Phytochemical and antioxidant potential of *Jatropha* species

ANJALI, A.
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A thesis submitted to

Avinashilingam Institute for Home Science and Higher Education for Women,

Coimbatore- 641 043.

In partial fulfillment of the requirements for the Degree of

Master of Science in Biotechnology

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Signature of the
Head of the Department

10/5/13

Signature of the
Supervisor

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"*God is infinite, but people try to count the letters of his name*"

- Thomas Szasz

This work would not have been possible unless he shower His abundant blessings. I humbly place my profound gratitude to ***GOD ALMIGHTY*** *for everything he has done to me.*

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1.0 INTRODUCTION

In recent years, the studies on “oxidative stress” and its adverse effects on human health have become a subject of considerable interest. It is a well-documented fact that exposure of organisms to exogenous and endogenous factors generates a wide range of reactive oxygen species (ROS), resulting in homeostatic imbalance (Halliwell, 1994; Sies, 1997; Halliwell and Gutteridge, 1999). ROS can induce alterations and loss of structural/functional architecture in the cell, leading directly to cytotoxicity and/or indirectly to genotoxicity (Girotti, 1994; Esterbauer, 1996; Sies, 1997; Halliwell and Gutteridge, 1999). Therefore, the factors that shift the physiological process, in the homeostatic balance are of great interest (Sies, 1997).

Oxygen free radicals or reactive oxygen species (ROS) are well recognized to play a dual role in biological systems, because they can be either harmful or beneficial to living systems (Huang *et al*., 2005; Valko *et al*., 2004). Actually, beneficial effects of ROS involve physiological roles in cellular responses to noxia. However, the high concentration of ROS can also induce the damage of cell structures, including lipids and membranes, proteins and nucleic acids, which lead to a number of diseases (Antolovich *et al*., 2002; Valko *et al*., 2007), such as cancer, atherosclerosis, cardiovascular diseases, inflammatory lung diseases, immune dysfunctions and neurodegenerative disorders.

Antioxidants are a diverse group of compounds that act against oxidative damage induced in the body. Antioxidants quench reactive free radicals, prevent the oxidation of other molecules and play a significant role in the prevention of degenerative diseases like cancer, cardiovascular diseases, cataract formation, the aging process, inflammatory diseases and a wide range of neurological disorders (Biglari *et al*., 2008; Silva *et al*., 2007, Choe and Min, 2009). The effectiveness of antioxidants to scavenge free radicals depends on the bond dissociation energy of the bond involved in hydrogen donation, pH related to the acid dissociation constant, reduction potential and delocalization of the antioxidant radicals (Choe and Min, 2009).

To prevent or reduce the oxidative stress induced by free radicals, sufficient amounts of antioxidants need to be consumed or added to foods. The antioxidants can be of synthetic or natural origin. However, some toxic effects of synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tert-butylhydroquinone (TBHQ) have been reported (Choi *et al*., 2000; Dapkevicius *et al*., 1998). Therefore, many researchers have focused on the investigation for natural compounds with antioxidant properties. A multitude of natural antioxidants have already been isolated from different kinds of plant materials such as oilseeds, cereal crops, vegetables, fruits, leaves, roots, spices, and herbs (Ramarathnam *et al*., 1995). Among these natural antioxidants, phenolic antioxidants are in the forefront as they are widely distributed in the plant kingdom. Its antioxidant mechanism is believed to quench oxygen-derived free radicals as well as the substrate-derived free radicals by donating a hydrogen atom or an electron to the free radical (Wettasinghe and Shahidi, 1999; Wanasundara and Shahidi, 1996).

The importance of medicinal plants is being highlighted as a source of natural antioxidant and functional foods (Vaidya and Devasagayam, 2007). Among various attributes of functional foods, antioxidant property is considered the best, as it reduces the oxidation processes in the body (Krishnaih *et al*., 2007) and plays an important role in maintaining health by protecting against reactive oxygen species (ROS) (Lan *et al*., 2007). Recently, natural antioxidants have attracted considerable attention of users and researchers owing to adverse toxicological reports on some synthetic antioxidants and growing awareness among consumers (Ramalakshmi *et al*., 2007). In this context, medicinal plants are being viewed as an easily available and potent source of antioxidants. However, detailed screening for chemical composition and antioxidant activity of most of the known medicinal plants has yet to be carried out worldwide.

Even though our body is safeguarded by natural antioxidant defense, there is always a demand for antioxidants from natural sources (Rimbach *et al*., 2005). Polyphenolic substances possess many biological effects which are mainly attributed to their antioxidant activities in scavenging free radicals, inhibition

peroxidation and chelating transition metals (Bahman *et al*., 2007). The scientific basis for the statement that plants and their active constituents play an important role in the prevention of chronic and degenerative diseases is continuously advancing. In fact, the origin of many therapeutic substances is due to secondary metabolism in the plant (Maganha *et al*., 2010). Epidemiological studies have shown that consumption of plant foods is beneficial to health and contributes to the prevention of degenerative processes, hence lowering the incidence and mortality rate from cancer and cardio and cerebro-vascular diseases (Halliwell and Gutteridge, 1997). Both edible and non-edible plants and plant-derived products are known to contain a complex mixture of phenolic compounds that possess multiple biological effects including antioxidant activity. Plants (fruits, vegetables, medicinal herbs) contain a wide variety of free radical-scavenging molecules, such as phenolic compounds, nitrogen compounds, vitamins, terpenoids and some other endogenous metabolites, that are rich in antioxidant activity (Zheng and Wang, 2001; Cai *et al*., 2003).

Phenolic compounds are bioactive substances widely distributed in plants and are important constituents of the human diet. Plants, the main sources of antioxidants, comprise a great diversity of compounds including flavonoids (anthocyanins, flavonols, flavones, etc.) and several classes of non-flavonoids (phenolic acids, lignins, stilbenes, terpenoids, etc.) as phenolics. These compounds vary in structure, the number of phenolic hydroxyl groups and their position, leading to variation in their antioxidative capacity (Bai *et al*., 2010)

Extensive investigation of natural sources of efficient radical-scavenging compounds is receiving high attention in health research. A wide variety of methods have been developed for the antioxidant assessment, including the ferric reducing antioxidant power (FRAP) assay (Benzie and Strain, 1996), the 2,20-azino-bis(3-ethylbenzothiazoline-6-sulphonate) radical cation (ABTS) assay (Miller *et al*., 1993), the oxygen radical absorbance capacity (ORAC) assay (Cao *et al*., 1993; Glazer, 1990), the cupric reducing antioxidant capacity (CUPRAC) assay (Apak *et al*., 2004), the electrochemical estimation of total reducing

capacity (Chevion *et al.*, 2000) and the 1,1-diphenyl-2 picrylhydrazyl radical (DPPH) assay (Bondet *et al.*, 1997; Brand-Williams *et al.*, 1995).

The genus *Jatropha* that belongs to tribe Joannesieae in the Euphorbiaceae family contains approximately 170 known species. The name *Jatropha* is derived from the Greek word ‘‘jatros’’ (doctor) and ‘‘trophe’’ (food), which implies its medicinal uses (Kumar and Sharma, 2008). *Jatropha* plants and is well known for its toxicity. *Jatropha* species for which the toxicity has been widely studied are *Jatropha curcas, Jatropha elliptica, Jatropha glauca, Jatropha gossypifolea, Jatropha aceroides, Jatropha tanoresisi, Jatropha macarantha, Jatropha integerrima, Jatropha glandulifera, Jatropha podagrica* and *Jatropha multifida* (Devappa *et al*., 2010).

To the best of our knowledge there is paucity of information regarding the bioactive compounds and *in vitro* antioxidant activity of *Jatropha* species. The plant ethnopharmacological applications are well known, but much of the information is empirical and lacking in scientific validation.

Therefore, the main **objective** of the study was designed to evaluate the

1. Antioxidant activities of ethanolic and aqueous extracts of seven *Jatropha* species by various *in vitro* model systems.
2. Phytochemical constituents in *Jatropha* species.

2.0 REVIEW OF LITERATURE

The review of literature pertaining to the present study is discussed under the following headings:

2.1 ROS and oxidative stress

2.2 *Jatropha* and its medicinal properties

2.3 Biological activity of *Jatropha* species

2.4 Secondary metabolites in plants

2.5 *In vitro* antioxidant scavenging assays

2.6 *In vitro* antioxidant activity of *Jatropha*

2.1 ROS and oxidative damage

The concept of reactive oxygen species (ROS) has gained significant recognition over the past several years by various studies in laboratories worldwide. ROS are chemically reactive molecules containing oxygen, including superoxide anion radical ($\bullet O_2^-$), hydroxyl radical (•OH), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), and nitric oxide (NO•). ROS form as a natural by-product of the normal metabolism of oxygen and play important roles in cell signaling and homeostasis. Reactive oxygen species (ROS), such as hydroxyl radicals, superoxide anions and hydrogen peroxide, are frequently generated spontaneously in the living cell during metabolism and play an important role in cell signalization. However, excessive amount of ROS can induce oxidative stress, resulting in significant damage to cell structures and macromolecules, including proteins, lipids, and nucleic acids. Oxidative stress is a key causative factor for cancer, cardiovascular disorders, and neurodegenerative diseases, including Parkinson's disease and Alzheimer's disease (Valko *et al*., 2007).

2.2 *Jatropha* and its medicinal properties

The genus *Jatropha* is extremely old and may have already existed 70 million years ago on the ancient continent ''Gondwanaland'' before it split up to form the individual continents. It is considered to be the most primitive member

of the large genus Euphorbiaceae. The genus *Jatropha* consists of 165–175 species. *Jatropha* is a large genus of diverse growth forms and are attractive monoecious or dioecious plants. These species are woody trees, shrubs and subshrubs of disjunct distribution in the seasonally dry tropics of the Old and the New World. Two distinct groups were recently recognized, subgenus *Jatropha* that includes the African, Indian, South American, Antillian and two of the relict North American taxa, and species of subgenus curcas which are predominant in Mexican with a few extending into Texas and Arizona (Dehgan, 1982). *Jatropha* species are used in traditional folklore medicine to cure various ailments in Africa, Asia and Latin America (Burkill, 1994), as ornamental plants and energy crops (Heller, 1996). Their usage as traditional health remedies is the most popular for 80% of the world population in Asia, Latin America and Africa and is reported to have minimal side effects (Cowan, 1999). Several known species from genus *Jatropha* have been reported for their medicinal uses, chemical constituents and biological activities such as *Jatropha curcas* Linn., *J. chevalieri* Beille, *J. elliptica* Muell. Arg., *J. gaumeri* Greenm., *J. glandulifera* Roxb., *J. gossypiifolia* Linn., *J. grossidentata* Pax et. Hoffm., *J. integerrima* Jacq., *J. macrantha*, *J. mahafalensis* Jum and H. Perrier, *J. multifida* Linn., *J. nana* Dalz, *J. podagrica* Hook, *J. pohliana* Muell. Arg., *J. tanjorensis* Ellis and Saroja, *J. unicostata* and *J. weddelliana* Baillon.

