African Journal of Pharmacy and Pharmacology

Volume 8 Number 11, 22 March, 2014
ISSN 1996-0816

Academic Journals

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Vol. 8(11), pp. 292-303, 22 March, 2014
DOI 10.5897/AJPP2014.3933
ISSN 1996-0816

http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Review

The search for new hypoglycemic agents from plants

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Received 6 January, 2014; Accepted 14 February, 2014

Diabetes mellitus is a serious endocrine disorder that causes millions of deaths worldwide. The conventional drugs are associated with a number of adverse effects and limitations. In the search for better alternatives, many medicinal plants have been investigated and a variety of compounds have also been isolated. In the present review, medicinal plants selected from those that have been investigated for their antidiabetic potential between the year 2000 and 2013 are presented. The most common families of plants presented are the Asteraceae, Euphorbiaceae and Gentianaecae. The structures of some previously isolated compounds with antidiabetic potential are presented. Most of the isolated antidiabetic principles are alkaloids, flavonoids, amino acid, steroids and organic acids. It was however discovered that most of the investigations are preliminary in nature. More detailed investigations into the efficacy, mode of action and safety profile of these plants and the isolated compounds in preclinical and clinical studies are recommended.

Key words: Antidiabetic plants, hyperglycemia, hypoglycemia, medicinal plants review.

INTRODUCTION

Diabetes mellitus is a chronic disorder characterized by elevated blood glucose levels and disturbance in carbohydrate, fat and protein metabolism (Aguwa, 2004). Diabetic patients experience various vascular complications such as, atherosclerosis, diabetic nephropathy, retinopathy and neuropathy (Sheetz, 2002). The 2012 report by the International Diabetes Federation (IDF) showed that more than 371 million people (8.3% of the world's population) had diabetes and the number of people with diabetes was increasing in every country, while 4.8 million people died and 471 billion USD were spent due to diabetes in 2012 (IDF, 2012).

The currently available therapy for diabetes includes insulin and various oral anti-diabetic agents such as the sulfonylureas, biguanides, thiazolidinediones and α-glucosidase inhibitors. These drugs are used as monotherapy or in combination to achieve better glycemic control. Each of the oral antidiabetic agents is however, associated with a number of serious adverse effects (Moller, 2001; Nwaegerue et al., 2007). Plant-based drugs have been known to be safe and cheaper. Before the discovery of insulin by Banting and Best (1922), the only options were those based on traditional practices (Ribnicky et al., 2009). Thus the search for safer and easily available antidiabetic agents among medicinal plants continues. According to world ethnobotanical information reports, almost 800 plants possess antidiabetic potential (Alarcon-Aguilara et al., 1998).

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Recently, an ethnobotanical survey of the plants used in the treatment of diabetes mellitus was conducted in some areas of South-Western Nigeria. The survey revealed the use of about 132 different plants species belonging to 56 families in the treatment of diabetes mellitus (Soladoye et al., 2012). Though these plants are claimed to possess hypoglycemic properties, most claims are anecdotal and few have received adequate medical or scientific evaluation (Bailey and Day, 1989). Several reviews on the plants used in the management of diabetes have been reported in the past (Bnouham et al., 2006; Kavishankar et al., 2011; Akah et al., 2002). However, information on the nature and source of the putative hypoglycemic active agents of some of the plants are scattered. Plant products are known to be rich in phenolic compounds, flavonoids, terpenoids, coumarins and other constituents which reduce blood glucose levels (He et al., 2005; Jung et al., 2006). There is need therefore to update the current knowledge as more plants are being investigated and to highlight the molecular structures and nature of some of the isolated hypoglycemic agents from plants. Here we present a list of selected plants which have been investigated for their hypoglycemic potentials between years 2000 to 2013. Also presented are the molecular structures and sources of some of the potential hypoglycemic compounds which have been isolated from medicinal plants.

Some plants investigated for antidiabetic activity

The first part of the present review work was conducted by searching the PubMed, Medline and Google scholar for medicinal plants that have been investigated between 2000 and 2013. Only some of the plants were selected based on their ethno-botanical importance and the depth of research on them. The second part of the work involves the hypoglycemic or antidiabetic plants with their active principles isolated. Unlike the first part of the work, the compounds were not necessarily identified in the period 2000 to 2013. The botanical, family and the common names of the medicinal plants that have been investigated for their antidiabetic potential are presented in Table 1. The most commonly occurring family of plants listed include Asteraceae (6), Euphorbiaceae (5), Gentianeacea (5), Brassicaceae (3), Caesalpiniaceae (3), Lamiaceae (3), Myrtaceae (3), Asclepiadaceae (2), Convolvulaceae (2), Cucurbitaceae (2), Oxalidaceae (2) and Papillionaceae (2). The investigations carried out on the plants have employed several plant extracts (aqueous, other solvents) in various models such as *in vitro* techniques involving enzyme inhibition or isolated cells, *in vivo* techniques involving administration (through oral or parenteral route, in various doses) in normal, chemical (alloxan, streptozotocin)-induced or in genetically modified diabetic animals (mice, rabbits, rats and dogs) and oral glucose tolerance test (OGTT). The experiments in animals were of acute (within 24 h) or chronic (a few days to few months) duration. Few of the studies have been carried out in humans. Toxicity studies and investigations on the mode of action of the plants are limited.

Chemical structures of isolated compounds from antidiabetic plants

The active compounds from the antidiabetic medicinal plants with their sources are shown in Figure 1. Twenty eight (28) compounds from different medicinal plants are shown. They have varied structures but most of them are alkaloids (11) or flavonoids (10) in nature. Others are amino acids (2), steroids and organic acid.

DISCUSSION

In this review, selected plants which have been investigated for antidiabetic potentials between year 2000 and 2013 are presented. The present work and earlier reviews on this subject show that a lot of research work has been performed in recent times in the search for antidiabetic agents from plants. However, not all the listed plants from ethnobotanical surveys are fully explored and most of the investigations have been preliminary studies. More detailed researches are therefore advocated in the search for more efficacious and safer hypoglycemic agents from plants. In addition, their long-term benefits in diabetic complications need to be evaluated in controlled studies.

The variety of phytoconstituent classes and the wide differences in the molecular structure of the isolated compounds suggest the possibility of different mechanisms of action in lowering blood glucose. Some have been shown to inhibit α-amylase with others potentiating the action or enhancing the release of insulin. Alkaloids inhibit α-glucosidase and decrease glucose transport through the intestinal epithelium. Polysaccharides increase the level of serum insulin, reduce the blood glucose level and enhance tolerance to glucose. Flavonoids suppress the glucose level, reduce plasma cholesterol and triglycerides significantly and increase hepatic glucokinase activity probably by enhancing the insulin release from pancreatic islets. Saponins stimulate the release of insulin and block the formation of glucose in the bloodstream (Patel et al., 2012; Bhushan et al., 2010). The detailed investigation into the actual mechanism of action of many of the plants and the isolated compounds is however, lacking. Further investigations to establish the actual mode of action of these plants and the isolated compounds are needed.

Besides efficacy and mode of action, the majority of the plants extracts and isolated compounds have not been subjected to thorough toxicological studies in animal models

Table 1. Medicinal plants with investigated antidiabetic potentials.

S/no.	Botanical name	Family	Significant bioactivity in relation to hypoglyceamia
1	*Abelmoschus moschatus* Medik	Malvaceae	The active principle of this plant, myricelin, improves insulin sensitivity in rats (Liu et al., 2007)
2	*Achiliea santolina L.*	Asteraceae	Exhibits hypoglycemic and antioxidant activities (Yazdanparast et al., 2007)
3	*Achyrocline satureioides* (Lam.) DC	Asteraceae	A new prenylated dibenzofuran, achyrofuran, derived from the plant significantly lowers blood glucose levels when administered orally at 20 mg/kg q.d (Carney et al., 2002)
4	*Ajuga iva* L. Schreberr (Medit)	Lamiaceae	Exhibits strong hypoglycemic effect in diabetic rats (aqueous extract at 10 mg/kg) (El Hilaly and Lyoussi, 2002)
5	*Annona squamosa* L.	Annonaceae	Isolated juercetin-3-O-glucoside from the leaves exhibits anti-hyperglycemic and antioxidant activities in animals (Panda and Kar, 2007)
6	*Anthocleista djalonensis* A. Chev (cabbage tree)	Gentianeacea	Extracts show α-amylase and *in vivo* hypoglycemic activity in rats (Olubomehin *et al*., 2013)
7	*Anthocleista Schweinfurthii*	Gentianeacea	Hypoglycemic (Schweinfurthiin, a new steroid and two known compounds, bauerenone and bauerenol were isolated) (Mbouangouere et al., 2007)
8	*Anthocleista vogelii* Planch	Gentianeacea	Extracts show α-amylase (Olubomehin et al., 2013)
9	*Artemisia dracunculus L.*(dragon herb)	Asteraceae	Hypoglycemic comparable to metformin (Ribnicky et al., 2009)
10	*Averrhoa bilimbi* L	Oxalidaceae	Hypoglycemic (leaf extract, 125 mg/kg, OGTT in normal and streptozotocin (STZ)-induced diabetic rats) (Pushparaj et al., 2001)
11	*Bauhinia candicans* Benth	Leguminosae	hypoglycemic (20 % dried leaf infusion in alloxan-induced diabetic rats but not in normal) (Fuentes et al., 2004)
12	*Biophytum sensitivum* (L) DC.	Oxalidaceae	Hypoglycemic (leaf extract in alloxan-induced diabetic rabbits, OGTT) (Puri, 2001)
13	*Bixa orellana* L.	Bixaceae	Hypoglycemic (normal and STZ-induced diabetic dogs) (Russell et al., 2008)
14	*Boerhaavia diffusa L.*	Nyctaginaceae	Decreases blood glucose level and increases plasma insulin levels, antioxidant (Pari et al., 2004)
15	*Brassica nigra* (L) Koch	Brassicaceae	Hypoglycemic (200 mg/kg aqueous extract to diabetic animals daily once for one month) (Anand et al., 2007)
16	*Butea manosperma* (Lam)	Caesalpiniaceae	Anti-hyperglycemic (Somani et al., 2006)
17	*Capparis spinosa* L.	Capparidaceae	Hypoglycemic (aqueous extract at 20 mg/kg in STZ-diabetic rats, acute and chronic treatments; no effect on normal animals) (Eddouks et al., 2004)
18	*Carum carvi L.*	Apiaceae	Potent anti-hyperglycemic (Eddouks et al., 2004)
19	*Cassia auriculata* L.	Caesalpinaceae	Hypoglycemic and enhances the activity of hepatic hexokinase, phosphofructokinase, suppresses glucose-6-phosphatase and fructose-l,6-bisphosphatase in diabetic animals after 15 day treatment (400 mg/kg) (Gupta et al., 2010)
20	*Cichorium intybus* L.	Asteraceae	Hypoglycemic in acute and chronic studies (125 mg/kg daily for 14 days to diabetic rats attenuates serum glucose by 20%, triglycerides by 91% and total cholesterol by 16% (Pushparaj et al., 2007)
21	*Clausena anisata* (Willd) Benth.	Rutaceae	Hypoglycemic (800 mg/kg, p.o., normal and diabetic rats) (Ojewole, 2002)
22	*Cocos nucifera* Linn. (Coconut palm)	Palmae	Neutral detergent fiber from the plant tested in rats fed 5%, 15% and 30% glucose causes significant lowering in glycaemia and serum insulin (Sindurani and Rajamohan, 2000)
23	*Cogniauxia podoleana*	Cucurbitaceae	Hypoglycemic and anti-hyperglycemic (Diatewa et al., 2004)
24	*Commelina communis* L.	Conimelinaceae	Anti-hyperglycemic, management of non-insulin-dependent diabetes (Youn et al., 2004)
25	*Curcuma longa* L.	Zingiberaceae	Hypoglycemic, plays a role in PPAR-gamma activation (Kuroda et al., 2005)

Table 1. Cont'd.

