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Research Article

PHYTOCHEMICAL SCREENING, GC-MS ANALYSIS, ANTIOXIDANT ACTIVITY AND *IN VITRO* ANTICANCER ACTIVITY OF LEAF EXTRACT OF *SEMECARPUS ANACARDIUM. LINN* (ANACARDIACEAE)

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ABSTRACT

The aim of the present study was to screen the phytochemicals, characterize the active bio components from the acetone leaf extract of *Semecarpus anacardium* Linn through GC-MS analysis and to further study their inhibition percentage against the reactive oxygen species using Nitric oxide radical scavenging assay. Cytotoxic activity was also carried on the leaf extract against Vero cells and HepG2 cells using MTT assay. The phytochemical screening showed the presence of various phytochemicals. The GC-MS analysis revealed the presence of ten bioactive components. The antioxidant activity of *S. anacardium* leaf extract showed an inhibition percentage of 42.28 ± 0.069 at 5 µg/ml and 53.03 ± 0.069 at 25 µg/ml respectively while that of the standard ABA showed 47.04 ± 0.069 at 5 µg/ml and 56.79 ± 0.069 at 25 µg/ml, both showing a significance value less than one. The *in-vitro* anticancer activity of *S. anacardium* on normal cell lines and liver cancer cell lines (HepG2) showed a decline in cell viability percentage with the increase in sample concentration.

Keywords: *Semecarpus anacardium* leaves, Phytochemicals, GC-MS, Antioxidant and Anticancer

1. INTRODUCTION

Liver cancer, also called Hepatocellular carcinoma (HCC) is the sixth most common fatal disease induced by the hepatocellular damage by reactive oxygen species and sustained chronic inflammation leads to carcinogenesis [1]. Plants sources are one of the important aspects in the treatment of these fatal diseases and they play a vital role in the traditional system of medicine [2]. Plants and their products are a major support system in primary health management. The defense mechanism is often associated with the secondary metabolites, which in turn protects the human from pathogens and this makes the plant and their metabolites to pave way for drug discovery. Phytochemicals are known to have several therapeutic, nutritive and immune modulative properties [3]. In a normal cell, there are sufficient antioxidants and this balance can be shifted when a state of oxidative stress occurs. Antioxidants from medicinal plants help to overcome the oxidative stress caused by several factors and serve as a natural reservoir of antioxidants for human [4]. Plant derived antioxidative agents are potent inhibitors of cancer cells. Though treatments like chemotherapy and radiotherapy are available, they situate the patients under lot of pain and stress. To counterpart these manmade stress anticancer agents from medicinal plants can be employed for the treatment of cancer [5]. For the present study, a highly potent medicinal plant *Semecarpus anacardium* Linn from Ayurvedic and siddha system of medicine was selected to understand the potentiality of the plant against cancer. The plant *Semecarpus anacardium* Linn (Family: Anacardiaceae) commonly known by its trade name Bhallatak or marking nut is well-known to the world for its medical properties. The plant growing naturally in a tropical and dry climate is a deciduous tree having a height of about 10 to 25m [6]. The family Anacardiaceae contains 700 species distributed among 60 genera. A close survey of the literature shows that widespread work is being carried out on Nut as compared to other plant parts of *S. anacardium*, though the plant has a unique place in Ayurveda and Siddha medicine. Since ancient time, *Semecarpus anacardium* Linn is used as both single and compound form to treat most of the ailments. Ayurveda and siddha system of medicine consider *S. anacardium* as a 'Panacea' [7]. The fruit and nut extracts of *S. anacardium* shows, antioxidant, antimicrobial, anti-inflammatory,

anti-reproductive, CNS stimulant, hypoglycemic, antiatherogenic, anticarcinogenic and hair growth promoter. The most significant components of the *S. anacardium* are bhilwanols, phenolic compounds, biflavonoid, sterols, anacardoside, semecarpetin, nallaflavanone, jeediflavanone, semecarpuflavanone, galluflavanone, anacarduflavone, bhilawanol- A, bhilawanol-B, amentoflavone, tetrahydroamentoflavone, semicarpol, anacardic acid, tetrahydrobustaflavone, O-hexamethylbichalcone B2, and O- tetramethylbiflavanone C [8].

