Nigerian Journal of Pharmaceutical and Applied Science Research, 9(3), 52-58; September 2020 (ISSN 1485-8059. Available at www.nijophasr.com)

Cytotoxic, phytotoxic and insecticidal assessment of the crude extract and fractions of leaves of *Conyza sumatrensis* (Retz.) E. Walker Asteraceae.

Ikpefan, E.O[1]*, Ukwubile C.A [2] and Nwankwo L.U[1]

[1]Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmacy, Delta State University Abraka, Nigeria.

[2] Department of Pharmacognosy, Faculty of Pharmacy, University of Maiduguri, P.M.B. 1069, Maiduguri, Nigeria

ABSTRACT.

This work focused on investigating the cytotoxic, phytotoxic and as well as the insecticidal activities of the extract and fractions of *Conyza sumatrensis*. The solvent-solvent partitioning of the extract obtained by cold maceration gave two fractions (aqueous and dichoromethane fractions) which were screened phytochemically along with the extract. The cytotoxic potential was evaluated using brine shrimp (10-1000 µg/mL) and tadpole mortality assays (20-400 µg/mL), while the phytotoxicity was done using *Lemna minor* frond (10-1000 µg/mL) and *Sorghum bicolor* radicle (1-30 mg/mL) inhibition assays as well as insect contact assay for insecticidal activity. While the DCM-fraction recorded 100 % mortalities on brine shrimp and tadpoles at the maximum concentrations (1000 and 400 µg/mL) it also recorded higher insecticidal activity of 80.62 % on *Rhyzopertha dominica*. However, the aqueous fraction was observed to give a higher phytotoxic activity as it gave complete inhibition at 20 and 30 mg/mL as well as 100 and 1000 µg/mL against the radicle of *Sorghum bicolor* and fronds of *Lemna minor* respectively. The DCM and aqueous fractions of the plant have expressed higher cytotoxic, phytotoxic and insecticidal activities over the crude extract, which is an indication of potent cytotoxic, phytotoxic and insecticidal compounds which require further investigation.

Keywords : *Conyza sumatrensis*, insecticidal, cytotoxicity, radicles, tadpoles, guinea corn, *Lemna minor*.

INTRODUCTION

The genus *Conyza* (horseweed, butterweed or fleabane) comprises about 50 species of flowering plants in the family Asteraceae, and it's a native to tropical and sometimes warm temperate of the world. The plant *Conyza sumatrensis* belongs to the genus. It is an annual or biennial tall herbaceous plant which grows profusely in Nigeria, especially in the Niger Delta region (Thebaud *et al*., 1996). Some reported ethnomedicinal values of this plant include its use in management of back pains, diarrhea, dysentery, pimples, postpartum pains, stomachache and toothache (Njoroge *et al*., 2006). Researchers such as Asongalem *et al.*, (2004), Okorosaye-Orubite, (2008) Kamdem, (2013), Mabrouk *et al*., (2013), Boniface and Anirban, (2013), Jack and have reported the analgesic, anti-inflammatory, allelopathic, antimicrobial, anti-malaria and anticonvulsant of this plant. The increasing interest in the medicinal properties of plants is as a result of their active pharmacological activities as well as their low toxicity to users (Chew *et al.*, 2012). The screening of natural products for biological activities involves selecting the appropriate bioassay which are usually inexpensive, simple, rapid and highly sensitive (Atanasov *et al*., 2015) thus, there is need to further determine other undisclosed potentials of this plant, hence, this study is therefore aimed at investigating various biological activities of this plant using different bioassay techniques. Bioassays which ensures the screening of biological samples (e.g plant extract) for biological activities also helps in identifying unidentified medicinal active substances. In the selection of the right bioassay methods in screening biological samples, certain requirements such as the simplicity, rapidity and sensitivity in detecting small amount of active principles in the sample being tested must be taken into consideration (Panuganti, 2015). The work is thus aimed at evaluating the cytotoxic, phytotoxic and insecticidal potentials of wowas to evaluate the antimicrobial activity of extract and fractions of *C. sumatrensis* using some simple bench-top assay techniques.

*Corresponding author ; e-mail: ikpefanemmanuel@delsu.edu.ng, Tel: +2348062366928

MATERIALS AND METHODS

Collection, processing and extraction of the leave sample.