Jatropha species have been used as medicinal plants by native people in the tropical and subtropical countries (Openshaw, 2000). *Jatropha* species are famous for the purgative effect of the seed oil. This purgative effect has been directed to cure digestive system symptoms i.e. diarrhoea, dysentery, vomiting, retching and stomachache. Contrast to its purgative effect, Lioglier (1990) reported that seeds of *Jatropha* species are highly toxic and advised that they not be used in herbal medicine. Besides seed oil, leaf of some *Jatropha* species also have similar purgative effect. The leaf of *J. integerrima* is reported to possess a high purgative effect which provoked vomiting and caused dehydration that its consumption as herbal medicine was not advised (Mongkolvisut *et al*., 2006). In addition, some parts of *Jatropha* plants are employed to heal skin-related

ailments. The seed oil, latex, leaf, stem bark or root of *Jatropha* plants are pounded and applied on infected skin i.e. eczema, itches, carbuncles, mouth blisters, wounds and swelling (Kirtikar and Basu, 1980; Banerji *et al*., 1993; Burkill, 1994; Heller,1996). They are also believed to cure venereal diseases and urinarydischarge (Kirtikar and Basu, 1980; Banerji *et al*., 1993). The roots of *J. gossypiifolia* and *J. multifida* have long been applied on people suffering from leprosy and gonorrhea, respectively (Kirtikar and Basu, 1980; Burkill, 1994). Such usage suggested that *Jatropha* plants may contain compounds with antimicrobial properties.

Medicinal uses of *Jatropha* species

Species	Parts	Medicinal uses	References
J. curcas	Whole	Wounds; allergies; burns; cuts	Heller(1996), Kaushik and Kumar (2004)
J. elliptica	Whole	Abdominal complaints	Flores and Ricalde (1996)
J. glandulifera	Leaf	Asthma; bronchitis; as analgesic; emmenagogue; scorpion-sting	Nayak and Patel (2009)
J. gossypifolia	Leaf	Constipation; vertigo; diarrhea; purgative; skin diseases; mouth blister; cancer Stomachache; eczema; carbuncles; itches; swelling; venereal disease; blood purifier	Burkill (1994) Banerji *et al*. (1993)
J. integerrima	Leaf	Purgative	Mongkolvisut *et al*. (2006)
J. multifida	Bark/leaf	Neurodermatitis; eczema; itches	Shu *et al*. (2008)
J. podagrica	Whole	Antibiotic; tumor; insect antifeedant	Aiyelaagbe and Gloer (2008)

2.3 Biological activity of *Jatropha* species

J.curcas

Simultaneous administration of the methanolic fraction at doses 100 and 200 mg/kg, p.o significantly inhibited the metastatic colony formation of the melanoma in lungs by 47.54 and 69.52%, respectively, with increase in the

survival rate of the metastatic tumor bearing animals, as compared to the untreated animal (Balaji *et al*., 2009). The crude ethanolic, methanolic and water extracts of the stem bark inhibited the growth of *Staphylococcus aureus, S. epidermidis, Pseudomonas aeruginosa, Escherichia coli, Streptococcus faecalis, Shigella dysentriae, Micrococcus kristinae, Klebsiella pneumonia, Bacillus cereus, B. subtilis, Proteus vulgaris* and *Serratia marcescens* (Igbinosa *et al*., 2009). The latex significantly reduce the clotting time of human blood. The blood diluted with latex did not clot at all even at high dilutions and prolonged clotting time (Osoniyi and Onajobi, 2003). The methanolic extract of the roots exhibited systemic and significant anti-inflammatory activity in acute carrageenan-induced rat paw edema (Mujumdar and Misar, 2004). The methanolic extract of the leaves assayed on pyrolus ligation and aspirin-induced gastric ulcers in Wistar rats displayed counter-action to gastric lesions indicating antiulcer activity Kannappan *et al*. (2008).

J. multifida

The hexane, ethyl acetate, chloroform and methanol extracts of the roots of *J. multifida* yellow root bark, red root bark and root wood effectively inhibited the growth of *B. subtilis* and *S. aures* at concentration of 200 µg/disk (Aiyelaagbe, 2000). The leaf exudates afforded acceleration of wound healing process on injuries made in rats which displayed maturing in that area Buch *et al*. (2008).

J. gossypiifolia

The alcoholic extract of the roots of *J. gossypiifolia* showed significant inhibitory activity in vitro against cancer cells derived from human carcinoma of the nasopharynx and *in vivo* against four standards of animal tumor systems Kupchan *et al*. (1970). The methanolic and chloroform extracts of the leaves showed activity against *S. typhi, S. aureus, P. aeroginosa* and *C. albicans* Ogundare (2007). Oral administration of the methanolic and petroleum ether extracts of dried aerial parts at doses of 100 and 200 mg/kg/day of body weight to healthy animal reduced the carrageenan-induced paw edema in rats. The methanolic extract exhibited significant activity than petroleum ether extract in

the treatment of pain and inflammatory Panda *et al*. (2009a). Potential hepatoprotective action against carbon tetrachloride induced hepatic damage in rats. The petroleum ether extract was shown to possess maximum protectivity and methanolic extract showed the minimum activity (Panda *et al*., 2009b).

2.4 Secondary metabolites in plants

Natural antioxidants are powerful substances that rare capable of scavenging ROS or neutralize free radicals before they damage the body's cells. Phenolic compounds are made up of a group of secondary metabolites, which are synthesized by plants as a result of a plant's adaptation to biotic and abiotic stresses. In recent years, phenolic compounds have attracted the interest of researchers because of their antioxidant capacity; to protect the human body from free radicals, whose formation has been associated with the natural metabolism of aerobic cells. The antioxidant activity of phenolics is mainly due to their redox properties, which gives them the capacity to act as reducing agents, hydrogen donors, metal chelators, free radical scavenger and singlet oxygen quenchers. The antiradical activity of flavonoids and phenols is majorly based on the structural relationship between the functional groups on their chemical structure (Dauq *et al*., 2011).

Polyphenols are a wide and complex group of secondary plant metabolites which are essential for the physiology of plants, having functions in growth, structure, pigmentation, pollination, allelopathy, and resistance for pathogens and predators (Harborne, 1986; Bravo, 1998; Manach *et al*., 2004). Polyphenols have attracted the interest of the researchers because of their antioxidant capacity. They have long been recognised to possess anti-allergic, anti-inflammatory, antiviral and anti-proliferative, anticarcinogenic and antioxidant (Harborne, 1994; Frankel *et al*., 1993).

2.5 *In vitro* antioxidant scavenging assays

The scavenging assays have been extensively studied as models for the peroxidative damage in biomembrane. A number of methods have been developed to measure the efficiency of antioxidants. These methods focus on different mechanisms of the antioxidant defense system.

2.5.1 DPPH radical scavenging assay

DPPH· assay is considered as a valid and easy assay to evaluate radical scavenging activity of antioxidants, since the radical compound is stable and does not have to be generated as in other radical scavenging assays. In DPPH assay, the antioxidants were able to reduce the stable radical DPPH to the yellow coloured diphenyl picrylhydrazine. This method is based on the reduction of DPPH in alcoholic solution in the presence of a hydrogen donating antioxidant due to the formation of the non radical form DPPH-H in the reaction. DPPH is usually used as a reagent to evaluate free radical scavenging activity of antioxidants (Oyaizu, 1986).

2.5.2 ABTS radical scavenging assay

$ABTS^{\bullet+}$ radicals are more reactive than DPPH radicals and unlike the reactions with DPPH radical, which involve H-atom transfer, the reactions with $ABTS^{\bullet+}$ radicals involve an electron transfer process. Generation of the ABTS radical cation forms the basis of one of the spectrophotometric methods that have been applied to the measurement of the total antioxidant activity of pure substances, aqueous mixtures and beverages (Miller 1996). A more appropriate format for the assay is a decolorization technique, in which the radical is generated directly in a stable form prior to reaction with putative antioxidants. The improved technique for the generation of $ABTS^{\bullet+}$ described here involves the direct production of the blue/green $ABTS^{\bullet+}$ chromophore through the reaction between ABTS and potassium persulfate. $ABTS^{\bullet-}$, the oxidant, was generated by potassium persulfate oxidation of $ABTS^{\bullet+}$ and the radical cation is measured

spectrophotometrically. This is a direct generation of a stable form of radical to create a blue green $ABTS^{\bullet+}$ chromophore prior to the reaction with antioxidant (Donald and Wicks, 2006). Bleaching of a preformed solution of the blue green radical cation $ABTS^{\bullet+}$ has been extensively used to evaluate the antioxidant capacity of complex mixtures and individual compounds. The reaction of the preformed radical with free radical scavengers can be easily monitored by following the decrease of the sample absorbance at 734 nm.

2.5.3 DMPD radicals scavenging assay

The principle of $DMPD^{\bullet+}$ assay is that at acidic pH and in the presence of a suitable oxidant solution, DMPD can form a stable and coloured radical cation ($DMPD^{\bullet+}$). The UV visible spectrum of $DMPD^{\bullet+}$ shows a maximum absorbance at 505 nm. Antioxidant compounds which are able to transfer a hydrogen atom to $DMPD^{\bullet+}$ quench the color and produce a decolouration of the solution. This reaction is rapid and the end point, which is stable, is taken as a measure of the antioxidative efficiency. Therefore, this assay reflects the ability of radical hydrogen donors to scavenge the single electron from $DMPD^{\bullet+}$ (Apak *et al*., 2006).

2.5.4 Hydroxy radical scavenging assay

The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells (Hochestein and Atallah, 1988). This radical has the capacity to join nucleotides in DNA and cause strand breakage which contributes to carcinogenesis, mutagenesis and cytotoxicity. Hydroxyl radical scavenging capacity of an extract is directly related to its antioxidant activity (Babu *et al*., 2001). The highly reactive hydroxyl radicals can cause oxidative damage to DNA, lipids and proteins. The removal of hydroxyl radical is therefore probably one of the most effective defences of a living body against various diseases.

2.5.5 Reducing power

The reduction capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Antioxidant compounds are able to donate electrons to reactive radicals, reducing them into more stable and unreactive species (Gulcin *et al.*, 2007). Antioxidant compounds reduce Fe^{3+}-ferricyanide complexes to the ferrous (Fe^{2+}) form. The prussian blue coloured complex is formed by adding $FeCl_3$ to the ferrous (Fe^{2+}) form. Therefore, the amount of reduction can be determined by measuring the formation of perl's prussian blue at 700 nm (Chung *et al.*, 2002). In this assay, the yellow colour of the test solution changes to green or blue depending on the reducing power of the antioxidant. A higher absorbance indicates higher ferric reducing power.

$$K_3Fe(CN)_6 \xrightarrow{\text{Reductant}} Fe(CN)_6^{4-}$$

$$Fe(CN)_6^{4-} + FeCl_3 \longrightarrow Fe_4[Fe(CN)_6]_3$$

2.5.6 Ferric reducing antioxidant power assay

The reducing capacity was investigated by measuring Fe^{3+}-Fe^{2+} conversion. The reducing capacity of a compound may serve as a significant indicator of its potential antioxdant activity (Meir *et al.*, 1995). The antioxidant activites of putative antioxidants have been attributed to various mechanisms such as prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued proton abstraction and radical scavenging (Diplock, 1997).

FRAP assay provides a simple and effective method for measuring the ability of antioxidants in plant samples to act as reducing agents. The ferric reducing ability of plasma (FRAP) assay uses antioxidants as reductants in a redox linked colorimetric reaction, reducing a ferric-tripyridyltriazine (Fe (III)-TPTZ) complex to the ferrous, Fe(II) form (Benzie and Strain, 1996), forming an intense blue colour complex which can be measured colorimetrically.

