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GC-MS analysis, Antibacterial activity and Brine Shrimp Lethality Analysis of *Anacardium occidentale* Linn.

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Abstract

Fruit of Anacardium occidentale Linn. was subjected to extraction of essential oil by hydro distillation in Clevenger apparatus. The composition of essential oil so collected were determined by GC-MS system and showed the presence of 15 different compounds. The most abundant were Propham (30.85%), Probenazole (17.37%), DBP (11.94%), Endrin (6.11%) and Triadimefon (5.61%). Antibacterial activities of the essential oil of A. occidentale were studied. Essential oil exhibited moderate antibacterial activity. The LC_{50} of the sample was found to be 125.01 μg/mL in Brine shrimp lethality assay.

***Keywords**: Anacardium oc cidentale Linn., Essential oil, GC-MS, Antibacterial, Brine Shrimp*

Introduction

Anacardium occidentale Linn. (family Anacardiaceae) is a tropical tree indigenous to Brazil, which is now extensively cultivated in India and east Africa[1]. It is commonly called cashew nut and called as Kaaju in Nepali. The cashew is small tree with obovate or elliptic leaves apex. Inflorescence is panicles terminal. Fruits are long, fleshy, dark coloured[2]. The fruit of the tree consists of epicarp, endocarp and a strongly vesicant cashew nut-shell liquid (CNSL). The CNSL present between the epicarp and pericarp[3].

CNSL is a unique natural source of meta-alkyl phenols with a variable degree of unsaturation attached to the benzene ring. A typical solvent-extracted material contains anacardic acid, cardol, cardanol, and traces of 2-methyl cardol[3]. Cashew nuts are a good source of proteins (20%), carbohydrates (23%) and fats (45%). Of the fat, 61% is oleic acid (ω-9) and 17% is linoleic acid (ω-6)[4].

CNSL is an important agricultural by-product. Industrial application of CNSL-based products are brake linings, paints and primers, foundry chemicals, lacquers, cements, speciality coatings and transformed cardanol for gasoline stabilization[1]. Consumption of cashew nuts may prevent from cardiovascular diseases[4]. Consumption of nuts has been associated with a lower risk of body weight gain, obesity and type two diabetes. Consumption of nuts show cholesterol-lowering effect, anticancer, and antioxidant effects by a number of different mechanisms[5]. Roots are considered as purgative. Barks and leaves are useful in odontalgia and ulitis. Gum bark is recommended in leprosy, ringworm, corns and obstinate ulcers. Fruits are useful in skin diseases, dysentery and anorexia[2].

Experimental Methods

Collection of Plant Materials

The fruit of cashew plant was collected from local Market of Kathmandu, Nepal (Imported to Nepal from Kerala, India) and identified by Department of Botany, Amrit Campus, Lainchour, Kathmandu, Nepal.

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Extraction of Essential Oil

300 g of *A. occidentale* fruits were subjected to hydro distillation in a Clevenger apparatus for about four hours. By this process about 2ml of essential oils were collected and stored in a sealed glass vials at low temperature (0-4° C) prior to analysis.

GC-MS Analysis

The essential oils sample of Cashew was subjected to GC-MS analysis. GC-MS analysis was performed on a gas chromatography mass spectrometer GCMS-QP2010 under the following condition: injection volume 1μL with split ratio 1:50; Helium as a carrier gas with a Rtx-5MS column of dimension 30m×0.25mm×0.25μm, temperature programmed at 40, 200 and 280^o C with a hold time of 2.0, 3.0 and 4.0 min identification was accompanied by comparison of MS with those reported in NIST 05 and FFNSCI.3 libraries. It was performed in Department of Food Technology and Quality Control, Nepal Government, Babarmahal, Kathmandu, Nepal.

Antibacterial activity

First of all MH agar was measured and dissolved in autoclaved water. Then mixture was autoclaved at 121^o C for minutes and cooled up to 45^o C. MH media was poured in 14cm diameter petriplates and allowed it to solidify. After that sterile paper disks were loaded with plant oil. These disks were kept on the surface of MH agar media, which was freshly spread with the overnight grown broth culture of microbial target strain. The plates were incubated at 37^o C for 6 hours. After incubation the diameter of zone of inhibition was measured.

Brine Shrimp Lethality Assay

Ten nauplii were exposed to each of different concentrations of the plant extract and number of survivors were calculated the percentage of death after 24 hours.

Results and Discussion

GC-MS Analysis

GC-MS analysis of essential oils of fruits of *A. occidentale* shows the presence of 15 different compounds. The chemical compounds identified in essential oils of the fruits are presented below. The major constituents present in the essential oils sample were Propham (30.85%), Probenazole (17.37%), DBP (11.94%), Endrin (6.11%) and Triadimefon (5.61%). Constituents of essential oils of *A. occidentale* are tabulated as follows.