2. MATERIALS AND METHODS

2.1. Sample Preparation

The mature leaves of *Semecarpus anacardium* Linn was collected from Mavelikara, Kerala, India. The leaves were washed, shade dried and coarsely powdered. 250g of powdered sample was soaked in 750ml of Acetone and then extracted using the cold extraction method. The excess solvent was removed using a rotatory evaporator and the extract was used for further studies.

2.2. Phytochemical screening

A standardized procedure was followed for the screening of preliminary phytochemicals present in the plant leaves [9].

2.3. GC-MS analysis

GC/MS analysis was performed with a JEOL GCMS-Mate-II model gas chromatograph-mass spectrometer equipped with an AOC-20i auto injector. Column: HP-5, 30 m x 0.25 mm ID x 0.25 µm film thickness. Temperature program: from 80°C (2 min) to 250°C (10 min.) at 10°C/min. Injection temperature: 250°C. Injection volume: 1.0 µL. Inlet pressure: 37.1 kPa. Carrier gas: He, linear velocity (u): 32.4 cm/sec. Injection mode: split (10:1). MS interface temp.: 250°C; MS mode: EI; 70 eV, detector voltage: 2 kV; mass range: 50-400 u; scan speed: 769 u/s; interval: 0.50 s (2 Hz). Data handling was made through JEOL software and matched with NIST library [10].

2.4. Nitric oxide radical scavenging activity

Nitric oxide radical scavenging activity of *Semecarpus anacardium* Linn leaf extract was determined by Griess Ilosvay reaction using sodium nitroprusside with different concentrations (5, 10, 15, 20, 25 µg/ml) of leaf sample and incubated for 150 min at 25°C. The absorbance of the pink colour of the solution was read at 540 nm. The percentage of nitric oxide inhibition was calculated using the following equation:

Percentage (%) of nitric oxide radical scavenging assay = $[(A_0-A_1)/A_0] \times 100$.

Where A_0 was the absorbance of the control, and A_1 was the absorbance of the treated sample [11].

2.5. MTT assay

Dulbecco's Modified Eagle's Medium was used as a trypsinizer and the homogenised cells was added to 24 well plate with different concentrations of serial diluted test samples (1.5325 to 200 µg/ml), incubated at 37°C. The cytotoxicity assay was carried out using (3- (4, 5-dimethyl thiazol-2yl)-2, 5-diphenyltetrazolium bromide (MTT) [12]. After 48 h incubation, the wells were added with MTT and left for 3 h at room temperature. All wells have removed the content using pipette and 100µl in DMSO were added to dissolve the formazan crystals, absorbance was read in Read Well Touch micro plate reader at 570 nm.

3. RESULTS AND DISCUSSION

3.1. Phytochemical screening

The results of the phytochemical screening of *S.anacardium* exploits the presence of metabolites like alkaloids, flavonoids, phenolic compounds, saponins, tannins, glycosides, coumarins.The results of the phytochemical screening of acetone extract from *S.anacardium* leaves are shown in Table 1.

Table 1: Phytochemical screening of leaf extract of *S.anacardium* Linn

Phytoconstituents	Test Sample
Alkaloids	+
Flavanoids	+
Coumarin	+
Saponins	+
Steroids	+
Tannins	+
Terpenoids	+
Glycosides	+
Phenol	+

This result set forth a way in screening the essential bioactive molecules from the leaf extracts. In previous studies, the Petroleum ether nut extract of *Semecarpus anacardium* has revealed the presence of various metabolites like alkaloids, Flavonoids, glycosoids, phenols, Saponins, steroids, Triterepenoids and Anthraquinones [13]. Also, methanolic extract of *S.anacardium* nuts showed the presence of alkaloids, glycosides, phenolic compounds, Saponins, steroids,

carbohydrates, tannins, flavonoids [14]. Earlier studies on oil and nuts of *S.anacardium* (petroleum ether, chloroform, ethanol and aqueous extracts) showed the presence of preliminary phytochemicals along with ascorbic acid, fixed fats and oils, gums, anthraquinones [15]. The phytochemical screening of methanolic leaf extract of *Kedrostis foetidissima* indicated the presence of alkaloids, flavonoids, phenols, tannins, steroids, triterpenoids, saponins and glycosides [16]. The phytochemical screening test carried on *Andrographis serpyllifolia* methanolic leaf extracts revealed the presence of bioactive compounds such as alkaloids, flavonoids, phenols, saponins, tannins, amino acids, oils, and resins while carbohydrates were absent in the methanolic extracts [17].