The fresh leaves of *C. sumatrensis* were collected in Sabongida-Ora both in Edo State, Nigeria and were properly and authenticated at Forest Research Institute where an herbarium number (FHI 108339) was obtained. The collected leaves of *C. sumatrensis* were allowed to air-dry on the laboratory table after which they were further dried in an oven maintained at 45 °C. They were subsequently grinded using a laboratory electric milling machine, weighed and stored. The powdered sample (1.0 kg) was subsequently extracted with 80 % methanol and filtrate reduced to dryness *in vacuo*.

Fractionation of Plant extract

A total of 30g of the leaves extract of *C. sumatrensis* was dissolved in equal amount of CH_3OH: H_2O (1:1 {100 mL}) and was exhaustively partitioned with dichloromethane (300 mL × 3). The aqueous fraction as well as the dicloromethane fractions were collected separately, dried and stored in an air-tight jar. for use.

Phytochemical screening of the extract and fractions

The extract and fractions of *C. sumatrensis* were screened for the presence of the secondary metabolites using standard methods previously described by Khandewal (2008).

Experimental.

In this study, the extract and fractions of *C. sumatrensis* were assessed for cytotoxicity using Brine shrimp and tadpole mortality assays. While the phytotoxic studies were performed using the *Lemna minor* and guinea corn radicle inhibition assays, the insecticidal activity was done using the insect contact assay method.

*Cytotoxicity Assay***:** Brine Shrimp cytotoxicity: This was carried out adopting the method of Meyer *et al*., (1982) and Saima *et al*., (2017) whose protocol of the shrimp egg hatching was followed. The stock solution was made by adding 2 mL of distilled water to 20 mg of the extract which gave concentrations of 10, 100 and 1000 µg/mL which were allowed to stand overnight followed by addition of 5 mL seawater solution (38 g/L) to each vial. After 36 h, 10 matured larvae were placed in each vial applying a Pasteur pipette. The vials were then kept at room temperature (25-27°C) under lighting and the positive controls comprises (add s) vials supplemented with brine solution (McLaughlin, (1991) and the LC_{50} was calculated using the following regression equation.

$Y = ax + b$

Cytotoxicity using *Raniceps ranninus* (Tadpoles): The procedures of Ikpefan and Ayinde, (2016) was adopted. Selected newly hatched tadpoles scooped from stagnant pool of water around Abraka Motel Beach were properly identified and ten of equal sizes were selected with the aid of Pasteur pipette into beakers containing 30 mL of the stagnant natural water from the tadpole's source. This was made up to 49 mL with distilled water. The mixture was made up to 50 mL with 20, 40, 100, 200 and 400 µg/mL of the extract in 5% DMSO (Obuotor and Onajobi, 2000). The experiment was repeated for the aqueous and dichloromethane fractions all in triplicate.

Growth inhibitory/antiproliferative assays:

(i) Phytotoxicity Assay using *Lemna minor*: The effects of the various extracts on fronds of *Lemna minor* were carried out at various concentrations. The preparation of the media was done by dissolving E-medium in 100 mL of distilled water and pH was maintained at 6.0-7.0 by the addition of KOH solution. The media were autoclaved at 121°C, 15 psi for 15 min in an autoclave (75X, 39 liters, WISCONSIN, U.S.A). The stock solution was prepared by dissolving 10 mg of the extract in 1 mL of ethanol. Three concentration replicates (10,100 and 1000 µg/mL) were made in flasks from the stock solution. The solvent was evaporated from the flask in the aseptic environment. On each container, 20 mL of the autoclaved medium together with ten plants each possessing a rosette of three fronds, were added. The percent (%) growth inhibition was analyzed with a recommendation to the negative and positive control (Atta-ur-Rahman, 1991). The experiment was repeated for the aqueous and dichloromethane fractions all in triplicate.