26	*Cynodon dactylon* Pers. (Bermuda grass)	Poaceae	Anti-hyperglycemic (Jarald et al., 2008)
27	*Eclipta alba* (L) Hassk.	Asteraceae	Leaf suspension (2 and 4 g/kg, p.o.) for 60 days produces hypoglycemia and decreases the activities of glucose-6- phosphatase and fructose-1,6-bisphosphatase, and increase the activity of liver hexokinase (Ananthi et al., 2003)
28	*Enicostemma littorale* Blume	Gentianaceae	Dried plant equivalent extract of 1.5 g/100 g causes hypoglycemia in diabetic rats without toxic effect (Maroo et al., 2003)
29	*Eruka sativa*	Brassicaceae	Hypoglycemic, antioxidant and improved lipid profile (after daily oral admin of oil of the seeds 2 weeks before or after diabetes induction with alloxan) (El-Missiry et al., 2000)
30	*Gentiana olivieri* L.	Gentianaceae	Hypoglycemic, anti-hyperlipidemic (Sezik et al., 2005)
31	*Ginkgo biloba* L.	Ginkgoaceae	Hypoglycemic (OGTT in humans), increases pancreatic beta-cell in NIDDM (Sugiyama et al., 2004; Kudolo et al., 2001)
32	*Glycyrrhiza uralensis* Fish.	Papilionaceae	PPAR-gamma ligand-binding activity, decreases the blood glucose levels (Kuroda et al., 2003)
33	*Gongronema latifolium* Benth.	Asclepiadaceae	Antidiabetic and antioxidant (aqueous and ethanol extract of leaf, p.o.) (Ugochukwu and Babady, 2003; Ugochukwu and Babady, 2002)
34	*Gymnema montanum* Hook	Asclepiadaceae	Anti-peroxidative, antioxidant (Ramkumar et al., 2005)
35	*Helicteres isora* L., As.	Sterculiaceae	Hypoglycemic comparable with insulin and metformin, antioxidant and hypolididemic (Suthar et al., 2009)
36	*Hintonia standleyana*	Rubiaceae	Anti-hyperglycemic (Guerrero-Analco et al., 2005)
37	*Hordeum vulgare* L. (Barley)	Gramineae	Glycemic responses in healthy and Type II diabetic patients show that barley is a suitable cereal for diabetic patients (Shukla et al., 2001)
38	*Ibervillea sonorae* S.	Cucurbitaceae	Hypoglycemia in acute and chronic studies (Alarcon-Aguilar et al., 2005)
39	*Ipomoea aquatic* Forsk.	Convolvulaceae	Boiled whole extract exhibits hypoglycemic effect with optimum dose of 3.4 g/kg and optimum activity observed 2 h after admin (Malalavidhane et al., 2003)
40	*Ipomea batata* Linn (Sweet potato)	Convolvulaceae	Hypoglycemia and reduction in hyperinsulinemia in rats (p.o.) in chronic studies, results comparable to troglitazone (Kusano and Abe, 2000)
41	*Lepidium sativum* L.	Brassicaceae	Aqueous extract (10 mg/kg/h) causes potent hypoglycemia in normal and diabetic rats (Eddouks and Maghrani, 2008)
42	*Loranthus micranthus* Linn	Loranthaceae	Weakly acidic fraction of methanol extract (250 and 500 mg/kg) shows activity in alloxanized rats; (Osadebe et al., 2010).
43	*Morus indica*. L.	Moraceae	Hypoglycemic (Devi and Urooj, 2008)
44	*Musa sapientum* Kuntz (Banana)	Musaceae	Hypoglycemia in OGTT; chloroform extract of the flowers at 1.5, 0.2 and 0.25 g/kg for 30 days (p.o.) causes a decrease in blood glucose and glycosylated haemoglobin level (Pari and Umamaheswari, 2000)
45	*Ocimum sanctum* Linn. *(*Tulasi)	Lamiaceae	Shows antidiabetic, antioxidant and other activities in diabetic rats (Vats et al., 2004)
46	*Origanum vulgare* L.	Lamiaceae	Aqueous extract of exhibits anti hypergly-cemic activity in STZ rats without affecting basal plasma insulin concentrations (Lemhadri et al., 2004)
47	*Phyllanthus amarus* Schum. Thonn	Euphorbiaceae	Oral administration of ethanolic leaf extract (400 mg/kg) for 45 days resulted in a significant ($p<0.05$) decline in blood glucose and significant recovery in body weight of diabetic mice (Shetty et al., 2012)
48	*Phyllanthus niruri* L.	Euphorbiaceae	Methanol extract of aerial parts shows antidiabetic activity in normal and alloxan-induced rats (Okoli et al., 2009)
49	*Phyllanthus sellowianus* Mull. Arg.	Euphorbiaceae	Hypoglycemic (Hnatyszyn et al., 2002)
50	*Piper longum*	Piperacea	The aqueous extract at a dosage of 200 mg/kg is found to possess significant antidiabetic activity (Nabi et al., 2013)

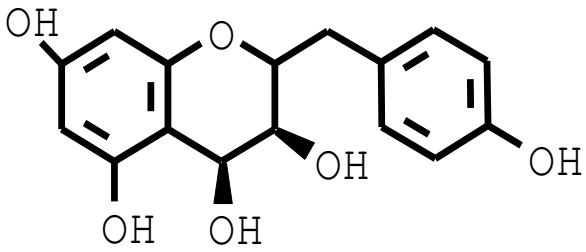
Table 1. Cont'd.

51	*Psidium guajava* L.	Myrtaceae	Leaf extract inhibit the increase of plasma sugar level in alloxan- induced diabetic rats during OGTT; leaf extracts also shows significant inhibitory effect on glucose diffusion in vitro (Mukhtar et al., 2004; Basha and Kumari, 2012)
52	*Punica granatum* L. (pomegranate)	Lythraceae	Hypoglycemia (aqueous-ethanolic extract of flowers in normal and hyperglycaemic rats (400 mg/kg) (Jafri et al., 2000)
53	*Retama raetam (RR)* (Forssk) Webb.	Papilionaceae	Aqueous extract possess significant hypoglycemic effect in normal and STZ rats (Maghrani et al., 2005)
54	*Sambucus nigra* L.	Adoxaceae	Insulin-releasing and insulin-like activity (Gray et al., 2000)
55	*Sanguis draxonis*	Apocynaceae	Increase insulin sensitivity and improve the development of insulin resistance in rats (Hou et al., 2005)
56	*Sclerocarya birea* (A. Rich)	Anacardiaceae	Hypoglycemic (Ojewole, 2003)
57	*Scoparia dulcis* L.	Scrophariaceae	Hypoglycemic, antihyperlipidemic, antidiabetic (Beh et al., 2010)
58	*Spergularia purpurea*	Caryophyllaceae	Hypoglycemic (aqueous extract in normal and diabetic rats at 10 mg/kg) (Jouad et al., 2000; Eddouks et al., 2003)
59	*Suaeda fruticosa (SF)* Euras	Chenopodiaceae	Hypoglycemic (aqueous extract in normal and diabetic rats at 192 mg/kg but no effect on plasma triglycerides in both groups (Benwahhoud et al., 2001)
60	*Syzygium alternifolium* (Wt) Walp	Myrtaceae	Hypoglycemic, antihyperglycemic and antihyperlipidemic (Rao and Rao, 2001)
61	*Tamarindus indica* L.	Caesalpinaceae	Hypoglycemic and hypolipidemia in STZ- diabetic rats (aqueous extract of seed in a chronic study) (Maiti et al., 2005)
62	*Terminalia bellirica* (Gaertn)	Combretaceae	Stimulates insulin secretion. Enhances insulin action andinhibits both protein glycation and starch digestion (Kasabri et al., 2010)
63	*Terminalia chebula* Retz.	Combretaceae	Dose-dependent hypoglycemic, antidiabetic and renoprotective,decreases hepatic and skeletal muscle glycogen content, increases insulin release from the pancreatic islets (Rao and Nammi, 2006)
64	*Tinospora cordifolia* Miers.	Menispermaceae	Hypoglycemic (aqueous root extract orally in alloxan rats, 400 mg/kg equivalent to 1 unit/kg of insulin) (Sengupta et al., 2009)
65	*Urtica pilulifera* L.	Urticaceae	Hypoglycemic (Kavalali et al., 2003)
66	*Vernonia amygdalina* Del.	Astereaceae	Extract improves biochemical and heamatological parameters in diabetic rats; combination of extract with metformin at various ratios shows that the ratio of 1:2 (extract: metformin) causes the most significant ($p<0.05$) reduction in blood sugar (66.07%) compared to control (Akah et al., 2009; Adikwu et al., 2010)
67	*Withania soimifera* (L) Dunal	Solanaceae	Hypoglycemic, antioxidant, diuretic and hypocholesterolemic (Adallus and Radhika, 2000)
68	*Zygophyllum gaetulum* Emb and Maire	Zygophyllaceae	Hypoglycemic, increases plasma insulin levels (Jaouhari et al., 2000)

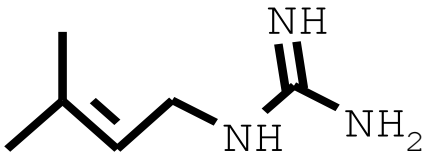
let alone in clinical settings. Isolating the compounds is a necessary step in the search for a new hypoglycemic agent. The safety of the isolated compounds is also of importance as it is possible that the isolated compound could be more toxic than when present in the plant in association with other agents. For instance, *Galega officinals* which is rich in guanidine was traditionally used in the management of diabetes in Europe. However, guanidine proved too toxic to be used in clinical practice. Metformin, a biguanide and the current drug of choice in the management of type 2 diabetes was later developed from the guanidines (Sterne, 1969; Bailey, 1988). Those plants with promising antidiabetic potential as well as the isolated compounds therefore need to be subjected to detailed toxicological evaluation.

Conclusion

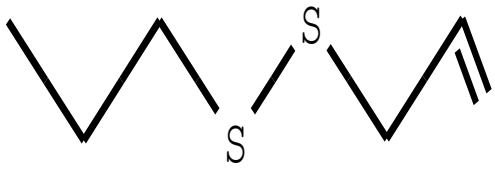
The present review has indicated that there is currently great interest in the search for anti-diabetic agents from plants and many potential compounds have been isolated. However, most of the investigations have been preliminary in nature. There is urgent need therefore to fully explore these promising plants by carrying out further



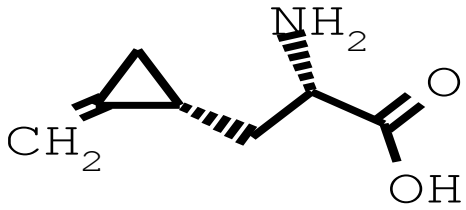
Cis-3,4-Leucopelargonidin
(from *Fiscus bengalensis;* Cherian et al., 1993)



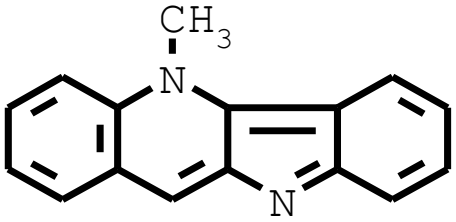
Galegine
(from *Galega officinalis;* Hadden, 2005)



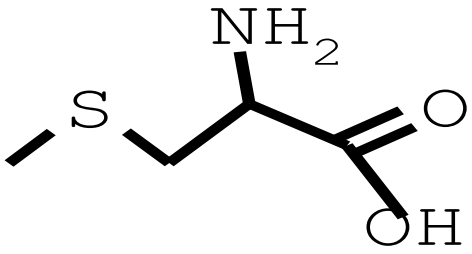
Poly allyl disulphide
(from *Allium cepa*; Romas-Ramos et al., 1995)



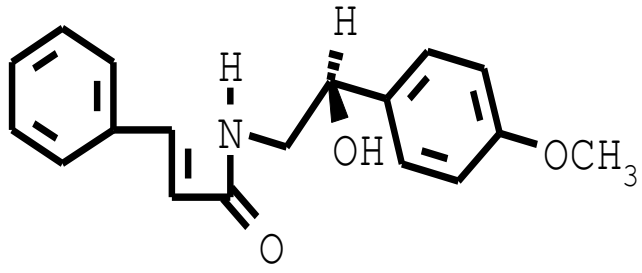
Hypoglycin
(from *Blighia sapadia* Chen et al., 1957)



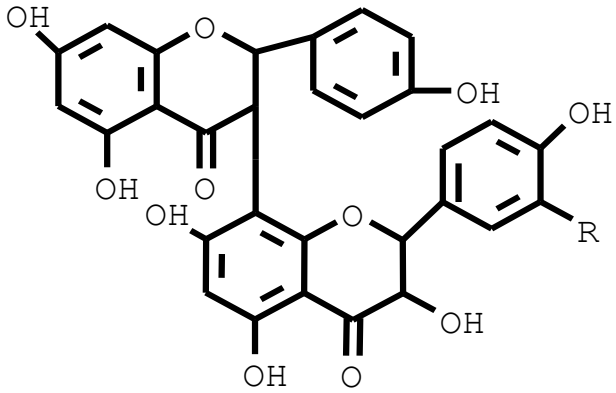
Cryptolepine
(from *Cryptolepis sanquinolenta*; Luo et al., 1998)



