Pak. J. Biochem. Mol. Biol. 2016; 49 (2): 48-52

Phytochemical and antioxidative characterization of *Trigonella foenum-graecum*, *Andrographis lineata* and *Eclipta alba*

*Arif Malik[1], Javed Iqbal[2], Syed Saeed-Ul-Hassan[2], Sulayman Waquar[1], Abdul Basit[1], Mahmood Husain Qazi[3]

[1]Institute of Molecular Biology and Biotechnology, The University of Lahore-Lahore-Pakistan, [2]Faculty of Pharmacy, The University of Lahore-Lahore-Pakistan, [3]Center for Research in Molecular Medicine (CRiMM)

Abstract: Aim of the study was to use plants secondary metabolites as it has medicinal values and show therapeutic potential like anti-viral, anti-inflammatory and immune-modulatory effects on hepatocytes proved to be crucial in chronic hepatitis. The secondary metabolites isolated from plants possess a wide range of therapeutic activities and also serve as model compounds for the synthesis of new drugs. These active compounds have been isolated as a result of detailed phytochemical screening of plant extracts. Antioxidants have always been associated with the health benefits of human beings and much attention has been devoted to the natural antioxidants during last few years. Plants have always been a powerful source of natural antioxidants. Due to the ability of donating hydrogen plant extracts may inhibit radical formation and may counteract with ROS. The best solvent medium for the growth of these plant extracts was petroleum ether that shows the maximum growth hence study reveals the antioxidant activity of the petroleum ether which is of prime importance in the medicinal field.

Keywords: ROS, anti-viral, anti-inflammatory, immune-modulatory, antioxidants
Received: June 05, 2016 **Accepted:** June 18, 2016
Author for Correspondence: arifuaf@yahoo.com

INTRODUCTION

Liver is the largest internal organ by percent weight in the human body and has crucial functions, including cholesterol production, intermediary metabolism, hormone synthesis, bile and urea production. The functional cells of the liver are eosinophilic cells referred to as hepatocytes. The liver is organized into structural units called lobules. Each lobule is centered on a terminal hepatic venule. Radiating from the central venule are plates of hepatocytes separated by wide vascular channels called sinusoids. Sinusoids carry blood from the portal vein that is responsible for carrying blood from gastrointestinal tract (GIT) which is rich in nutrients while hepatic artery that is responsible for carrying blood from the lungs containing blood rich in oxygen[1]. Sinusoids thus allow blood to have intimate contact with hepatocytes, allowing for exchange of nutrients and metabolic products. Liver, being a metabolic organ, exhibit various functions to maintain the steady state of the body and most of the metabolic syndromes are linked with the performance of the liver. It is responsible for the production of the substances that metabolize the fats; it converts glucose into glycogen for storage in the body and also stores vitamins and minerals. It is involved in the production of urea and few amino acids. Moreover, it filters harmful material from the blood. Cholesterol is also produced by the liver.

Various diseases are directly related to the liver including hepatitis, cirrhosis and liver cancer. Alcohol is directly involved in altering the normal metabolism of the liver. Like other substances or molecules are metabolized by the liver but consumption of alcohol over long periods of time show detrimental results. Liver is commonly injured by xenobiotics and any toxins and lead to the weakening of the liver and hepatic disorders. Including xenobiotics, the metabolism and excretion of drugs are accomplished by the liver. The drugs given in any disorder have some side effects and may affect the hepatocytes like Tagfur, Cytoxan (anti-cancer drugs), Serzone as well as medicines given for the treatment of diabetes[2]. The major sapogenin found in *Trigonella Foenum-graceum* is Diosgenin and its 25-β epimer named Yamogenin. Another compound named trigonellagenin was isolated that was considered to be a mixture of these two epimers. When the extraction of powdered seeds of plants was carried out using petroleum ether, fixed oils were detected among which majority was of squalene hydrocarbons. The various sterols isolated from plant included β-sitosterol and cholesterol[3].