Table 1: List of compounds in essential oils of *A. occidentale*

S. N.	Name of the compounds	Molecular Formula	Molecular Weight	Retention Time	Area %	Height %
1.	Triadimefon	$C_{14}H_{16}ClN_3O_2$	293	5.557	5.61	8.97
2.	Triadimenol	$C_{14}H_{18}ClN_3O_2$	295	5.633	2.00	2.58
3.	Probenazole	$C_{10}H_9NO_3S$	223	6.292	17.37	26.26
4.	Oryzalin	$C_{12}H_{18}N_4O_6S$	346	6.667	4.25	1.88
5.	OH-DMP	$C_{12}H_{20}O_6$	260	7.969	2.92	2.25
6.	Triflumizole	$C_{15}H_{15}ClF_3N_3O$	345	12.483	2.89	1.88

7.	Benfuresate	$C_{12}H_{16}O_4S$	256	13.915	1.53	2.64
8.	Propham	$C_{10}H_{13}NO_2$	179	13.993	30.85	25.54
9.	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	16.720	11.94	11.49
10.	Molinate	$C_9H_{17}NOS$	187	17.473	1.94	3.46
11.	Oxpoconazole	$C_{19}H_{24}ClN_3O_2$	361	23.601	4.48	1.67
12.	Simazine	$C_7H_{12}ClN_5$	201	23.675	1.56	2.00
13.	Molinate	$C_9H_{17}NOS$	187	23.764	4.76	5.45
14.	Endrin	$C_{12}H_8Cl_6O$	378	24.358	6.11	2.17
15.	Cyanazine	$C_9H_{13}ClN_6$	240	24.601	1.79	1.79
					100.00	100.00