(ii) *Guinea corn radicle inhibition assay*: The capacity of the extract and fractions of *C.sumatrensis* to inhibit growth was evaluated using seed radicle inhibition assay of guinea corn (*Sorghum bicolor*) previously described by Ikpefan (2018) and Ayinde *et al* (2011). The guinea corn seeds were obtained from Abraka small market (in Ethiope-East Local Government of Delta State) and were cleansed with absolute alcohol and air dried before use. Viability test involving the use of simple floatation test was performed on the dried seeds and the viable seeds which submerged were selected. Subsequently, 10 mL of 1, 5, 10, 20 and 30 mg/mL of the methanol extract containing 5% Dimethyl sulphoxide (DMSO) in water was poured into 9 cm wide Petri dishes laid with cotton wool and filter paper (Whatman No 1). Twenty (20) viable seeds were spread on each and

the lengths (mm) of the radicles emerging from the seeds were taken at 24, 48, 72, and 96 hours. The control seeds were only treated with 5% DMSO in distilled water containing no extracts. The experiments were carried out in triplicates and was repeated for the fractions.

(iii). *Insecticidal activity (Contact Toxicity method)*: This was carried out by adopting the method of Ghazala and Shameel (2005), where insects which were obtained from the Department of Zoology, University of Karachi were exposed to the test compound by direct contact toxicity method using filter paper impregnated with the test sample (3mL of extract/fractions equivalent to 1mg/mL) as reported by Ahn *et al*, (1995). Subsequently, ten adult insects of various types and the same age were moved into Petri dishes containing the filter papers. A comparison batch of negative and positive control was treated with solvent (for determination of solvent effect) and a standard drug (Permethrin). The experimental setup was kept without food for 24 h and mortalities were recorded. At the end of 24 h, the number of survivals for each species was counted and the percentage mortality calculated.

Statistical Analysis

The LC_{50} and FI_{50} values of the extract and fractions against brine shrimp and fronds of *Lemna minor* were obtained from the best-fit line by regression analysis. One-way ANOVA was carried out using GraphPad Prism version 7.0 (UK) was used.

RESULTS

The yield of extract/fractions of *C.sumatrensis*

The 1.0 kg powdered leave sample recorded a yield of 52.68g which is equivalent to 5.27 % while the dichloromethane and aqueous fractions recorded as 5.02 and 12.16 g respectively corresponding to 0.50 and 1.22 % respectively (Table 1).

Table 1: Yield of the extracts and their respective percentages

Extracts	Weight (g)	Yield (%)
Leaves	52.68	5.27
	Fractions of the leaves	
Aqueous	5.02	0.50
Dichoromethane	12.16	1.22

Phytochemical screening.

The phytochemical screening of the extract and fractions suggested the presence of various phytochemical groups among the extract and fractions in varying intensities (Table 2).

Table 2: Results of the phytochemical constituents in the extract and fractions of *C.sumatrensis*

Phytochemical Groups	Extract	AQ fraction	DCM fraction
Anthraquinones	-	-	-
Alkaloids	+	+	-
Tannins	+	+	+
Flavonoids	+	+	+
Saponins	+	+	+
Cardiac glycosides	+	+	+
Terpenes	+	-	+
Steroids	+	-	+

Key: +: detected; - : not detected. AQ= Aqueous; DCM= Dichloromethane

Results of cytotoxicity of the methanol extracts on Brine Shrimp (*Artemia salina*)

The extract and fractions exhibited a concentration dependent activity. Applying the criteria of Ali *et al*., (2014) which recommended that a test sample could show low (30-40 %), moderate (50-59 % lethality), good (60-70 %) or significant activity (>70 % lethality), the extracts of *C. sumatrensis* at 1000 µg/mL gave 56.30 % (good activity) with LC_{50} of 467.74 µg/mL. However, the DCM fraction at the same concentration recorded a significant cytotoxic activity of 86.33 % mortality with LC_{50} of 33.88 µg/mL. While the aqueous fraction recorded little or no activities, the standard drug (etoposide) produce LC_{50} of 7.46 µg/mL (Table 2).

Table 2: Results of the extract and fracions of *C.sumatrensis* on Brine Shrimp.