Cysteine
(from *Allium cepa*; Kumari et al., 1995)

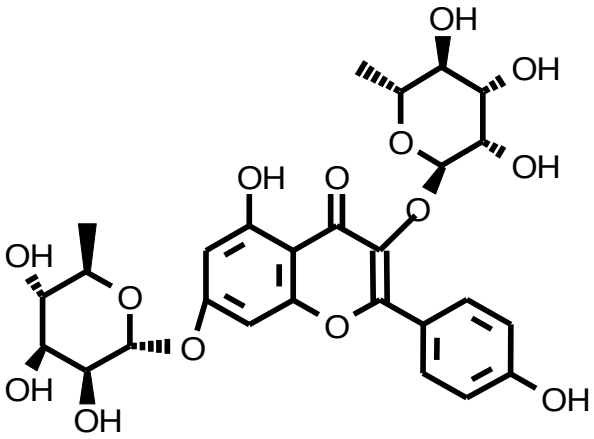


Aegeline
(from *Aegle marmelose;* Narender et al., 2007)

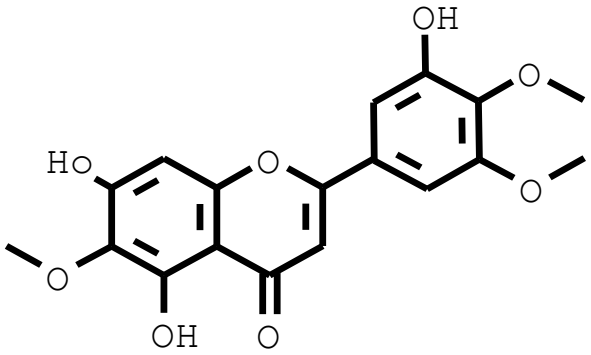


Kolaviron
(from *Garcina kola*; Iwu et al., 1990)

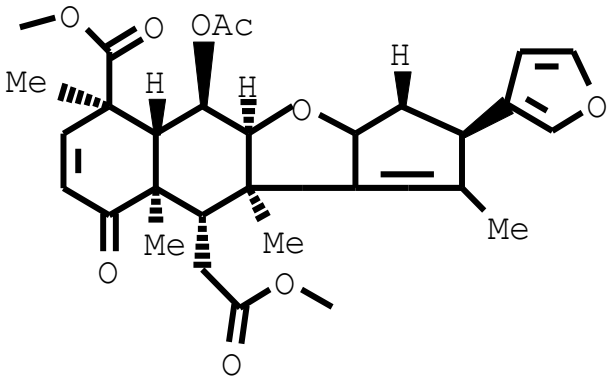
Figure 1. Chemical structures of some antidiabetic principles isolated from plants.



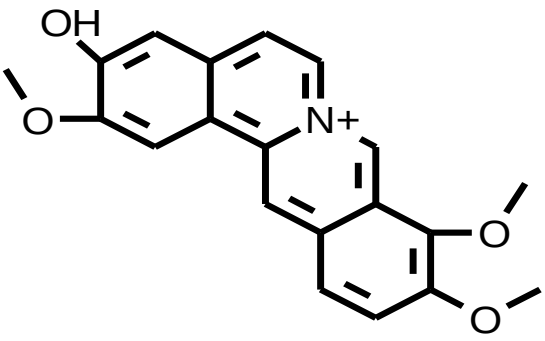
Kaemferitrin
(from *Bauhinia forficate*; De Sousa et al., 2004)



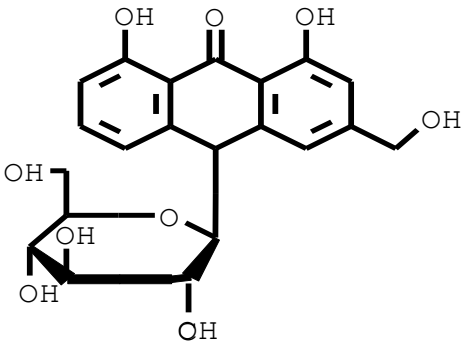
5, 7, 3-Trihydroxy 3, 6, 4-trimethoxyflavone
(from *Brickelia veronicaefolia*; Perez et al., 2000)



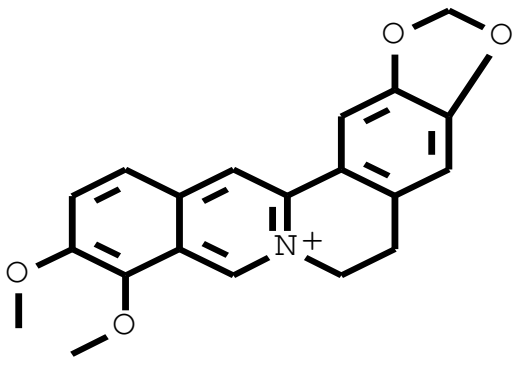
Nimbidin
(from *Azadirachta indica*; Waheed et al., 2006)



Jatrorrhizine
(from *Berberis aristata* Sadiq et al., 2013; Atta-ur-Rahman, 1989)

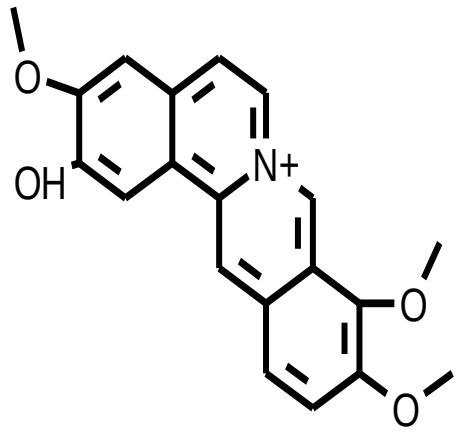


Isobarbaloin
(from *Aloe vera ;* Akira *et al*., 2009)

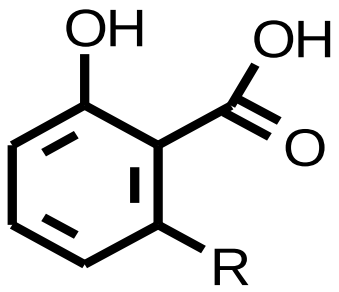


Berberine
(from *Berberis aristata*; Chen et al., 1986; Handa et al., 1989)

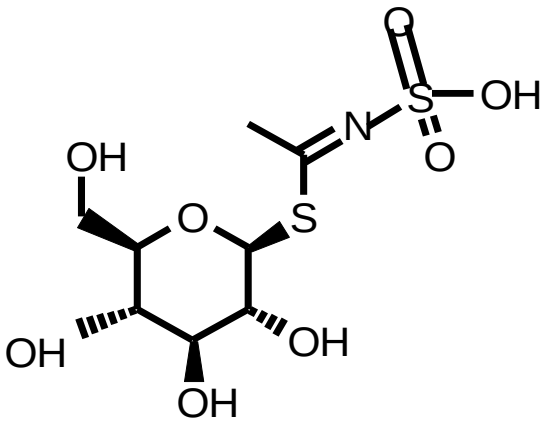
Figure 1. Cont'd.



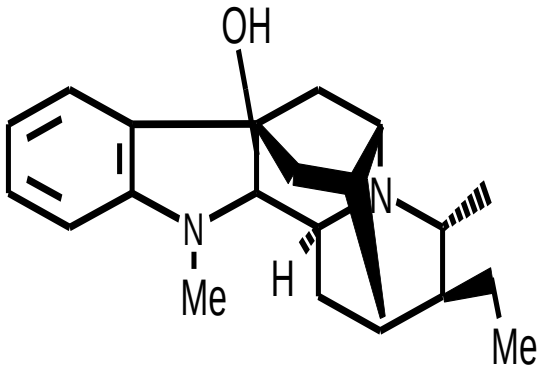
Columbamine
(from *Berberis aristata*; Handa et al., 1989)



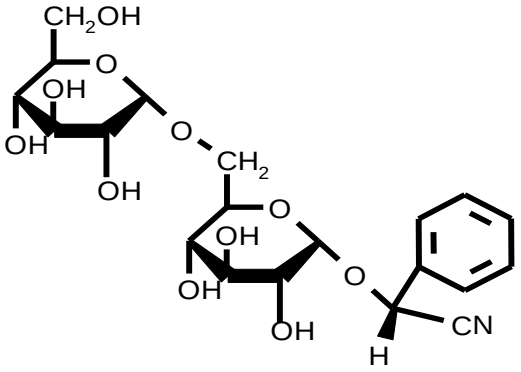
Anacardic acid
(from *Anacardium occidentale*; Tedong et al., 2010)



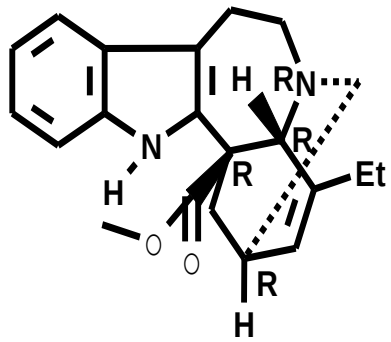
Glucocapparin
(from *Capparis sepiaria*; Juneja et al., 1970)



Ajmaline
(from *Rauwolfia serpentine*; Chatterjee et al., 1960)

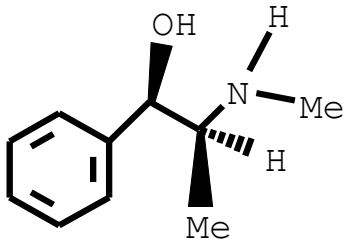


Amygdalin
(from *Prunus persica*; Mirmiranpour *et al*., 2012)

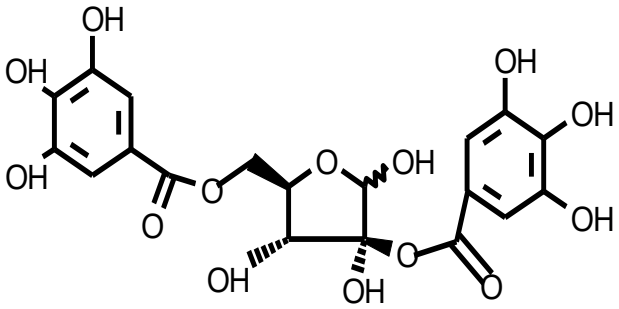


Catharanthine
(*Catharnthus roseus*; Handa et al., 1989; Atta-ur-Rahman, 1989)

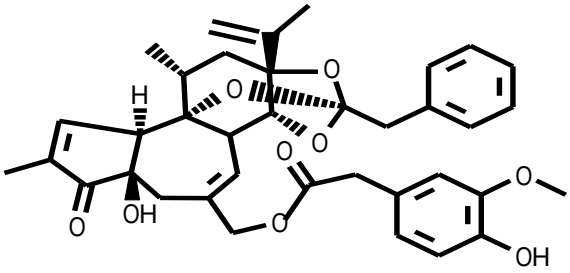
Figure 1. Cont'd.