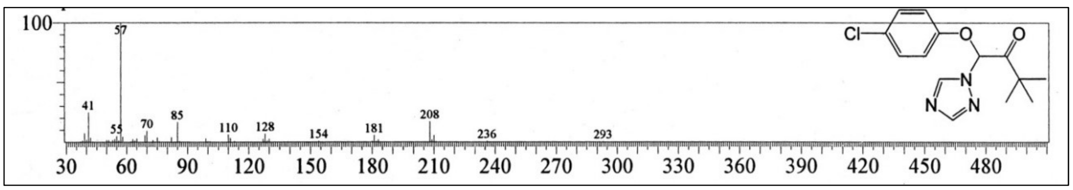

Figure 1: Mass spectral data of Triadimefon

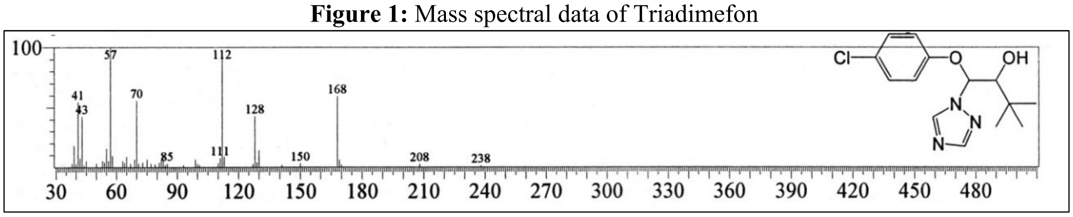

Figure 2: Mass spectral data of Triadimenol

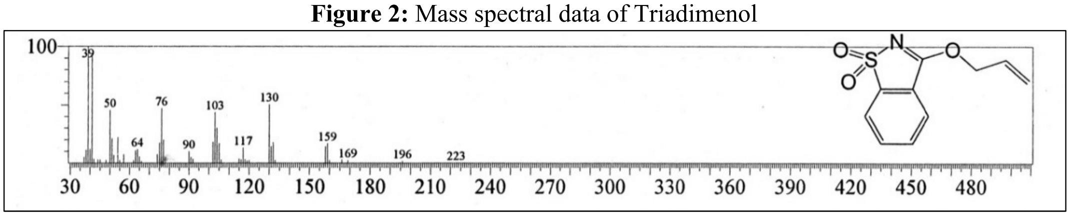

Figure 3: Mass spectral data of Probenazole

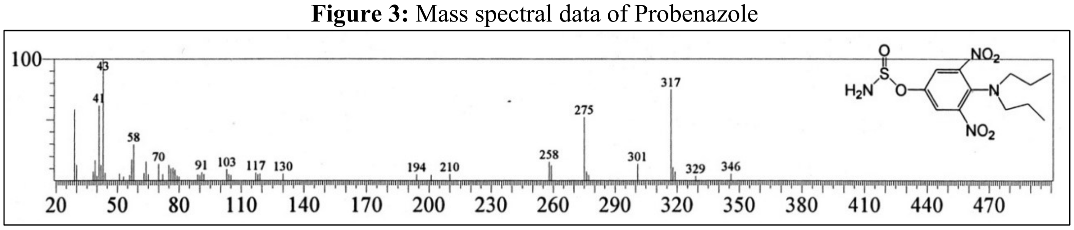

Figure 4: Mass spectral data of Oryzalin

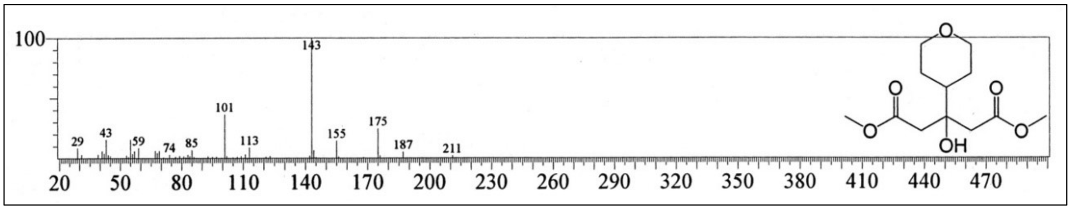

Figure 5: Mass spectral data of OH-DMP

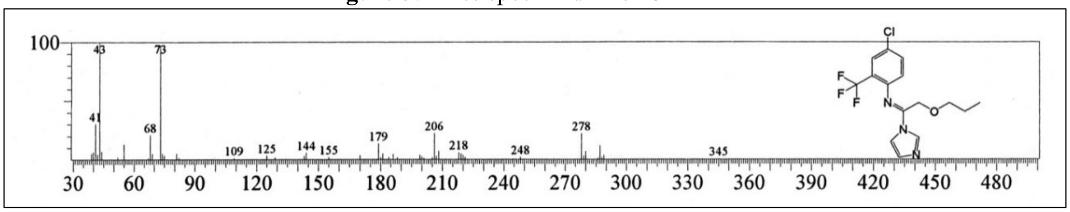

Figure 6: Mass spectral data of Triflumizole

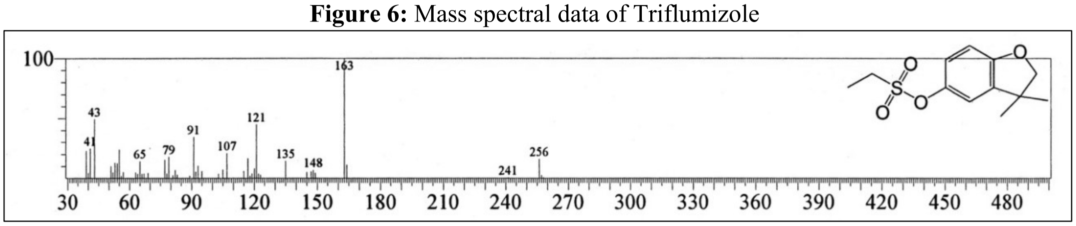

Figure 7: Mass spectral data of Benfuresate

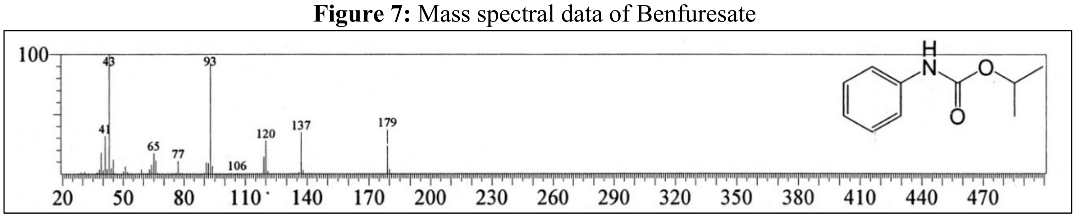

Figure 8: Mass spectral data of Prophan

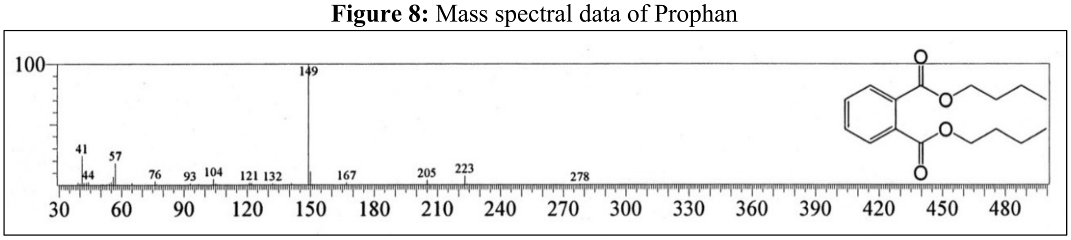

Figure 9: Mass spectral data of DBP

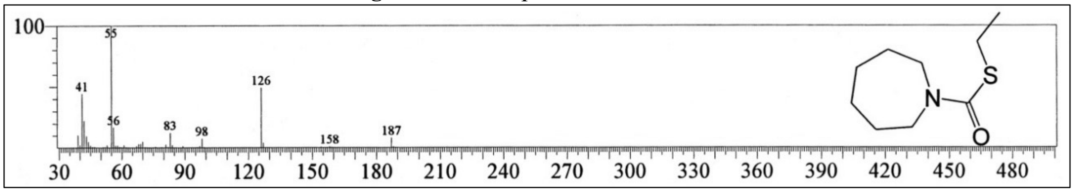

Figure 10: Mass spectral data of Molinate

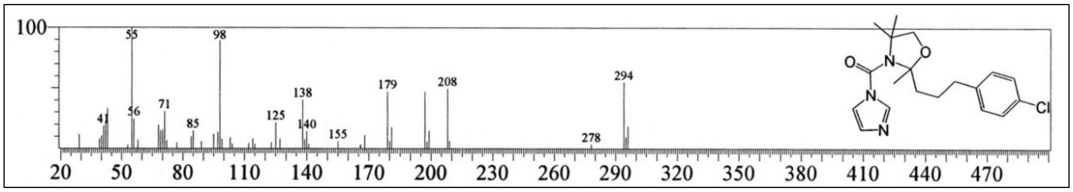

Figure 11: Mass spectral data of Oxpoconazole

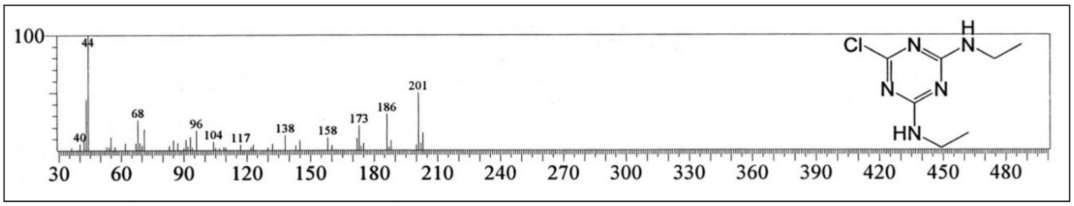

Figure 12: Mass spectral data of Simazine

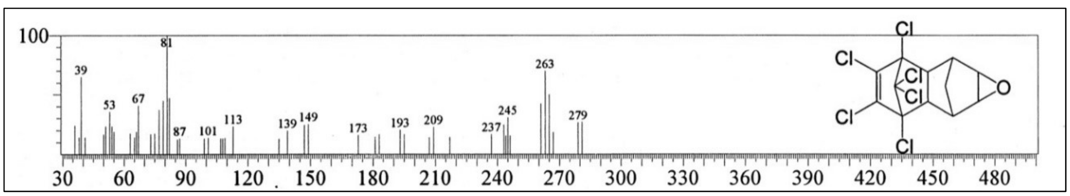

Figure 13: Mass spectral data of Endrine

