www.sphinxsai.com

PharmTech
International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.2, No.1, pp 649-655, Jan-Mar 2010

# Evaluation of Phytochemical standards and *In Vitro* Antioxidant Activity of tannins rich fraction of Stem Bark of *Bridelia retusa (Li*).

**A.U.Tatiya*, A.K. Saluja[1]**

***Dept. of Pharmacognosy, R.C.Patel College of Pharmacy, Shirpur-425405,**

**Dist-Dhule ,M.S.,India.**

**[1]A.R college of Pharmacy, Vallabh Vidhyanagar, Gujarat,India.**

***Corres.author: anil120171@yahoo.com***
***Mob No. : 9923070789***
***Fax No. : 02563-255189***

**Abstract:** *Bridelia retusa syn:Bridelia airy shawii* Li. bark is known to have various medicinal properties. Plants containing polyphenols have been reported to possess strong antioxidant properties. The bark of *Bridelia retusa* was screened for estimation of phytochemical standards like: phenolic, flavonoid, tannins, carbohydrates and mucilage content. In addition to its acetone fraction (ACE) i.e. tannins rich fraction, are also subjected to *in vitro* antioxidant activity using standard procedures. Results of Phytochemical standards analysis of *B.retusa* revealed the presence of bioactive components comprising Phenolics (2.92-3 g of GAE/100 g of dry sample), total tannins (33-43 % w/w as tannic acid equivalence), proanthocyanidin content (2.98-4 % w/w as catechin equivalent), ellagic acid (0.135 % w/w), mucilage (3-4 % w/w), flavonoids (257 mg of Rutin equivalence/100 g dry plant) and carbohydrates. (7 – 7.1g /100g of plant). The ACE exhibited $IC_{50}$ values of 47.20, 110.76, 48.81 44.15 and 50.42 µg/ml, respectively in DPPH, ALP, hydroxyl, hydrogen peroxide and nitric oxide radical inhibition assays while ACE had effective reducing power. These values were compared with ascorbic acid and BHA used as internal standard. These results suggest strong antioxidant potentials of the ACE of *Bridelia retusa.* These bioactive compounds may be responsible for antioxidant properties of *B.retusa* that form the basis of their use in herbal medicine in India,
**Key words**: *Bridelia retusa*, Phenolics, proanthocyanidin, flavonoids, DPPH, Reducing power, TBARS, $H_2O_2$, hydroxyl.

## Introduction

Free radicals have significant interest among scientists in the past decade. Their broad range of effects in biological system has drawn on the attention of many experimental works[1].Cells are impaired by an imbalance between ROS generating & scavenging systems. Thus ROS plays an important role in the pathogenesis of clinical human diseases including neurodegenerative disorders, cardiovascular diseases & mutagenesis[2, 3].Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and repercussion injury of many tissues, a central nervous system injury, gastritis and cancer. Due to environmental pollutants, radiation, chemicals, toxins, deep fries and spicy foods as well as physical stress, free radicals cause depletion of the immune system antioxidants, the change in gene expression and induce abnormal proteins. The oxidation process is one of the most important routs for producing free radicals in food, drugs, and even living systems.

The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators.. A number of synthetic

antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxyl toluene (BHT) have been extensively added to foodstuffs, although their use has begun to be questioned because of their toxicity, so there is considerable interest in preventive medicine and in the food industry in the development of natural antioxidants obtained from botanical sources, especially herbal plants[4].

The plant of *Bridelia retusa* Spreng. Syn:*Bridelia airy shawii* (Family: Euphorbiaceae, Sanskrit: Asan, Hindi: Khaja) is a small to moderate sized deciduous tree, spinous when young with the grey bark, found through out India up to altitude of 1000 m except in very dry regions. In Pharmacological trials the bark of *Bridelia retusa* exhibited anti-viral, hypoglycemic and hypotensive properties[5].According to Ayurveda, the bark is good for removal of urinary concretions, useful in lumbago and hemiplegia. The bark is used as liniment with gingelly oil in rheumatism[6] .The bark is documented to be used ethno botanically to promote antifertility. The presence of triterpene ketone [4-desmethyl eupha 7, 24 (28) - diene-3-one] in the bark has been reported. The bark contains 16-40% of tannins[7] .Phenolics including tannins are the natural products present in abundant amount & possess various biological properties related to antioxidant mechanisms[8]. Literature survey revealed that there is no systematic approach has been made on the plant *Bridelia retusa* so it was therefore need to investigate the phyto-constituents of these plants and antioxidant property.