2.5.7 CUPRAC assay

The CUPRAC assay was developed for a reducing power assay. This method is simultaneously cost effective, rapid, stable, selective and suitable for a variety of antioxidants regardless of chemical type or hydrophilicity. Cuprac chromogenic redox reaction is carried out at a pH (7.0) close to the physiological pH and the method is capable of measuring thiol type antioxidants such as glutathione and non protein thiols, unlike the widely applied FRAP test, which is non responsive to -SH group antioxidants (Apak *et al*., 2006).

2.5.8 Lipid peroxidation inhibition assay

Lipid peroxidation, which has been reported as an important event in cellular damage, is a typical free radical oxidation and is strongly associated with aging, carcinogenesis and other diseases (Halliwell *et al*., 1992). Thus, testing the content of malondialdehyde (MDA) not only can reflect the lipid peroxidation extent directly but also can reflect the cellular damage extent indirectly. This model has been used extensively for the study of lipid peroxidation *in vitro*. Fe^{2+}-Vitamin C, a common free radical inducer, could increase the content of MDA when adding into a homogenate of liver of rats (Li *et al*., 2006).

Initiation of lipid peroxidation by ferrous sulphate takes place either through ferryl-perferryl complex or through hydroxyl radical by Fenton's reaction. The inhibition could be caused by absence of ferryl-perferryl complex or by scavenging the hydroxy radical or the superoxide radicals or by changing the Fe^{3+}/Fe^{2+} or by reducing the rate of conversion of ferrous to ferric or by chelating the iron itself. Iron catalyses the generation of hydroxyl radical from hydrogen peroxide and superoxide radicals. The hydroxy radical is highly reactive and can damage biological molecules, when it reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids, lipid hydroperoxides produced (Valentao *et al*., 2002). Lipid hydroperoxide can be decomposed to produce alkoxy and peroxy radical they eventually yield

numerous carbonyl products such as malondialdehyde (MDA). The carbonyl products are responsible for DNA damage, generation of cancer and aging related diseases (Okhawa *et al*., 1979). Thus the decrease in the MDA level with increase in the concentration of the extracts indicates the role of the extracts as an antioxidant.

2.5.9 β-carotene linoleic acid assay

This is one of the rapid method to screen antioxidants, which is mainly based on the principle that linoleic acid, which is an unsaturated fatty acid, gets oxidized by ‘‘Reactive Oxygen Species’’ (ROS) produced by oxygenated water. This method is determined by measuring the inhibition of the production of volatile organic compounds and the formation of conjugated diene hydroperoxides due to linoleic acid oxidation, which bleach the β-carotene in the emulsion. The reaction mechanism involves the bleaching of carotenoids via heat-induced oxidation and the resultant discolouration being inhibited or diminished by antioxidants that donate hydrogen atoms to quench radicals. Absorbance of β-carotene is measured at 470 nm (Ndhlala *et al*., 2010). Thus, the reaction mechanism can be described as follows (Kaur and Geetha, 2006):

β-carotene–H(orange) + ROOH $\longrightarrow$ β-carotene$^{\cdot}$(discoloured) + ROOH

β-carotene–H(orange) + ROOH + AH $\longrightarrow$ β-carotene-H(orange) + ROOH + A$^{\cdot}$

2.5.10 Phosphomolybdenum assay

Total antioxidant capacity assay is a spectroscopic method for the quantitative determination of antioxidant capacity, through the formation of phosphomolybdenum complex. The phosphomolybdenum method is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds and the formation of green phosphate/Mo (V) complex with the maximal absorption at 695 nm. The assay being simple and independent of other antioxidant measurements commonly employed, its application was extended to plant polyphenols (Prieto *et al*., 1999). Higher absorbance indicates a higher antioxidative activity.

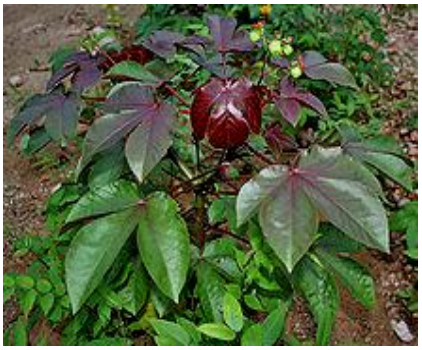
Mo(VI)(yellow) + e^{-} $\longrightarrow$ Mo(V)(blue)

2.6 *In vitro* antioxidant activity of *Jatropha*

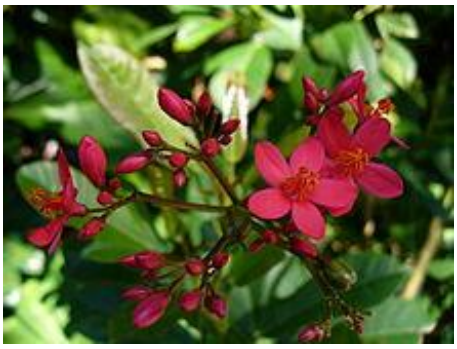
Antioxidant activity of Jatropha plants was shown by *J. gaumeri*,*J. macrantha* and *J. unicostata* assayed using DPPH radical and β-carotene bleaching.. Mothana (2011) reported that the methanol extract of *J.unicostata* showed a total antioxidant activity of 43.8%. Desmarchelier *et al*. (1997) reported the antioxidant activity of the methanol and dichloromethane extracts of *J. macrantha* roots by the quenching of luminal-enhanced chemiluminance. The methanol extract of the leaves of *J. gaumeri* showed promising antioxidant activity (Sãnchez-Medina *et al*., 2001).

The antioxidant capacity of crude extract, ethyl acetate, dichloromethane, butanolic fractions of *Jatropha isabellei* was evaluated by the DPPH method and inhibition of thiobarbituric acid reactive substances (TBARS). All fractions demonstrated antioxidant capacity against the DPPH radical and LPO inhibition. These fractions also showed the presence of phenolics, flavonoids and condensed tannins, exhibiting a positive relation between the phenolic content and antioxidant activity (Fröhlich *et al*., 2012). Antioxidant properties of free and bound phenolic extract of the leaves of *Jatropha tanjorensis in vitro* demonstrated potent but dose-dependent free radical scavenging activity against hydroxyl, DPPH radicals, Fe(II)-chelating and ferric reducing properties, inhibitory effect against lipid peroxidation induced by Fe^{2+} in rat's brain and liver.

J.curcas



J.gossypifolia



J.integerrima

J.multifida

J.tanjorensis

J.podagrica

J.villosa

3.0 MATERIALS AND METHODS

The present investigation entitled **"Phytochemical and antioxidant potential of *Jatropha* species**" was executed as follows:

3.1 Collection of sample

The leaves of *J. carcus*, *J. tanjorensis*, *J. podagrica*, *J. multifida*, *J. integerrima*, *J. gossypiifolia* and *J. villosa* were collected from Forest College and Research Institute, Tamil Nadu Agricultural University, Coimbatore.

3.2 Preparation of the extract

The leaves of different species of *Jatropha* were dried for 24 h at 70° C and powdered. The dried samples were extracted with ethanol and water. 1 g of the sample was extracted with 10 ml of corresponding solvent at 25°C for 24 h in a shaker. Filtered the extracts through Whatman No.4 filter paper. The residues were re-extracted with two additional 10 ml portions of the solvent, as described above. The combined extracts were evaporated under low temperature at 40° C in an incubator to dryness. The extract thus obtained was used directly for the estimation of bioactive constituents and also for the assessment of antioxidant activity through various *in vitro* assays.

3.3 *In vitro* scavenging assays

3.3.1 DPPH radical scavenging activity (Shimada *et al*., 1992)

Principle

DPPH radical is scavenged by antioxidants through the donation of a proton forming the reduced DPPH. The colour change from purple to yellow after reduction can be quantified by its decrease in absorbance at 517 nm.

Reagents

1. 0.2 mM DPPH
2. 80% Methanol
3. Butylated hydroxyl anisole

4. Ascorbic acid

Procedure

Different concentrations of plant extracts (2-10 mg/ml) (4.0 ml) were mixed with 1.0 ml of methanolic solution containing DPPH, resulting in the final concentration of DPPH being 0.2 mM. The mixture was shaken vigorously, left for 30 min, at room temperature and the absorbance was measured at 517 nm. Ascorbic acid and BHA (0.02-0.10 mg/ml) were used as positive controls. The DPPH radical scavenging activity was calculated as follows:

Scavenging activity = $[(A_0-A_1/A_0)] \times 100$, Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract.

EC_{50} value (mg extract/ml) was the effective concentration at which DPPH radicals were scavenged by 50% and were obtained by interpolation from linear regression analysis.

3.3.2 ABTS radical scavenging activity (Re *et al*., 1999)

Principle

ABTS decolourisation assay involves the generation of the $ABTS^+$ chromophore by the oxidation of ABTS with ammonium persulphate. It is applicable for both hydrophilic and lipophilic compounds. The scavenging activity of various polysaccharide extracts on ABTS radical cation were measured at 734 nm.

Reagents

1. 7 mM ABTS
2. 2.45 mM Ammonium per sulphate
3. ABTS solution: 7 mM of ABTS was mixed with 2.45 mM ammonium per sulphate and the mixture was allowed to stand in dark at room temperature for 12-16 h before use. $ABTS^+$ solution were diluted to an absorbance of 0.7 ± 0.05 with ethanol at 734 nm.
4. Ethanol

Procedure

The reaction was initiated by the addition of 1.0 ml of diluted $ABTS^{\cdot+}$ to 10 µl of different concentration of plant extracts (20-100 mg/ml) or 10 µl of methanol as control. The absorbance was read at 734 nm after 6 min and the percentage inhibition was calculated. Ascorbic acid and BHA (0.1-0.5 mg/ml) were used as positive controls. The inhibition was calculated according to the equation $I = A_0\text{-}A_1/A_0 \times 100$, where A_0 is the absorbance of control reaction, A_1 is the absorbance of test compound.

EC_{50} value (mg extract/ml) was the effective concentration at which ABTS radicals were scavenged by 50% and were obtained by interpolation from linear regression analysis.

3.3.3 DMPD scavenging activity (Fogliano *et al*., 1999, Gulcin, 2010)

Principle

In the presence of Fe^{3+}, a coloured DMPD radical cation is generated; antioxidant compounds transfers a hydrogen atom to $DMPD^{\bullet+}$ cause a decolouration of the solution measured by the decrease in absorbance at 505 nm.

Reagents

1. 0.1 M Acetate buffer, pH 5.3
2. 0.05 M Ferric chloride
3. 100 mM $DMPD^{\bullet+}$ solution : 20.9 mg of $DMPD^{\bullet+}$ was dissolved in 1.0 ml of deionised water and 1 ml of this solution was added to 100 ml of 0.1M acetate buffer (pH 5.3), and the colored radical cation ($DMPD^{\bullet+}$) was obtained by adding 0.2 ml of a solution of 0.05 M ferric chloride. Prepared fresh daily is constant up to 12 h at room temperature.

Procedure

Different concentrations of plant extracts (4-20 mg/ml) were added in test tubes and the total volume were adjusted with distilled water to 0.5 ml. 10 min later, the absorbance was measured at 505 nm. 1.0 ml of $DMPD^{\bullet+}$ solution were directly added to reaction mixture and its absorbance at 505 nm was measured. Ascorbic acid and BHA (0.04-0.20 mg/ml) were used as positive controls. The

buffer solution was used as a blank sample. The $DMPD^{\bullet+}$ scavenging activity was calculated using the following equation: scavenging activity (%) = (A_0- A_1/A_0) x 100, where A_0 is the absorbance of the initial concentration of $DMPD^{\bullet+}$, A_1 is the absorbance of the remaining concentration of $DMPD^{\bullet+}$ in the presence of the extract.

