INTERNATIONAL JOURNAL OF PHARMACEUTICS & DRUG ANALYSIS
VOL.4 ISSUE 10, 2016; 480 – 479 ; http://ijpda.com; ISSN: 2348-8948

Research Article

ANTIOXIDANT ACTIVITY AND PRELIMINARY PHYTOCHEMICAL ANALSYSIS OF *LORANTHUS ELASTICUSOF LORANTHACEAE*

T. Krishnaveni, R. Valliappan[1], R. Selvaraju [2], P. Nagendra prasad [3]

Department of Chemistry
[1]Chemistry Wing, DDE,
[2]Physics Section, FEAT
Annamalai University, Annamalai Nagar 608 002, Tamil Nadu, India.
[3]Department of Biomedical Engineering, Noorul Islam University, Kumarakoil, Nagerkoil, Tamil Nadu, India.

Date Received: 4th October 2016; Date accepted: 15th September 2016; Date Published: 19th October 2016

E-mail: rmvs1962@yahoo.com

Abstract
In the present study the extracts prepared using plant materials of *Loranthuselasticus*of Loranthaceae grown on the Peepal Tree (in Tamil ArasaMaram – *Ficusreligiosa*) were evaluated for their antioxidant activity by using 1,1-diphenyl-2-picrylhydracyl (DPPH), ferric ion reducing power antioxidant activity (FRAP), Nitrc oxide (NO) scavenging assay and the Total phenolic and flavonoid content are studied. The hydro-methanolic extract of the plant material was found to possess significant antioxidant activity. The preliminary phytochemical screening of the plant material was done and the results are discussed.

Key Words: Antioxidant, DPPH method, FRAP, NO scavenging assay, phytochemical analysis.

Introduction:
Plants and plant products have always had meaning in many parts of human life. The use of plants as medicines predates written human history. Knowledge of plant use was widespread in ancient civilization. Until the middle of the 19th century, plants were the main therapeutic agents used by humans and even today their role in medicine is still relevant. One can argue forever, what precise percentage of the world's population use local and traditional medicine. These herbal or mineral or fungal or occasionally animal medical products from systems of knowledge and practice that have been transmitted over centuries and which continually change[1]. In developing countries and rural societies, the use of medicinal plants is both a valuable resource and necessity and furthermore, it provides a real alternative for primary health care systems[2].

In the present study the plant material chosen as *Loranthuselasticus* of*Loranthaceae*family plant grown in the Peepal Tree (in Tamil – ArasaMaram) – *Ficusreligiosa* tree. The members of *Loranthaceae*are about 75 genera and most of them are globally known as mistletoes[3,4]. Historically, the mistletoe, whose name is believed to be derived from the Celtic word for "all-heal", was used for a variety of treatments. The various types of *LoranthaceaeLoranthus*plants used as therapeutic agents[5,6]. Based on the above results the plant chosen for the study is the *Loranthuselasticus* grown on the Peepal tree (*Ficusreligiosa*).

MATERIALS AND METHODS

The plant material was collected from the peepal Tree (*Ficusreligiosa*) in Aannamalai Nagar, Chidambaram, Cuddalore district, Tamil Nadu. The plant was taken to the Department of Botany, Annamalai University for authentication. After authentication, a voucher sample of the plant was kept in the herbarium of the Department of Botany, Annamalai University.

Extraction of plant material
The plant material *L. elasticus* was rinsed with water to remove contaminants, followed by drying in a ventilated oven at 40°C for 3 days. On day 4, the plants were ground into fine powder (pass through a 30 mesh sieve) using a blender, boiled in sterile distilled water for 20 min and subsequently cooled to 25°C before filtration through a Whatmann No. 1 filter paper. The resulting crude extract was evaporated to dryness in vacuo using a rotary evaporator at 40°C to obtain a brownish precipitate.

Antioxidant Activity
The plant samples were analysed for the antioxidant property. 100 grams of accurately weighed powdered leaves are defatted with petroleum ether (60-80°C) (3 x 200 ml) and then extracted with 250ml of methanol and water separately in a Soxhlet apparatus for 48 hours and the extract were condensed and used for the antioxidant assay.