## Experimental

The bark of *Bridelia retusa* was collected from hilly region of Toranmal Dist-Nandurbar, India. The plants was identified and authenticated by Dr. D.A. Patil, Taxonomist, Department of Botany, SSVPS science college Dhule, India. The voucher specimens were deposited in the department.

### Preparation of plant for extraction of crude tannins

The bark of *Bridelia retusa* was air-dried at the laboratory for 10 days and then ground into a uniform powder using a pulverizer. (DRONE 9500). The powdered materials were stored in airtight bottles for chemical and biological analysis. Defatted bark powder was extracted with acetone: water mixture (70:30) to obtained ACE i.e. total tannins rich fraction.

### Physico chemical evaluation Or Quantitative estimation of chemical standards:

***Fat-Lipid soluble sample***

25 g of the bark sample was extracted with 100 mL of petroleum ether (60-80) using a Soxhlet apparatus for 6 h.

***Total phenolic content***

Total soluble phenolic levels were analyzed by a modified procedure adapted from Yen chang by using the Folin–Ciocalteu reagent. Briefly, extract (1mg/ml) was diluted with 3 mL distilled water. A volume of 0.5 mL of Folin–Ciocalteu reagent was added and the mixture allowed standing for 3 min at room temperature. Then 2ml of 20 % $Na_2CO_3$ was added and the mixture allowed to stand for 90 min, after which absorbance was measured at 765 nm after cooling in darkness. Ethanol was used as blank. Total phenolic contents were expressed as mg gallic acid/100 g, based on a standard curve of 0.2–1 mg of gallic acid/mL[9].

***Estimation of Total Tannins:***

***a) Titrimetric method***

Defatted bark powder boiled with 300ml of double distilled water for 2 hr, cooled and diluted up to 500 ml and filtered. Measured 25 ml of filtrate in to 2 liter conical flask add 20 ml indigo solution and 750 ml of double distilled water and Titrate with standard 0.1 N potassium permanganate ($KMNO_4$) solution, until blue solution changes to green, then add a few drops at a time until solution became golden yellow colour. Similarly, titrate the mixture of 20 ml indigo carmine solution and 750 ml of double distilled water. Multiplied the difference between two titration by the factor to obtained value of total tannins. Factor: Each ml of 0.1 N potassium permanganate is equivalent to 0.004157 g of total tannins. Quantity of total tannins (%) = $[(T_2-T_1)$ X actual normality X 0.004157 X 1000] / W X 0.1

**b) *Animal Hide Powder Method*:**

Dried bark powder (20 g) in to a conical flask containing 150 ml water, heated on a boiling water bath for 30 min. and cooled. Transferred the mixture to a 250 ml volumetric flask and diluted it with water up to the mark. Allow the solid material to settle and filtered the liquid through the filter paper, discarded the first 50 ml of the filtrate. Determined the total amount of material extractable in to water, by evaporating 50 ml of the bark extract to dryness and dried the residue in an oven at 105 $^oC$ for 4 h and weighed **($T_1$)**. Determined the amount of bark extract not bind to the hide powder extractable in to water, taken 80 ml of the bark extract, added 6.0 gm of hide powder and shaken well for 60 min. Filtered and evaporated 50 ml of the clear filtrate to dryness. Dried the residue in an oven at 105 $^oC$ and weighed **($T_2$).** Determined the solubility of hide powder, taken 6 gm of hide powder, added 80 ml of water and shaken well for 60 min. Filtered and evaporated 50 ml of the clear filtrate to dryness. Dried the residue in an oven at 105 $^oC$ and weighed **($T_0$).** Percentage of total tannins is calculated by the formula: % of total tannins= $[T_1-(T_2+T_0)]$ X 500 /w, Where, W= Weight of the bark powder[10].

