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Chemical profiling and bioactivities on the leaf extracts of *Vitex neugundo*

Hina Gul[1], Nyla Jabeen[2], Samra Irum[2], Rehana Kausar[3], Huma Ajab[4] and Muhammad Gulfraz[4*]

[1]Department of Plant Sciences Faculty of Biological Sciences Quaid-i-Azam University, Islamabad, Pakistan
[2]Department of Biotechnology and Bioinformatics International Islamic University, Islamabad, Pakistan
[3]Department of Botany, AJ& K University, Muzaffarabad
[4]Department of Chemistry COMSATS, Institute of Information Technology, Abbottabad, Pakistan

Abstracts: The *Vitex negeundo* is a widely used medicinal plant which has not been fully investigated in the past. We assessed the *in vivo* hepatoprotective and *in vitro* antioxidant, antibacterial, cytotoxicity and anti proliferative study of leaf extracts of *V. neugundo.* The chemically profiled using HPLC, three flavonoids were quantified and GC-MS analysis revealed the presence of two new compounds those were not reported earlier from the leaf extract of *V. neugundo*. The animal study was conducted on mice treated with CCl4 using methanolic and chloroform extracts (100, 200 and 300mg/kg b.w), with silymarin as a positive control. Hepatoprotective effects were determined by analyzing blood for liver marker enzymes, direct bilirubins and hematological parameters (RBC, WBC and platelets). The methanolic extract (300mg/kg b.w) has shown the stronger hepatoprotective effects against abnormalities produced by CCl4. The *in vivo* hepatoprotective effects correlated well with the *in vitro* antioxidant, cytotoxicity and antiproliferative activities and with high levels of flavonoids and other organic compounds analyzed from plant extracts. The leaf extracts of this plant could be good candidates for lead compound required for the development of antioxidant/ anticancer drugs.

Keywords: *Vitex negeundo,* leaf extracts, secondary metabolites, antioxidant, hepatoprotective, antiproliferative activity.

INTRODUCTION

Tradition knowledge and practices of plants attracts the attention of people for curing chronic ailments (Nazif, 2007). Besides significance advances in modern medicine, plants still make an important contribution to health care and about 25% of the pharmacological drugs were isolated from plants in the developed countries ,those are being used against many human infections. Much interest, in medicinal plants however, emanates from their long use in folk medicines as well as their prophylactic properties, especially in developing countries (Dillard and German, 2000). Although flavonoids possess many biochemical properties but the best described property of flavonoids is their capability to act as antioxidants and to prevent the damaging processes caused by oxidative stress (Cefarelli *et al*., 2006). The phytochemicals research is very useful in drug discovery, those play a significant role in the prevention and treatment of human diseases.

The disproportionate between oxidants and antioxidants levels in the body ultimately favor development of oxidative stress (Abbasi *et al*., 2015). Various environmental factors particularly air pollutants as well as cigarette smoke produces reactive oxygen species (ROS) in cellular metabolism of living organisms. Reactive oxygen species break down structure of molecules like carbohydrates, nucleic acid, lipids and proteins that ultimately weaken and damage cells in the body. Organisms depends on oxygen supply incorporated antioxidant systems and effectively block harmful effects of ROS. Oxidative stress contributes for progression of many diseases like cancer, atherosclerosis, hypertension diabetes, acute respiratory distress syndrome pulmonary disease and asthma. Oxidative stress also involved in modification of cell component like carbohydrates, lipids and DNA (Valko *et al*., 2006). Release of enzymes usually follows their respective concentration gradients between liver, and the blood compartment, however in case of acute liver injury mechanisms of actions of many enzymes still not cleared (Dufour *et al*., 2000; Peltenburg, 1989).