EC_{50} value (mg extract/ml) was the effective concentration at which DMPD radicals were scavenged by 50% and were obtained by interpolation from linear regression analysis.

3.3.4 Hydroxy radical scavenging assay (Smirnoff and Cumbes, 1989)

Principle

Hydroxy radicals were generated from $FeSO_4$ and hydrogen peroxide and detected by their ability to hydroxylate salicylate and the hydroxylated salicylate complex was measured at 562 nm.

Reagents

1. 1.5 mM Ferrous sulphate
2. 6 mM Hydrogen peroxide
3. 20 mM Sodium salicylate

Procedure

The reaction mixture 3.0 ml contained 1.0 ml of 1.5 mM $FeSO_4$, 0.7 ml of 6 mM hydrogen peroxide, 0.3 ml of 20 mM sodium salicylate and different concentrations (2.0-10 mg/ml) of plant extracts. After incubation for 1 h at 37°C, the absorbance of the hydroxylated salicylate complex was measured at 562 nm. Ascorbic acid and BHT (0.10-0.50 mg/ml) were used as positive controls. The percentage scavenging effect was calculated as, scavenging activity = [1-(A_1-A_2) / A_0] x 100, where A_0 is absorbance of the control (without extract) and A_1 was the absorbance in the presence of the extract, A_2 was the absorbance without sodium salicylate.

EC_{50} value (mg extract/ml) was the effective concentration at which hydroxyl radicals were scavenged by 50% and were obtained by interpolation from linear regression analysis.

3.3.5 Reducing power assay (Oyaizu, 1986)

Principle

Reducing power was measured by direct electron donation in the reduction of $Fe^{3+}(CN^-)_6$ to $Fe^{2+}(CN^-)_6$. The product was visualized by the addition of free Fe^{3+} ions after the reduction reaction, by forming an intense prussian blue colour complex, $Fe_4{}^{3+}[Fe^{2+}(CN^-)_6]_3$ and quantified by measuring the absorbance at 700 nm.

Reagents

1. 0.2 M Phosphate buffer, pH 6.6
2. 1% Potassium ferricyanide
3. 10% TCA
4. 0.1% Ferric chloride

Procedure

The reaction mixture contained 2.5 ml of different concentrations of plant extracts (2.0-10 mg/ml), 2.5 ml of 1% potassium ferricyanide and 2.5 ml of 0.2 M sodium phosphate buffer. The control contained all the reagents except the sample. The mixture was incubated at 50°C for 20 min and was terminated by the addition of 2.5 ml of 10% (w/v) of trichloroacetic acid, followed by centrifugation at 3000 rpm for 10 min. 5.0 ml of the supernatant was mixed with 5.0 ml of deionized water and 1.0 ml of 0.1% ferric chloride. The absorbance was measured at 700 nm against blank that contained distilled water and phosphate buffer. Increase in absorbance indicates increased reducing power of the sample. Ascorbic acid and BHA (0.2-1.0 mg/ml) were used as positive controls.

EC_{50} value (mg extract/ml) was the effective concentration at which the absorbance is 0.5 for reducing power and was obtained by interpolation from linear regression analysis.

3.3.6 FRAP assay (Benzie and Strain, 1996)

Principle

The total antioxidant potential of sample was determined using ferric reducing ability of FRAP assay as a measure of antioxidant power. FRAP assay measures the change in absorbance at 593 nm owing to the formation of a blue coloured Fe II-tripyridyl triazine compound from colourless oxidized Fe III form by the action of electron donating antioxidants.

Reagents

1. 10 mM 2, 4, 6-tripyridyl-s-triazine (TPTZ)
2. 40 mM HCl
3. 20 mm Ferric chloride
4. 0.3 M Acetate buffer, pH 3.6
5. Trolox
6. FRAP reagent: It contains 2.5 ml TPTZ solution, 2.5 ml ferric chloride solution and 25 ml acetate buffer. It was freshly prepared and warmed at 37°C.

Procedure

The stock solution of 10 mM 2, 4, 6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl, 20 mM $FeCl_3.6H_2O$ and 0.3 M acetate buffer (pH 3.6) were prepared. 900 µl FRAP reagent was mixed with 90 µl water and 30 µl different concentrations of plant extracts (4.0-20 mg/ml) /methanol/distilled water/standard antioxidant solution. The reaction mixture was then incubated at 37°C for 30 min and the absorbance was recorded at 595 nm. An intense blue color complex were formed when ferric tripyridyl triazine (Fe^{3+}-TPTZ) complex were reduced to ferrous (Fe^{2+}) form. The absorption at 540 nm was recorded. Ascorbic acid and BHA (0.02-0.10 mg/ml) were used as positive controls.

EC_{50} value (mg extract/ml) was the effective concentration at which the absorbance is 0.5 for ferrous ions reducing power and was obtained by interpolation from linear regression analysis.

3.3.7 CUPRAC assay (Apak *et al*., 2006; Karman *et al*., 2010)

Principle

The CUPRAC method has also been used to determine the reducing power of antioxidant compounds. This method is based on the reduction of Cu^{2+} to Cu^{+} by antioxidants in the presence of neocuproine.

Reagents

1. 10 mM $CuCl_2$
2. 7.5 mM ethanolic neocuproine
3. 1 M ammonium acetate buffer, pH 7.0

Procedure

0.25 ml $CuCl_2$ solution (0.01 M), 0.25 ml ethanolic neocuproine solution (7.5×10^{-3}M) and 0.25 ml CH_3COONH_4 buffer solution (1 M) were added to a test tube, followed by mixing with different concentrations of plant extracts (1.0-5.0 mg/ml). Then, total volume was adjusted to 2.0 ml with distilled water and thoroughly mixed. The tubes were stoppered and kept at room temperature. Absorbance was measured at 450 nm against a reagent blank after 30 min. Ascorbic acid and BHA (0.04-0.20 mg/ml) were used as positive controls. Increased absorbance of the reaction mixture indicates increased reduction capability.

EC_{50} value (mg extract/ml) was the effective concentration at which the absorbance is 0.5 for cupric ions reducing power and was obtained by interpolation from linear regression analysis.

3.3.8 P*hosphomolybdenum reducing antioxidant power (PRAP) assay* (Falcioni *et al*., 2002)

Principle

The sample is treated with phosphomolybdic acid to produce a greenish blue colour and the absorbance measured at 600 nm.

Reagents

1. 10% phosphomolybdic acid in ethanol
2. Quercetin

Procedure

Various concentrations of the plant extracts (300 µl) were mixed with 10 ml of 10% phosphomolybdic acid solution in ethanol (w/v). The solution was incubated at 80°C for 30 min and the absorbance was measured at 600 nm and compared to that of quercetin as the reference.

3.3.9 Phenanthroline assay (Szydłowska-Czerniaka *et al*., 2008)

Principle

The method is used to determine the reducing power of antioxidant compounds. This assay measures the change in absorbance at 510 nm based on the formation of ferrous-phenanthroline complex.

Fe(III)-1,10-phenanthroline + antioxidant → Fe(II)-1,10-phenanthroline + oxidized antioxidant

Reagents

1. 0.5% 1, 10-phenanthroline in methanol or acetone
2. 0.2% ferric chloride in methanol or acetone
3. BHT

Procedure

0.10 ml of various concentrations of the polysaccharide extracts and BHT, 0.50 ml of 0.2% ferric chloride solution and 0.25 ml of 0.5% 1, 10-phenanthroline solution were mixed and made up the volume with methanol to 5 ml. The reaction mixture was then incubated at 30°C in the dark for 20 min and the absorbance orange red solutions was measured at 510 nm against a reagent blank. Calibration curve was prepared using working solutions of ferrous sulfate in the range of 0.09–0.72 µmol/ml.

EC_{50} value (mg extract/ml) was the effective concentration at which the absorbance is 0.5 for iron reducing power and was obtained by interpolation from linear regression analysis.

3.3.10 Lipid peroxidation assay in rat liver homogenate (Yen and Hsieh, 1998; Banerjee *et al*., 2005)

Principle

Malondialdehyde has been identified as the product of lipid peroxidation that reacts with thiobarbituric acid to give a red colour absorbing at 535 nm.

Reagents

1. 0.5 mM $FeCl_2$
2. 0.5 mM H_2O_2
3. 20% TCA
4. 0.8 % TBA

Procedure

1.0 ml of different concentrations of plant extracts (2.0-10 mg/ml) was mixed with 1.0 ml of 1% liver homogenate, then 0.05 ml of 0.5 mM $FeCl_2$ and 0.5 mM H_2O_2 were added to initiate lipid peroxidation. After incubation at 37°C for 60 min, 1.5 ml of 20% TCA and 1.5 ml of 0.8% TBA solution (0.8%, w/v) were added to quench the reaction. The resulting mixture was heated at 100°C for 15 min and then centrifuged at 4000 rpm for 10 min. The absorbance of the upper layer was measured at 532 nm. Ascorbic acid and BHA (0.02-0.10 mg/ml) were used as positive controls. The inhibition effect on lipid peroxidation was calculated as follows: Inhibition effect (%) = $[1 – (A_1 - A_{2)} /A_0] \times 100$, where A_0 was the absorbance of the control (water instead of sample), A_1 is the absorbance of the sample, and A_2 was the absorbance of the sample only (water instead of liver homogenate).

EC_{50} value (mg extract/ml) was the effective concentration at which liver lipid peroxidation inhibition by 50% and was obtained by interpolation from linear regression analysis.

3.3.11 Lipid peroxidation inhibition assay in egg homogenate (Ruberto *et al*., 2000)

Principle

A modified thiobarbituric acid reactive species (TBARS) assay was used to measure the lipid peroxide formed, using egg yolk homogenates as lipid rich media. Malondialdehyde (MDA), a secondary end product of the oxidation of polyunsaturated fatty acids reacts with two molecules of thiobarbituric acid (TBA) yielding a pinkish red chromogen with an absorbance maximum at 532 nm.

Reagents

1. 0.8% TBA
2. $FeSO_4$ (0.07 M)
3. 20% acetic acid (pH adjusted to 3.5 with NaOH)
4. 20% TCA
5. n-Butanol
6. 10 % Egg homognate

Procedure

0.5 ml 10% egg homogenate and 0.1 ml of different concentrations of plant extracts (2.0-10 mg/ml) were mixed in a test tube and the volume was made up to 1.0 ml by adding distilled water. Finally 0.05 ml $FeSO_4$ (0.07 M) was added to the above mixture and incubated for 30 min to induce lipid peroxidation. Thereafter, 1.5 ml of 20% acetic acid (pH adjusted to 3.5 with NaOH) and 1.5 ml of 0.8% TBA (prepared in 1.1% sodium dodecyl sulphate) and 0.05 ml 20% TCA were added, vortexed and then heated in a boiling water bath for 60 min. After cooling, 5.0 ml of n-butanol was added to each tube and centrifuged at 3000 rpm for 10 min. The absorbance of the organic upper layer was measured at 532 nm. Ascorbic acid, trolox, BHT and quercetin (0.02-0.10 mg/ml) were used as positive controls.

3.3.12 β-Carotene bleaching assay (Yae *et al*., 2003)

Principle

The antioxidant activity of the extract was measured by the bleaching of the β-carotene linoleate system and the absorbance was measured at 470 nm.