***Total mucilage content***

Dried stem bark was powered by using pulverizer passed through sieve no 80. The dried powder of (100 g) was defatted with pet ether (40-60 o) for 24hr. and defatted marc was extracted twice with hot water at temp.60-70$^0$ for 3-4 h. The mixed hot aqueous extract was centrifuged at 10000 rpm for 30 min., decanted and passed through 0.45 µm syringe Millipore filter to remove any debris. The filtered aqueous extract was added with 3 volumes of acetone and allowed to stand overnight. The precipitate was then collected by centrifugation and dried in vacuum at room temp. Further purification was done with small quantity of hydrogen peroxide. Mucilage was bleached completely and then washed successively with ethanol & acetone, dried in oven at 50$^0$C and the percentage yield was calculated[11].

***Determination of Total Flavonoids Content***

Total flavonoids content was determined by the Aluminium chloride colorimetric assay.An aliquot (l ml) of standard solution of rutin (20, 40, 60, 80,100 µg/ml) was added to 10 ml volumetric flask containing 4 ml of 5 % $NaNO_2$. After 5 minute 0.3 ml of 10 % $AlCl_3$ was added into above solution. At 6$^{th}$ minute 2 ml of 1 molar NaOH was added and the total volume was made up to 10 ml with distilled water. Same dilutions were prepared with the test solution. Blank determination was done by using methanol in place of test or standard solutions. Mixed well and absorbance was measured at 358 nm against blank. The total flavonoids content of methanolic extract of *B.retusa* bark was expressed as µg rutin equivalent[12].

***Proanthocyanidin content***

Total proanthocyanidin were determined using vanillin-$H_2SO_4$ method. One ml of
extract (50 & 100 µg/ml) was mixed with two ml of freshly prepared vanillin solution (1% vanillin in 70% of $H_2SO_4$) and maintained for 15 min at 20 $^0$C. The absorbance was measured at 500 for epicatechin (8-40 µg/ml) and 280 nm for Catechin (30-120 µg/ml).Calibration curved was drawn. The results were given in table. All experiments were performed in triplicate and mean values were used for calculation. Spectrophotometrically determinations were performed using on Shimadzu UV-2450 spectrophotometer.[13]

***Determination of ellagic acid***

10 mg of *B.retusa* tannin in 2N $H_2SO_4$ (5mL) were put into constricted test tubes and frozen. The tubes were vacuum-walled and heated for 24h at 100°C. Tubes were cooled, opened and the filtered content made up to 10 mL with pyridine. Then 1.1mL of pyridine and l mL of sample were mixed in a dry test tube, 0.1 ml of concentrated HCl was added to it. The sample was brought to 30°C. The sample was quickly mixed after addition of 0.1mL of 1% (w/v) $NaNO_2$in $H_2O$, and the absorbance at 538 nm was immediately recorded. After a 36 min incubating period at 30°C, the absorbance was again recorded. The difference between the initial absorbance and the absorbance at 36 min. (A538) was proportional to the ellagic acid concentration. The measured absorbance obeys the relationship: A538=[0.0074 × (mg of ellagic acid)] – 0.0296. Ellagic acid (10-50 mcg/ml) was used as a standard and the data were based on experiments carried out in triplicate. [14]

***Carbohydrates determination***

1 g of the powdered bark was weighed and transferred to a boiling tube. The sample was hydrolyzed by keeping it in a boiling water bath for three hours with 50 ml of 2.5N hydrochloric acid. Then the mixture was cooled and neutralized with sodium carbonate until the effervescence ceases. The volume was made up to 100 ml and centrifuged. The supernatant was collected and 1ml aliquot was taken for analysis.

1 ml solutions of different concentrations (20, 40, 60, 80, 100 µg/ml) of glucose were prepared and taken in each test tube. Then 1ml of 5 % phenol solution was added to each tube including solutions of samples. 5ml of 96 % sulphuric acid was added to sample solution as well as in solutions of standards and mixed well. After 10 minutes the contents were shaken in the tubes and placed in water bath at 25-30$^0$C for 20minutes.The absorbance of the solutions was measured at 490nm.The amount of total carbohydrates present in sample was calculated from standard curve [15].