Determination of Total Phenolic content
Phenolics content and reducing the power of the extracts is often determined using the Folin-Ciocalteu method.

Equal volumes of Folin-Ciocalteu reagent and given quantity (mg) of plant extracts of different concentrations (e.g. 0.4, 0.3, 0.2, 0.1 and 0.05 mg/ml) are often mixed in different sets of test tubes shaken thoroughly, and left to stand for 1 min. Ten per cent of $NaHCO_3$ is then added and the mixture once again allowed to stand for 30 minutes, after which the absorbance (725nm) is measured spectrophotometrically. Gallic acid (0.05-0.5 mg/ml) is often used to produce a standard calibration curve and the total phenolic content expressed as mg equivalent of gallic acid (mg GAE) per gram dry weight of the extract by computing with standard calibration curve[7].

Total Flavonoids

The Total flavonoids assay is to find out the amount of flavonoids present in the sample. In this assay Quercetin (0.1 mg/ml) was taken as the standard compound. Different aliquots of the standard were taken in different test tubes and $AlCl_3$ (1 g/10ml) 100µL, $KOOCCH_3$ (0.98 g/10mL) 100µL were added. The volume of the solution was made up to 3 ml with double distilled water. The test tubes were then incubated for 30 minutes. After 30 minutes of incubation absorbance was noted at 416nm. Similarly, it was repeated for the other samples. Methanol was taken as the blank. The presence of total flavonoids was calculated from the standard graph.

DPPH radical scavenging Assay

Most common spectrophotometric assay methods applied is the DPPH radical scavenging system in which the hydrogen or electrons donation ability of plant extracts are measured from bleaching of purple methanol solution of 2, 2'-diphenyl-1-picryl hydrazyl (DPPH) free radical. This spectrophotometric assay uses the stable radical DPPH as a reagent. DPPH absorbs at 517nm, and as its concentration is reduced by the existence of an antioxidant, the absorption gradually disappears with time. A 2-ml aliquot of a suspension of the ethanol extracts is mixed with 1 ml of 0.5 mM 2,2-diphyenyl-1-picrylhydrazyl (DPPH) solution and 2 ml of 0.1 M sodium acetate buffer (pH 5.5). After shaking, the mixture is incubated at ambient temperature in the dark for 30 minutes, following which the absorbance is measured at 517nm using a UV-160A spectrometer. A solvent such as ethanol can be used as negative control. Radical scavenging activity is often expressed as percentage inhibition and is often calculated using the formula:

$$\% \text{ radical scavenging activity} = [(A_{control} - A_{test})/A_{control}] \times 100$$

where $A_{control}$ is the absorbance of the control (DPPH solution without test sample) and A_{test} is the absorbance of the test sample (DPPH solution plus antioxidant).

Ferric Ion Reducing Antioxidant Power (FRAP)

For determination of reducing power of plant extracts, the ferric reducing/antioxidant power (FRAP) assay method can be applied. The assay is based on the reducing power of a compound (antioxidant). A potential antioxidant reduces the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}); the later forms a blue complex (Fe^{2+}/2, 4, 6-tripyridyl-*s*-triazine (TPTZ)), which increases the absorption at 593nm. Stronger absorption at this wavelength indicates higher reducing power of the phytochemical, thus higher antioxidant activity. Reaction mixture containing test extract sample at different concentrations (10-100µl) in phosphate buffer (0.2 M, pH 6.6) and equal amounts of 1% (w/v) potassium ferricyanide are incubated at 50ºC for 20 minutes and then the reaction terminated by the addition of equal volumes of 10% (w/v) tricarboxyllic acid (TCA) solution and the mixture centrifuged at 3000rpm for 20 minutes.Thesupernatantis mixed with equal volume of distilled water and 0.1 % (w/v) ferric chloride solution and the absorbance measured at 700nm. Increased absorbance of the mixture with concentration indicates the reducing power of extract[8].