Vitex negundo is belong to Verbenaceae family is commonly known as Nirgundi is an aromatic large shrub or small slender tree of about 3 meters in height with quadrangular branches. It is found in moist area often on banks of rivers throughout Indo Pak up to an altitude of 1500 meter, also grown in Mediterranean countries and Central Asia. Various medicinal properties are attributed to it particularly in the treatment of anti-inflammatory, fungal diseases, antioxidant and hepatoprotective disorders (Movileanu *et al*., 2005). Therefore, antioxidant, antibacterial, cytotoxic anti proliferative and hepatoprotective activities of leaf extracts of *V. negundo* were carried out along with HPLC and GC-MS analysis.

**Corresponding author:* e-mail: gulfrazsattie@ciit.net.pk

MATERIALS AND METHODS

Collection and Preparation of leaves samples

Leaves samples of *V. Negundo* were collected from Kotli Sattian (Rawalpindi) areas during May and June 2016. The samples were properly identified by expert taxonomist. A specimen has been deposited (Voucher no. 141) at Herbarium, faculty of biological sciences Quaid-i-Azam University Islamabad for future reference.

Preparation of crude extracts

The samples were washed several times with distilled water to remove traces of impurities. The samples were shade and sun dried followed by oven drying for overnight at 60º C. The dried samples were ground by using electrical grinder, sieve (80 msh) and saved in fine plastic bags for further uses. Total 100 grams of leaf samples were dissolved in distilled water methanol, ethanol chloroform and n- hexane and were extracted by using soxhlet apparatus and rotary evaporator procedure.

Determination of phytochemicals

Total flavonoids, phenols and tannins were estimated using a modified (Folin-Ciocalteu colorimetric) procedure. Alkaloids and saponins were determined by using method as described earlier (Harbone, 1998; Movileanu *et al*., 2005).

HPLC analysis

HPLC analysis was performed using a Shimadzu HPLC system (Tokyo, Japan), C18 column (25 mm × 4.5mm, 5µm) and UV/visible detector. The compounds were eluted using a gradient of acetonitrile and 0.1% phosphoric acid (36:64).The injection volume for all samples was 20µl. Flavonoids were monitored at 280 nm and 285nm at a flow rate of 1 ml/min. Quercetin was used as a standard and all determinations were performed in triplicate (Dehghan and Khoshkam, 2012).

GC-MS Analysis

GC-MS (Shimadzu), capillary column RTx- 5MS, 30m x 0.25mm x 0.25µm. Split injection at 250ºC; helium carrier gas, column flow 1.2mL/min at a constant linear velocity mode. Column oven temperature program programmed at 4ºC/ min to 150ºC. The temperature injector was 275ºC, carrier gas N_2 (1.0mL/min), 0.2µl injection volume and split ratio 50: 1. Compounds were identified from spectral data base of NIST library and amount (%) of each compound was calculated by comparing its peak area to that of the total peak areas.

Determination of antioxidants capacity of leaf extracts.

The scavenging ability of leaf extracts was assessed by using the 1,1 diphenyl 1-2 -picryl-hydrazyl (DPPH) assay (Moon and Shibamoto, 2009) and the 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) assay, hydroxyl radical, superoxide and Fe chelating assays were conducted (Cefarelli *et al*., 2006; Beanchamp and Fridovich, 1971). IC_{50}values were calculated from dose response curves.

Estimation of antibacterial activity of leaf extracts

The leaf extracts of *V. Negundo* were screened to determined antibacterial potential by using agar well diffusion assay against four bacterial strain, *Staphylococcus auresu* (ATCC 6538), *Escherichia Coli* (ATCC15224), *Klebsiella pneumonia* (MTCC618) and *Bacillus subtilis* (ATCC6633). Standard antibiotic (Cefixime) was used in this study and OD was determined at 420 nm with UV/visible spectrophotometer. The MIC was estimated as the lowest concentration of the extracts that blocked the bacterial growth after 24 hours of incubation period (Upadhyay, 2015).

Brine shrimps cytotoxic assay

Brine shrimps assay was carried out to evaluate the cytotoxic effects of leaf extracts of *V. Negundo* (Ruch *et al*., 1989).