Reagents

1. β-carotene
2. Tween 80
3. Linoleic acid

Procedure

A solution of β-carotene was prepared by dissolving 2 mg of β-carotene in 10 ml of chloroform. 2.0 ml of this solution were pipetted into a 100 ml round bottom flask. After the chloroform was removed at 40°C under vacuum, 40 mg of linoleic acid, 400 mg of Tween 80 emulsifier and 100 ml of distilled water were added to the flask with vigorous shaking. 4.8 ml of this emulsion were transferred into different test tubes containing 0.2 ml of different concentrations of plant extracts (4.0-20.0 mg/ml). The tubes were shaken and incubated at 50°C in a water bath. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm. A blank, devoid of β-carotene, was prepared for background subtraction. β-carotene bleaching inhibition was calculated using the following equation: (β-carotene content after 2 h of assay/initial β-carotene content) x 100. EDTA (0.2-1.0 mg/ml) were used as positive controls.

EC_{50} value (mg extract/ml) was the effective concentration at which β-carotene bleaching inhibition by 50% and was obtained by interpolation from linear regression analysis.

3.3.13 Phosphomolybdenum assay (Prieto *et al*., 1999)

Principle

The assay is based on the reduction of Mo(VI)-Mo(V) by the extract and subsequent formation of green phosphate/Mo(V) complex at acidic pH. The absorbance of the solution was measured at 695 nm against a blank.

Reagents

1. 0.6 M Sulphuric acid
2. 28 mM Sodium phosphate
3. 4 mM Ammonium molybdate

Procedure

0.1 ml of plant extracts were dissolved in water was taken in screw capped tubes and added 1.0 ml of the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a thermal block at 95°C for 90 min. After cooling to room temperature, the absorbance of the aqueous solution in each tube was measured at 695 nm against a blank. Ascorbic acid and gallic acid were used as standards and the total antioxidant capacity is expressed as equivalents of ascorbic acid (AAE) or gallic acid (GAE).

3.4 Bioactive components

3.4.1 Assay of total phenolics (Singleton and Rossi, 1965)

Principle

The phenolic compounds are oxidized to phenolates by the folin ciocalteau reagent at alkaline pH in a saturated solution of sodium carbonate resulting in a blue molybdenum-tungstate complex. The colour developed is measured at 650 nm against a reagent blank.

Reagents

1. 35% Sodium carbonate
2. Folin Ciocalteau's reagent
3. Gallic acid (0.01-0.1 mM)

Procedure

1.0 ml of plant extracts was mixed with 1.0 ml of folin ciocalteau's phenol reagent. After 3 min, 1.0 ml of saturated Na_2CO_3 (~35%) was added to the mixture and made up to 10 ml by adding distilled water. The reaction was kept in the dark for 90 min, after which its absorbance were read at 725 nm. A calibration curve was constructed with different concentrations of gallic acid,

caffeic acid, ferrulic ac``id and catechol. The results were expressed as mg of gallic acid (GAE), caffeic acid (CAE), ferrulic acid (FAE) and catechol equivalents (CE) per g of extract.

3.4.2 Assay of total flavonoids (Jia *et al*., 1999)

Principle

The sample was mixed with a reagent containing aluminium chloride and sodium nitrite and pink coloured flavanoid aluminium complex was formed in alkaline medium and the coloured complex can be estimated calorimetrically at 510 nm against a reagent blank.

Reagents

1. 5% Sodium nitrite
2. 10% Aluminium chloride
3. 1 M Sodium hydroxide
4. Stock standard: 10 mg of catechin or rutin/10 ml of distilled water.

Procedure

Added 0.5 ml of plant extracts into a test tube containing 1.25 ml of distilled water. Then added 0.075 ml of 5% sodium nitrite solution and allowed to stand for 5 min. Added 0.15 ml of 10% aluminium chloride, after 6 min, 0.5 ml of 1.0 M sodium hydroxide were added and the mixture were diluted with another 0.275 ml of distilled water. The absorbance of the mixture at 510 nm was measured immediately. The flavonoid content was expressed as milligram of catechin (CE), quercetin (QE) and rutin equivalents (RE) per g extracts.

3.4.3 Assay of total condensed tannins (Sun *et al*., 1998)

Principle

The vanillin reagent will react with any phenol that has an unsubstituted resorcinol or phloroglucinol nucleus and forms a coloured substituted product which is measured at 500 nm.

Reagents

1. 1% Vanillin reagent: Mixed vanillin in glacial acetic acid: HCl solution (92:8).
2. Catechin stock standard: 1 mg/ml in ethanol.

Procedure

To 0.2 ml of plant extracts, 1.0 ml of freshly prepared vanillin reagent was added. After incubation for 20 min at 30°C, the absorbance was measured at 500 nm against a reagent blank. Catechin ranging from 50 to 250 µg/ml were used for the preparation of the calibration curve The results are expressed as mg catechin equivalents (CAE) per g of the extract.

3.4.4 Assay of total phenolic acids (Szaufer-Hajdrych, 2004)

Principle

Total phenolic acid estimation was carried out according to the Arnov method.

Reagents

1. Arnov reagent (10 g sodium molybdate and 10 g sodium nitrite dissolved in 100 ml of distilled water).
2. 1 M NaOH
3. 0.5 M HCl
4. Caffeic acid stock standard: 1 mg/ml in methanol.

Procedure

1.0 ml of the plant extracts was mixed with 5.0 ml of distilled water, 1.0 ml 0.5 M HCl, 1.0 ml of Arnov reagent and 1.0 ml 1 M NaOH and made up the volume to 10 ml with distilled water. The absorbance was measured at 490 nm. Caffeic acid ranging from 40 to 200 µg/ml were used for the preparation of the calibration curve. The total phenolic acid content was expressed as caffeic acid equivalent (CAE) per g of the extract.

3.5 Statistical analysis`

Each experiments were performed in triplicate. The data are reported as the mean ± standard deviation and were analysed by SPSS (version 17.0 SPSS Inc.). ANOVA and Student's t-test were performed. Significant differences between the means were determined by Duncan's Multiple Range test. $P<0.05$ was considered significant.

EC_{50} values was calculated by linear regression analysis and correlation (r) between phenol, flavonoid and various *in vitro* scavenging assays was calculated using free statistical regression calculator (http://easycalculation.com/statistics/regression.php).

4.0 RESULTS AND DISCUSSION

The present investigation entitled **"Phytochemical and antioxidant potential of *Jatropha* species"** was discussed under the following headings:

4.1 *In vitro* antioxidant scavenging activity

The various polysaccharide extracts were investigated for antioxidant activity by several methods *in vitro*, such as DPPH, ABTS, DMPD and OH radical scavenging assay, reducing power, FRAP, CUPRAC, PRAP, phenanthroline assay, lipid peroxidation inhibition assay, β-carotene bleaching assay and phosphomolydenum assay.

4.1.1 Scavenging effect on DPPH

The DPPH free radical is a stable free radical and can accept an electron or hydrogen radical to become a stable diamagnetic molecule, which has been widely accepted as a tool for estimating the free-radical scavenging activities of antioxidants (Hu *et al*., 2004). Alcoholic solutions of DPPH have a characteristic absorption maximum at 517 nm. The method of scavenging DPPH is based on the reduction of DPPH ethanol solution in the presence of a hydrogen donating antioxidant, resulting in the formation of the non-radical form DPPH-H by the reaction (Li *et al*., 2007). It can accommodate many samples in a short period and is sensitive enough to detect active ingredients at low concentrations (Sanchez-Moreno, 2002).

Table 1 and Fig. 1 showed that ethanolic and aqueous extracts of *Jatropha* species exhibited dose dependent DPPH radical scavenging activities. At concentrations of 0.5-2.5 mg/ml, the scavenging abilities of ethanolic extracts of *J.carcus*, *J.tanjorensis*, *J.podagrica*, *J.multifida*, *J.integerrima*, *J.gossypiifolia* and *J.villosa* on DPPH radicals were between 87.99-99.77%, 84.11-90.45% 18.34-96.00%, 25.79-91.09%, 28.46-98.35%, 84.12-93.97% and 93.79-96.00%. The scavenging abilities of aqueous extracts were between 27.29-55.00%, 1.48-18.59%, 4.67-80.07%, 7.34-30.79%, 3.25-20.55% and 1.97-36.34%. respectively

at 1-5 mg ml^{-1}. EC_{50} values of the DPPH radical scavenging activity of ethanolic and aqueous extracts ranged from 0.15 to 1.04 and 1.36 to 7.09 mg/ml respectively.

At 0.02-0.10 mg/ml, the radical scavenging ability of positive controls ascorbic acid and BHA were between 89.69-94.77% and 89.76-95.72% respectively. Positive controls exhibited higher free radical scavenging activity than those of plant extracts.

A significant difference ($p<0.05$) in DPPH radical scavenging activity was observed with different sample concentrations and between various *Jatropha* species. This radical scavenging activity of various extracts could be related to the nature of phenolics, thus contributing to their electron transfer/hydrogen donating ability.

4.1.2 $ABTS^{\bullet+}$ radical scavenging activity

$ABTS^{\bullet+}$ radicals are more reactive than DPPH radicals and unlike the reactions with DPPH radical, which involve H-atom transfer, the reactions with $ABTS^{\bullet+}$ radicals involve an electron transfer process. Generation of the ABTS radical cation forms the basis of one of the spectrophotometric methods that have been applied to the measurement of the total antioxidant activity of pure substances, aqueous mixtures and beverages (Miller, 1996). A more appropriate format for the assay is a decolorization technique, in which the radical is generated directly in a stable form prior to reaction with putative antioxidants.

The improved technique for the generation of $ABTS^{\bullet+}$ described here involves the direct production of the blue/green $ABTS^{\bullet+}$ chromophore through the reaction between ABTS and potassium persulfate. Bleaching of a preformed solution of the blue green radical cation $ABTS^{\bullet+}$ has been extensively used to evaluate the antioxidant capacity of complex mixtures and individual compounds. The reaction of the preformed radical with free radical scavengers can be easily monitored by following the decrease of the sample absorbance at 734 nm.

As shown in Table 2 and Fig. 2 ethanolic and aqueous extracts of *Jatropha* species is an effective $ABTS^{\bullet+}$ radical scavenger in a concentration dependent manner. At concentrations of 1-5 mg/ml, the scavenging abilities of ethanolic extracts of *J.carcus*, *J.tanjorensis*, *J.podagrica*, *J.multifida*, *J.integerrima*, *J.gossypiifolia* and *J.villosa* on ABTS radicals were between 68.57-95.10%, 58.51-95.42%, 61.60-91.80%, 58.50-90.00%, 58.51-93.83%, 58.61-98.12% and 48.69-92.66%. The scavenging abilities of aqueous extracts were between 69.37-98.50%, 67.60-97.10%, 64.70-95.80%, 70.70-92.70%, 67.62-95.81% and 69.60-94.53%. EC_{50} values of the ABTS radical scavenging activity of ethanolic and aqueous extracts ranged from 0.69 to 0.86 and 0.43 to 0.57 mg/ml respectively.

The ABTS radical scavenging ability of positive controls ascorbic acid and BHA were between 21.92- 95.32% and 61.25-96.78% at 0.1-0.5 mg/ml respectively.

A significant difference ($p<0.05$) in ABTS radical scavenging activity was observed with different sample concentrations and between various *Jatropha* species.