Nitric oxide scavenging assay

Nitric oxide radical inhibition was estimated by the use of GriessIllosvory reaction. In this investigation, GriessIllosvory reagent was modified by using Napthyl ethylene diaminedihydrochloride (0.1%w/v) instead of the use of 1-napthylamine (5%). The reaction mixture (3ml) containing 2ml of 10mM sodium nitroprusside, 0.5ml saline phosphate buffer and 0.5ml of standard solution or aqueous and ethanolic extract of 500 -1000µg/ml were incubated at 25°C for 150min. After incubation, 0.5ml of the reaction mixture was mixed with 1ml sulfanilic acid reagent (0.33% in 20%glacial acetic acid) and allowed to stand for 5min for the completion of diazotization reaction. To this 1ml of the napthyl ethylene diaminedihydrochloride was added, mixed and was allowed to stand for 30min at 25°C. The concentration of nitrite was assayed at 546nm and was calculated with the control absorbance of the standard nitrite solution (without extracts or standards, but the same condition was followed).The blank was buffered and the Ascorbic acid and Quercetin (10 -50 µg/ml) was taken as the standard. The percentage inhibition was calculated using the formula:

$$\% \text{ scavenging Activity} = \frac{A_{control} - A_{test} \text{ or } A_{Std}}{A_{control}} \times 100$$

PHYTOCHEMICAL SCREENING

Preliminary qualitative phytochemical analysis were carried out for the methanol extract as per the standard method followed[9, 10].

The plant parts are dried immediately either in an artificial environment at a low temperature (50-60°C) or dried, preferably in the shade so as to bring down the initial large moisture content to enable its prolonged storage life. The dried berries are pulverised by mechan-

ical grinders and the oil is removed by solvent extraction. The defatted material is then extracted in a soxhlet apparatus or by soaking in water or alcohol (95% v/v). The resulting alcoholic extract is filtered, concentrated in vacuo or by evaporation, treated with HCl (12N) and refluxed for at least six hours. This can then be concentrated and used to determine the presence of phytoconstituents.

RESULTS AND DISCUSSION

Antioxidant activity

DPPH radical scavenging activity

The present study examines antioxidant activity of *L. elasticus* present in the ArasaMaram and showed a concentration dependent antiradical activity Table 1 by inhibiting DPPH radical with EC_{50} value of 250.50 µg/ml (BHT – Butyrated hydroxyl toluene) as the control with EC_{50} values of 140.5 µg/ml) DPPH is a purple coloured free radical which on reaction with the plant extract changes to a yellow coloured stable compound and the extent of the reaction is depending on the hydrogen releasing capacity of the antioxidant. So the plant *L. elasticus* of ArasaMaram – has the ability to stop the chain reactions of free radicals by forming the stable compounds. Also in the present study, the plant showed better activity in competing with oxygen to react with nitric oxide and thus the inhibition of a generation of anions by having an EC_{50} value of 460.25 µg/ml as the positive control with EC_{50} value of 140.5µg/ml. Nitric oxide is responsible for the production of peroxynitrite, a highly toxic substance which is causing irreversible damage to the cell membranes b oxidizing low density lipoproteins.

Table 1 DPPH free radical scavenging assay of *L. elasticus* – *Arasu*

Samples tested	Concentration (µg/ml)	Percentage inhibition	EC_{50} (µg/ml)
PPM – Arasu	50	28.31	
	100	37.52	
	250	45.28	
	500	66.27	250.25
	1000	74.25	
BHT			140.05

PPM-Powdered plant material: BHT: Butyrated hydroxyl toluene

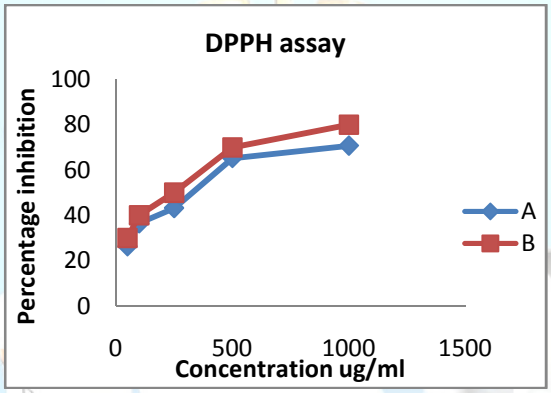

Table - 2 Ferric Reducing Antioxidant Power (FRAP) assay

Samples tested	Concentration (µg/ml)	Percentage inhibition	EC_{50} (µg/ml)
PPM – Arasu	50	26.31	
	100	36.42	
	250	43.18	
	500	65.20	250.20
	1000	70.70	
BHT			130.05