Antiproliferative activity

Activity of the leaf extracts against human hepatocellular carcinoma (HepG2, ATCC HB-8065) cell lines was measured using a sulforhodamine B(SRB assay), which estimates cell number by staining total protein with SRB dye (Abidemi *et al*., 2015). The plates were incubated for 48 hours and wells layered with chilled 50% TCA to produce a final concentration of 10%. Plates were dried and SRB dye was added to each well and incubated at room temperature for 30 minutes. Unbound SRB dye was removed by washing five times with 1% acetic acid, followed by drying. Total 100µl of Tris buffer (0.01M, pH 10.4) was added and shaken for 5 minutes, and the optical density was recorded on an ELISA reader at 515 nm. The inhibition of the extracts (ethanol, methanol and chloroform) was determined.

Hepatoprotective study

Thirty albino mice of either sex (body weight 55.2±2.5g, National Institute of Health, Islamabad) were housed in standard conditions and fed with commercial chow (Feed Mills, Islamabad). Experiment work on animal model was conducted by approval of ethical committee of University constituted for animal studies. Acute toxicity was evaluated (Gulfraz *et al*., 2008) by measuring body weight and observing behavioral changes before and after the treatment period.

Experimental design. Animals were divided randomly into 10 groups of 5. Group I: untreated control; group 11: vehicle control (animals given 1mL of olive oil with feed for 14 days); group III: disease control group (treated with 1mL/kg b.w CCl_4i/p for 14 days); groups IV, V and VI, test groups (administered 100, 200 and 300mg/kg methanol extract respectively, after treatment with CCl4);

groups VII, VIII and IX (treated with 100, 200 and 300 mg/kg chloroform extract), and group X, the positive treatment control (100mg/kg b.w of silymarin). All extracts were administrated by gavage. Animals were sacrificed on day 15, blood collected from the heart and serum separated by centrifugation at 3000 rpm for 10 minutes. Blood and serum were stored at -20^{o}C before analysis and body weights of animals were recorded before and after treatment.

Analysis of Blood. The biochemical activities of serum ALT, AST, ALP and bilirubin were analyzed by using procedure described by manufacture, AMS diagnostic kits. Level of various blood parameters were also determined (Lowry *et al*., 1951; Mirsa and Fridovich, 1972; Flohe and Gunzler, 1984).

Ethical approval

Experiment work on animal model was conducted by approval of Ethical Committee of University constituted for animal studies.

STATISTICAL ANALYSIS

Data obtained after triplicate analysis were analyzed by using One way ANOVA with the help of graph pad prism 7 and level of significance was P≤0.05.

RESULTS

Determination of Phytochemicals

Methanolic leaf extracts of *V. negundo* has provided higher amount of total flavonoids (120.16±2.19mg GA/100g), total phenols (150.38±1.73mg GA/100g), alkaloids (18.82±0.28mg/g), tannins (20.53±0.38 mg GA/100g) and saponins (7.27±0.25mg/100g) and results were significant (P<0.0 5) (table 1).

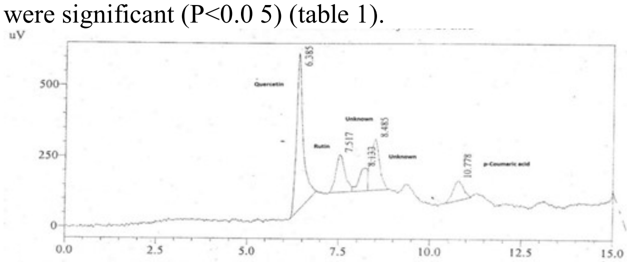

Fig. 1: HPLC Chromatogram of flavonoids from leaf extract of *V. neugundo.*

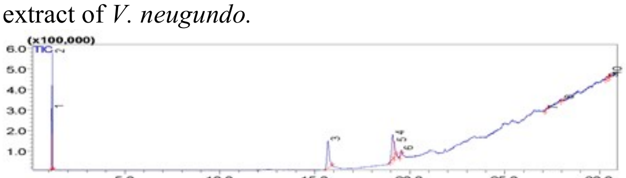

Fig. 2: GC-MS analysis of methanolic leaf extracts of *V. Negundo* .