	% Mortality				
Test samples	10 µg/mL	100 µg/mL	1000 µg/mL	R2	LC_{50} (µg/mL)
Extract	13.33 ±0.30	38.03 ±3.97	56.30 ± 8.33	0.05	467.74
AQ-fraction	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	-	> ∞1000
DCM -fraction	30.33 ±0.33	56.07 ±0.17	86.33 ± 0.09	0.94	33.88
Distilled water	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	-	> ∞1000

LC_{50} of Etoposide (standard drug) = 7.4625 µg/mL, LC_{50} was calculated using the regression equation Y= ax +b, a=x variable, b=intercept. n =3, Values are Mean ± standard error of mean

Result of the cytotoxic effects of the methanol extracts on tadpoles

At 400µg/mL which was the maximum concentration used, a mortality of 73.30 ± 1.20 % was produced for *C. sumatrensis*, the methanol extracts gave maximum mortalities of 100 % at 200 and 400 µg/mL at a time of 150 min and 46 min respectively. Also, for *C. sumatrensis*, the dichloromethane fraction gave mortalities of 6.67, 33.33 and 96.67 % at 40, 100 and 200 µg/mL respectively. At the maximum concentration of 400 µg/mL, 100 % mortality was observed within 46 min with an LC_{50} of 87.20 µg/mL. However, the aqueous fractions over the entire incubation period were observed to be inactive (Table 3).

Table 3: Cytotoxicity of the extract and fractions of *C. sumatrensis* on tadpoles

	% Tadpole Mortality				
Test samples	20 µg/mL	100 µg/mL	200 µg/mL	400 µg/mL	LC_{50} (µg/mL)
Extract	0.00 ±0.00	3.33 ±1.06	25.16 ±3.33	63.33±5.18	446.68
AQ-Fraction	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	23.33±2.97	>1000
DCM- fraction	6.33 ±0.16	63.33 ±2.06	100 ± 0.00	100 ±0.00	87.20
Distilled water	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	>1000

LC_{50} was calculated using the regression equation Y= ax +b, a=x variable, b=intercept. n =3, Values are Mean ± standard error of mean

Phytotoxicity

The phytotoxicity was noted to be dose dependent and the results were recorded as low (% inhibition (≤40 %), moderate (% inhibition = 40) and significant (50-100%) as prescribed by Ullah *et al*., (2012). At the maximum concentration of 1000 µg/mL, the aqueous fraction recorded a significant growth inhibition of 93.20 % while the dichloromethane fraction as well as the extract gave 37 and 63 %, respectively (Table 4).

Table 4: Inhibitory effects of the extract and fractions of *C. sumatrensis* on *Lemna minor*

	% Frond Inhibition				Paraquat
Test samples	10 µg/mL	100 µg/mL	1000 µg/mL	FI_{50} (µg/mL)	0.015 (µg/mL)
Extract	19.66 ±1.33	26.10 ±1.90	63.30± 3.68	436.52	
AQ-Fractions	50.46± 0.14	100± 0.00	100 ± 0.00	10.72	100 %
DCM -fraction	9.33 ±0.17	21.66 ±0.26	37.33± 0.49	>1000	
Distilled water	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	>1000	

Values are expressed as the mean ± SEM of three independent observations. Conc. Standard drug (Paraquat) 0.015 µg/mL= 100 % inhibition. Incubation conditions = 28 ± 1 °C. FI_{50} =frond inhibition at 50 %
AQ= Aqueous; DCM=Dichloromethane.

Growth Inhibitory effects of the extract and fractions of *C. sumatrensis*

A concentration dependent effects of the extract and fractions of *C. sumatrensis* on the radicle length of *S.bicolor* was observed. At 24 h, control seeds showed an average radicle length of 3.84 ± 0.6 mm compared to average length of 0.72 ± 0.20 mm (81 % inhibition) and 0.38 ± 0.12 mm (90 % inhibition) shown by the seeds treated with 20 and 30 mg/ml of the leaf extract of *C. sumatrensis*. After 96 h, the control seeds produced an average length of 51.30 ± 6.57 mm whereas the seeds treated with 20 and 30 mg/ml of the extract produced average lengths of 1.10 ± 0.25 and 0.48 ± 0.12 mm respectively. These implied 98 % and 99% reduction in length compared to the control seeds. The aqueous fractions were observed to suppress the growth of the radicle length with increase in concentrations more than the extract and dichloromethane fraction. For example, at 24 hour, the average radicle length of the control seeds was 4.50.00 ± 0.63 mm while seeds pre-treated with 1, 5 and 10 mg/mL of the aqueous fraction produced average lengths of 1.68±0.63, 0.98 ± 0.34, and 0.77± 0.28 mm which implied 63, 78 and 83% reduction in radicle length compare to the control seeds. At 20 and 30 mg/mL concentrations there was no sign of germination. After 96 hours, the control had an average length of 57.33 ± 9.63 mm while seeds pre-treated with 1, 5 and 10mg/ml showed an average lengths of 12.65 ± 4.84 mm (78% inhibition) 4.08 ± 2.01 mm (93 % inhibition) and 1.83 ± 0.75 mm (97 % inhibition) respectively. Throughout the period of

incubation, 20 and 30 mg/mL completely inhibited the germination of the seeds (Fig. 1).