Fenugreekine is a new C27 steroidal sapogenin peptide ester isolated from fenugreek seeds[4]. The silica gel chromatography and spectral analysis of seeds of Fenugreek plant resulted in the isolation of seven different compounds named N,N′ dicarbazyl, glycerol monopalmitate, stearic acid, beta-sitosteryl glucopyranoside, ethyl-alpha-D-glucopyranoside, D-3-O-methyl-chiroinositol and sucrose[5]. Fenugreek gum was isolated from fenugreek seeds that contains a mannose backbone grafted with galactoseunits. Different flavonoids isolated from the whole plant of *andrographis*

lineata include pentamethoxy flavone, tri-methoxychalcone and dihydroskullcapflavone[6]. This plant has been traditionally used as anti-diabetic, anti-pyretic, anti-inflammatory, in jaundice, snake bite and various skin diseases, as hepato-protective and is an important component of traditional system of Indian medicine. *Eclipta alba* contains large amounts of resins, alkaloids, reducing sugars, stigmasterol, nicotine, triterpene, saponin, a-amyrin, ursolic acid and oleanolic acid. The aerial parts contain β-amyrin, luteolin 7-o- glucoside, apigenin, cinnaroside, and sulphur compounds. Seeds are rich in sterols. Roots contain stigmasterol and ecliptal. The major alkaloid found in *Eclipta alba* is ecliptine. On further extraction and fractionation, eight steroidal alkaloids were isolated from *Eclipta alba*, six of them were absolutely new natural alkaloids.

Eclipta alba acts as hepato-protective, anti-diabetic, anti-cancer, anti-inflammatory, antioxidant and lipid lowering plant. It possesses antimicrobial activity against various bacteria and viruses and is active against hepatitis B virus. *Eclipta alba* is the active ingredient of various formulations for the treatment of liver ailments in traditional system of medicines. It is used as diuretic and used as a tonic in spleen enlargement. It is used for the treatment of various skin diseases and in jaundice. It is also used to improve appetite and digestion. It is considered to be anti-aging agent and is used as a tonic to treat general debility. It is used in inflammation and is used in the treatment of minor cuts and burns. It is also used in eye and ear infections[7]. In past few years, many researchers investigated the incredible effects of many herbs like *Trigonella Foenum-Graecum* (Trigonelline), *Andrographis Lineata* (Andrographolide) and *Eclipta Alba* (Wedelolactone) alone on curing of liver injury. However, no such evidence was presented on the hepato-protective role of these herbs administered in various combinations which may possible to have a synergistic role against liver damage. In this connection, the present work was designed to evaluate the hepato-protective role *Trigonella foenum-graecum* (Trigonelline), *Andrographis lineata* (Andrographolide) and *Eclipta alba* (Wedelolactone) extracts alone and in different therapeutic combinations to assess the key processes, which are responsible to ameliorate the damaging effect of dimethyl nitrosamine.

MATERIALS AND METHODS

The scavenging activity of the peel extract and standard compound were analyzed by the DPPH assay. Such assay have 100 µl of 0.01% methanolic whereas 2,2-diphenylpicrylhydrazyl (DPPH) was inserted on 50µl of the extract in a microtiter plate and then it was incubated for the time of 30 minutes in dark environment. There was discoloration in the positive samples from purple to yellow range and these ranges were then used for quantitative analysis. In quantitative assay,[8] 0.2ml of extract at a concentration of 25, 50, 75 and 100 µg/mL was combined with 2.7ml of methanol and 1ml of 0.1 % methanolic DPPH, again incubated for 30 minutes in same dark condition and absorption then measured at 517nm. The control contained all reagents except that of extract fraction while methanol employed as blank. 0.16% of Butylated Hydroxy Toluene (BHT) was used as the standard. Plant extracts were believed to undergo various phytochemical tests that help in depicting their active contents which may be present in the crude extracts when they were obtained within different solvents. Qualitative presence of alkaloids, carbohydrates, glycosides, flavonoids, tannins, phenolics and saponins were analyzed. The slightly modified method of Okerulu and Ani[9] was used.

Extraction by methanol

The dried parts of plants were considered to their size reduction mechanically however then these powdered forms were extracted under the action of methanol by using specialized apparatus. Solvents were then removed either by the process termed as evaporation and thus the extract is then later concentrated by vacuum evaporator.