3.2. GC-MS analysis

GC-MS spectral analysis of *Semecarpus anacardium* Linn leaf extract exposed the peaks that revealed the presence of difference compounds Ethanol-1- methoxy acetate, TATP. 2-4-(4- MethoxyBenzy) Phenyl-Propan-2-ol, Phytol, Trans decalin 2 methyl, 4-acetoxy-1,2,3,5,6,7,8,8A Octahydrazulene, 1-hexyl- 1-nitrocyclohexane, Hexadeca-2,6,10,14-tetraen-1-ol, 3,7,11,16-tetramethyl-, (e,e,e)-, 1-bromoeicosane (Fig 1). The spectral data of the compounds identified using the data library along with their molecular weight is listed in Table 2. Among the ten bioactive compounds, Phytol shows a high retention value of 16.604 with the chemical formula $C_{20}H_{40}O$ and molecular weight of 296 g/mol. Phytol is a constituent of chlorophyll and is an active precursor of synthetic Vitamin E and vitamin K. Phytol has been reported to have antimicrobial, antioxidant, anti-inflammatory, anti-convulsant, cytotoxicity, anxiolytic, immunomodulators, induction of apoptosis and protective autophagy also several other pharmacological importance [18]. Previous investigations in diethyl ether extract of *S.anacardium*fruits showed the presence of eleven compounds [19].

Table 2: Bioactive compounds identified in acetone extract of *S. anacardium* Linn leaves

RT	Area	Compound Name	Mol. formula	Mol.Wt (g/mol)
7.525	10,119,352.0	Ethanol, 1-methoxy - acetate	$C_5H_{10}O_3$	118
8.256	483,865.2	Ethanol, 1-methoxy - acetate	$C_5H_{10}O_3$	118
8.351	1,092,925.2	TATP	$C_9H_{18}O_6$	222
12.798	3,936,211.0	2-[4-(4-methoxybenzyl)phenyl]propan-2-ol	$C_{17}H_{20}O_2$	256
16.604	7,229,759.5	Phytol	$C_{20}H_{40}O$	296
16.894	2,416,692.2	Trans-decalin, 2-methyl	$C_{11}H_20$	152
17.119	3,842,492.5	4-acetoxy-1,2,3,5,6,7,8,8a-octahydroazulene	$C_{20}H_{40}O$	296
19.825	440,301.6	1-hexyl- 1- nitrocyclohexane	$C_{12}H_{23}O_2N$	213
24.497	1,116,554.8	Hexadeca-2,6,10,14-tetraen-1-ol, 3,7,11,16-tetramethyl-, (e,e,e)-	$C_{20}H_{34}O$	290
26.338	631,471.2	1-bromoeicosane	$C_{20}H_{41}Br$	360

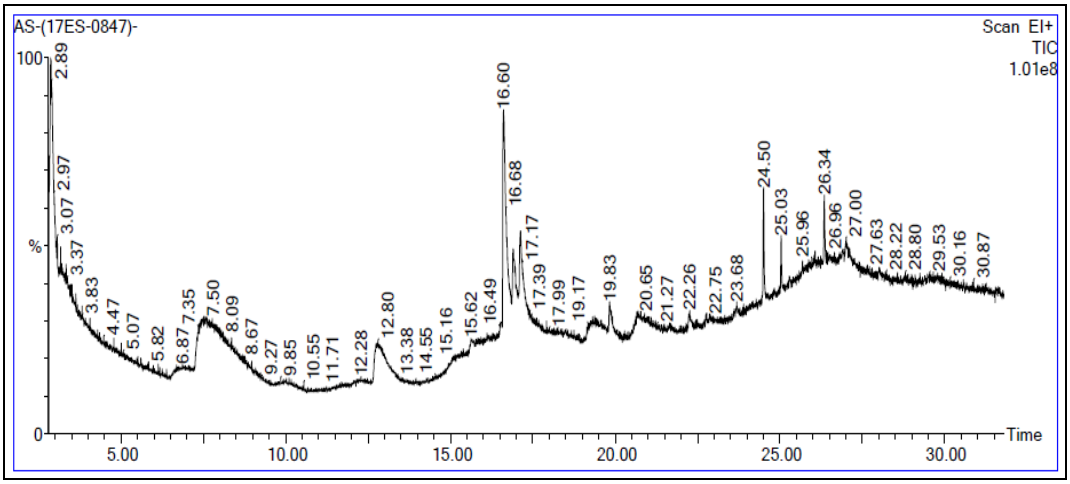


Fig. 1: GC – MS Chromatogram of bioactive constituents in acetone extract of *S. anacardium*