4.1.3 $DMPD^{\bullet+}$ radical scavenging activity

The principle of the $DMPD^{\bullet+}$ assay is that, DMPD can form a stable and coloured radical cation ($DMPD^{\bullet+}$) at acidic pH and in the presence of a suitable oxidant solution. The UV visible spectrum of $DMPD^{\bullet+}$ shows a maximum absorbance at 505 nm. Antioxidant compounds, which are able to transfer hydrogen atom to $DMPD^{\bullet+}$, quench the colour and produce a decolouration of the solution. This reaction is rapid, and the end point, which is stable, is taken as a measure of the antioxidative efficiency. Therefore, this assay reflects the ability of radical hydrogen donors to scavenge the single electron from $DMPD^{\bullet+}$ (Fogliano *et al*., 1999; Gulcin, 2008).

The ethanolic extracts of *J.carcus*, *J.tanjorensis*, *J.podagrica*, *J.multifida*, *J.integerrima*, *J.gossypiifolia* and *J.villosa* on were shown to scavenge the DMPD radicals to different extent over a concentration range of 0.1-0.5 mg ml^{-1}

with an inhibition percentage of 2.10-75.81%, 7.44-81.54%, 3.78-75.59%, 1.45-77.65%, 2.17-69.15%, 3.43-80.17% and 24.16-84.74% respectively. The aqueous extracts showed an inhibition percentage of 23.76-86.53%, 19.56-89.34%, 18.24-79.31%, 18.92-86.54%, 5.43-89.00% and 20.16-84.56% respectively (Table 3 and Fig. 3). EC_{50} values of the DMPD radical scavenging activity of ethanolic and aqueous extracts ranged from 0.25 to 0.32 and 0.23 to 0.33 mg/ml respectively.

At 0.04-0.20 mg/ml, the DMPD radical scavenging ability of positive controls ascorbic acid and BHA were between 20.67-82.09%, 65.58-83.48%, 38.42-69.59% and 26.54-83.19% respectively.

A significant difference ($p<0.05$) in ABTS radical scavenging activity was observed with different sample concentrations and between various *Jatropha* species.

4.1.4 Hydroxyl radical scavenging activity

The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells (Hochestein and Atallah, 1988). This radical has the capacity to join nucleotides in DNA and cause strand breakage which contributes to carcinogenesis, mutagenesis and cytotoxicity. Hydroxyl radical scavenging capacity of an extract is directly related to its antioxidant activity (Babu *et al*., 2001).

The hydroxyl radical, more likely to be produced *in vivo*, is considered to be the most reactive and poisonous free radical in organisms because it can nonspecifically oxidize all classes of biological macromolecules including lipids, proteins and nucleic acids (Ozyurek *et al*., 2008). Therefore, it is used extensively as the free radical to evaluate effectiveness of antioxidants.

Most hydroxyl radicals are produced from the decomposition of hydro peroxides (ROOH). In this experiment, hydroxyl radicals were generated from 2,3- dihydroxybenzoic acid, generated by sodium salicylate in this reaction

system. Table 4 and Fig. 4 showed that ethanolic and aqueous extracts of *Jatropha* species exhibited hydroxy radical scavenging activity in a dose dependent manner. At concentrations of 0.5-2.5 mg/ml, the scavenging abilities of ethanolic extracts of *J.carcus*, *J.tanjorensis*, *J.podagrica*, *J.multifida*, *J.integerrima*, *J.gossypiifolia* and *J.villosa* were between 47.85-.78.83%, 8.73-73.92%, 16.25-98.46%, 14.11-85.88%, 30.67-93.55, 11.34-96.62% and 32.82-92.63% respectively. EC_{50} values of hydroxyl radical scavenging abilities of various polysaccharide extracts ranged from 0.63 to 6.31 mg/ml. The scavenging abilities of aqueous extracts were between 63.19-96.01%, 11.23-84.96%, 24.53-92.33%, 19.63-83.74%, 30.67-93.55% and 10.12-76.07%. respectively at 1-5 mg ml^{-1}. EC_{50} values of the OH radical scavenging activity of ethanolic and aqueous extracts ranged from 0.71to 1.67 and 0.26 to 1.63 mg/ml respectively.

The hydroxy radical scavenging ability of positive controls ascorbic acid and BHT were between 2.53-96.01% and 40.18- 95.09% respectively at 0.1-0.5 mg/ml.

A significant difference ($p<0.05$) in hydroxy radical scavenging activity was observed with different sample concentrations and between various *Jatropha* species. These results suggest that the extracts are capable of scavenging hydroxyl radicals and could help prevent or ameliorate oxidative damage.

The production of •OH is dependent on the content of Fe^{2+} and H_2O_2 according to the Fenton reaction. It was widely reported that the scavenging activity of hydroxyl radical of antioxidant was due to its inhibition of hydroxyl radical generation by chelating ions such as Fe^{2+} and Cu^{2+} (Wang *et al*., 2010) and the reaction of reductones in it with precursors of peroxide which thus prevented peroxide formation (Qi *et al*., 2005; Zhang *et al*., 2011).

4.1.5 Ferric cyanide (Fe^{3+}) reducing antioxidant power

The reduction capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Antioxidant compounds are able to donate electrons to reactive radicals, reducing them into more stable and unreactive

species (Gulçin *et al*., 2007). Antioxidant compounds reduce Fe^{3+} ferricyanide complexes to the ferrous (Fe^{2+}) form. The prussian blue coloured complex is formed by adding $FeCl_3$ to the ferrous (Fe^{2+}) form. Therefore, the amount of reduction can be determined by measuring the formation of Perl's Prussian blue at 700 nm (Chung *et al*., 2002). In this assay, the yellow colour of the test solution changes to green or blue depending on the reducing power of the antioxidant. A higher absorbance indicates higher ferric reducing power.

The antioxidant activity has been reported to have a direct, positive correlation between with the reducing power (Osman *et al*., 2004). The reducing properties are generally associated with the presence of reductones, which could donate a hydrogen atom and exert antioxidant action by breaking the free radical chain (Gordon, 1990). Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation. The antioxidant activity was concomitant with the reducing power (Duh *et al*., 1999). The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.

In order to elucidate the relationship between the antioxidant activity and the reducing power of various plant extracts, we investigated the Fe^{3+}-Fe^{2+} transformation in the presence of extracts. The ferric cyanide (Fe^{3+}) reducing antioxidant power of ethanolic and aqueous extracts of *Jatropha species* increased as the concentration increased from 0.4 to 2.0 mg/ml (Table 5 and Fig. 5). The reducing power of ethanolic extracts of *J.carcus*, *J.tanjorensis*, *J.podagrica*, *J.multifida*, *J.integerrima*, *J.gossypiifolia* and *J.villosa* were 0.313, 0.322, 0.116, 0.143, 0.135, 0.271 and 0.201 at 0.4 mg ml^{-1} and the extracts showed reducing power of 0.957, 0.982, 0.450, 0.680, 0.607, 0.642 and 0.811 at 2 mg ml^{-1}, respectively. The aqueous extracts showed an inhibition 0.126, 0.183, 0.057, 0.101, 0.082 and 0.148 at 0.4 mg ml^{-1} and the extracts showed reducing power of 0.680, 0.502, 0.133, 0.253, 0.233, and 0.330 at 2 mg ml^{-1}. The EC_{50} values of ethanolic and aqueous extracts in the ferric cyanide (Fe^{3+}) reducing antioxidant power assay ranged from 0.65 to 2.87 and 1.53 to 10.60 mg/ml respectively.

The ferric cyanide (Fe^{3+}) reducing antioxidant power of positive controls ascorbic acid and BHA were between 0.295 and 0.113 at 0.2 mg/ml and the extracts showed an excellent reducing power of 1.213 and 1.173 at 1.0 mg/ml respectively. All the tested samples presented much lower reducing powers than those of positive controls, which indicates that synthetic antioxidants have better reducing ability than the plant extracts.

A significant difference ($p<0.05$) in ferric cyanide reducing antioxidant power was observed with different sample concentrations and between various *Jatropha* species. The reducing properties of plant extracts are generally associated with the presence of reductones which could react with the radicals to stabilize and terminate free radical reactions.

The results reported in the present study are in agreement with the result previously reported by Yen and Duh (1993). They found that reducing power is associated with the antioxidant activity. Our data on reducing power of various plant extracts showed that the polysaccharides an act as electron donors and can react with free radicals to convert them to more stable products and thereby terminate radical chain reactions.

4.1.6 Ferric-reducing antioxidant power

The FRAP assay treats the antioxidants contained in the samples as reductants in a redox linked colorimetric reaction, and the value reflects the reducing power of antioxidants. The procedure is relatively simple and easy to standardise. This assay is also commonly used for the routine analysis of single antioxidant and total antioxidant activity of plant extracts (Xu *et al*., 2009). The antioxidant potentials of different samples were estimated by their ability to reduce the TPTZ-Fe(III) complex to the TPTZ-Fe(II) complex with an maximum absorption at 593 nm. The reduction of absorbance is proportional to the antioxidant content (Benzie and Strain, 1996).

The FRAP of various ethanolic and aqueous extracts of *Jatropha species* are shown in Table 6 and Fig. 6. The antioxidant capacities of all the samples correlated well with increasing concentration. The FRAP of ethanolic extracts of

J.carcus, *J.tanjorensis*, *J.podagrica*, *J.multifida*, *J.integerrima*, *J.gossypiifolia* and *J.villosa* on were between were between 0.348, 0.374, 0.282, 0.246, 0.236, 0.308 and 0.423 at 1 mg ml^{-1} and 0.786, 0.688, 0.706, 0.778, 0.883 and 0.988 at 5 mg ml^{-1} respectively. The aqueous extracts showed an inhibition 0.318, 0.347, 0.206, 0.267, 0.220 and 0.231 at 1 mg ml^{-1} and the extracts showed reducing power of 0.658, 0.618, 0.538, 0.508, 0.540, and 0.515 at 5 mg $ml^{-1.}$. The EC_{50} values of ethanolic and aqueous extracts ranged from 2.10 to 3.78 and 3.14 to 5.06 mg/ml respectively.

The FRAP assay of positive controls ascorbic acid and BHA were between 0.452 and 0.337 at 0.02 mg/ml and 1.316 and 0.987 at 0.10 mg/ml respectively.

A significant difference ($p<0.05$) in ferric reducing antioxidant power was observed with different sample concentrations and between various *Jatropha* species.

4.1.7 Cupric ion (Cu^{2+}) reducing CUPRAC assay

The CUPRAC method has also been used to determine the reducing power of antioxidant compounds (Apak *et al*., 2004). This method is based on the reduction of Cu^{2+} to Cu^{+} by antioxidants in the presence of neocuproine (Gulcin, 2008). In this assay, a higher absorbance indicates higher cupric ion (Cu^{2+}) reducing ability.

Cu^{2+} reducing capability of ethanolic and aqueous extracts of *Jatropha species* was found to be concentration dependent (1-5 mg/ml). At 1-5 mg ml^{-1}, cupric ion (Cu^{2+}) reducing ability of ethanolic extracts of *J.carcus*, *J.tanjorensis*, *J.podagrica*, *J.multifida*, *J.integerrima*, *J.gossypiifolia* and *J.villosa* were between 0.241-0.762, 0.352-0.930, 0.276-0.863, 0.297-0.779, 0.243-0.722 and 0.439-0.985 respectively (Table 7 and Fig.7). The aqueous extracts showed reducing ability between 0.499-0.592, 0.426-0.588, 0.225-0.608, 0.476-0.557, 0.394-0.546 and 0.454-0.590. The EC_{50} values of ethanolic and aqueous extracts the cupric ion (Cu^{2+}) reducing antioxidant power assay ranged from 0.10 to 1.89 and 1.41 to 4.23 mg/ml respectively.