PPM-Powdered plant material: BHT:

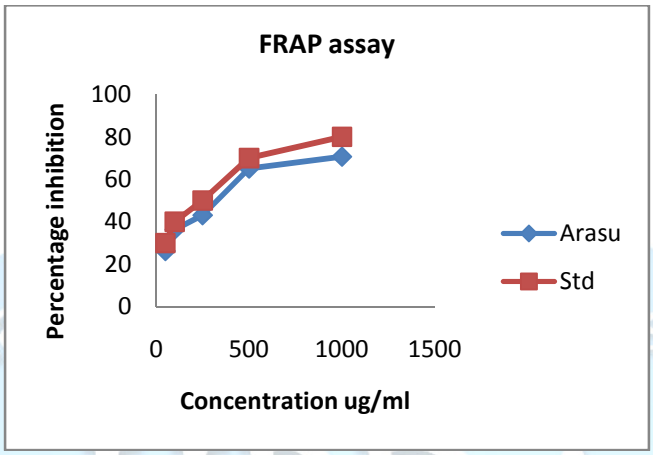

Table 3 Nitric oxide radical scavenging activity

Samples tested	Concentration (µg/ml)	Percentage inhibition	EC_{50} (µg/ml)
PPM – Arasu	50	18.20	
	100	26.40	
	250	37.11	
	500	56.25	452.25
	1000	68.73	
BHT			130.05

PPM-Powdered plant material: BHT

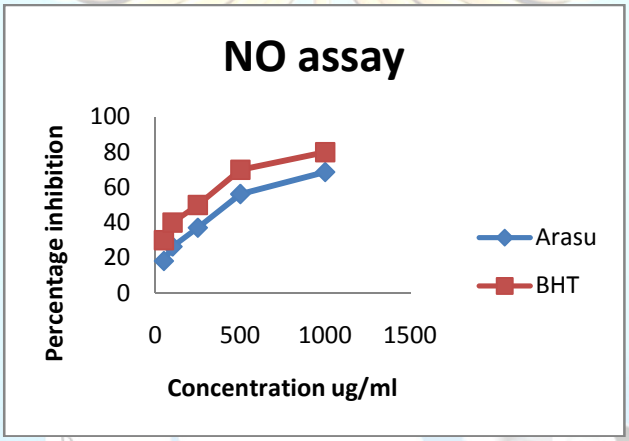

Table 4: Physicochemical analysis and extractive values of leaf powder of Loranthuselasticus present in ArasaMaram

S. No.	Parameter	*L. elasticus*
	Organoleptic characteristics	**Powder**
1	Appearance	Powder
	Colour	Greenish brown
	Smell	Aromatic
	Taste	Pungent with astringent
2	Loss on drying at 105°C	6.2-8.2
3	Total Ash value	12.90
	Water soluble Ash	5.80
`	Acid insoluble ash	1.10
	Sulphated ash	3.66

4	Moisture content	8.20
5	Foreign organic matter	7.20
6	Crude fibre content	2.80
7	Solubility values	
	Alcohol	7.09
	Water	6.26

Table 5 Percentage extractive values of Arasu

S. No.	Extractive values in	Arasu
1	Petroleum ether	11.216
2	Benzene	2.200
3	Chloroform	3.320
4	Ethanol	14.200
5	Water	9.200