HPLC analysis revealed presence of higher quantity of quercetin followed by rutin and p- cumaric acid in methanolic leaf extracts of *V. negundo* (fig. 1).

GC-MS analysis of leaf extracts of V. Negundo

Methanolic leaf extracts *of V. negundo* were subjected to GC-MS analysis and results are presented in fig. 3 and table 2.

Various organic compounds detected by GC-MS analysis

Total 10 organic compounds were detected by GC-MS analysis and among that 7 compounds were dominating in methanolic leaf extracts (fig. 2 table 2). By comparing chromatogram of GC-MS, peak areas of compounds were calculated and other required information were obtained from NIST library data base. Peak 1. Indicates Nickel tetracarbonyl (0.105%) C_4NIO_4, Peak 2. Propanone (0.228%), C_3H_6O, peak 3 Ascorbic acid (0.294%) $C_{38}H_{68}O_8$ peak 4 Oleic acid (0.189%). $C_{18}H_{34}O_2$, peak 5. Octadecenoic acid (0.104%), $C_{18}H_{34}O_2$ peak 6 Stearic acid (0.031%), $C_{18}H_{36}O_{2,}$ peak 7 Dimethylphosphinomethyl (0.011%), $C_{11}H_{27}P_3$, peak 8 n-Butyl-2 methyl-trans-decahydro (0.012%) $C_{14}H_{27}NO$, peak 9 Fumaric acid (0.016%) $C_{23}H_{40}O_4$ and peak 10 Fumaric acid (0.008%) $C_{26}H_{46}O_{4.}$ Therefore compounds represented by peaks 7 and 8 were not reported earlier from plant extracts especially from leaf extracts of *V. negundo*. The analysis of these compounds form leaf of *V. negundo* could be first investigation because these information are not available in the recent literature.

Determination of antioxidant capacity in vitro

The antioxidant activities of various leaf extracts of *V. Negundo* were determined. According to results, lowest IC50 value (12.23±0.7µg/ml) of methanolic leaf extracts of *V. Negundo* represented highest free radical scavenging activity as compared to other extracts tested (table 3).

According to antibacterial assays higher zone of inhibition was provided by methanolic leaf extracts for *S. aureus* (22.16±0.4 mm) followed by ethanolic extracts (19.21 ±0.5 mm) and chloroform extract (18.3±0.7 mm) (table 4).

Minimum inhibitory concentration indicates significant antimicrobial potential of *V. negundo*. Resulting regarding MIC values (table 5) revealed the inhibitory potential of extracts for given bacterial strains. The lowest MIC value was observed for methanol extracts perhaps due to its purity or solubility of plant materials in this solvent.

Brine shrimps lethality assay

Three different dilutions of leaf extracts (10,100 and 1000 µg/ml) were made to check brine shrimps cytotoxicity assay. The results revealed the better brine shrimps larvicidal potential and lethality was maximum at maximum concentration of leaf extracts and was concen tration dependent. It was assumed that extracts might be

Table 1: Quantification of various secondary metabolites from leaf extracts of *Vitex negundo*

Extracts	Total Flavonoids (mg GA/100g	Total phenols (mg GA/100g	Alkaloids (mg/100g)	Tannins (mg GA/100g)	Saponins (mg/100 g)
Methanolic extracts	120.16 ± 2.19Þ	150.38 ± 1.73[a]	18.82 ±0.28[a]	20.53±0.38Þ	7.27 ±0.25Þ
Ethanolic Extracts	114.12± 1.54[a]	136.21 ± 1.18[a]	16.35±1.61Þ	18.35±1.79[a]	6.25±1.16Þ
Chloroform extracts	91.32 ± 0.24Þ	106.56 ± 1.5Þ	15.13 ±0.24[a]	14.18±0.78[a]	5.31 ±0.44[a]
Aqueous extract	62.18 ±0.58	96.8± 0.56	9.65 ± 0.45	11.52±0.48	3.75±0.08

