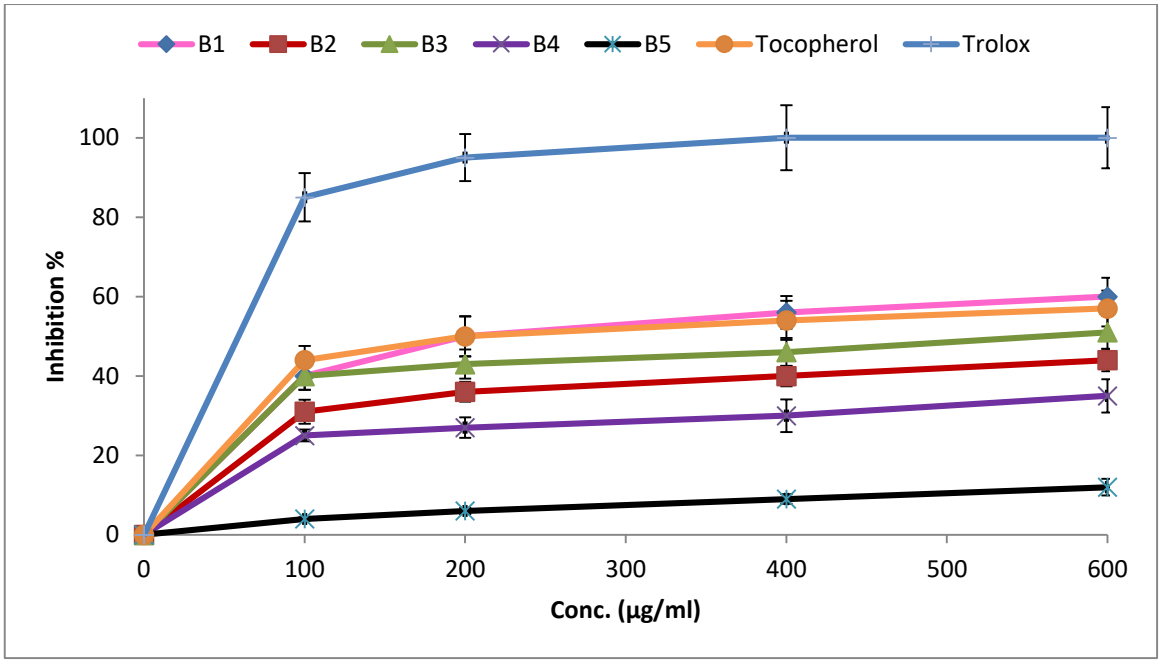

Figure 1: Metal chelating activity of *Anisosciadium lanatum* extracts.

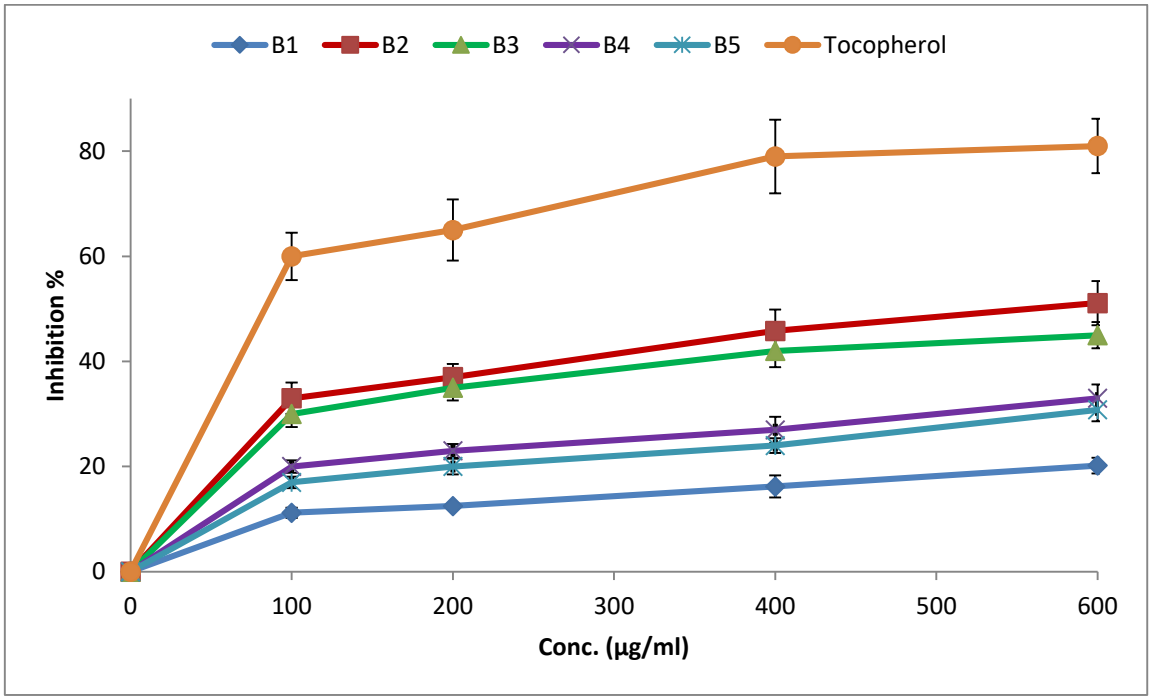

Figure 2: Free radical scavenging activity of *Anisosciadium lanatum* extracts.

killing the cells. Modulation of cancer cell signaling, and triggering apoptosis and cell cycle arrest could be held responsible for the reported anticancer activities of the extracts but these assumptions need further studies for confirmation. Extract IV with highly polar compounds discerned itself by acting specifically against colon cells which cannot be explained but other studies showed that highly polar glycosides, aglycons and flavonoids target colon HT29 with specificity and efficacy[29].

CONCLUSIONS

The n-hexane extract showed a remarkable metal chelating and anticancer activities. This extract will be fractionated and the active constituents will be identified. The non-polar constituents deserve more attention. The phytochemical and antioxidants studies did not precisely predict the potential anticancer activity of extracts or at least what is performed in the current study. Collectively, the antimutagenic activities of the extracts along with the reductions in mutant frequency reported correlates well with the anticancer activities. Therefore, we believe that the antimutagenic activity along with phytochemical analysis could serve as a plausible surrogate in the

prediction of potential anticancer activity in the process of screening botanical extracts.

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CONFLICT OF INTERESTS
None declared.

AUTHOR CONTRIBUTION
AA and MA identified and collected the plants and performed the extraction. WE and WH planned and carried out all biological experiments. WE performed the statistical analyses and drafted the manuscript.

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