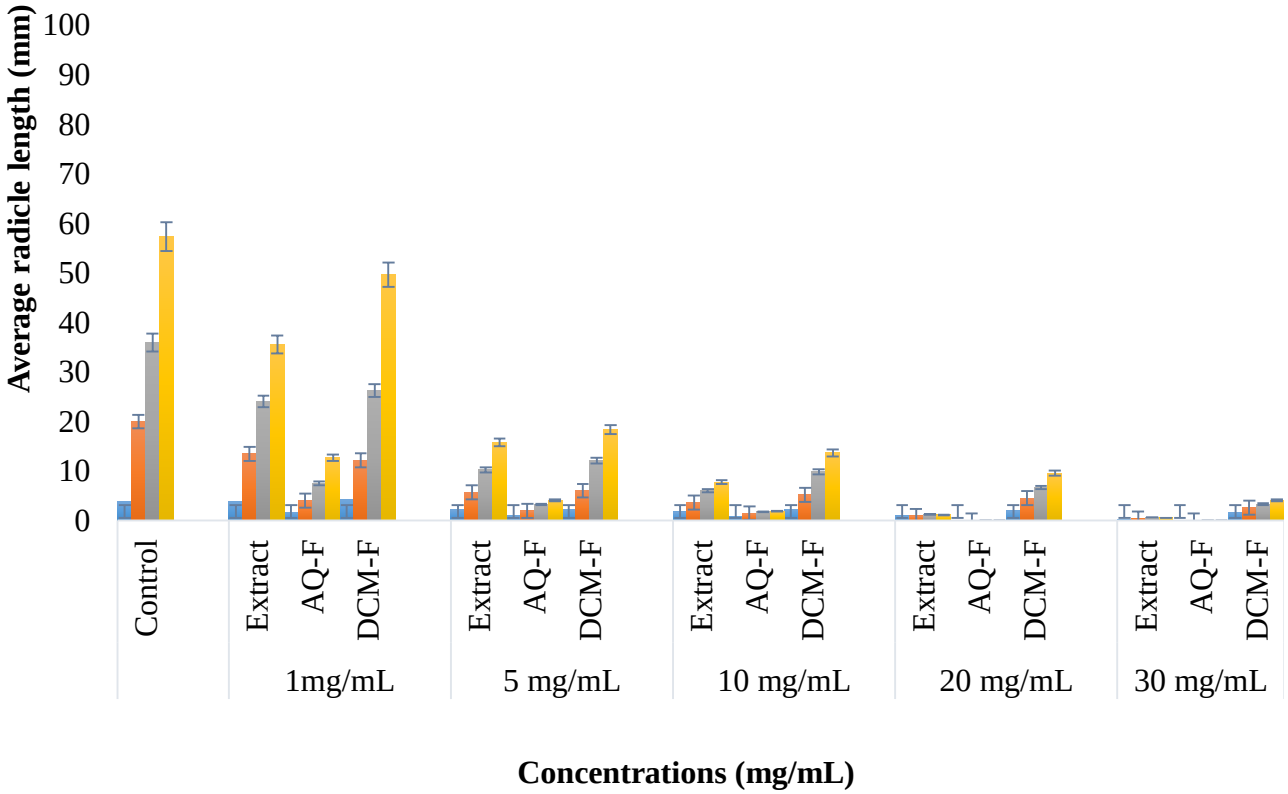

Figure 1. Effect of the extract and fractions on germinating radicles of *Sorghum bicolor* Values are Mean ± S.E.M, n =20. AQ-F =Aqueous fraction; DCM-F= *Di*chloromethane fraction.

Control

20mg/mL extract on seeds

20mg/mL of DCM-fraction on seeds

20mg/mL of Aqueous-fraction on seeds

Figure 2: Some selected plates showing the effects of the extract and fractions of *C.sumatrensis* on radicle length at 96 h.