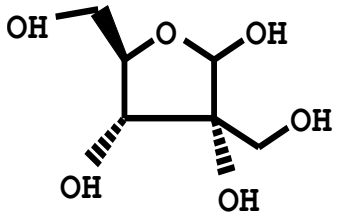
Ephedarn
(from *Ephedra distachya*; Handa et al., 1989)



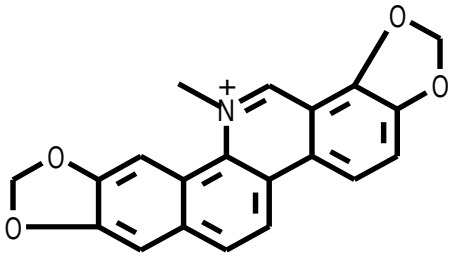
Hamamelitannin
(from *Hamada salicornica*; Ajabnoor et al., 1984)



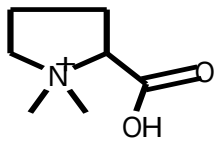
Euphorbol
(from *Euphorbia prostrate*; Alarcon-Aguilara et al., 1998)



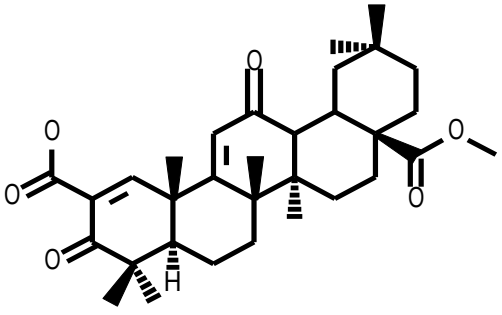
Hamamelose
(from *Hamada salicornica*; Ajabnoor et al., 1984)



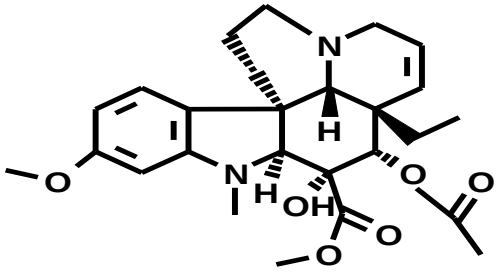
Sanguinarine
(from *Fumaria* parviflora; Hilal et al., 1989)



Stachydrine
(from *Capparis sepiaria*; Juneja et al., 1970)



Tormantic acid
(from *Poterium ancisroides*; Ivorra et al., 1988)



Vindoline
(from *Catharanthus roseus*; De and Saha, 1975)

Figure 1. Cont'd.

research geared towards identifying and exhaustively evaluating the putative phytochemicals with more emphasis on their pharmacological and toxicological profile. The list of plants in this review is not exhaustive of all the plants investigated for hypoglycemic effects. However, it is hoped that the list of medicinal plants presented here

will further broaden the knowledge base on the various medicinal plants available for the management of diabetes mellitus. The studies already performed and highlighted the need for more studies in this direction.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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academicJournals

Vol. 8(11), pp. 304-310, 22 March, 2014
DOI: 10.5897/AJPP2013.3413
ISSN 1996-0816

http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Evaluation of students' satisfaction with professor performance in a pharmacology and clinical pharmacy course

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Received 9 January, 2013; Accepted 20 February, 2014

Student satisfaction has become an increasingly important component of institutional reports as a means of accountability to educational stakeholders. This study was carried out to evaluate students' satisfaction with the teacher´s performance during a pharmacology and clinical pharmacy course. The 30 students enrolled in the pharmacology course were asked to complete an anonymous survey instrument, using the following rating scale: poor (0.0 to 4.9), appropriate (5.0 to 6.9), good (7.0 to 8.4) and excellent (8.5 to 10.0). The survey instrument consisted of 15 items grouped in three sections: planning (3 items), development (8 items) and results (4 items). The survey instrument was developed and approved by the San Jorge University Technical Quality Unit involved in ensuring the quality of the subject taught at the university. An open-ended response section asked students to identify strengths and weaknesses in the teacher's performance. Twenty-two of the 23 students enrolled in the pharmacology course returned their survey instrument, resulting in a 95.6% response rate. The majority of students indicated an adequate satisfaction with the pharmacology teacher´s performance in all sections evaluated. Qualitative analyses of comments showed that most students expressed more time for the development and presentation of the final project seminar and replace pharmacoepidemiology issues by experimental pharmacology practices. This survey was a good start in identifying areas where professor development is needed and which teaching behaviors should be continued and need improvement.

Key words: Clinical pharmacy, pharmacology, pharmacy student, teacher performance.

INTRODUCTION

Universities and governments are increasingly interested in using quality measures that provide evidence that can be used to improve the quality of student learning as well as for benchmarking and funding decisions. Standard scales for assessing the students' experiences of their learning and of the teaching they receive during their studies are growing in popularity and use (Calvo et al., 2012).

Student satisfaction has become an increasingly important component of institutional reports as a means of

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accountability to educational stakeholders. The measures and models for this vary across higher education institutions. Some models try to understand how different perceptions of quality areas impact student satisfaction, while others use more complex relationships that integrate factors such as student learning outcomes and student persistence intentions (Duque, 2011).

The use of standardized questionnaires for measuring student satisfaction in higher education is motivated by theories predicting a close relationship between students' satisfaction and learning outcomes. The reliability and validity of the course experience questionnaire as an indicator of teaching performance have been established by factor analysis in earlier studies from several countries (Indrehus, 2003). In Spain, the program DOCENTIA developed by the National Agency for Quality Assessment and Accreditation (ANECA in Spanish) (ANECA, 2007) has the main objective of evaluating faculty teaching in accordance with its established guide-lines by ensuring compliance with basic quality standards in the performance of university teaching activity. It also allows for the evaluation of teachers with a view to their selection and promotion, as it is fundamental to make a reasoned judgment about their teaching competence. The implementation of the program DOCENTIA is part of the internal quality assurance of the San Jorge University (USJ in Spanish). This program allows the University to prepare reports on the merits of its faculty teachers individually, which can be provided as a credit to request access to the university's faculty within the framework of academia as well as in the evaluation and accreditation of teachers prior to their employment or admission to university faculty.

Following the adoption of pharmaceutical care as the primary mission for Pharmacy Practice, the Bachelor of Pharmacy (BScPharm) degree at the San Jorge University in Spain was designed. The San Jorge University School of Pharmacy is the only pharmacy school in Spain that incorporates pharmaceutical care as a compulsory and trunk subject in the curriculum.

Pharmacology is an intrinsically multidisciplinary subject. The challenges in teaching pharmacology are to retain subject-based discipline integrity within integrated pharmaceutical science curricula and to improve skills and knowledge of pharmacology graduates, thus escalating the quality and employability of future graduates. Pharmacists must possess comprehensive pharmacology knowledge, which involves an understanding of the scientific principles underpinning medications as well as the ability to contextualize medication management to patient needs (Stupans, 2012). Some studies have been conducted to evaluate the student attitude towards teaching and learning pharmacology teaching (Mohanbabu, 2012; Shankar, 2003) and assessment methods in the subject of pharmacology (Dinesh et al., 2010), innovation and improvement of pharmacology teaching (Anuradha, 2010; Izazola-Conde, 2006; Li, 2004) in contrast to a lower reference about the satisfaction of pharmacy students with teacher performance in the pharmacology teaching process.

The aim of this paper is to determine satisfaction level of the students with the teacher´s performance during a Pharmacology and Clinical Pharmacy course in Pharmacy Degree at the San Jorge University in Zaragoza, Spain.

METHODOLOGY

Design of the course

Pharmacology course was a 6-European Credit Transfer Scheme (ECTS) offered to third year pharmacy students at the San Jorge University in Zaragoza (Spain). These ECTS represent a total of 150 h elective name, 70 h of classroom instruction and 80 h of student self study, according to the Bologna Agreement (Terry, 2012) in which each learning outcome is expressed in terms of credits, with a student workload ranging from 1,500 to 1,800 h for an academic year, and one credit generally corresponds to 25 to 30 h of work. Forty-three students enrolled in the course during the first semester of the third year September, 2011 to February, 2012. The Pharmacology and Clinical Pharmacy I is a basic course in which principles underlying the actions of drugs are presented, including pharmacokinetics, drug-receptor interactions and drug metabolism. In addition, factors modifying drug action and therapeutic outcome, clinical pharmacology and drug adverse reaction topics are explained. General educational outcomes, topics and specific learning objectives for the pharmacology course are listed in Table 1.

The Pharmacology course included didactic lectures, seminaries, in-class activities, discussions of cases and current articles, small group sessions, written essays and 2 examinations (these 2 examinations constituted 50% of the course grade). The evaluation process is carried out throughout the course, after consulting the participants, as well as at the end of the course with a written examination. Approximately half of each examination was composed of multiple-choice questions, with the remainder being a short-answer format.

Lecture notes were offered and the textbook "*Flórez, J. Farmacología humana, 4a ed. 2003*" was required for the course. Readings from the current literature were assigned to provide background, emphasis and relevance to the lecture's conceptual topics. Additional readings for written assignments were required to ensure the application of knowledge and to enhance students' critical thinking and analytical skills.

There is no patient contact or on-call responsibility. The course schedule typically includes 2 classroom days each week as two 90 min time allocated for self-directed learning. The course is a full-time course where students are also expected to devote much time to studies of their own. The instructor was available to the allotted office hours, by appointment, or via email or telephone. A series of seminars are essential parts of the course. The themes for the final seminar project works are chosen by the students themselves after consulting their professor.

The professor uses a Microsoft PowerPoint-based slide presentation and handout as a backbone for the material presented. All slides are also uploaded to the Blackboard Learning System and are available to the students on their computers at any time. The format of the lectures consists of an introduction to the learning objectives and a discussion of the relevant studied in the different topics. The Evidence-Based Pharmacotherapy component consisted of a lecture on critical appraisal of literature in pharmacotherapy and a working workshop on drug promotion.

Table 1. General educational outcomes, topics and specific learning objectives for the Pharmacology and Clinical Pharmacy I Course.

S/No	Specific learning objectives
	After completing the course students should be able to:
1	Explain the rationale underlying the more general mechanism of action of the drug in the patient, based on the drug interaction - receptor
2	Assess pharmacological activity, depending on the factors that may modify it.
3	Apply the principles of clinical pharmacology to drug therapy management under the patient´s necessities
4	Analyze the basis of adverse events related to the drug, and the relevance of pharmacist in promoting rational drug use
5	Understanding the process of evaluation of drugs and the social consequences of its use through the analysis of the most general principles of pharmacoepidemiology for their rational use
	General topics for the Pharmacology course:
1	Historical Perspective. Current Practices and Trends. Drug names (generic/trade). Biosocial Aspects of Pharmacotherapy.
2	Metabolism of drugs. Pharmacokinetics and Pharmacodynamics. Mechanisms of drug action. The actions and effects of drug. The significance of receptor in the drug action affinity, and intrinsic activity
3	The factors of influence on drug action. Geriatric, pregnancy and paediatric Pharmacology
4	Noncompliance / Nonadherence. The pathological factors of influence on drug action. Metabolic disturbances. Pathological states or presence of disease
5	Clinical pharmacology. Therapeutic Drug Monitoring. Information about drugs. Adverse Reactions and Drug Interactions
6	Pharmacoepidemiology. Drug Utilization studies. Large Data Base Research
7	Introduction of autonomic nerve system pharmacology. Adrenergic and Adrenergic Blockers. Cholinergic and Anticholinergics. Receptors α and β. Drugs related
	Pertinent San Jorge University School of Pharmacy Educational Outcomes:
1	Provide patient-centered pharmaceutical care
2	Promote health improvement and disease prevention
3	Identify and implement strategies to encourage patient adherence to therapeutic interventions
4	Design risk reduction strategies to ensure patient safety and prevent medication errors and adverse drug events
5	Design strategies to monitor patients' drug regimens for therapeutic and toxic effects of medications

Research setting and sample

The 30 students enrolled in the Pharmacology course were asked to complete an anonymous survey instrument, using the following rating scale: poor (0.0 to 4.9), appropriate (5.0 to 6.9), good (7.0 to 8.4) and excellent (8.5 to 10.0). The survey instrument consisted of 15 items grouped in three sections: planning (3 items), development (8 items) and results (4 items). The survey instrument was developed and approved by the San Jorge University Technical Quality Unit involved in ensuring the quality of the subject taught at the university. An open-ended response section asked students to identify strengths and weaknesses in the teacher's performance.

Data collection procedures

The data was collected using the standard questionnaire designed to evaluate teaching staff imparts teaching and research materials in Programs Grade and integrates into the Manual Evaluation and Improvement of Teaching Activities Teachers, developed under the DOCENTIA Program by National Agency for the Evaluation of Quality and Accreditation (ANECA). The evaluation includes the four dimensions of the Deming's (Clerghon et al., 1996) model for continuous improvement cycle (Plan, Do, Check, Act): Planning of teaching (Plan), development of teaching (Do), results of teaching (Check) and improvement of teaching (Act). In addition each of the dimensions has a number of specific elements or sub-domains defined in Table 2. The teacher evaluation survey was administered by an internet survey tool. The time commitment to complete the survey instrument was approximately 5 min. The questionnaire was administered at the end of the course in compliance with the standards established by the San Jorge University for the development of teacher evaluation process.

Data analyses

Statistical analysis of survey results was conducted using PASW Statistics 18.0 software licensed to be used at the San Jorge University. Descriptive statistics were tabulated. Data relating to the teacher's assessment summary are taken from the survey sent to teachers which only includes the results of descriptive statistics. Demographics data are not referred.

RESULTS

Twenty-two of the 23 students enrolled in the Pharmacology course returned their survey instrument, resulting in a 95.6% response rate. Table 3 reports students' ratings pertaining to the major sections survey of teacher evaluation process,

Table 2. Dimensions and sub-dimensions used in the evaluation process of the teacher activity.