Table 2: GC-MS analysis of organic compounds from methanolic leaf extracts of *V. negundo*

Peak	R.T	Area (%)	Molecular Formula	Molecular weight	Name of compound	NIST Lab no.
1	1.135	0.105	C_4NiO_4	170	Nickel tetracarbonyl	1l Lib
2	1.177	0.228	C_3H_6O	58	Propanone	11 s Lib
3	15.688	0.294	$C_{38}H_{68}O_8$	652	Ascorbic acid	11 Lib
4	19.098	0.189	$C_{18}H_{34}O_2$	282	Oleic acid	11 Lib
5	19.185	0.104	$C_{18}H_{34}O_2$	282	Octadecenoic acid	11s Lib
6	19.536	0.031	$C_{18}H_{36}O_2$	284	Stearic acid	11s Lib
7	27.155	0.011	$C_{11}H_{27}P_3$	252	Dimethyl Phosphinomethyl	11s Lib
8	28.005	0.012	$C_{14}H_{27}NO$	225	n-Butyl-2 methyl –trans-decahydro	11 Lib
9	30.335	0.016	$C_{23}H_{40}O_4$	380	Fumaric acid	11 Lib
10	30.524	0.008	$C_{26}H_{46}O_4$	422	Fumaric acid	11Lib

Table 3: Antioxidant potential of various leaf extracts of *V. negundo* (IC 50 values µg/ml).

Extracts	DPPH	H_2O_2	ABTS	Reducing power assay	Superoxide	Iron chelating assay	Ascorbic acid	Gallic acid
Ethanol	18.13±0.2[a]	36.61±0.8a[a]	31.30±0.5[a]	95.43± .16[a]	92.54±1.4Þ	35.37± 1.4[a]	12.44±0.8[a]	12.19±0.2Þ
Methanol	12.23±0.7Þ	27.67±1.3[a]	25.45±0.3[a]	91.51± 1.5Þ	79.13±2.5Þ	31.07± 2.8 Þ	11.19±1.9[a]	10.12±0.3Þ
Chlorofor m	21.35±0.4Þ	41.32±0.9[a]	34.01±0.5[a]	97.54± 2.5Þ	96.34±1.1Þ	38.39± 0.1 Þ	18.52±0.3Þ	15.13±0.4Þ
Aqueous	31.05±0.3[a]	46.13±0.4 Þ	41.43±0.6[a]	108.54±1.4[a]	102.65±1.3[a]	45.64± 1.2[a]	24.35±0.1Þ	22.21±0.6Þ

Means ± SD, (n = 3), where as [a] = p<0.01,Þ= p<0.05

Table 4: Antibacterial activities of leaf extracts of *V. negundo*. Zone of inhibition in mm.

Extracts	*S. aureus*	*E. coli*	*K. pneumonia*	*B. subtilis*	Cefixime
Methanol	22.16±0.4	16.4±1.9	17.6±1.3	9.16±0.4	23.5±0.3
Ethanol	19.21±0.5	17.3±0.8	15.6±0.6	8.15±0.5	21.4±0.8
Chloroform	18.3±0.7	13.2±0.3	14.2±0.4	7 .28±0.5	22.7±0.9
Aqueous	11.4±0.8	11.6±0.9	9.3±0.5	5.16±0.3	9.4±0.8

Table 5: Minimum inhibitory concentration (µg/ml) of bacterial strains

Extracts	*S. aureus*	*E. coli*	*K. pneumonia*	*B. subtilis*	Cefixime
Methanol	1.2±0.3	1.6.±0.3	1.5 ±0.3	2.1±0.4	0.5.±0.1
Ethanol	1.7 ±0.5	1.7.±0.8	1.7±0.6	3.5±0.5	1.4±0.7
Chloroform	1.8.±0.3	1.3.±0.3	1.8±0.4	4 .8±0.5	1.7±0.6
Aqueous	5.4±0.9	3.6±0.7	5.3±0.6	8.6±0.3	2.4±0.5

Results mean ± S D after triplicate analysis (n=3).