Insecticidal activity of *C.sumatrensis* leave extract and fractions.

The extract as well as the respective fractions of *C.sumatrensis* recorded insecticidal activity in varying degree. The sensitivities of the test samples were observed to be more on *R. dominica* with the dichloromethane fraction recording the highest insecticidal activity of 80.6 % while the extract and aqueous fraction gave 9.0 and 6.67 %, respectively (Table 5).

Table 5: Insecticidal activity of extract and fractions of *C .sumatrensis*

	% Mortality of insects				
Test Samples	TS	SA	RD	CA	TG
Extract	1.60 ±0.02	1.33 ±0.00	9.00 ±3.33	0.00 ±0.00	0.00 ±0.00
AQ-F	0.00 ±0.00	0.00 ±0.00	6.67 ±0.18	0.00 ±0.00	0.00 ±0.00
DCM-F	0.00 ±0.00	30.30 ±3.41	80.62 ±1.82	40.10 ±5.11	50.60±1.02
-ve Control (Saline)	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00
Std drug (+ve control)	100	100	100	100	100

TS=*Tribolium castaneum*, SA=*Sitophilus aryzae*, RD= *Rhyzopertha dominica*, CA=*Callosbruchus analis*, TG=*Trogoderma granarium* n=10, Conc. *of std drug*=2.39.5µg/cm^2

DISCUSSION

The secondary metabolites present in plants plays a vital role in the observed biological activities of plants (David and Emma, 2011). The phytochemical screening conducted showed variations in the presence of alkaloids, flavonoids, terpenoids, steroids, tannins and saponins among the extract and fractions of *C.sumatrensis*, which could explain the differences in the observed biological activities. The crude extract containing various phytochemical groups having various polarities recorded higher yield over the fractions. However, the yield of the aqueous fraction was observed to be higher than dichloromethane fraction. This could be as a result of the higher polarity the aqueous solvent possessed over that of the dichloromethane. Also, the variation of the phytochemical groups detected in the extract as well as the fractions could be as a result of their solubility in the various solvents. The result of the cytotoxic studies showed the dichloromethane fraction as having a higher cytotoxic potential over the aqueous fraction and extract of *C.sumatrensis* as it recorded LC_{50} of 33.8 and 82.72 µg/mL against brine shrimp and tadpoles respectively. The cytotoxic properties of extracts and fractions on brine shrimp have previously been reported. For example, Wakawa and Fasihuddin (2017) reported the higher activity of the dichloromethane fraction of *Abrus precarious* leaves and root extracts over the other fractions as they exhibited LC_{50} value of 19.14 µg/mL against 226.05 µg/mL. Similarly, Costa *et al*, (2013) has also reported that the Dichloromethane fraction of *Platonia insignis* seed higher cytotoxicity (IC_{50} = 24.89 µg/mL) over the ethyl acetate fraction (IC_{50} = 129.0 µg/mL). These results correspond with the findings of the present study. The results of the brine shrimp cytotoxicity studies were also supported by the tadpole and insecticidal mortality assays of this plant where the DCM fraction recorded the highest activities. In the phytotoxicity study, the aqueous fraction of *C.sumatrensis* showed higher inhibitory effect against *Lemna minor* in all test concentrations (10–1000 µg/mL) with FI_{50} 10.72 µg/mL while the crude extract was found less effective as it recorded FI_{50} of 436.52 µg/mL. However, the DCM fraction recorded FI_{50} greater than 1000 µg/mL.

CONCLUSION

The plant, particularly the aqueous and dichloromethane fractions of *C.sumatrensis* have demonstrated remarkable cytotoxic, phytotoxic and insecticidal potentials more effectively than the extract. These variations in activities demonstrated by the aqueous and dichloromethane factions could be due to the nature of constituents they contain (polar and less polar constituents). Hence, the leaves of this plant could possibly be used as natural weedicides and insecticides with little or no toxicity to the environment. Also, the anti-tumor activity of the active fractions could further be research on.

Acknowledgment

The authors wish to thank NAM S &T Center for providing research grant used in carrying out this work and also Prof **Muhammad Iqbal Choudhary**, Director, International Center for Chemical and Biological Sciences (ICCBS) for making available the necessary facilities for a successful completion of this work.

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