Extraction by petroleum ether and ethylacetate

Similarly extracts of plants those with ethyl acetate were collected by the procedure as described by Jayasekhar *et al*[10] and Seikel[11]. These powdered plant extracts were then allowed to de-fat by the help of certain organic solvents that may be even petroleum ether (40 ^{o} C-60 ^{o} C) they tend to eradicate the phenols and other nonpolar substances from the dried mass. After defatting was done, these extracted were later separated with 95% ethanol. It doesn't stop here further these dried ethanol extracts were again extracted with ethyl acetate and are concentrated with trace amounts of phenolic materials present in them.

Extraction by ethanol

Wells were loaded with about 50µl of extracts especially ethanolic ones at their different concentrations taken as 25ug/disc, 250ug/disc and 500ug/disc. Then the controls were used with (10 mcg/disc) and amphotericin B (100 units/disc). They were then incubated at the temperature $28^{O}C$ for about 2days, and then their region of Inhibition was measured. Extracts with their various concentrations exhibit different degree of their action.

RESULTS

In the first phase of the experiment, the crude extract of *Trigonella foenum-graecum, Andrographis lineata, Eclipta alba* were extracted in different solvent systems *e.g.,* methanol, ethanol, chloroform, ethyl acetate, petroleum ether. The results (Table 1) show that the maximum extraction was observed in petroleum ether (11.67%) followed by (5.78%), (5.76%), (4.87%) and (5.78%) by chloroform, ethanol, methanol respectively. The lowest recovery was recorded by ethyl acetate (4.78%). In second phase, petroleum ether extract was evaluated to estimate total phenolic, tannin, alkaloid, flavonoid and carotenoids contents. The maximum phenols (13.65±1.45mg of GAE/g of extract) were recorded in *Trigonella foenum-graecum* followed by (10.87±1.76 mg of GAE/g of extract) and (9.45±2.87 mg of GAE/g of extract) from *Eclipta alba and Andrographis lineata* respectively.

The maximum Tannins (48.87±6.76 mg of GAE/g of extract) were recorded in *Andrographis lineata* followed by (33.67±4.87 mg of GAE/g of extract) and (21.67±2.87 mg of GAE/g of extract) from *Trigonella foenum-graecum and Eclipta alba* respectively. The maximum alkaloids (54.56±6.89 mg of GAE/g of extract) were recorded in *Trigonella foenum-graecum*followed by (33.87±3.67 mg of GAE/g of extract) and (16.87±4.87 mg of GAE/g of extract) from *Andrographis lineataanda* and *Eclipta alba* respectively. The maximum flavonoids (70.67±9.56 mg of QE/g of extract) were recorded in *Trigonella foenum-graecum* followed by (61.98±4.45 mg of QE/g of extract) and (39.09±4.98 mg of QE/g of extract) from *Eclipta alba and Andrographis lineata*respectively. The maximum carotenoids (34.76±4.98 mg of GAE/g of extract) were recorded in *Trigonella foenum-graecum* followed by (19.76±2.76 mg of GAE/g of extract) and (18.78±1.87 mg of GAE/g of extract) from *Andrographis lineata and Eclipta alba* respectively.

Figure.1 supplementary data shows that the petroleum ether extracts of *Trigonella foenum-graecum, Andrographis lineata, Eclipta alba* has anti-radical activity by inhibiting DPPH radical with the IC_{50} value of (69.04±3.65%, 71.76±6.99% and 83.23±9.62% respectively comparable with ascorbic acid standard (53.99±4.88%). IC_{50} value is considered as an effective concentration especially in which activity of antioxidant is recorded to be 50%. DPPH is administered as a substrate which would later depicts the anti-oxidative activity of antioxidant. Whereas method should be dependent on reduction of methanol DPPH solution. There may be such antioxidants present which are able to donate hydrogen under the formation of non-radical from DPPH-H by the reaction. Plant extract was capable to reduce the radical of DPPH until it gets to the yellow colored diphenyl picrylhydrazine. Hence it is determined that cysteine, glutathione, ascorbic acid, tocopherol, poly-hydroxy aromatic compounds (hydroquinone, pyrogallol, gallic acid, etc.) reduce and was responsible for the decolonization of DPPH by their hydrogen donating ability. It appears that extracts of *Trigonella foenum-graecum, Andrographis lineata and Eclipta alba* possess hydrogen donating abilities to act as an antioxidant.