Dimension		Sub-dimension
Plan	Activity planning teaching	Knowledge of objectives and content of the degree Organization of teaching-learning activities and evaluation Coordination and planning with the teaching staff
Do	Development of activity teaching	Management and use of materials and resources Addressing the needs of students Punctuality and compliance of schedule Flexibility and problem solving Participation in working groups
Check	Activity results teaching	Compliance with planned learning objectives Student satisfaction with the results
Act	Improvement of teaching activity	Teaching Innovation Review and teaching improvement Improving the degree

planning, development and results. The majority of students indicated an adequate satisfac-tion with the pharmacology teacher´s performance in all sections evaluated. Most students indicated an acceptable planning of the course, similarly the majority of students considered appropriate the use of the blackboard learning system. Many students voiced their pleasure with the course development, the closeness of the teacher, his/her availability for advice and clarification of doubts, and his/her influence on the morale of the students for learning were positively valued. The assessment of the teacher's activity referred to the results of the course has been satisfactory, most students indicated agreed with the evaluation criteria and system of assignment applied by the professor. Qualitative analyses of comments showed that most students expressed more time for the development and presentation of the final project seminar and replaced pharmacoepidemiology issues by experimental pharmacology practices.

DISCUSSION

Based on the aforementioned international normalization, those universities that opt for the implementation of homogeneous systems of evaluation of the teaching quality will broaden their possibilities and those of their students. In this regard, the general structure of the model of evaluation presented in this article has many possibilities of being implemented internationally once specific contextual amendments have been agreed, since it has already been agreed and tried on several major Spanish universities. Its design is based on the specifications and requirements established both in the European Union regulations regarding the Bologna system and in the European Space for Higher Education regulations.

This fact guarantees the high level of normalization of the model, allowing its implementation by different universities in order to adapt to the protocols and procedures of many other European universities (Rosendo-Ríos and Messía de la Cerda, 2013). The discussion of the results shown in this paper includes the phases or analyzable extensions of the teaching process (planning of education, development of the lessons learned, and results achieved). The overall results of this 15-item survey used to evaluate the professor performance in the Pharmacology course showed general agreement (appropriate evaluation) between students on 10 of the 15 items evaluated. Students were somewhat satisfied with the professor’s performance.

Planning of the teaching

Students appeared to be less satisfied with their professor’s performance in the area of planning, especially on methodologies and resources, suitability of digital and print materials as well as the relationship between theoretical and practical activities. Perhaps students wanted to receive all course support materials, specifically the slides presented by the teacher before receiving lectures, without feeling motivated to consult textbooks planned for the course, combined with the practical activities of reproductive character which is only necessary to repeat knowledge received in lectures, as usual in most courses taught in this context. Similar results were reported by Garg et al. (2004). Professor´s performance evaluation ratings were significantly higher on 5 of 15 survey items related to the use of the blackboard

Table 3. Pharmacy students' responses to survey items regarding the quality of the teacher performance.

	Survey Items	n	Average	SD*
Planning	I understand the teacher's instructions when complete the proposed activities	23	5.8	3.0
	The teacher prepares, organizes and structures well activities that we must make	23	6.2	3.0
	The teacher follows the Course Guide of matter	23	6.1	2.9
Development	The teacher uses appropriate methodologies and resources.	20	5.1	3.0
	The print and digital materials provided by the Professor are suitable.	23	5.1	3.3
	The teacher makes good use of the Blackboard Learning System	23	7.0	2.2
	The practical activities complement the theoretical matter	23	5.4	3.1
	Professor resolves doubts and guides students in and outside the class	22	7.8	1.9
	The teacher is available between the hours of tutoring	23	6.2	2.8
	The teacher encourages class participation	23	6.2	3.1
	The teacher is punctual at the start and end of our classes	23	7.6	2.5
Results	The evaluation criteria used by the teacher are consistent with those set out in the teaching guide	21	7.9	2.3
	The system of assessment (examinations, tests, papers practical projects, etc.) is suitable for the subject	22	7.1	2.7
	This course has given me the knowledge, skills and values for my personal and professional performance	23	6.1	3.4
	I am satisfied with the teacher's teaching	23	5.9	3.2

*Standard deviation.

blackboard learning system, the teacher´s punctuality and especially on the evaluation criteria used in the course.

Changes in pharmacology teaching are being driven by various pressures such as changes in the discipline itself from the professional load, from student, from changes in teaching styles and opportunities by academic staff. Such changes or transitions will require an alteration in the knowledge, skill and attitudes of teachings by academic pharmacologist. Various innovations are an effort directed towards meeting the learning needs at under graduate level. In fact the need of the hour is teaching reformations from the same old traditional methods of teaching (Biggs, 1999). Each of these cognitive activities promotes deep rather than surface learning. Lectures are no more monotonous for the students. Students are more attentive, there is transformation of the process of passive learning to active learning. Such reformations in educational pharmacology learning promote deep learning along with surface learning of the subject. Similar to other study (Jaykaran and Preeti, 2010), student seminars were not popular.

Development teaching

Satisfaction levels reported by students in the planning and development areas of the course may be influenced by the application of methods such as cooperative learning (CL), which were mainly applied in the seminars. CL most often involves small groups of students who contribute to each other's learning and encourage students to work together to achieve success rather than compete for a grade (Seifert et al., 2009). However, in these areas lower levels of satisfaction were indicated in points related to methodologies, print and virtual materials and instructions offered by the professor. Accordingly, our results suggest that a right guidance from teachers is necessary to overcome the reluctance to adopt the novel idea of learning. Thus teachers need to assist the students as they attempt to unravel the learning issues and act like a coach or facilitator for the student. Actually, the professor provided ongoing explanation and support to

students via the methodological guide in the blackboard. Moreover, the CL topic and progress arrangement should be adequately prepared in advance considering students' interest and ability. It is likely that students need some guidance in working in a group. It is essential for the educator to work with the students to guide their reflection on CL (Jun et al., 2012).

Currently, student's feedback represents the primary means used by most programs to assess their methodology (Victoroff and Hogan, 2006). Nevertheless, feedback about the performance of the teacher in the course is conducted only at the end, for this reason it is not possible to enable corrective action and comparing levels of student satisfaction at different times of course, this could be one of the limitations of this study. It is important to know what our students need and whether they feel comfortable with the ever-expanding course with limited duration of time. Frequent feedbacks may help teachers plan the curriculum and improve upon the teaching and assessment methods (Dinesh, 2010).

Results teaching

However, students appeared to be more satisfied with their professor's performance, especially in the area of course results providing an adequate evaluation system and contributing to the students' professional future (average 7.9 and 7.1, respectively). These results suggest that students are able to reflect both on what and how they are learning; which is consistent with results reported by other authors, referring to the attitude of pharmacology professor at the paradigm shifts of pharmacology teaching. According to Markham et al. (1998), many of the pharmacology teachers are aware of the nontraditional teaching and learning methods and believe that they are appropriate to discipline and effective in producing learning gains in student. Students' interest can be understood from the poll as they demanded the introduction of some special topics like experimental pharmacology. More time to the final seminar presentation show the student´s acceptance level to the seminar experience in Pharmacology course, designed to improve oral communication skills.

It is satisfying to note that most of the students felt satisfied pharmacology professor´s performance during the course. As the subject program is taught by first time, its practical importance and the professor´s performance perhaps cannot be highlighted to the maximum at that time. We feel that a more clinically oriented innovative teaching program with a broader view of pharmacology as science practical experiences showing the relevance of knowledge related to pharmacoepidemiology and rational use of drugs will allow greater satisfaction students' level with professor´s activity. It is just a matter of time before we all put our heads together and set the ball rolling for a revised pattern of teaching pharmacology which is learner-centred and more clinically oriented.

CONSTRAINTS AND LIMITATIONS

Our study has some limitations. First, all information on teacher performance was derived from student reports and not from actual monitoring and observation of teacher´s activity. Little information is known about other factors that could be affecting the student´s satisfaction. Thus, we do not know if other unobserved characteristics influenced both student reports of teaching practices and their satisfaction with the quality of teacher performance.

Second, our study did not examine the association between student satisfaction with teacher' teaching style and their subsequent physiotherapist performance. It is not possible to know from these data what impact the identified instructional practices, satisfaction levels, and other aspects of teaching process have on the future abilities of students. Nonetheless, we underscore that our focus was on student satisfaction with the teacher' performance, not with the teaching process quality as a whole.

Concerning the survey, the structure of each question should be easy and should be drafted soon, addressing only one aspect, or too detailed or too general and the appearance of the questionnaire should be attractive. Similarly, issues not valuing the teacher´s performance or not within his/her jurisdiction should be removed, these limitations are consistent with DOCENTIA program disadvantages showed by Martín and Fraile (2008). Despite these limitations, our study offers insight into the importance of a specific system to teacher´s performance evaluation. In a period in which faculty time is scarce, this study identifies points by which professors can enhance their perceived effectiveness with students. Encouraging the use of this evaluation instrument as teaching strategy is likely to raise satisfaction and the quality of Pharmacy education.

Conclusion

This survey identified the perceptions of students about professor performance in a Pharmacology and Clinical Pharmacy I course in the areas of planning, development and course results. To improve the quality of experiential education, we must evaluate teacher performance and create programs to guide them in their development and encourage their continual development. We must also make students aware of their right to get feedback and evaluation from professors. This survey was a good start in identifying areas where professor development is needed and which teaching behaviors should be continued and which need improvement. Furthermore, a formal professor development program is needed to resolve the teaching problems identified by this survey.

Conflict of Interest

The authors declare that there are no conflicting interests.

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Vol. 8(11), pp. 311-320, 22 March, 2014
DOI 10.5897/AJPP2011.856
ISSN 1996-0816

http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Effect of fractionation on *In vitro* antiradical efficacy of acetone extract of *Terminalia chebula* Retz.

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Received 23 December 2011; Accepted 20 February, 2014

The chemical diversity of antioxidants in complex matrices makes it difficult to separate and quantify them in natural form. Therefore, it is enviable to establish methods that can measure the total antioxidant capacity of extracts. In the present study, the different assays, especially most widely used: deoxyribose, reducing, chelating power, lipid peroxidation and DNA nicking assays have been used to assess the antioxidant capacity of acetone extract/fractions of *Terminalia chebula*. The extract was prepared by maceration method and further fractionated with ethyl acetate and water. It was observed that the radical scavenging activity of fractions was comparatively more as compared to crude extract, and ethyl acetate fraction showed maximum effect in all assays. The percent inhibition with ethyl acetate fraction of acetone extract was observed to be 79.2, 85.9, 90.1 and 88.9% in chelating power, lipid peroxidation, site specific and non-site specific deoxyribose scavenging assays, respectively at maximum concentration tested. The results of present work indicate that ethyl acetate fraction (EAF) might be the potential antioxidant for application in food products.

Key words: *Terminalia chebula,* antioxidants, lipid peroxidation assay, DNA nicking assay, reducing power assay.

INTRODUCTION

Ample generation of reducing oxygen species (ROS) proceeds to a variety of pathophysiological disorders such as arthritis, diabetes, inflammation, cancer, arteriosclerosis, ischemia-reperfusion injury, liver disease, diabetes mellitus, inflammation, renal failure, aging and genotoxicity (Kourounakis et al., 1999; Gulcin et al., 2002; Tanea, 2011; Zapico and Ubelaker, 2013). Compounds that can scavenge free radicals are effective in ameliorating the progression of these related diseases are called antioxidants. Phenolics or polyphenols have received considerable attention because of their physiological functions, including antioxidant, antimutagenic and antimutagenic and antitumour agents (Saliva et al., 1991; Kono et al., 1995). They can undergo auto-oxidation to produce hydrogen peroxide in the presence of metals and are capable of modulating certain cellular enzyme activities (Huang and Ferraro, 1992).

Phenolic compounds are ubiquitous in plants and have been associated with the sensory and nutritional quality of fresh and processed plant foods (Stoclet et al., 2004; Proestos and Komaitis, 2013). During the last few years, researchers and food manufacturers are increasingly interested in these compounds which may be exploited for the development of functional foods or in the

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chemoprevention. Fortification of foods with materials rich in phenolic compounds has been shown to impart anti-mutagenic, antiinflammatory and antioxidant properties which may be exploited for the development of health foods (Friedman, 1997). These justify the overwhelming interest in finding naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants (Parr and Bolwell, 2000). It has generally been recognized that traditional oriental medicines have unique therapeutic roles in the prevention and treatment of many human diseases related to excess free radicals. In addition, there is considerable evidence that polyphenols isolated from medicinal plants are potential therapeutic agents (Castillo et al., 1989; Robak and Marcinkiewicz, 1995; Inoue and Jackson, 1999; Packer et al., 1999; Middleton et al., 2000; Rodrigo et al., 2011).