Table 6 Fluorescence analysis of leaf powder of L. elasticus – Arasu

S. No.	Powder + Reagent used	L. elasticus - Arasu	
		Daylight	UV light
1	Powdered plant material	Pale green	Light green
2	Powder + 1M H_2SO_4	Light black	Dark black
3	Powder + dil. HNO_3	Brown	Brownish yellow
4	Powder + 50% HNO_3	Orange red	Brownish green
5	Powder + Acetic acid	Yellowish green	Brown
6	Powder + 1M HCl	Light green	Greenish yellow
7	Powder + picric acid	Light green	Yellowish green
8	Powder + Iodine solution	Green	Green
9	Powder + $FeCl_3$ (5% solution)	Green	Dark green
10	Powder + 1M NaOH	Green	Green
11	Powder + 25% NH_4OH	Green	Greenish yellow
12	Powder + CH_3OH	Green	Orange red

Table – 7 Qualitative phytochemical screening of leaf powder of L. elasticus in Arasu

S. No.	Test applied	L. elasticus - Arasu					
		Reagents used	Pet ether	Benzene	Chloroform	Alcohol	Water
1	Alkaloids	Mayer's	-	-	-	-	+
		Wagner's	-	-	-	+	+
		Hager's	-	-	-	+	+
		Dragondroff's	-	-	-	+	+
2	Amino acids	Ninhydrin's	+	+	+	+	+
3	Anthocyanins		+	+	+	+	+
4	Carbohydrates	Fehling's	-	-	-	+	+
		Benedict's	-	-	-	+	+
5	Cardial glycoside		-	+	-	+	+
6	Coumarins		+	+	+	+	+
7	Diterpenes	Copper acetate	+	+	+	+	-
8	Emodins		-	-	-	+	-
9	Fatty acids		+	+	+	+	+

10	Flavonoids		-	-	-	-	+
11	Fixed oils and fats	Spot test	+	+	-	-	-
12	Glycosides		+	+	+	+	+
13	Gum and mucilage	Alcoholic precipitation	-	-	-	+	+
14	Leucoanthocyanin		-	-	-	-	-
15	Phenolic	$FeCl_3$	+	+	+	+	+
16	Phlobatannin		-	-	-	-	-
17	Phytosterols	L.B. Test	+	+	+	+	+
18	Proteins	Xanthophoretic	+	+	+	+	-
19	Saponins	Foam test	-	-	-	+	+
20	Steroids		+	+	+	+	-
21	Tannins and Phenols	10% lead acetate	-	-	-	+	+
22	Terpenoids		+	+	+	+	-

'+' presence of compounds; '-'absence of compounds

Ferric Reducing Antioxidant Power (FRAP) assay

FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyhridyltriazine (Fe^{3+}-TPTZ) complex and producing a coloured ferrous tripyridyltriazine (Fe^{2+}-TPTZ). Generally, the reducing properties are associated with the presence of compounds which exert their action by breaking the free radical chain by donating a hydrogen atom. FRAP assay treats the antioxidants in the sample as a reductant in a redox-linked colorimetric reaction. In the present study, the trend for ferric ion reducing activities of Arasu and BHT are shown in Fig. 2. The absorbance of Arasu clearly increased, due to the formation of the Fe^{2+}-TPTZ complex with increasing concentration. Hence, they should be able to donate electron to free radicals stable in the actual biological and food system.

Nitric oxide radical scavenging activity

The *L. elasticus* of Arasu showed better activity in competing with oxygen o react with nitric oxide and thus the inhibition of a generation of anions by having an EC_{50} value of 452.25 µg/ml (BHT as the positive control with EC_{50} value of 130.25 µg/ml). Nitric oxide is responsible for the production of peroxynitrite, a highly toxic substance, which is causing irreversible damage to the cell membranes by oxidizing low density lipoproteins.

Physicochemical Analysis

Physicochemical Analysisof the plant materials was analysed and the tests like total ash, water-soluble ash, acid-insoluble ash, sulphated ash, loss on drying, moisture content, foreign organic matter, crude fibre content and solubility in alcohol, and water extracts values of the leaf of *Loranthuselasticus* plant present in Peepal Tree - *Ficusreligiosa* (ArasaMaram) is given in Table 4.