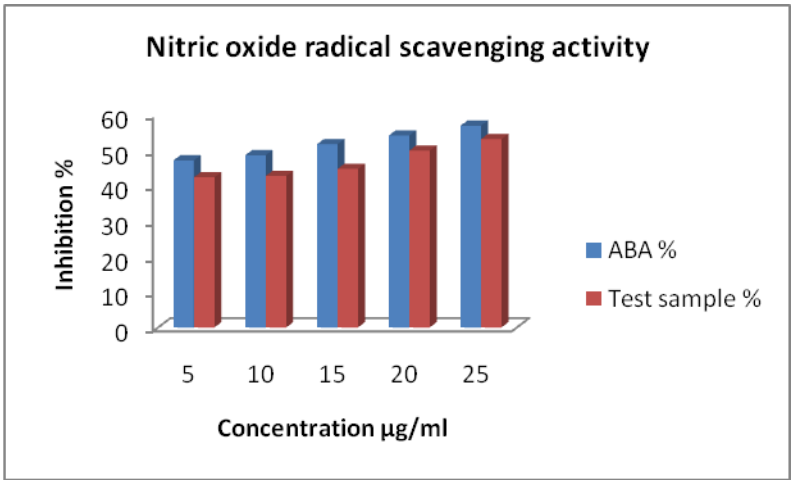
3.3.Nitric oxide radical scavenging activity

Reactive oxygen species (ROS) results in oxidative stress causing excessive damage to cellular biomolecules contributing to the increased risk of several chronic disorders. Antioxidants are an essential defence mechanism to protect our body against free radical damage and protect us from diseases like cancer, Alzheimer's disease, diabetes, and aging. In the present study, the acetone extract of *Semecarpus anacardium* leaves showed significant activity (42.28±0.069 µg/ml at 5 to 53.03±0.069 at 25µg/ml) when compared to the standard Ascorbic acid. The results were statistically analyzed using one way ANOVA and are tabulated in Table 3. The results showed a significance value less than 0.05 and hence the leaves are highly potential against the reactive oxygen species. From the past investigations done in the nuts of *S. anacardium*, it is evident that the petroleum ether extract and ethanol extract showed remarkable antioxidant activity estimated by DPPH assay [20]. Earlier studies have been carried out in the acetone, chloroform, ethanol and aqueous extract of nuts and leaves of *S. anacardium* by ABTS and DPPH assay, in which the ethanol extract showed activity in comparison to other extracts [21].

Table 3: One way ANOVA result of N_2O_2 activity of *S. anacardium* Linn leaves

Conc (µg/ml)	ABA %			Test Sample %		
	Mean ± SD	**F value**	**P value****	**Mean ± SD**	**F value**	**P value****
5	47.04 ± 0.069	9901.269		42.28 ± 0.069	14015.01	
10	48.48 ± 0.069	9901.269		42.64 ± 0.069	14015.01	
15	51.59 ± 0.069	9901.269	0.000**	44.64 ± 0.069	14015.01	0.000**
20	54.03 ± 0.069	9901.269		49.80 ± 0.069	14015.01	
25	56.79 ± 0.069	9901.269		53.03 ± 0.069	14015.01	

*P value ** indicates that the observed values were significantly different ($p < 0.05$); Absorbance of Control: 0.83467 ± 0.00058*



Graph 1: Nitric oxide radical scavenging activity of Acetone extract of *S.anacardium Linn* leaves

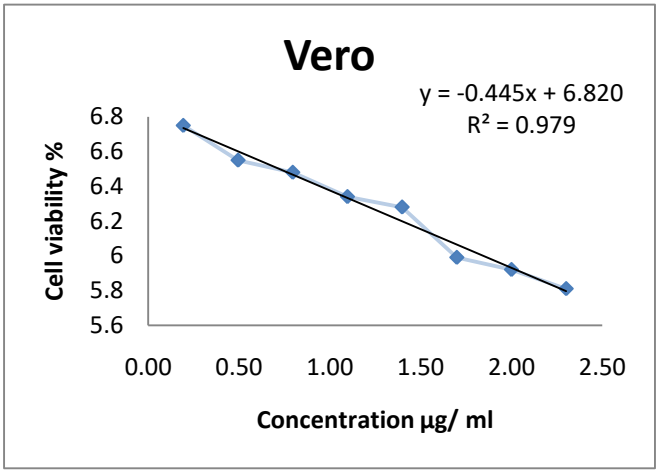
3.4 Anticancer effect on Normal cell lines and HepG2 cancer cell lines