***Anti oxidant activity***

***1,1 Diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging activity***

The free radical scavenging activity of ACE *Bridelia retusa* and ascorbic acid were measured in terms of hydrogen donating or radical scavenging ability using the stable radical of DPPH (1,1-diphenyl-2-picryl-hydrazyl), according to method of Blois [16,17]. To 3 ml of different concentrations (20-100µg/ml) of extract, 1 ml of 0.1mM solution of DPPH in ethanol was added. 30 min later, absorbance was recorded at 517 nm in triplicate and % scavenging was calculated to the corresponding blank reading. The antioxidant activity of the extract was expressed as the $IC_{50}$ value which was defined as concentration (in µg/ml) of extracts required to scavenge 50 %.DPPH free radical. DPPH scavenging effect (%) = $(A_0-A_1/A_0)$ x100, where, $A_0$ is the absorbance of the control reaction &$A_1$ is the absorbance presence in the different samples.

***Total reduction capability by $Fe^{3+}$ -- $Fe^{2+}$ transformation***

In this assay, the yellow color of the test solution changes to various shades of green & blue depending

upon the reducing power of each antioxidant samples. ACE in 1.0 ml of deionized water was mixed with phosphate buffer (2.5ml, 0.2M, pH 6.6) and 1 % potassium ferricyanide ($K_3Fe(CN)_6$ ,2.5ml). The mixture was incubated at 50ºC for 20 min. Aliquots of trichloroacetic acid (TCA, 2.5 ml, 10%) were added to mixture, which was then centrifuged at 3000 rpm for 10 min at 1000g. The upper layer of solution (2.5 ml) was mixed with 2.5 ml of freshly prepared $FeCl_3$ solution (1%) in distilled water and absorbance was measured at 700 nm. Using ELISA micro plate reader. Increased absorbance of the reaction mixture indicates increased reducing power. [18, 19]

**Thio barbituric acid reacting substances Assay**

Rat liver homogenate 10 % was prepared according to the procedure described by Tripathi *et. al.* The mixtures containing 0.5 ml of homogenate (10%), 1 ml of 0.15 M KCl and 0.5 ml of different concentrations (20-100µg/ml) of ACE were prepared. Lipid peroxidation was initiated by adding 100 µl of 1 mM ferric chloride. The reaction mixture was incubated for 30 minutes at 37ºC. After incubation, the reaction was stopped by adding 2 ml of ice-cold 0.25 N HCl containing 15 % trichloroacetic acid (TCA) and 0.38% thiobarbituric acid (TBA), and 0.2 ml of 0.05% Butylated hydroxyl toluene (BHT). These reaction mixtures were heated for 60 minutes at 80ºC, cooled and centrifuged at 6900rpm for 15 min. The absorbance of supernatant was measured at 532 nm against blank, which contained all reagents except liver homogenate and drug. Identical experiments were performed to determine the normal (without drug and $FeCl_3$) and induced (without drug) lipid peroxidation level in the tissue [20,21].The percentage of anti-lipid Peroxidation effect (% ALP) was calculated by following formula.

$$\% \text{ ALP} = \frac{FeCl_3 \text{ O.D - Sample O.D.}}{FeCl_3 \text{ O.D - Normal O.D.}} \times 100$$

***Scavenging activity against Metal Ion-Dependent of Hydroxyl radical (OH˙)***

The scavenging capacity for hydroxyl radical was measured according to the modified method of Halliwell *et al,* 1987. The assay was performed by adding 0.1 ml EDTA (1 mM), 0.01 ml $FeCl_3$ (10mM), 0.1 ml of $H_2O_2$ (10 mM), 0.36 ml of deoxyribose (10 mM) in each tube. Transferred 1.0 ml of MEBR extract (20-100 µg/ml) in distilled water, 0.33 ml of Phosphate buffer (50 mM, $P^H$ 7.4) and 0.1 ml of ascorbic acid (1 mM) in sequence. The mixtures were incubated at 37 ºC for 1 h. After incubation, an equal volume of 10 % trichloroacetic acid (TCA) and 0.5% thiobarbituric acid (TBA) (in 0.025M NaOH containing 0.025 % BHA) was added and the mixtures were boiled at 100 for 15 min. The pink chromogen measured at 532 nm.The hydroxyl radical scavenging activity of the extract is calculated by % inhibition of deoxyribose degradation[22].