At 0.02-0.10 mg/ml, cupric ion (Cu^{2+}) reducing ability of positive controls of ascorbic acid and BHA were between 0.475-1.632 and 0.344-0.824 respectively.

A statistically significant difference ($p<0.05$) in cupric ions reducing ability was observed with different sample concentrations and between various *Jatropha* species.

4.1.8 Phenanthroline assay

The Phenanthroline method is based on the ability of antioxidants to reduce Fe(III) to Fe(II). Reducing power of ethanolic and aqueous extracts of *Jatropha* species was investigated and showed in Table 8 and Fig. 8. The reducing capacities of ethanolic and aqueous extracts of *Jatropha* species increased with the increase of sample concentration. At the concentration of 1-5 mg/ml, the reducing capacity of ethanolic extracts of *J.carcus*, *J.tanjorensis*, *J.podagrica*, *J.multifida*, *J.integerrima*, *J.gossypiifolia* and *J.villosa* were 0.447, 0.569, 0.584, 0.562, 0.551, 0.677 and 0.590 at 1 mg ml^{-1} and the extracts showed reducing power of 0.608, 0.709, 0.726, 0.718, 0.710, 0.783 and 0.730 at 5 mg ml^{-1}. The aqueous extracts showed reducing power of 0.409, 0.496, 0.568, 0.468, 0.578 and 0.532 at 1 mg ml^{-1} and 0.543, 0.654, 0.692, 0.624, 0.722, and 0.718 at 5 mg ml^{-1}. The EC_{50} values of ethanolic and aqueous extracts phenanthroline assay ranged from 0.34 to 2.70 and 0.39 to 4.15 mg/ml respectively.

At 0.2-1.0 mg/ml, reducing ability of BHT was between 0.554-1.435. The EC_{50} values of various polysaccharide extracts in reducing Fe(III) ranged from 2.47 to 14.05 mg/ml.

A statistically significant difference ($p<0.05$) in iron reducing ability was observed with different sample concentrations and between various *Jatropha* species.

4.1.9 Phosphomolybdenum reducing antioxidant power (PRAP) assay

The sample is treated with phosphomolybdic acid to produce a greenish blue colour and the absorbance measured at 600 nm. The reduction of absorbance is proportional to the antioxidant content.

PRAP of ethanolic and aqueous extracts of *Jatropha* species are shown in Table 9 and Fig. 9. The reducing power increased with increase in concentration. The PRAP of ethanolic extracts of *J.carcus*, *J.tanjorensis*, *J.podagrica*, *J.multifida*, *J.integerrima*, *J.gossypiifolia* and *J.villosa* on 0.118, 0.199, 0.201, 0.186, 0.184, 0.183 and 0.221 at 1 mg ml^{-1} and the extracts showed reducing power of 0.641, 0.629, 0.737, 0.624, 0.657, 0.636 and 0.665 at 5 mg ml^{-1}. The aqueous extracts showed an inhibition 0.109, 0.086, 0.198, 0.098, 0.105 and 0.199 at 1 mg ml^{-1} and the extracts showed reducing power of 0.587, 0.542, 0.533, 0.478, 0.556, and 0.654 at 5 mg ml^{-1}. The EC_{50} values of ethanolic and aqueous extracts ranged from 3.17 to 3.98 and 3.58 to 5.05 mg/ml respectively.

The PRAP of positive controls of ascorbic acid was 0.411 at 0.06 mg/ml and 1.089 at 0.30 mg ml^{-1} respectively.

A significant difference ($p<0.05$) in reducing antioxidant power was observed between different sample concentrations and various *Jatropha* species.

4.1.10 Lipid peroxidation inhibition assay in egg yolk

Lipid peroxidation involves the formation and propagation of lipid radicals with numerous deleterious effects, including destruction of membrane lipids, metabolic disorders and inflammation, and production of malondialdehyde (MDA) is a hallmark of this process. Inhibition of lipid peroxidation was assessed by the amount of MDA produced. Lipids in egg yolk undergo rapid nonenzymatic peroxidation in the presence of ferrous sulphate. Various plant extracts showed a dose dependent lipid peroxidation inhibition.

The LPO inhibition in egg homogenate of ethanolic extracts of *J.carcus*, *J.tanjorensis*, *J.podagrica*, *J.multifida*, *J.integerrima*, *J.gossypiifolia* and *J.villosa* on Lipid peroxidation inhibition assay in egg yolk radicals were between 44.82-95.40%, 50.11-91.26%, 47.12-90.14%, 38.16-90.57%, 43.21-

92.41%, 28.73-88.50% and 37.24-87.35%. The LPO inhibition of aqueous extracts were between 81.87-98.22%, 82.20-97.08%, 79.12-98.38%, 75.72-97.89%, 77.66-98.05% and 77.18-96.60%. (Table 10 and Fig. 10). EC_{50} values of the LPO inhibition in egg homogenate of ethanolic and aqueous extracts ranged from 1.12 to 2.75 and 0.41 to 0.48 mg/ml respectively.

The egg yolk LPO inhibition of positive controls ascorbic acid and BHT were between 21.05 and 23.08 at 0.02 mg/ml and 68.21 and 55.90 at 0.10 mg/ml respectively..

A statistically significant difference ($p<0.05$) in LPO inhibition was observed with different sample concentrations and between *Jatropha* species.

4.1.11 Lipid peroxidation inhibition in rat liver homogenate

Lipid peroxidation, a process induced by free radicals, leads to oxidative deterioration of polyunsaturated lipids. LPO inactivates cellular components and there in plays a key role in oxidative stress in biological systems. Several toxic byproducts of LPO can damage other biomolecules, including DNA, although these biomolecules are distant to the site of their generation (Box and Maccubbin, 1997). Transition metal ions, such as iron and copper, are known to stimulate LPO through various mechanisms (Halliwell and Gutteridge, 1984). These metal ions may generate $OH^{\cdot}$ to initiate the LPO process and/or propagate the chain process via decomposition of lipid hydroperoxide (Braughler *et al*., 1987). Hence, the inhibitory activity of the polysaccharide extracts on LPO was evaluated.

The LPO of rat liver was triggered by Fe^{2+}/ascorbate and the end products of the process were measured in terms of thiobarbituric acid reactive substances (TBARS) formed. The ethanolic and aqueous extracts of *Jatropha* species inhibited lipid peroxidation in a concentration dependent manner (Table 11 and Fig. 11). The LPO inhibition of ethanolic extracts of *J.carcus*, *J.tanjorensis*, *J.podagrica*, *J.multifida*, *J.integerrima*, *J.gossypiifolia* and *J.villosa* were between 18.36-97.95%, 2.36-35.71%, 2.58-92.85%, 10.88-74.14%, 1.20-12.95%, 53.74-92.85% and 5.44-79.25% at 1-5 mg ml^{-1} respectively. The LPO inhibition

of aqueousc extracts were between 3.80-88.89%, 53.07-92.85%, 35.71-96.93%, 3.31-69.33%, 2.57-19.72%, and 1.42-14.96% at 0.5-2.5 mg ml^{-1} respectively. EC_{50} values of the LPO inhibition of ethanolic and aqueous extracts ranged ranged from1.11 to 8.80 and 1.26 to 5.30 mg/ml respectively. The extracts could inhibit lipid peroxidation by scavenging the OH^- or O_2^- radicals or by chelating the iron itself. The mechanism of the inhibitory effect by which plant extracts protect against a lipid peroxidation might involve radical scavenging activity and reducing capacity (Li *et al*., 2006).

At 0.02-0.10 mg/ml, LPO inhibition of positive controls ascorbic acid, and BHA were between 51.42-71.43% and 48.57-71.43% respectively.

A statistically significant difference ($p<0.05$) in LPO inhibition was observed with different sample concentrations and between various *Jatropha* species.

4.1.12 β-carotene bleaching inhibition

In the β-carotene linoleic acid assay, the free radical linoleic acid attacks the highly unsaturated β-carotene, and the presence of different antioxidants can hinder the extent of β-carotene bleaching by neutralising the linoleate free radical and other free radicals formed in the system. The absorbance decreased rapidly in samples without antioxidant, whereas in the presence of an antioxidant the colour was retained for a long time.

In the β-carotene bleaching assay, linoleic acid produces hydroperoxides as free radicals during incubation at 50°C. Linoleic acid hydroperoxides attack the β-carotene molecule and, as a result, it undergoes rapid decolorization. The corresponding decrease in absorbance can be monitored spectrophotometrically. The presence of antioxidant extracts can hinder the extent of β-carotene bleaching by acting on the free radicals formed in the system (Jayaprakash *et al*., 2001).

Table 12 and Fig. 12 shows the antioxidant activity of ethanolic and aqueous extracts of *Jatropha* species as measured by the inhibition of bleaching the β-carotene linoleate system. At 1-5 mg ml^{-1}, β-carotene bleaching inhibition

of ethanolic extracts of *J.carcus*, *J.tanjorensis*, *J.podagrica*, *J.multifida*, *J.integerrima*, *J.gossypiifolia* and *J.villosa* were between 64.90-85.00, 82.33-88.09, 75.94-83.83, 57.45-84.07, 72.86-84.67, 80.69-89.03 and % respectively. The aqueous extracts exhibited an inhibition between 54.24-70.47, 67.44-88.37, 78.78-86.90, 60.22-68.69, 72.59-79.37 and 68.63-92.10%. EC_{50} values of the β-carotene bleaching inhibition of ethanolic and aqueous extracts ranged from 0.11-0.26 and 0.23-0.42 mg/ml respectively.

At 0.2-1.0 mg/ml, the β-carotene bleaching inhibition of positive controls ascorbic acid, trolox, BHA and quercetin were between 61.16-85.99%, 71.67-94.59%, 87.22-94.47% and 84.52-89.64% respectively.

A statistically significant difference ($p<0.05$) in β-carotene bleaching inhibition was observed with different sample concentrations and between *Jatropha* species.

4.1.13 Phosphomolybdenum assay

The phosphomolybdenum method is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds and the formation of green phosphate/Mo (V) complex with the maximal absorption at 695 nm. The assay being simple and independent of other antioxidant measurements commonly employed, its application was extended to plant polyphenols (Prieto *et al.*, 1999). Higher absorbance indicates a higher antioxidative activity. The total antioxidant capacity was expressed as number of equivalents of ascorbic acid (AAE)/g extract.

The total antioxidant activity of ethanolic and aqueous extracts of *Jatropha* species is depicted in Table 13. The plant extracts exhibited various degrees of antioxidant capacity. The total antioxidant capacity of ethanolic and aqueous extracts of *Jatropha* species ranged from 17.99-21.85 and 9.48-19.58 mg/g AAE respectively.

Table 13
Total antioxidant capacity (Phosphomolybdenum assay) in ethanolic and aqueous extracts from *Jatropha* species.[#]

Sample	Total antioxidant activity µM (AAE)/g	
	Ethanol	Aqueous
J. carcus	21.80 ± 0.61[bc]	19.58 ± 0.54[f]
J. tanjorensis	20.72 ± 0.70[b]	13.11 ± 0.38[c]
J. podagrica	20.83 ± 0.60[bc]	9.48 ± 0.36[a]
J. multifida	17.99 ± 0.46[a]	14.93 ± 0.50[d]
J. integerrima	21.51 ± 0.60[bc]	10.61 ± 0.29[b]
J. gossypiifolia	21.85 ± 0.61[c]	17.31 ± 0.50e
J. villosa	20.95 ± 0.70[bc]	

There are many different methods for determining antioxidant function each of which depends on a particular generator of free radicals, acting by different mechanisms (Huang *et al*., 2005). Antioxidants may act in various ways such as scavenging the radicals, decomposing the peroxides and chelating the metal ions (Cam *et al*., 2009).