Terminalia chebula a native plant in India and Southeast Asia is extensively cultivated in Taiwan, and is rich in polyphenolic compounds. According to Indian mythology, this plant originated from the drops of ambrosa (Amrita), which fell on the earth when Indra was drinking it (Srikanthmurthy, 2001). The fruits of *T. chebula* are known as black myroblan are being used for the treatment of different types of diseases and disorders since antiquity. The plant has been studied for its antioxidant, antimicrobial and antimutagenic properties (Saleem et al., 2002; Chen et al., 2003; Bag et al., 2013). It is also reported that oral administration of the extracts from *T. chebula* reduced the blood glucose level in normal and in alloxan-diabetic rats (Sabu and Kuttan, 2002; Akhand et al., 2013). Keeping in view the immense importance of the plant, the present study was planned to evaluate the antioxidant activity of acetone extract/fractions of fruits of *T. chebula*.

MATERIALS AND METHODS

Chemicals

Deoxyribose was purchased from Lancaster. Thiobarbituric acid was purchased from Sigma Aldrich USA. Other chemicals like ferrozine, Folin-Ciocalteu (FC) reagent, potassium ferricyanide, ferric chloride, ethylenediaminetetraacetic acid (EDTA), hydrogen peroxide, L-ascorbic acid, sodium hydroxide, BHA, trichloroacetic acid and other solvents were procured from CDH and were of analytical grade.

Extraction/fractionation procedure

The fruits of *T. chebula* were purchased locally from the market. These were washed with tap water, dried in oven at 40°C and ground to a fine powder. To 1000 g of fruit powder 1500 ml of acetone was added. The supernatant was collected, filtered by using Whatman sheet no.1 and evaporated through rotary evaporator to have the dry crude acetone extract. This dry crude acetone extract was further fractionated. For the fractionation, the crude acetone extract (AI) was redissolved in acetone and after some time the precipitates were formed. The precipitates (AP) and supernatant (AII) were separated and dried at room temperature separately. The dried supernatant (AII) was dissolved first in water and then in ethyl acetate, resulted in formation of two layers: ethyl acetate fraction (EAF) and water fraction (WF). These layers were separated and dried at room temperature (Flow chart 1).

Spectroscopic analysis of extract/fractions

The acetone extract/fractions of *T. chebula* were analyzed by ^{1}H NMR and ultra violet (UV) spectroscopy. For nuclear magnetic resonance (NMR) spectroscopy, 1 mg acetone crude extract (AI) was dissolved in spectroscopic grade dimethyl suphoxide (DMSO), filtered and transferred to NMR tubes. The tubes were spun at 20 to 30 Hz about its vertical axis and interpretation was done. For UV analysis, the solution of acetone extract/fractions was prepared in spectroscopic grade methanol in the concentration of 1 mg/10 ml and a spectrum was recorded on UV-visible spectrophotometer (Shimadzu-1601).

Antioxidant testing

The acetone crude extract (AI), supernatant (AII), precipitates (AP), and two fractions that is, ethyl acetate fraction (EAF) and water fraction (WF) were tested for their antioxidant potential by using the following *in vitro* assays.

Deoxyribose assay

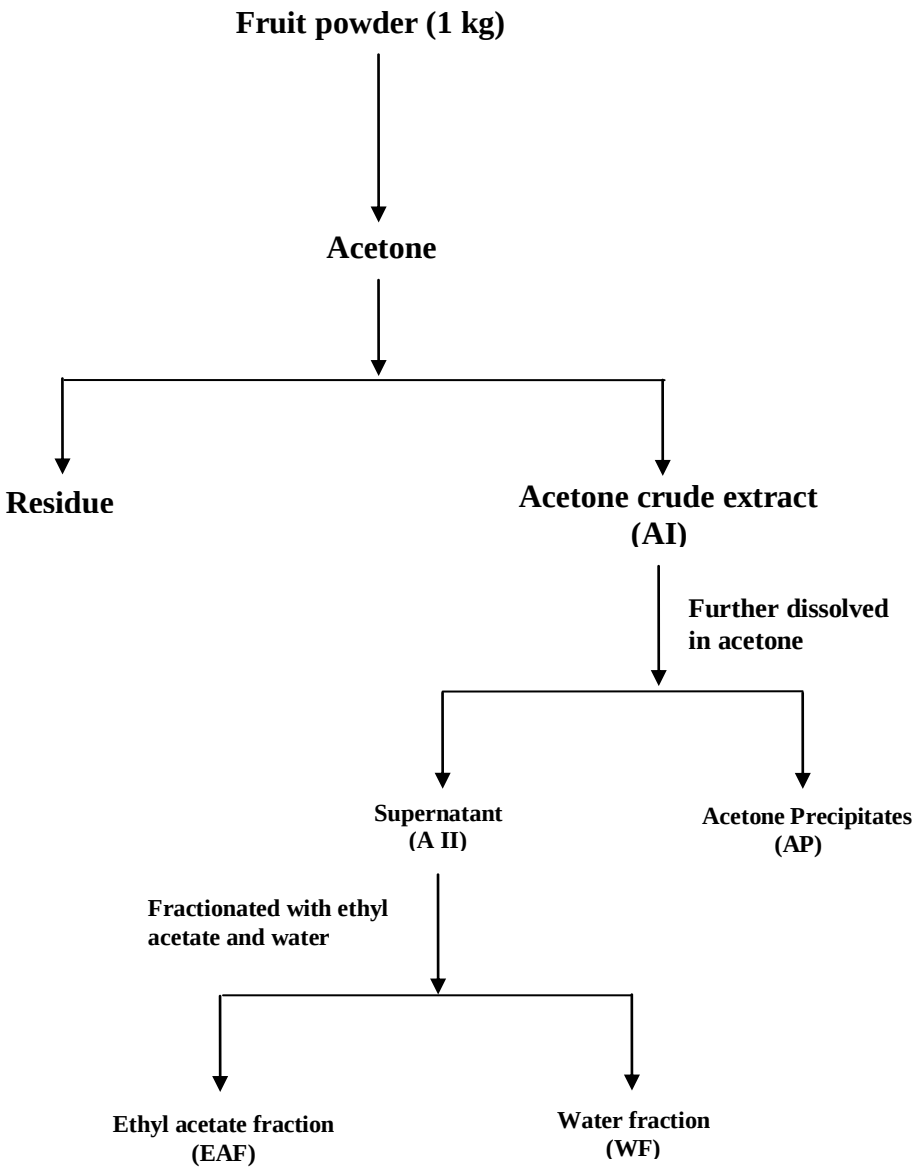
This method was used to measure the hydroxyl radicals scavenging activity of extracts (Halliwell et al., 1987). This assay was performed by two ways that is, non-site specific and site-specific. In non-site specific deoxyribose assay, 0.1 ml of EDTA, 0.01 ml of $FeCl_3$, 0.1 ml of H_2O_2, 0.36 ml of deoxyribose, 1 ml of extract concentrations (10 to 100 µg/ml), 0.33 ml of phosphate buffer (50 mM, pH 7.4) and 0.1 ml of ascorbic acid were added in sequence. The mixture was incubated at 37°C for 1 h. 1 ml of the incubated mixture was mixed with 1 ml of 10% trichloroacetic acid and 1 ml of thiobarbituric acid (0.025 M NaOH) and heated for one hour on water bath at 80°C and pink chromogen developed, which was measured at 532 nm. In site-specific deoxyribose assay, EDTA was replaced with phosphate buffer.

Reducing power assay

This method is used to estimate the relative reducing activity of extracts (Oyaizu, 1986). 1 ml of extract/fractions (50 to 300 µg/ml) was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. 2.5 ml of 10% tricholroacetic acid was then added to the mixture and centrifuged at 3,000 rpm for 10 min. 1 ml of aliquot of supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (0.1%) and absorbance was measured at 700 nm. Increase in absorbance was interpreted as increased reducing activity.

Lipid peroxidation assay

In this method, thiobarbituric acid (TBA) reacts with malondialdehyde (MDA) to form a diadduct, a pink chromogen, which can be detected spectrophotometrically at 532 nm (Halliwell and Guttridge, 1989). Normal albino rats of the Wistar strain were used for the preparation of liver homogenate. The perfused liver was isolated, and 10% (w/v) homogenate was prepared using a homogenizer at 0 to 4°C with 0.15 M KCl. The homogenate was centrifuged at 3,000 rpm for 15 min, and clear cell-free supernatant



Flow chart 1. Extraction/fractionation procedure.

was used for the study of *in vitro* lipid peroxidation. Different concentrations of extracts mixed with 1 ml of 0.15 M KCl and 0.5 ml of rat liver homogenates were added to the test tubes. Peroxidation was initiated by adding 100 µl of 0.2 mM ferric chloride. After incubation at 37°C for 30 min, the reaction was stopped by adding 2 ml of ice-cold HCl (0.25 N) containing 15% trichloroacetic acid (TCA), 0.38% TBA and 0.5% butylated hydroxytoluene (BHT). The reaction mixture was heated at 80°C for 60 min. The samples were cooled and centrifuged, and the absorbance of the supernatants was measured at 532 nm.

Chelating power assay

In this assay, 1 ml of extract with different concentrations was mixed with 3.5 ml of methanol, and then the mixture was mixed with ferrous chloride (2 mM, 0.1 ml) and ferrozine (1 mM, 0.2 ml) for 10 min at room temperature. The absorbance was measured at 562 nm against a blank in which the extract was not added (Dinis et al., 1994).

DNA nicking assay

A DNA nicking assay was performed using supercoiled pBR322 plasmid DNA (Lee et al., 2002). Plasmid DNA (0.5 µg) was added to Fenton's reagents (30 mM H_2O_2, 50 µM ascorbic acid, and 80 µM $FeCl_3$) containing concentration of the extracts/fractions and the final volume of the mixture was brought up to 20 µl. The mixture was then incubated for 30 min at 37°C, and the DNA was analyzed on a 1% agarose gel followed by ethidium bromide staining.

Determination of total phenolic content

The total phenolic content of the extract was determined using Folin-Ciocalteu method (Yu et al., 2002). To 100 µl of extract/ fraction was added 900 µl of water. To this, 500 µl of FC reagent was

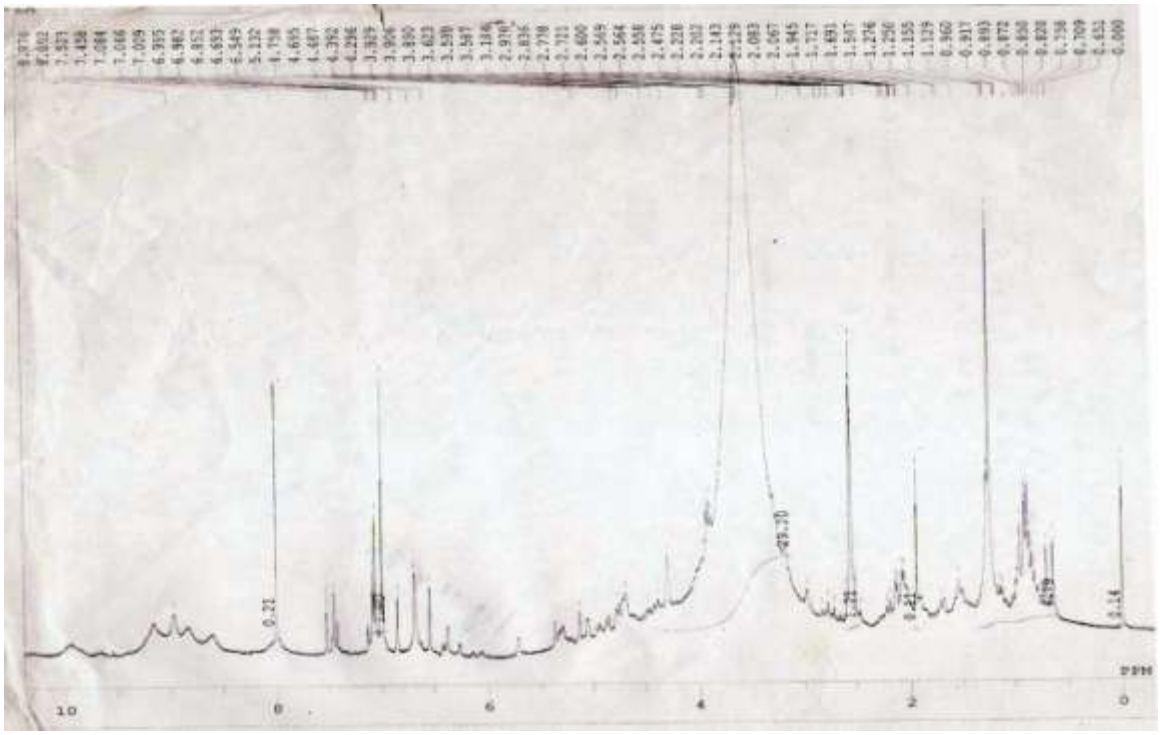


Figure 1. ^{1}H NMR spectrum of crude acetone extract (AI).

added. This was followed by the addition of 1.5 ml of 20% sodium carbonate. The mixture was shaken thoroughly and allowed to stand for 2 h. The volume of mixture was made up to 10 ml with distilled water and absorbance was observed at 765 nm. The phenolic content was calculated as gallic acid (mg/g) equivalents.