Table 6: Cytotoxicity screening of various concentration (µg/ml) leaf extracts of *V. negundo*

Extracts	10	100	1000	LC50
Methanol	32.1±0.6	41.1±0.6	45.8±0.3	<1000
Ethanol	35.6 ±0.5	43.6±0.5	48.7±0.3	700
Chloroform	39.8±0.3	46.9±0.3	49.2±1.4	100
Aqueous	47.4±0.3	51.6±0.7	59.7±0.3	270

Table 7: Antiproliferative activity of 100 µg/mL of leaf extracts against Hep Ga cell lines

Type of extract	Viability (%)	HepG2 IC_{50}value(µg/mL)
Methanol	65.31 ±1.35	19. 54±0.28
Ethanol	58.45±1.18	25.16±0.53
Chloroform	54.39±1.33	28.27±1.53

Table 8: Effects of leaf extracts on liver enzymes and bilirubin

Sr. No.	Group	ALT (U/L)	AST (U/L)	SALP (U/L)	Direct bilirubin (g/dl)
1	Normal control	41.5 ± 0.5	81.5 ±0.7	113.8 ±1.5	0.6±0.01
2	Olive oil	44.6±1.2	84.5±2.5	176.2±1.5	0.5±0.01
3	CCl_4	121.5±0.7	139.4 ±1.5	265.6±2.8	1.18±0.2
4	Methanolic 100 mg/kg b.w + CCl_4	88.7±3.5	118.5±1.7	206.7±3.5	1.1±0.1
5	Methanolic 200 mg/kg b.w + CCl_4	66.4±2.1	87.2±1.3	128.4±2.6	0.9±0.1
6	Methanolic 300 mg/kg b.w + CCl_4	49.5±3.1	85.1±2.9	115.3±1.4	0.7±0.02
7	Chloroform 100 mg/kg b.w + CCl_4	85.1±3.5	115.5±1.6	143.5±2.7	1.5±0.3
8	Chloroform 200 mg/kg b.w + CCl_4	69.2±2.1	95.7±2.6	127.5±2.6	1.3±0.4
9	Chloroform 300 mg/kg b.w + CCl_4	54.6±2.3	88.3±2.6	125.3±3.5	0.9±0.4
10	Silymarin (100 mg/kg b.w) + Cl_4	48.6 ±1.9	83.1±1.5	116.6±2.6	0.5±0.01

Mean + SD (n=3)

Table 9: Effects of various leaf extracts of *V. negundo* on blood cells of animals

Sr. No.	Groups	Red Blood cell 10^6 /µl	White Blood cell 10^3 /µl	Platelets count 10^3 /µl
1	Normal group	5.30±0.07	6.12±0.48	242.53 ± 3.8
2	Olive oil control	4.28±0.12	5.58±0.07	254.31 ±2.5
3	CCl_4 Control	1.92±0.07	2.65±0.15	106.29± 2.82
4	Methanolic 100 mg/kg b.w + CCl_4	2.82±0.06	2.18±0.36	138.32± 0.15
5	Methanolic 200 mg/kg b.w + CCl_4	4.38 ±0.16	4.92±0.05	195.71± 0.06
6	Methanolic 300 mg/kg b.w + CCl_4	4.95 ±0.36	5.48±0.06	235.62±0.028
7	Chloroforml 100 mg/kg b.w +CCl4	2.84±0.19	2.67±0.08	165.38± 0.34
8	Chloroforml 200 mg/kg b.w +CCl_4	3.92± 0.26	4.56±0.25	186.25± 0.18
9	Chloroforml 300 mg/kg b.w +CCl_4	4.12± 0.61	4.29±0.05	231.32± 0.01
10	Silymarine (100 mg/kg b.w)	4.91±0.15	4.96±0.06	242.35± 3.16

composed of antitumor components in the form essential phytonutrients. The extracts whose value i.e. LD50 <1000µg/ml was biologically active while LD50 >1000 µg/ml was biologically inactive (table 6).