Four mechanisms have been proposed to explain how phenolic antioxidants can play their role. The first one involves a direct hydrogen atom transfer (HAT) (Mayer and Rhile, 2004) from the antioxidant to the radical. The second mechanism, involves single electron transfer (SET) (Rojano *et al*., 2008) from the antioxidant to the radical, leading to indirect H-abstraction. The third has been termed sequential proton loss electron transfer (SPLET) (Klein and Lukes, 2007) and takes place once the anion has been formed. The fourth mechanism is metal chelating activity. Metals chelation may provide important antioxidative effects by retarding metal catalyzed oxidation (Gulcin *et al*., 2010). All four mechanisms may occur in parallel, but at different rates. It is very difficult to assess the antioxidant activity of a product on the basis of a single method because the antioxidant mechanism in biological matrices is quite complex and several different factors play a role in these mechanisms (Huang *et al*., 2005).

4.2 Bioactive components

4.2.1 Determination of total phenol, flavonoids, phenolic acid and tannin content

The phenolic and flavonoid contents can be used as an important indicators of antioxidant capacity which can be used as a preliminary screening for any product when intended as a natural source of antioxidants in functional foods. Phenolic compounds are known to be powerful chain-breaking antioxidants and may directly relate to the antioxidant action.

The total phenol content is expressed as mg of gallic acid equivalents (GAE) per gram of the extract (Table 14). The phenolic content of ethanolic and aqueous extracts of *Jatropha* species varied from 0.54 to 1.03 mg/g GAE and 0.14 to 0.58 mg/g GAE respectively.

The total flavonoid content expressed as mg of catechin (CAE) equivalents per gram of the extract (Table 14). The flavonoid content of ethanolic and aqueous extracts of *Jatropha* species varied from 2.23 to .3.47 mg/g CAE and 1.89 to 2.9059 mg/g CAE respectively.

The total phenolic acid content of ethanolic and aqueous extracts of *Jatropha* species varied from 123.26 to 196.48 mg/g GAE and 29.56 to 77.42 mg/g GAE respectively. The tannin content of ethanolic and aqueous extracts of *Jatropha* species varied from 1.80 to 3.84 and 0.31 to 1.97 mg/g respectively.

Many studies have shown that the consumption of diets rich in phenolic contents is associated with a decreased risk of cardiovascular diseases and certain cancers. These health effects have been attributed, in part, to the presence of phenolic compounds in dietary plants, which may exert their effects as a result of their antioxidant properties (Sarikurkcu *et al*., 2008). Therefore, the results of the present study suggest that the extracts of *Jatropha* might reduce oxidative damage in the human body and provide health protection.

5.0 SUMMARY AND CONCLUSION

Reactive oxygen species comprising of hydrogen peroxide, hydroxyl radical, nitric oxide peroxynitrite, superoxide anion are potential agents to initiate degenerative processes in human body. Though humans have excellent defense mechanism to overcome oxidative stress related diseases caused by ROS, a proper equilibrium between the ROS generations in humans and components of defense system is needed by supplementing antioxidants via diet or medicine to prevent diseases related to chronic oxidative damages in tissues and cells and are thus believed to protect against cancer, cardiovascular diseases and could delay the ageing process. Synthetic antioxidant supplementation are reported to exhibit tumour forming activities and hence carry health risk. While antioxidants from plant sources, such as vegetables, fruits, leaves, tree barks, roots, spices and herbs are more bioavailable, safe for human consumption and consequently explored. The antioxidant activity of plant products can be mainly ascribed to the presence of phenolic compounds. Plant synthesizes phenolic and flavonoid compounds for its own defense system against ROS.

Medicinal plants are reported to be rich in antioxidants, namely, polyphenols, flavonoids, vitamin A, C, E and several other constituents, which are necessary for maintaining good health and useful for therapeutic purposes against various disease. Medicinal plants are gaining a lot of importance as an alternate medicine against therapy and prevention from various diseases.

The main objective of the study was designed to evaluate the antioxidant activities of ethanolic and aqueous extracts of seven *Jatropha* species by various *in vitro* model systems and phytochemical constituents in *Jatropha* species.

The leaves of *J. carcus*, *J. tanjorensis*, *J. podagrica*, *J. multifida*, *J. integerrima*, *J. gossypiifolia* and *J. villosa* were collected from Forest College and Research Institute, Tamil Nadu Agricultural University, Coimbatore. The leaves of different species of *Jatropha* were dried, powdered and extracted with ethanol and water.

The plant extracts were investigated for antioxidant activity by several methods *in vitro*, such as DPPH, ABTS, DMPD, OH radical scavenging assay, ferric cyanide reducing power, FRAP, CUPRAC, PRAP, phenanthroline assay, lipid peroxidation inhibition assay, β-carotene bleaching inhibition assay and phosphomolybdenum assay. The phytochemical constituents were estimated in various plant extracts.

DPPH radical is scavenged by the antioxidants through the donation of a proton forming reduced DPPH. EC_{50} values of the DPPH radical scavenging activity of ethanolic and aqueous extracts ranged from 0.15 to 1.04 and 1.36 to 7.09 mg/ml respectively. The better scavenging ability of various extracts might be due to more hydrogen donating components extracted by plants. ABTS assay measures the relative antioxidant ability to scavenge the radical $ABTS^{+}$ and is an excellent tool for determining the antioxidant activity of hydrogen donating antioxidants and chain breaking antioxidants. EC_{50} values of the ABTS radical scavenging activity of ethanolic and aqueous extracts ranged from 0.69 to 0.86 and 0.43 to 0.57 mg/ml respectively. The good antioxidant activity on ABTS radicals may be attributed to a direct role in trapping free radicals by donating hydrogen atom or electron. DMPD assay reflects the ability of radical hydrogen donors to scavenge the single electron from $DMPD^{\bullet+}$. EC_{50} values of the DMPD radical scavenging activity of ethanolic and aqueous extracts ranged from 0.25 to 0.32 and 0.23 to 0.33 mg/ml respectively. The result confirms that components within the extracts are capable of participating in electron transfer reactions which may play a beneficial role in reducing reactive free radicals. Hydroxyl radical scavenging capacity of an extract is directly related to its antioxidant activity. EC_{50} values of the OH radical scavenging activity of ethanolic and aqueous extracts ranged from 0.71to 1.67 and 0.26 to 1.63 mg/ml respectively. These results suggest that the extracts are capable of scavenging hydroxyl radicals and could prevent or ameliorate oxidative damage.

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The EC_{50} values of ethanolic and aqueous extracts in the ferric cyanide (Fe^{3+}) reducing antioxidant power assay ranged

from 0.65 to 2.87 and 1.53 to 10.60 mg/ml respectively. The reducing properties of plants are generally associated with the presence of reductones and they exert antioxidant action by breaking the free radical chain by donating hydrogen atoms. FRAP assay measures the antioxidant effect of any substance in the reaction medium as reducing ability. The EC_{50} values of ethanolic and aqueous extracts ranged from 2.10 to 3.78 and 3.14 to 5.06 mg/ml respectively. The CUPRAC method has also been used to determine the reducing power of antioxidant compounds. The EC_{50} values of ethanolic and aqueous extracts the cupric ion (Cu^{2+}) reducing antioxidant power assay ranged from 0.10 to 1.89 and 1.41 to 4.23 mg/ml respectively. PRAP assay measures the antioxidant effect of any substance in the reaction medium as reducing ability. The EC_{50} values of ethanolic and aqueous extracts ranged from 3.17 to 3.98 and 3.58 to 5.05 mg/ml respectively. The reduction of absorbance is proportional to the antioxidant content. The phenanthroline method is based on the ability of antioxidants to reduce Fe(III) to Fe(II). The EC_{50} values of ethanolic and aqueous extracts phenanthroline assay ranged from 0.34 to 2.70 and 0.39 to 4.15 mg/ml respectively.

Lipid peroxidation, a process induced by free radicals, leads to oxidative deterioration of polyunsaturated lipids. LPO inactivates cellular components and therein plays a key role in oxidative stress in biological systems. EC_{50} values of the LPO inhibition in egg homogenate of ethanolic and aqueous extracts ranged from 1.12 to 2.75 and 0.41 to 0.48 mg/ml respectively. EC_{50} values of the LPO inhibition in liver homogenate of ethanolic and aqueous extracts ranged ranged from 1.11 to 8.80 and 1.26 to 5.30 mg/ml respectively. The various plant extracts could inhibit lipid peroxidation by scavenging the OH^- or O_2^- radicals or by chelating the iron itself. The mechanism of lipid peroxidation inhibiting effect may be relative to the membrane stabilization of phenolic compounds.

The β-carotene bleaching method estimates the relative ability of antioxidants compounds to scavenge the radical of linoleic acid peroxide that oxidizes β-carotene in the emulsion phase. EC_{50} values of the β-carotene bleaching inhibition of ethanolic and aqueous extracts ranged from 0.11-0.26 and

0.23-0.42 mg/ml respectively. The presence of different antioxidants in the extracts can hinder the extent of β-carotene bleaching by neutralizing the linoleate free radical and other free radicals formed in the system.

The phosphomolybdenum method is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds and the formation of green phosphate/Mo (V) complex. Plant extracts exhibited higher absorbance indicating a high antioxidant activity.

The phenolic content of ethanolic and aqueous extracts of *Jatropha* species varied from 0.54 to 1.03 mg/g GAE and 0.14 to 0.58 mg/g GAE respectively. The flavonoid content of ethanolic and aqueous extracts of *Jatropha* species varied from 2.23 to 3.47 mg/g CAE and 1.89 to 2.90 mg/g CAE respectively. The total phenolic acid content of ethanolic and aqueous extracts of *Jatropha* species varied from 123.26 to 196.48 mg/g GAE and 29.56 to 77.42 mg/g GAE respectively. The tannin content of ethanolic and aqueous extracts of *Jatropha* species varied from 1.80 to 3.84 and 0.31 to 1.97 mg/g respectively. The phenolic compounds act as reducing agent due to their hydrogen donating and single oxygen quenching ability, which not only prevent generation of oxidant free radicals and reactive species but also scavenge free radicals.

In conclusion, the results of the present study indicated that among various *Jatropha* species studied, all the species resulted in highest amount of phytochemical constituents and the strongest antioxidant activity. The ethanol and aqueous extracts showed a good antioxidant activity by the scavenging of the free radicals, strong reducing power and lipid peroxidation inhibition and this could be explained by the presence of phenolics, flavonoids and condensed tannins.

More research is needed to characterize the chemical compositions and structures that contribute to the total antioxidant activities of *Jatropha*. The results of this study is expected to provide sufficient baseline information for further exploration of this *Jatropha* extracts for nutraceutical purposes as well as for developing new, cheaper and safer pharmaceutical products.

However, further *in vitro* and *in vivo* data are warranted to better understand the therapeutic efficacy and mechanism of action of the active principles in these plant extracts, which could ultimately lead to their application in pharmaceutical formulations.

Suggestions for future research

1. Isolation of bioactive compounds and their *in vitro* free radical scavenging activity.
2. Structural elucidation of the bioactive compounds.

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