Table 01: Extractive values for different solvents used (% yield)

Selected medicinal plants	Methanol	Ethanol	Chloroform	Ethyl acetate	Petroleum ether
T. foenum-graecum	4.87	5.76	5.78	4.78	11.67
A. lineata	7.67	5.87	6.54	6.56	10.67
E. alba	6.87	6.34	3.98	3.87	8.91

Table 02: Total phenolics, tannins, alkaloids flavonoids and carotenoids contents in the plant extracts (petroleum ether)

Selected medicinal plants	Phenols mg of GAE/g of extract	Tannins mg of GAE/g of extract	Alkaloids mg of GAE/g of extract	Flavonoids mg of QE/g of extract	Carotenoids mg of GAE/g of extract
Trigonella foenum-graecum	13.65±1.45	33.67±4.87	54.56±6.89	70.67±9.56	34.76±4.98
Andrographis lineata	9.45±2.87	48.87±6.76	33.87±3.67	39.09±4.98	19.76±2.76
Eclipta alba	10.87±1.76	21.67±2.87	16.87±4.87	61.98±4.45	18.78±1.87

Table 03: Scavenging activity of DPPH (IC-50 %)

Plant Extract	Active compounds	%
T. foenum-graecum	Trigonelline	69.04±3.65
A. lineata	Andrographolide	71.76±6.99
E. alba	Wedelolactone	83.23±9.62
Ascorbic acid	Ascorbic acid	53.99±4.88

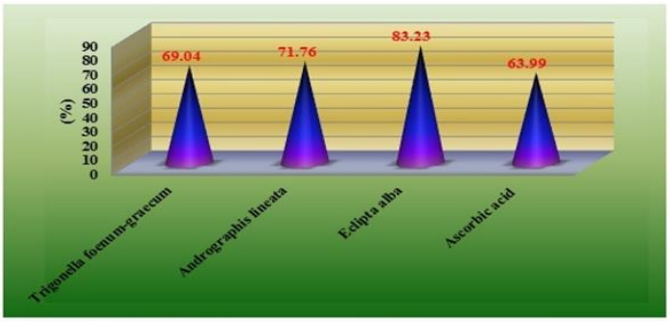

FIGURE 1: SCAVENGING ACTIVITY OF DPPH (IC-50 %)

DISCUSSION

The projected work was established to show the effect of several discussed plant extracts in respect to counter the effects of free radicals that may have several adverse effects in our body for the reason three prominent plant extracts as discussed in the literature were used individually and with the their possible combinations to see their effect in the body and to the vital organs that may be the reason for the aggregation of the disease for the reason these extracts were separated using specialized techniques and then proper monitoring of the serum and plasma levels were done whereas with the existing knowledge these extracts were stated with number of beneficial effects to our body like these extracts in term have proved to be hepato-protective and useful in treatment of number of diseases[7] for the reason the study was designed to see how these plants extracts are useful and how are they prepared easily in laboratory or industrially for the reason each of plant extract was being grown in several different solvent medias like petroleum ether, ethanol, Ethanol, Chloroform etc. and was determined that the best growth was shown by the petroleum ether and with the following cascade then petroleum ether was estimated for its contents that may include tannins, phenols, alkaloids, carotenoid etc.it was stated clear that these plant extracts namely *Trigonella foenum-graecum*, *Andrographis lineata* and *Eclipta alba* were found to have their antiradical activity especially by inhibiting DPPH radicals it also depicts that these plant extracts namely *Trigonella foenum-graecum*, *Andrographis lineata* and *Eclipta alba* have the special ability to donate Hydrogen hence they are used as antioxidants.

The results showed that *Trigonella foenum-graecum* (Trigonelline) maximally alleviate the deleterious effects of Dimethyl nitrosamine and improve the anti-oxidative status even in the presence of Dimethyl nitrosamine with a present increase of 47.61%in glutathione level. These results are in agreement with the previous work[12] of that *Trigonella foenum-graecum* (Trigonelline) which modulate the oxidative stress through decreasing lipid peroxidation and improve the immunity level in the body from incoming toxicant such as medication, drugs, xenobiotics such as paracetamol, Dimethyl nitrosamineetc. Another demonstration indicates that *Trigonella foenum-graecum* (Trigonelline) and *Andrographis lineata* (Andrographolide) are responsible for reduction of kidney cholesterol level but collectively both *Trigonella foenum-graecum* (Trigonelline) and *Andrographis lineata* (Andrographolide) administration have synergistic effect which means they have more capacity for lowering the cholesterol level in the kidney. But *Eclipta alba* (Wedelolactone) has no such effect in lowering the kidney cholesterol level but it was significant when used in combination with

Trigonella foenum-graecum (Trigonelline) and *Andrographis lineata* (Andrographolide) in suitable doses. These results are in complete agreement with the previous work of Kang and co-workers[13] that these phytochemicals could be used for the treatment against toxicity in kidney by inhibiting lipid peroxidation in the kidney which is affected by xenobiotics like dimethyl nitrosamine or other toxic chemicals with similar effects.