The results of physicochemical parameters are given in Table – 4. The total ash value, acid insoluble ash value and water soluble ash value were found to be 12.10, 0.92 ,5.80 and sulphated ash value of 3.66% respectively. The total ash value was relatively high which may be due to high content of carbonates, phosphates, silicates and silica. Ash value is useful in determining authenticity and purity of plant material and also these values are important quantitative standards[10]. Percent weight has on drying or moisture content could prevent bacterial, fungal or yeast growth[11].

Foreign organic matter in the powdered plant material was 7.20%, this may be contributing to the wildness of the plant, leading to the contamination in the course of its collection. The crude fibre content of the plant material was found to be 2.80%. Determination of crude fibre is useful in distinguishing between similar drugs or in the deduction of adulteration. It also helps to remove the more resistant parts of plant organs which can be used for microscopic examination. Alcohol soluble and water soluble extractive values were found to be 6.50% and 10.12 % respectively.

Bioassays can play an important role in the analysis of plant materials and their product of therapeutic purpose. Different physicochemical parameters are being used to determine the active constituents present in the plant material. The extractive values are used to determine the active constituents. The highest percentage of *L. elasticus* extract was found to be 14.20% in solvent ethanol. The analysis revealed the presence of fatty constituents. The ash values were analysed to check the possible presence of any foreign matters like soil, sand

and adherence of water soluble salts to the plant surface. The ash values of different plant materials may vary, but this difference lies within narrow limits in the case of the similar plant materials. The acid insoluble ash usually contains silica and therefore, high acid insoluble ash is an indicator of the contamination with earthy materials in the plant. The water soluble ash indicates the quality of inorganic elements in the sample. Therefore, the total ash values of the plant materials are not always reliable parameters because of the risk of the occurrence of non-physiological materials. Thus, acid insoluble ash was quantified, which revealed the lowest content in the extract. Moisture contents and Loss on drying (LOD) were used to find out the amount of moisture, including volatile contents of the tested plant material. The hydrolysis of the active ingredients of the plant material may occur due to higher moisture content in the plant material which leads to poor quality and efficacy. The final processes of dryness and removal rate of moisture contents are of great importance.

The total phenolic and flavonoid contents in the methanolic extract of *L. elasticus* were found to be 1.72 mg/g and 1.08 µg/g respectively. These results showed that the *L. elasticus* extract contains significant quantities of phenolic and flavonoid compounds.

The percent yields of different extracts are given in Table 5. The percent yields of petroleum ether, chloroform, ethanol and aqueous extracts were found to be 3.0, 2.0, 2.2 and 11.8 % respectively. The percent yield of alcohol and aqueous extracts by cold extraction were found to be 2.4 and 9.2 % respectively.

Study of powder

The Behaviour of powders of the leaf of the plant sample of Arasu on treatment with different chemical reagents and their fluorescence behaviouris given in Table 6. The leaf powder of Arasu treated with sulphuric acid to give a black colour in both UV and visible light. The leaves treated with Hydrochloric acid (HCl), Picric acid, Iodine (I_2), Ferric chloride ($FeCl_3$), and ammonium hydroxide (NH_4OH) gives almost green colour in both UV and visible light. The leaf powder Arasu gives brown colour in nitric acid (HNO_3) in visible light and brownish green in UV light whereas in CH_3OH produce dark green in daylight and orange red colour in UV light.

The fluorescent studies of the plant material using different chemical reagents are given in Table 6. Fluorescence is an important phenomenon exhibited by various chemical constituents present in plant material. Since constituents show fluorescence in the visible range to many natural products (e.g., alkaloids like beriberine, which, do not visibly fluoresce in day light. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives by applying different reagents hence some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognositical evaluation[12].

Qualitative Phytochemical analysis

The results of preliminary phytochemical analysis of different extracts are given in Table 7.Secondary metabolites were found in good proportion in ethanolic and aqueous extracts when compared with petroleum ether and chloroform extracts. These secondary metabolites may be responsible for various pharmacological effects of ethanolic and aqueous extracts of the plant material. Successive solvent extracts of leaves of the plant *L. elasticus* - Arasu was subjected to qualitative phytochemical screening and the values are given in Table 7.