***Hydrogen peroxide radical Scavenging activity:***

The hydrogen peroxide scavenging ability of ACE was determined according to the method of Ruch *et al* .A solution of hydrogen peroxide( $H_2O_2$) was prepared in phosphate buffer pH 7.4 .Different concentrations of ACE (20-100 µg/ml) were added to the $H_2O_2$ solution (0.6 ml, 40Mm). After 10 min absorbance of $H_2O_2$ was recorded at 230 nm against blank solution without $H_2O_2$.

***Nitric oxide scavenging assay***

Nitric oxide generated from sodium nitroprusside was measured by the Griess reagent by the method of Marcocci et al. (1994). Sodium nitroprusside (5mM) in standard phosphate buffer solution was incubated with different concentration of extracts was dissolved in standard phosphate buffer (0.025M, pH 7.4) and the tubes were incubated at 25°C for 5 hr. After 5 h, 0.5 ml of incubation solution containing nitrite was pipetted and mixed with 0.5 ml Griess reagent (prepared by mixing equal volume of 1% sulphanilamide in 2% phosphoric acid and 0.1% naphthylelene diamine dihydrochloride in water) and allowed to stand for 5 min for completing diazotization. The absorbance of chromophore formed was measured at 546 nm against the corresponding blank solutions using micro titer plate reader (BIO-Tek Power wave TM XS, Model-96well micro plate). The experiment was performed (in triplicate) and % scavenging activity was calculated using the formula 100-[100/blank absorbance × sample absorbance. The activity was compared with ascorbic acid, which was used as a standard antioxidant. $IC_{50}$ for test extracts was calculated[23].

**Statistical Analysis:**

Statistics of comparisons between groups were carried out by one way analysis of variance (one-way ANOVA) followed by student T-test using the Prism 4.03 (Graphic Pad Software, Inc., USA). Results with $P < 0.05$ were considered statistically significant.

## Results and Discussion

The Phytochemical standards of *Bridelia retusa* bark *is* shown in Table 1. Flavonoids in many plants have a wide range of actions. They are anti-inflammatory, anti-oxidant and are especially useful in maintaining healthy circulation The Flavonoids content was very high in *Bridelia retusa* 257 mg 100 g-1 Rutin equivalence of flavonoids. The biological functions of flavonoids include protection against allergies, platelet aggregation, microbes, ulcers, hepatoxins, viruses and

tumors. Flavonoids reduce the risk of estrogen-induced cancers by interfering with the enzymes that produce estrogen. Flavonoids significantly inhibit lysosomal enzyme secretion and arachidonic acid release from membranes by inhibiting lipoxygenase, cycloxygenase and phospolipase A2 [24,25].

Tannins content was more in *Bridelia retusa bark i.e.* 33 -38 g of tannins 100 g-1. Tannins and tannin like substances are widespread in nature and are probably present in all plant materials. Tannins could affect the inflammatory response via their radical scavenging activities. There is ample evidence supporting the connection between inflammation and free radical reactions. Lin and Lin reported that the galloyl group and the phenolic hydroxyl groups at the 3' position on EGCG (epigallocatechin gallate), which is a tannin found in green tea, were responsible for its anti-inflammatory properties [26,27].

Total phenolic content estimated in bark of *Bridelia retusa* was found to be 2.92 g/100g.The presence of phenols in these plants indicates that they could act as antioxidants, immune enhancers and anti inflammatory agents. Phenols have been responsible in having the ability to block specific enzymes that causes inflammatory disorders. Proanthocyanidins are currently of great interest because of their properties as anti-oxidants and radical scavengers Tannins occur in complex mixtures. Carbohydrates and mucilage content of *B.retusa* was also in good amount.