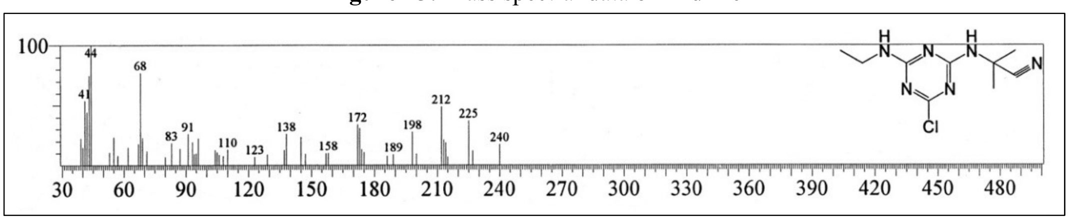

Figure 14: Mass spectral data of Cyanazine

Antibacterial activity

Table 2: Antibacterial activity of the *A. occidentale*

Test Organism	Zone of Inhibition(cm)
	A. occidentale oil
Pseudomonas aeruginosa	0.1

The essential oil of *A. occidentale* exhibited moderate antibacterial activity against tested bacterial organisms as compared to the standard. The study revealed that essential oil is effective against gram negative bacteria *Pseudomonas aeruginosa* (0.1cm).

Brine Shrimp Lethality Assay

The LC_{50} of the sample was found to be 125.01μg/mL in Brine shrimp lethality assay.

Conclusions

GC-MS analysis of essential oils of fruits of *A. occidentale* found 15 different compounds. The major constituents present in the essential oils sample were Propham (30.85%), Probenazole (17.37%), DBP (11.94%), Endrin (6.11%) and Triadimefon (5.61%). Essential oil of plant showed antibacterial activity against gram negative bacteria *Pseudomonas aeruginosa*. The LC_{50} of the sample was found to be 125.01 μg/mL.

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