Cytotoxic studies were conducted using normal cell lines and liver cancer cell lines. The Vero cells and HepG2 cells are exposed to serial diluted concentrations ranging from 1.5625 µg/ml to 200 µg/ml of the acetone leaf extract from *S. anacardium* and found 76.69% of cell viability at the highest concentration of 200 µg/ml with a IC 50 value of 4.11 µg/ml, whereas the HepG2 cells showed 54.36 % cell viability at 200 µg/ml with a IC 50 value of2.43µg/ml respectively. From the results, it is clear the crude extract of *S. anacardium* leaves exhibit a cytotoxic effect on liver cancer cells and when the concentration reaches 6.25µg/ml half of the cell proliferation is reduced. The study makes it clear; a minimal amount of the sample can control and inhibit cell proliferation. The results were statistically analyzed using Probit analysis and regression analysis and are tabulated in Table 4 and Graph 2 and Graph 3 show cell viability percentage plotted against their respective concentrations. In studies against HepG2, the methanolic extract of *Pleiogynium timorense* (Family: Anacardiaceae)

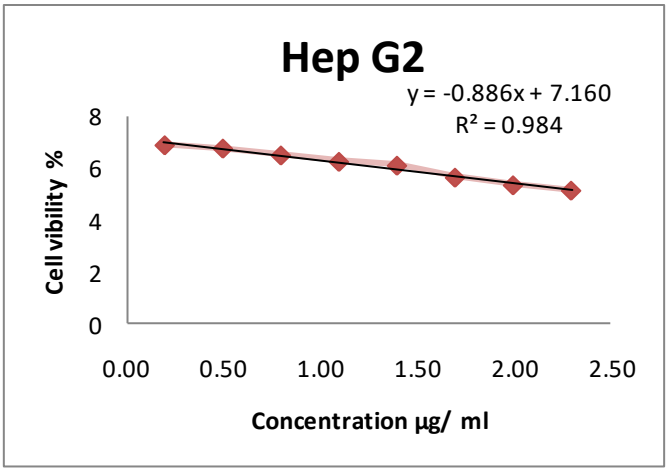
exhibited an IC50 value of 4.39 µg/ml [22]. The hydroalcoholic extract of *S.anacardium* showed cytotoxic activity against Hela and SiHa cells [23]. The methanolic extract of *S.anacardium* nuts on Vero (81.13%) and HepG2 (87.5%) showed antiproliferative effect with increase in concentration [24].

Table 4: Determination of Cell viability % by MTT assay

Conc. (µg/ml)	Normal cell lines - Vero	R^2	IC_{50}	Liver cancer cell lines – HepG2	R^2	IC_{50}
	Cell viability %			**Cell viability %**		
1.5625	96.97			97.46		
3.125	94.22			96.57		
6.25	93.61			93.44		
12.5	92.40	0.979	4.11	89.63	0.984	2.43
25	90.92			86.88		
50	84.53			73.23		
100	83.46			62.79		
200	79.69			54.36		



Graph 2: Effect of Different Concentrations of *S. anacardium* leaf extract on the viability of normal cell lines



Graph 3: Effect of Different Concentrations of *S. anacardium* leaf extract on the viability of Hep G2 lines

4. CONCLUSION

The objective of this study was to determine the phytochemical composition, GC-MS analysis, anti-oxidant activity and *in vitro* anti-cancer potential of acetone leaves extract of *Semecarpus anacardium* Linn. Phytochemical screening of leaves extracts revealed to be rich in essential metabolite compounds that are important for various physiological processes. The *in vitro* antioxidant activity of acetone leaves extract of *Semecarpus anacardium* revealed a significant antioxidant activity and its potential use in oxidative stress related diseases control. The study has also proved that acetone leaves extracts of *Semecarpus anacardium* Linn has an inhibitory action on HepG2 cancer cell lines and thus paving a way for future research to elucidate the compound that is significantly important in the treatment of liver cancer and attain a predominant position in traditional system of treatment and medicine against cancer and other illness.

5. ACKNOWLEDGEMENT

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