The scavenging effect of ACE and standard on the DPPH radical was 79.56 % at 100 µg/ml whereas $IC_{50}$ value 47.20 µg/ml. which was comparable to that of ascorbic acid & BHA 20.82 and 35.34 µg/ml respectively. (Table 2) For the measurement of the reductive ability, we investigated the $Fe^{3+--}$ Fe $^{2+}$transformation in the presence of ACE sample. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. As concentration of extract increases reducing power also increases. ACE of *Bridelia retusa* was effective in inhibiting the lipid peroxidation induced by $Fe2^{+}$ ascorbate system in rat liver homogenate .The generation of malondialdehyde (MDA) and related substances that react with thiobarbituric acid (TBARS) was found to be inhibited by the extract. This indicated the significant lipid peroxidation inhibiting activity of the extracts. The IC50 was found 110.76 µg/ml. The degradation of deoxyribose to TBARS by hydroxyl radical generated from Fe3+ -ascorbate- EDTA- $H_2O_2$ system was markedly decreased by the ACE .This indicated the significant hydroxyl radical scavenging activity of the extract. The effect ACE on the inhibition of free radical mediated deoxyribose damage was assessed by means of Iron-II dependent DNA damage assay. The $IC_{50}$ was found 48.84 µg/ml. The scavenging effect of crude tannins on hydrogen peroxide and the comparison with AA as standard in dose dependent manner. Crude tannins and AA exhibited 76.79 %, and 82.38 % scavenging activity on hydrogen peroxide at 100 µg/ml respectively. In other words, hydrogen peroxide scavenging activity of ACE was higher than ascorbic acid, suggesting that crude tannins had stronger hydrogen peroxide scavenging activity. ACE exhibited $IC_{50}$ 44.15 µg/ml which was comparable to and ascorbic acid (17.95 µg/ml). Hydrogen peroxide is a normal metabolite in living cells. Recent investigations have shown that the antioxidant properties of plants could be correlated with oxidative stress defense and different human diseases. In this respect flavonoids and other polyphenolic compounds have gained the greatest attention[28].

## Conclusions

The results of this study showed that tannins rich fractions of bark has strong antioxidant activity by inhibiting DPPH, ALP, reducing power, hydroxyl radical and hydrogen peroxide and nitric oxide scavenging when compared with standard.

In the present study, we demonstrate *Bridelia retusa* plant contain phenolic compounds which can serve as natural sources to develop free radical scavengers. The phenolic compound may contribute directly to the anti oxidant action it is suggested that poly phenolic compound may have inhibitory effects on mutagenesis & carcinogenesis in human. Phenolic are known as powerful chain breaking antioxidants because of hydroxyl group [29-32] and can be used as easily accessible source of natural. However, the components responsible for the antioxidant activity of tannins rich fraction of the bark of *Bridelia retusa* are currently unclear. Therefore, it is suggested that further work should be performed on the isolation and identification of the antioxidant components in tannins rich fraction.

**Table 1. Phytochemical standards composition of *Bridelia retusa bark* on dry weight basis (g /100g)**

| Phytochemical standards | Results |
|---|---|
| Fats, lipids | 0.5 |
| Phenolic | 2.92-3 |
| Flavonoids | 0.257 |
| Tannins | 33-40 |
| Proanthocyanidins | 2.98-4 |
| Ellagic acid | 0.135 |
| Mucilage | 3-4 |
| carbohydrates | 7-7.1 |

**Table 2. In vitro antioxidant data of *Bridelia retusa* bark**

| Test compound | % inhibition | | | | |
|---|---|---|---|---|---|
| | DPPH | ALP | OH | $H_2O_2$ | Nitric oxide |
| ACE | 79.56±1.69 | 45.74±0.321 | 74.79±4.27 | 76.79±5.31 | 71.02±2.67 |
| Ascorbic acid | 91.33±5.33 | 64.56±0.50 | 86.38±0.98 | 82.82±3.12 | 80.25±1.06 |
| BHA | 88.79±4.12 | 63.54±1.42 | 73.18±8.54 | 81.09±1.61 | 82.23±3.68 |
| $IC_{50}$ | 47.20 | 110.76 | 48.51 | 44.15 | 50.42 |

## Acknowledgement

The authors are very grateful to ISTAR, and Director SICART, Vallabh Vidhyanagar, Gujarat, India for providing required facilities for this work. Author(s) are also thankful to the Dr.D.A.Patil, Taxonomist, and Head of Depts. Botany Dhule (MS) for his valuable support in authentication of plant material for this study.