RESULTS AND DISCUSSION

The NMR spectrum of crude acetone extract (AI) of *T. chebula* showed signals spreading from 0.5 to 10 ppm. These pointed to the presence of multiple components in the extract. The signals between 0.5 to 3.0 δ pointed to the fatty esters or terpenoides. The presence of signals between 3.5 to 5.0 δ clearly showed the presence of glycosides. The number of signals between 6.0 to 8.0 ppm referred to polyphenolics, which may be present as glycosides. The large number of exchangeable and inter/intramolecularly H- bonded protons were also observed between 8.5 to 10.0 δ (Figure 1).

The acetone crude extract (AI) constituted 23.5% of fruit powder and was dark brown in colour. The UV analysis showed the presence of peak at λ_{max} = 362 nm in crude acetone extract which points towards the presence of polyhenols. The AII, AP, WF and EAF showed maximum absorbance λ_{max} at 363, 362, 363 and 368 nm, respectively, strappingly signifying the presence of glycosides of phenolic nature (Figure 2).

The results of acetone extract/fractions are depicted in Figures 3 to 8. It was observed that the fractions that is, water and ethyl acetate were more effective as compared to the crude acetone extract. The amount of total phenolics in extract/fractions ranged from 340 to 780 mg/g of gallic acid. The total phenolic content was maximum in ethyl acetate fraction (EAF) that is, 780 mg/g of gallic acid, which signify its highest antioxidant activity. The crude acetone extract (AI), supernatant (AII), acetone precipitates (AP), and water fraction (WF) had 437, 557, 340 and 64 7mg per gram phenolic content, respectively.

To date, the $^{\cdot}$OH is one of the most reactive free radical species known with damaging effects to almost every biological molecule found in living cells. It can be generated *in vivo* in the presence of both superoxide radicals and transition metals, such as iron or copper via the Haber-Weiss reaction (Castro and Freeman, 2001). In order to substantiate the free radical scavenging capacity of acetone extract/fractions in an *in vitro* Fenton-type assay system: non-site-specific (Fe^{2+} + H_2O_2 + EDTA) and site specific (Fe^{2+}+H_2O_2) was used, in which $^{\cdot}$OH radicals are generated in free solution that attack the deoxyribose substrate and fragmenting it into thiobarbituric acid reactive substances (TBARS). Figures 3 and 4 depicts the activity of acetone extract/fractions in non-site specific and site-specific deoxyribose assay, respectively. It was observed that the extract/fraction showed a dose response relationship up to 100 µg/ml.

Furthermore, a comparatively high activity was noticed in site-specific assay than in non-site specific assay

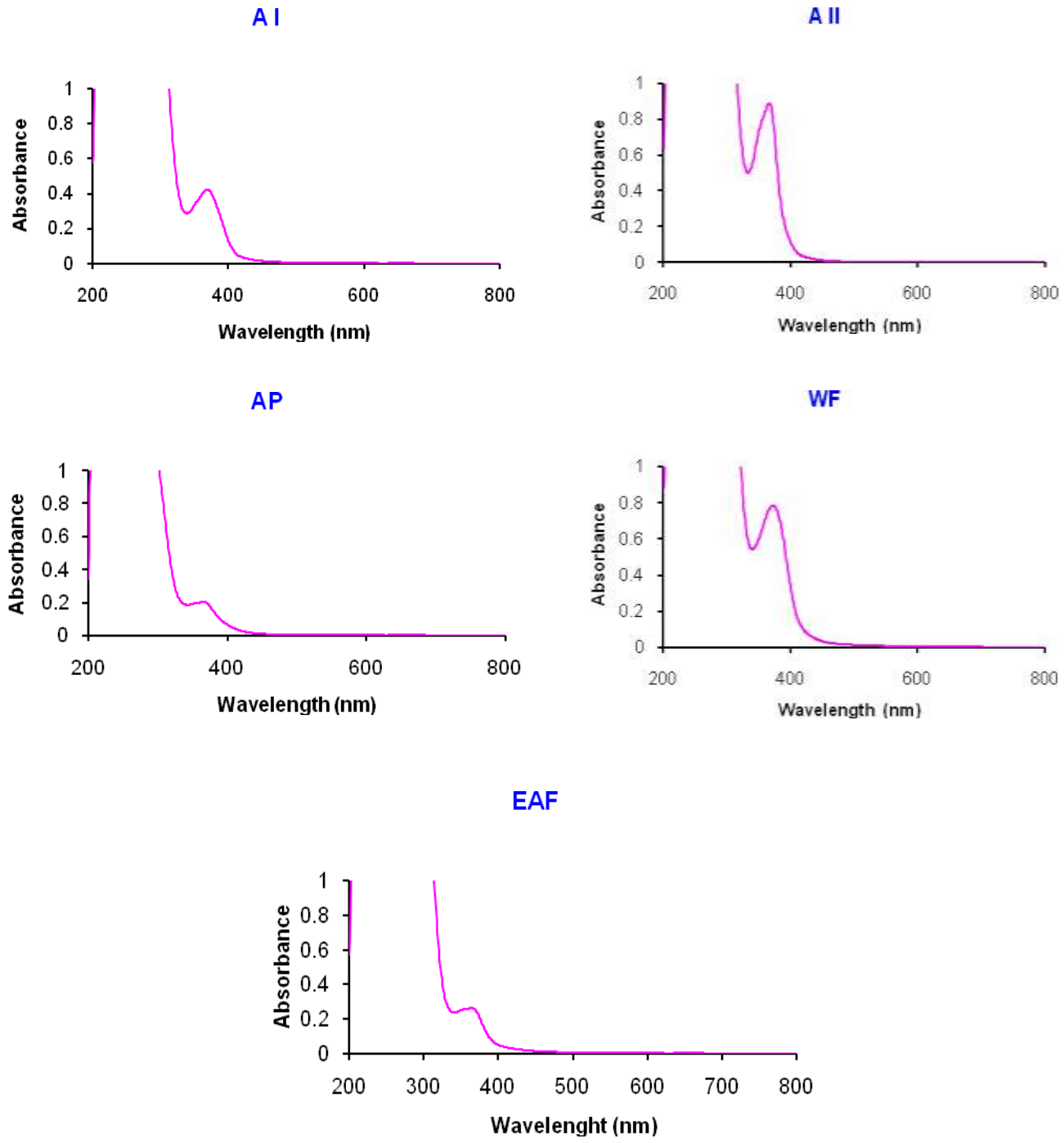


Figure 2. The UV-Visible spectra of acetone extract/fractions of *Terminalia chebula.*
A I: Crude extract, **A II**: Supernatant, **AP**: Precipitates, **WF**: Water fraction, **EAF**: Ethyl acetate fraction

indicating the high chelating activity of the extracts/fractions. The crude acetone extract (AI) showed 79.3 and 86.8% inhibition in non-site specific and site specific assay at 100 µg/ml of concentration, respectively. A maximum inhibition of 90.1 and 88.3% was shown by ethyl acetate fraction (EAF) in site specific and non-site specific assay, respectively at 100 µg/ml of concentration.

It was noticed that in both the assays, EAF showed maximum inhibition. The acetone precipitates (AP) showed the minimum inhibition that is, 62 and 60.1% in site specific and non-site specific assay, respectively. It is also clear from Figures 3 and 4 that the extract exhibited good antioxidant and chelating activity than standard antioxidant, that is gallic acid. The presence of phenolic

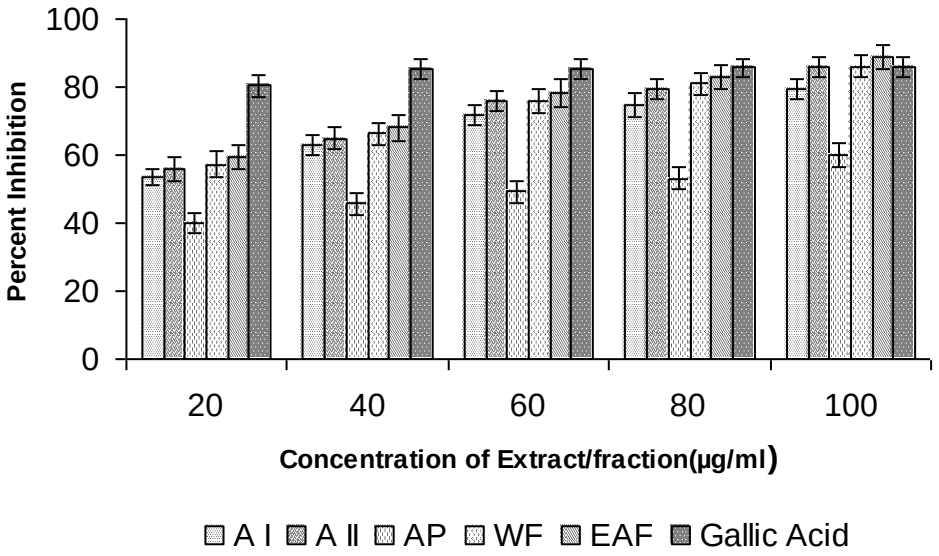


Figure 3. Histogram showing the pattern of inhibition by acetone extract/fractions in non-site specific deoxyribose assay.

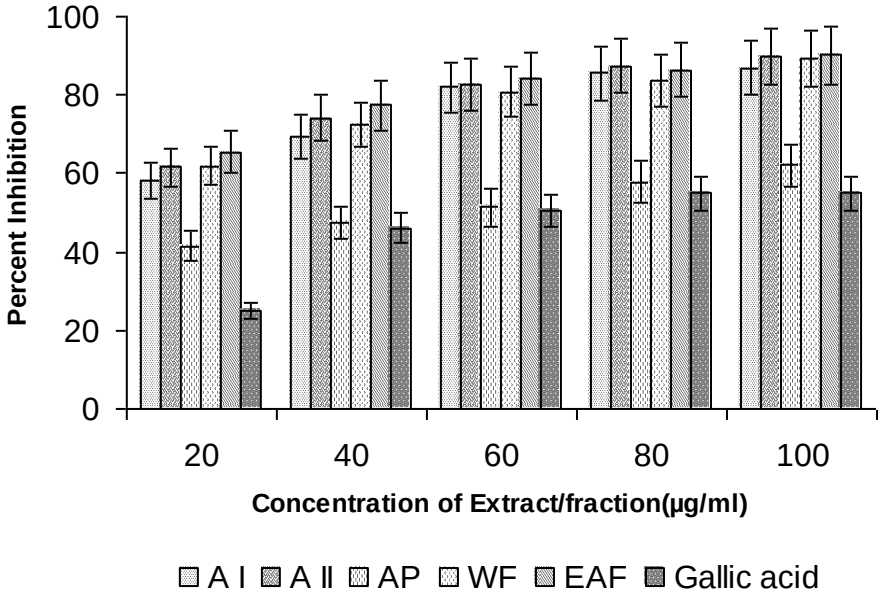


Figure 4. Histogram showing the pattern of inhibition by acetone extract/fractions in site specific deoxyribose assay.

groups in extract/fractions could be responsible for $^{\cdot}OH$ radical scavenging activity. The results indicated that the acetone extract/fractions has more hydrogen donating ability, which may be due to the presence of polyphenolic glycosides as indicated by ^{1}H NMR spectrum that showed major signals between 3.0 to 5.5 δ and UV analysis which indicated the presence of phenolic compounds. Earlier, numerous workers (Halliwell et al., 1987; Pin-Der-Duh et al., 1999) have employed this system to assess the biological activity of various natural plant derived biomolecules. One reported that the molecules that can inhibit deoxyribose degradation are those that can chelate iron ions and render them inactive or poorly active in a Fenton reaction which strengthens our result obtained in iron chelating assay (Smith et al., 1992). It is likely that the chelating effect of acetone extract/fractions on metal ions may be responsible for the inhibition of deoxyribose oxidation.