CONCLUSION

Following study explains the growth of *Trigonella foenum-graecum*, *Andrographis lineata* and *Eclipta Alba* which are of prime importance in their action against cellular stress in different mediums named as petroleum ether, chloroform, ethanol and methanol and to find out the maximum number of phenols in them. Hence it was observed that maximum growths of these extracts were explained in petroleum ether, then in chloroform, ethanol and methanol. While least recovery was observed within the ethyl acetate and maximum phenols were found in the *Trigonella foenum-graecum.*

Acknowledgements:
The authors thanks Prof. Dr. M. H. Qazi, Director, Centre for Research in Molecular Medicine (CRiMM) and Institute of Molecular Biology and Biotechnology (IMBB). The University of Lahore, for the technical/financial support provided in writing and critical review of this paper.

Conflict of interest:
The authors declare that they have no conflict of Interests.

REFERENCES

1. Valero V, Amini N, Spolverato G, Weiss MJ ,Hirose K, Dagher NN, Wolfgang CL, Cameron AA, Philosophe B, Kamel IR and Pawlik TM. Sarcopenia adversely impacts postoperative complications following resection or transplantation in patients with primary liver tumors. *J. Gastrointest. Surg*., 2015; 19: 272-281.
2. Pradhan SC and Girish C. Hepatoprotective herbal drug, silymarin from experimental pharmacology to clinical medicine. *Indian J. Med. Res*., 2006; 124: 491-504.
3. Benveniste P. Biosynthesis and accumulation of sterols. *Annu. Rev. Plant Biol*., 2004; 55: 429-457.
4. Srinivasan K. Fenugreek (*Trigonella foenum-graecum*): A review of health beneficial physiological effects. *Food Rev. Int.* 2006; 22: 203-224.
5. Djeridane A,Yousfi M, Nadjemi B, Boutassouna D, Stocker P and Vidal N. Antioxidant activity of some algerian medicinal plants extracts containing phenolic compounds. *Food Chem*., 2006; 97: 654-660.
6. Reddy MK, Reddy MV, Gunasekar D, Murthy MM, Caux C and Bodo B. A flavone and an unusual 23-carbon terpenoid from *Andrographis paniculata*. *Phytochemistry.* 2003; 62: 1271-1275.
7. Tabuti JRS, Lye KA and Dhillion SS. Traditional herbal drugs of Bulamogi, Uganda: plants, use and administration. *J. Ethnopharmacol*., 2003; 88: 19-44.
8. Blois MS. Antioxidant determinations by the use of a stable free radical. *Nature*. 1958; 181: 1199-1200.
9. Okerulu IO and Ani CJ. The phytochemical analysis and antibacterial screening of extracts of Tetra carpidium conophorum. *J. Chem. Soc. Nigeria*. 2001; 26(1): 53-55.
10. Jayasekhar P, Mohanan PV and Rathinam K. Hepatoprotective activity of ethyl acetate extract of *Acacia catechu. Indian J Pharmacol*. 1997; 29:426-428.
11. Seikel MK. Isolation and identification of phenolic compounds in biological materials. In: Harborne JB editor. Biochemistry of phenolic compounds. London: Academic press. 1964; 33-76.
12. Ying WX, Ming L, Dong LX and Ping H. Hepatoprotective and anti-hepatocarcinogenic effects of glycyrrhizin and matrin. Chemico-Biological Interactions. 2009; 181: 15-19.
13. Kang DG, Sohn EJ,Mun YJ, Woo WH and Lee HS.Glycyrrhizin ameliorates renal function defects in the early-phase of ischemia-induced acute renal failure. *Phytother. Res*., 2003; 17(8):947-951.