## References

1. Fejes S, Blazovics A, Lugasi A, Lemberkovics E, Petri G, Kery A, *J Ethnopharmacol*.,2000, 69 ,259-265.
2. Hou W.C, Lin R.D, cheng, K.T, *Phytomedicine,* 2003*,* 10,170-175.
3. Grice H.C, *Food Chemistry and Toxicology* ,1986*,* 24 ,1127-1130
4. Ljiljana Stanojević , Mihajlo Stanković , Vesna Nikolić , Ljubiša Nikolić, Dušica Ristić Jasna Čanadanovic-Brunet and Vesna Tumbas, *Sensors,* 2009, 5702-5714
5. Anonymous. *The Wealth of India*. 1988, 2 (B), 295-97.
6. Kirtikar KR, Basu BD, *Indian Medicinal Plants,* 1999, 3, 2212-14
7. Chopra RN, Nayer SL. *Glossary of Indian Medicinal plants*, 1992,162.
8. Christel Q D, Bernard G, Vasseur J, *J.ethanopharmacol, 2000,* 72, 35-42.
9. Yen Chaung, *Journal of Agricultural and Food Chemistry*, 2000, 48, 2760-65.
10. Anonymous,. *Indian Herbal Pharmacopoeia*. Indian Drug manufacturer's Association, Mumbai, Revised New edition, 2002, 1-11.
11. Heng-Long Wang, Kai-Wun Yeh, Peng-Ren chen, Chih-hao chang, *Biosci. Biotechnology. Biochem***,** 2006, 70(2), 551-553
12. Lin, J.-Y.; Tang, C.-Y,*Food Chem,* 2007, *101*, 140–147.
13. Olfa T, Chiraz T, Cherif B, sakly M. *Ind,J. Of Expt.Biol, 2006,* (44) 246-249
14. Garro GJM, Riedl B and Conner AH. *Holzforschung.* 1997,(51) 235-243
15. Dubois M, Gilles KA, Hamilton JK, Reberts PA. *Anal chem*, 1956,(28)350-356
16. Blois MS, *Nature.1958,* 181, 1199-1200.
17. Joaquin A., Sofia S., Mercedes Perez-Bonilla, Pablo J. Linares, Teris A. Van Beek, Manuel Nogueras, Aldolfo Sanchez, *Fitoterapia,* 2005, 76,348-351.
18. IIhami g . O I Kufrevioglu, Munir oktay, *J.ethanopharmacol, 2004,* 90, 205-15
19. Oyaizu M, *Japanion Journal of Nutrition.*1986, 44 ,307-15.
20. Yerra, Rajeshear, Senthil Kumar GP, Malaya Gupta, Upal Kanti Mazumdar. *European Bulletin of Drug Research. 2005,*13 ,31-38.
21. Tripathi, Y.B., Sing, A.V, *Indian J. Exp. Biol*., 2001,39 ,798.
22. Halliwell B., Gutteridge, J.M.C., Arnoma OL., *Anal Biochem*, 1987, 165,215-219.
23. Marcocci L, Packer L, Sckaki A, Albert GM. *Methods in Enzymology,* 1994, 234,462–475

24. Oboh, G.; Raddatz, H.; Henle, T, *J. Sci. Food. Agric,* 2008, *88*, 2486–2492
25. Konczak, I.; Okuno, S.; Yoshimoto, M.; Yamakawa, O, *J. Biomed. Biotechnol.* 2004, *5*, 287–292
26. Lin YL, Lin JK,*Mol. Pharmacology* 1997, *52,* 465-472.
27. Melanie Diane Jeffers, Advisor: Dr.Ann E.Hagerman,Thesis,2006, 6-7.
28. Soni KP.Suresh Kumar, Saraf MN, Indian *Journal of Pharmaceutical Sciences, 2003, 65* (1), 27-30.
29. Khlifi S, EI Hachmi Y, Khalil A, Es-Safi N, EI Abbouyi A,*Indian Journal of Pharmacology.* 2005,37 (4),227-31.
30. Soares JR, Dins TCP, Cunha AP,*Free Radical Research,1997,* 26,469-78.
31. Shahidi F, Wansundara PK, *Food Science and Nutrition,1992,* 32,67-103.
32. Hantano T, Edamatsu R, Mori A, *Chemical and Pharmaceutical Bulletin*. 1989, 37, 2016-21.

*****