The phytochemical analysis of the petroleum ether, benzene, chloroform, methanol and water extract of the plant *Loranthuselasticus* present in the Peepal tree was presented in the table 7. The result indicated that the phytochemical constituents, namely alkaloids, amino acids, anthocyanin, carbohydrates, cardial glycosides, coumarins, diterpenes, emodins, fatty acids, phlobatannin, phenols, saponin and terpenoids were present in methanol extract, whereas the flavonoids, glycosides, leucoanthocyanin, phytosterol, proteins, steroids and tannin were absent in methanol extract. The other phytochemical constituents present in the rest of the solvents are given. Small amounts of Saponins were noticed in alcohol and water extracts. In water extracts of the leaves shows moderate amounts of gums and mucilage.

Since the plants are said to be a biosynthetic laboratory for a multitude of chemical compounds. The secondary metabolites are compounds which are responsible for the therapeutic efficacy of the plant material. The results of preliminary screening of the phytochemicals in methanolic extract of *L. elasticus* showed the presence of carbohydrates, phenolic compounds, flavonoids, proteins, lipids, steroids and tannins. The phenolic and flavonoid compounds are important antioxidants which also include antimicrobial, anti-allergic, anti-inflammatory and anticancer agent, etc. Phenolic compounds are most widely distributed phytochemicals which are derivatives of pentose phosphate, shikimate and phenylpropanoid pathways in plants. These secondary metabolites play a vital role in reproduction and growth. These compounds also provide protection against harmful pathogenic microbes and predators. Therefore, qualitative analysis of such vital compounds is extremely significant to determine the quality of plant material.

REFERENCES

1. Heinrich, M. 2000. Ethanobotany and its role in drug development. Phytotheraphy Research, 14, 479-488.
2. Hayata S, Polat R, Selvi S. 2014. Traditional uses of medicinal plants in Elazig (Turkey), Journal of Ethnopharmacology 154, 613-623.

3. Barlow BA, 1965. Classification of the Loranthaceae and viscaceae. Proc Linn Soc New South Wales. 89:268- 72.
4. Calvin CL, Wilson CA. 2006. Comparative morphology of epicortical roots in old and new world *Loranthaceae* with reference to root types, origin, patterns of longitudinal extension and potential for clonal growth. Flora-Morphology, Distribution, Functional Ecology of Plants.201 (1):51-64.
5. Numan IT, Hamad MN, Hassan A. 2013 Hematopoietic toxicity of *loranthuseuropaeus*: in vivo study. Asian J Pharm Clin Res;6 (1):117-20.
6. Omar ZA, , Ibrahim M. Salmana IM, Siddiquib MJA, Yama MF, Sriramanenia RN, Sadikunb A, ZhariIsmailb, Shaha AM and Asmawi MZ. 2010 Pharmacological mechanisms underlying the vascular activities of LoranthusferrugineusRoxb. In rat thoracic aorta. J Ethnopharmacol 127: 19-25.
7. Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouna, D., Stocker, P. and Vidal, N. 2006.
8. Antioxidant Activity of Some Algerian Medicinal Plants Extracts Containing Phenolic Compounds. *Food Chemistry* 97: 654-660.
9. Jayasri, M.A., Mathew, L. and Radha, A. 2009. A report on the antioxidant activities of leavesand rhizomes of *Costuspictus*D. Don. International Journal of Integretive Biology. 5(1):20-26.
10. Trease GE and Evans WC 2009. Pharmacognosy16th ed. Elsevier Ltd. Pub. London, 42-44.
11. Kokate CK Practical Pharmacognosy, 4th ed. Pharma Med Press, Hyderabad, 2011
12. African Pharmacopoeia 1986, General methods for analysis. OAU/STRC Scientific Pub. Lagos 2 2), 1-5, 137-149, 223-237.
13. Ansari, SH 2006. Essentials of Pharmacognosy. 1st ed. Birla Pub. Pvt Ltd. New Delhi