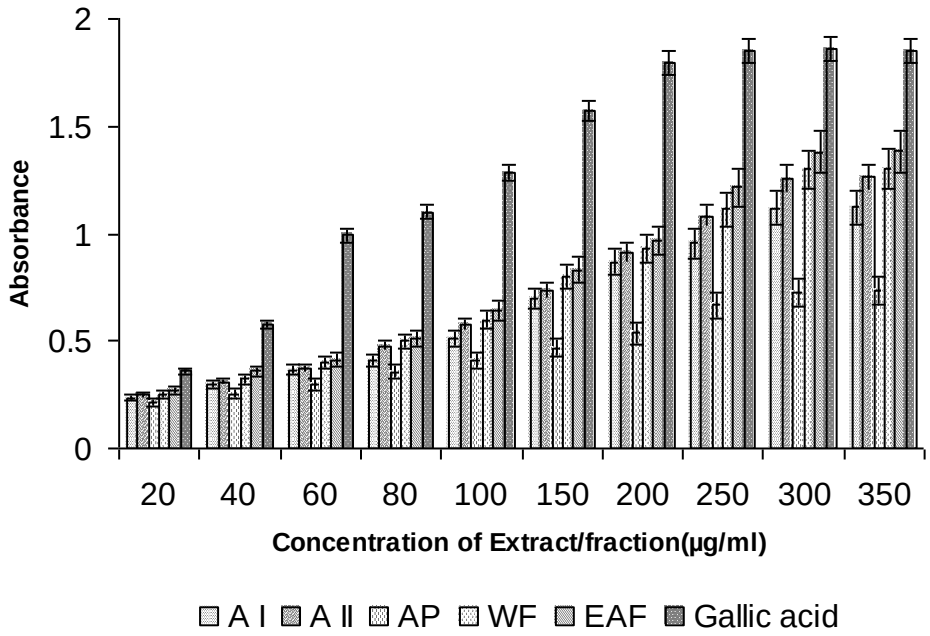


Figure 5. Histogram showing the pattern of absorbance by acetone extract/fractions in reducing power assay

Iron, a transition metal, is capable of generating free radicals from peroxides by the Fenton reaction and is implicated in many diseases (Halliwell and Gutteridge, 1990). Fe^{2+} has also been shown to produce oxyradicals and lipid peroxidation, and reduction of Fe^{2+} concentrations in the Fenton reaction would protect against oxidative damage.

The antioxidant activity of acetone extract/fraction was also discernible in the reducing power assay, which primarily evaluates hydrogen donating ability. Figure 5 depicts the reducing power of acetone extract/fractions and gallic acid, a known antioxidant. The reducing power of extract/fractions showed dose relationship up to 350 µg/ml of concentration. However, as anticipated, the reducing power of gallic acid was relatively more pronounced than that of acetone extract/fractions. In this, the minimum absorbance was shown by acetone precipitates (AP), that is 0.731 and maximum by ethyl acetate fraction, that is 1.385 at 350 µg/ml of concentration. Earlier authors (Tanaka et al., 1988; Yildirim et al., 2001) have also observed a direct correlation between antioxidant activity and reducing power of certain plant extracts. The reducing properties are generally associated with the presence of reductones which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Gordon, 1990).

Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation. The presence of reductants (that is, antioxidants) in the extract/fractions causes the reduction of the Fe^{3-}/ferricyanide complex to the ferrous form. Therefore, the Fe^{2-} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Our data on the reducing power of extract/fraction suggest that it is likely to contribute significantly towards the observed antioxidant effect.

In order to determine whether the extracts are capable of reducing *in vitro* oxidative stress, the traditional lipid peroxidation assay that determines the production of malondialdehyde and related lipid peroxides in living system was carried out. Peroxidation is important in food deterioration and in the oxidative modification of biological molecules particularly lipids. Inhibition of lipid peroxidation by any external agent is often used to evaluate its antioxidant capacity. Figure 6 gives the level of inhibition of lipid peroxidation in terms of TBARS produced in rat liver mitochondria induced by ferric chloride system in the presence of extract/fraction. The order of inhibition of peroxidation was EAF (85.9%) > WF (79.5%) > AII (70.5%) > AI (68.3%) > AP (60.3%) at 100 µg/ml of concentration. The increase in inhibition can directly be correlated with the increase in polyphenolic content. The total phenolic content of the ethyl acetate fraction (780 mg/g) reveals that there is direct relationship between amount of phenolic compounds and antioxidant activity. The UV analysis of EAF of acetone extract exhibited λ_{max} at 362 which revealed the presence of polyphenolic compounds. The comparison of results with

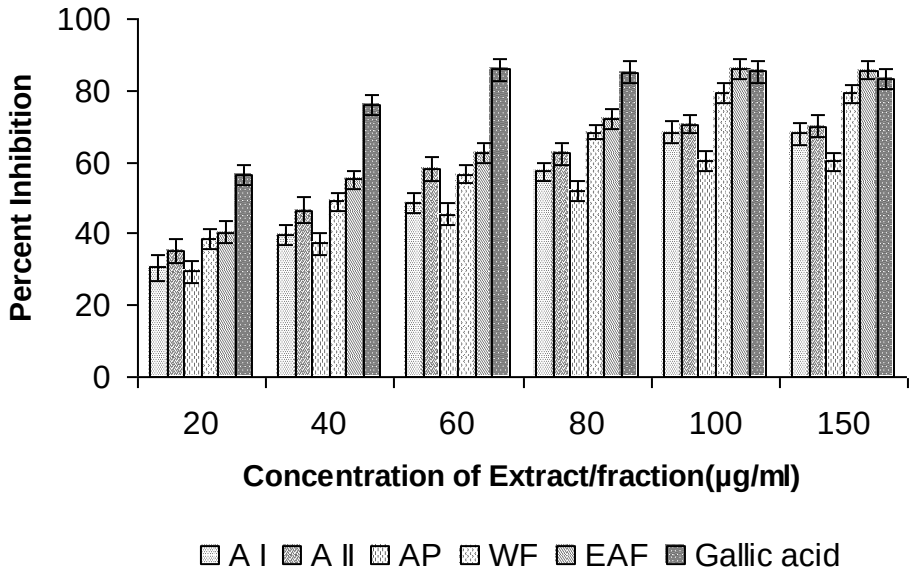


Figure 6. Histogram showing the pattern of inhibition by acetone extract/fractions in lipid peroxidation assay.

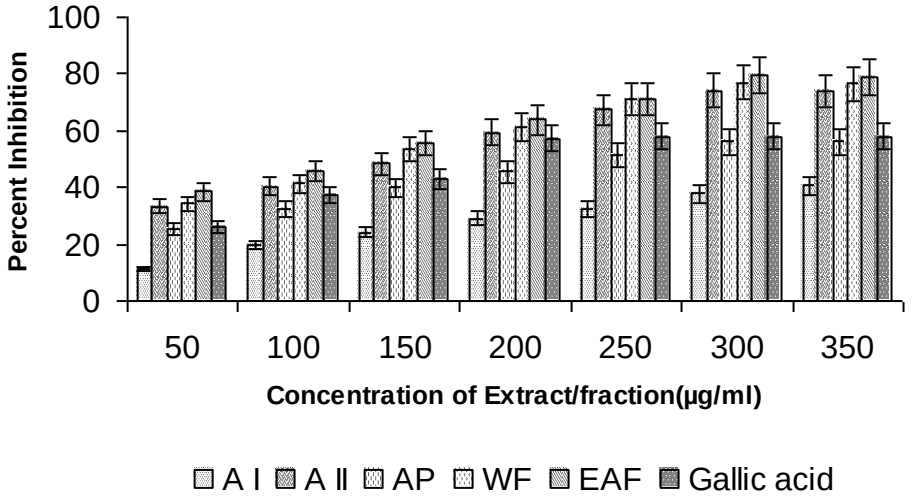


Figure 7. Histogram showing the pattern of inhibition by acetone extract/fractions in chelating power assay.

gallic acid indicated that the extract/fractions exhibited more or less the same inhibition.

Figure 7 depicts the chelating activity of acetone extracts/fractions. The maximum inhibition was shown by EAF, which was 79.2% and minimum with acetone crude (AI) that is, 40.7% at 350 µg/ml of concentration. The extract/fractions exhibited more chelating activity as compared to standard (gallic acid). Ferrous ions could stimulate lipid peroxidation by Fenton reaction, and also accelerate peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation (Halliwell, 1991). Chelating agents may serve as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the metal ions (Gordon, 1990). Since ferrous ions were the most effective pro-oxidants in food system, the moderate to high ferrous-ion chelating abilities of the various extract/fractions would be beneficial (Yamaguchi et al., 1988).

In the DNA nicking assay, antioxidative activity was assessed by measuring the degree of protection on DNA scission by acetone extract/fractions that was induced by the attack of $^{\cdot}OH$ radicals, which was shown by the agarose

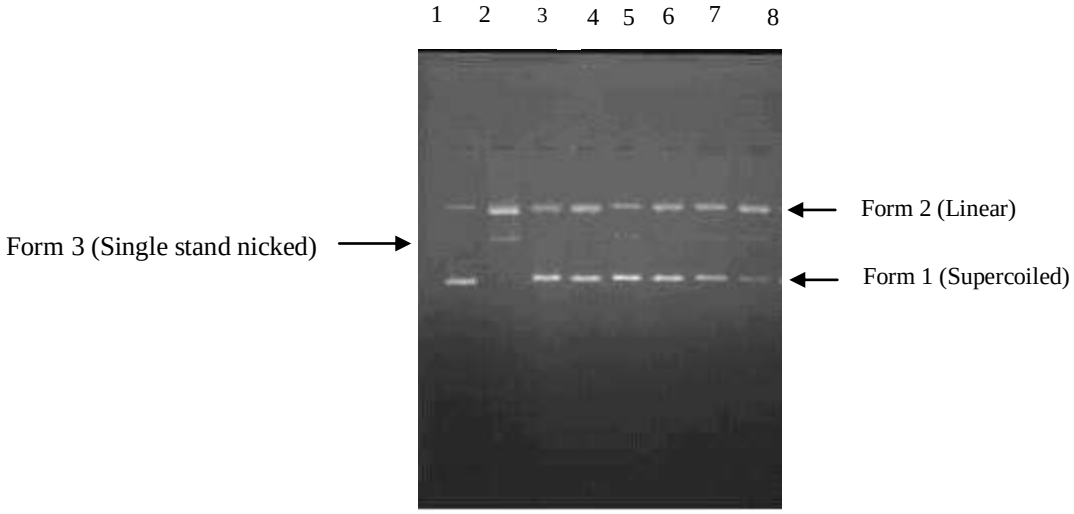


Figure 8. Inhibitory effects of acetone extract/fractions of *Terminalia chebula* at 250 µg/ml concentration on pBR322 DNA nicking caused by hydroxyl radicals. Lane 1, Native DNA; lane 2, DNA + Fenton reagent; lane 3, DNA + Fenton reagent +Gallic acid; lane 4, DNA + Fenton reagent + A I; lane 5, DNA + Fenton reagent + A II; lane 6, DNA + Fenton reagent + EAF; lane 7, DNA + Fenton reagent + WF; lane 8, DNA + Fenton reagent + AP.

agarose electrophoresis pattern. In this assay, when pBR322 plasmid DNA was exposed to Fenton reaction, it caused a change in DNA band from Form I (Native plasmid DNA) to Form II (single-stranded, nicked circular plasmid DNA) or to Form III (Linear plasmid DNA). The extract/fractions scavenge the $^{\cdot}OH$ radicals and protect the pBR322 plasmid DNA. Different concentrations were tried but at the concentration of 250 µg/ml, the extract/fractions showed the reduction in Form II and III, and increased in Form I which is a normal DNA. The extract/fractions showed comparable effect to gallic acid (Figure 8). The ethyl acetate fraction showed best result among all the extracts/fractions and precipitates showed the minimum effect.

Conclusion

On the basis of this study, it can be concluded that EAF of acetone extract from *T. chebula* showed strong antioxidant properties in deoxyribose assay, reducing power, ferrous ions chelating activity, lipid peroxidation. Furthermore, ethyl acetate fraction also exhibited comparatively more inhibition of $^{\cdot}OH$ radicals induced by Fe^{2+} in DNA Nicking assay as compared to other extract/fraction. The results of present work indicate that EAF might be the potential antioxidant for application in food products.

ACKNOWLEDGMENTS

We are grateful to CSIR, New Delhi, for financial support in the form of major research project.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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