Anti proliferative activity of leaf extracts

The SRB assay is recommended by the National Cancer Institute (NCI, USA), which suggests that 20 µg/mL is the upper IC_{50} limit considered promising for purification and further investigation. The methanol leaf extracts produced higher anti- proliferative activity (19. 54±0.28µg/mL) compared to other extracts (table 7).

Animal study

No mortality and noticeable behavioral changes was observed for animals of all groups. The methanolic leaves extracts of *V. negundo* were found to be safe up to 500mg/kg body weight as earlier reported (Gulfraz *et al*., 2008). The weight of mice was suddenly reduced (58.6±2.3 to 52.4±0.7) when CCL_4 was induced in animals ,however after treatment with plant extracts especially with 300 mg/kg b.w methanolic leaf extract increased in body weight was observed which was comparable with animals of normal group.

Effect of leaf extracts on liver enzymes

The levels of different biochemical markers (ALT, AST, ALP and direct Bilirubin) in serum of experiment animals were assessed after induction of CCl4. After administration of CCl_4, levels of various parameters were increased (table 8). However, after treatment with methanol and chloroform leaf extracts levels of all enzymes and bilibrubin, became normal and significant results were noted for 300 mg/kg b.w of methanolic extracts.

Hematological study

Effects of leaf extracts on level of RBC, WBC and Platelets were evaluated. After administration of CCl4, levels of RBC, WBC and Platelets were decreased. After treatment with methanolic and chloroform leaf extracts, levels of these parameter became closed to normal values and the improvement was dose depended manner (table 9). It was reported that rapid increase or decrease in hematological parameters causes weakness in immune systems and prolong untreated condition may results serious consequence.

DISCUSSION

Plants are unlimited source of secondary metabolites those due to their therapeutic properties are usually rectified after their phytochemicals screening and pharmacological testing. In current work, significant amount of flavonoids, phenols, alkaloids, tannins and saponins were quantified from leaf extracts of *V. Negundo.* Three organic compounds were quantified by HPLC like quercetin, rutin and p- cumaric acid. Where as GC-MS analysis has disclosed presence of ten organic, mostly fatty acids and two compounds were new (Dimethyl Phosphinomethyl and n-Butyl-2 methyl-trans-decahydro), those were not reported earlier from plant extracts especially from *V. Negundo.* Where as *in vitro* and *in vivo* study leaf extracts has shown higher antioxidant, antibacterial, cytotoxicity, antiproliferative and hepatoprotective potential of these plant extracts (Adedapo *et al*., 2009; Ashafa *et al*., 2010; Demirtas *et al*., 2009; Hussain *et al*., 2014). Various plant extracts revealed the inhibitory potential for both Gram+ve and Gram –ve bacterial strains, where as Brine shrimps assay exposed its lethality behavior which was promising and was dose depended (Newman and Cragg, 2012). The anti proliferative activity of plant extracts also provided promising results (Saha *et al*., 2011; Schwartsmann *et al*., 2002; Skehan *et al*., 1990), exposing its importance for utilization against various cancer infection, which exposed importance of leaf extract of *V. Negundo* .Further detail studies are recommended for isolation of lead compounds for development cost effective safer drugs for human population. The non toxic effects of plant extracts on behavior of animals and protective effects on liver marker enzymes as well as blood parameters, indicates suitability of this extracts to use for treatment of liver and blood cells relevant disorders (Wu *et al*., 2006). If a lead compound could be isolated from these plant extracts in future, it could be beneficial and might be required by pharmaceutical industries for the development of new drugs.

CONCLUSION

It is concluded that the in vivo and vitro bioactivities of leaf extracts of *V. negundo* correlate well with amount of flavonods as well as some new compound obtained during study, which has provided scientific evidence for use of this plant extracts in ethnomedicines

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