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Aqueous Extract of Betel Nut and Betel Leaf: Natural Resource for Potential Antioxidant

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Abstract

Plants used in traditional medicine have shown to be effective against various disorders with free radical involvement. Several plants being used in folklore medicine also show wonderful properties against free radical mediated disorders. Betel nut and betel leaves is closely related with India culture especially among Hindus and used in many traditional ceremonies. According to Ayurveda, betel nut and betel leaves is a post meal digestive stimulant, oral deodorant, natural antiseptic, astringent, diuretic, mood elevator, aphrodisiac and nerve tonic. The most popular spectrophotometric methods for determination of the antioxidant capacity of plant extract, foods, beverages and vegetable extracts because the radical compounds can directly react with antioxidant. In this present study most popular spectrophotometric method is used to determination of the antioxidant capacity of betel nut and betel leaf aqueous extract. In this present study indicates that the aqueous extract betel leaf found to contain a noticeable amount of total antioxidant capacity compared with betel nut extract by phosphomolybdenum method. The antioxidant activity of these extracts may be attributed to the polyphenolic compounds with potential application to reduce oxidative stress with health benefits.

Introduction

Oxygen is absolutely important for the life of aerobic organism but it may become toxic if supplied at superior concentrations level. Dioxygen in its ground state is relatively unreactive; its incomplete reduction gives rise to active oxygen species (AOS) such as singlet oxygen, super oxide radical anion, hydrogen peroxide etc. This is partly due to the oxidative diverse physiological role in the body. The body is constantly exposed to the negative and sometimes lethal effects of oxidants during normal physiological processes (Manmohan *et al.*, 2011). Generally, up to 5% of inhaled oxygen may be converted to reactive oxygen species (ROS). These ROS have the ability to bind to cellular structures, and have been implicated in number of pathological processes such as aging,

inflammation, reoxygenation of ischemic tissues, atherosclerosis, cancer and even Parkinson's disease in men (Setiadi *et al.*, 2003).

Plants used in traditional medicine have shown to be effective against various disorders with free radical involvement. Several plants being used in folklore medicine also show wonderful properties against free radical mediated disorders (Sachin *et al.,* 2010). The most popular spectrophotometric methods for determination of the antioxidant capacity of plant extract, foods, beverages and vegetable extracts because the radical compounds can directly react with antioxidant (Goncalves *et al*., 2005). In evaluating the potential antioxidant functions of components in natural plant extracts as prophylactic agents or food additives, it is important to employ a number of analytical techniques since the antioxidant potency can differ substantially according to the physical and chemical parameters of the systems used for their characterization (Zhou and Elias, 2013).

Betel leaf has an esteemed place in human society right from the dawn of civilization, particularly in India (Sharma *et al*., 1996). Generally betel nut and betel leaves is closely related with India culture especially among Hindus and used in many traditional ceremonies. According to Ayurveda, betel nut and betel leaves is a post meal digestive stimulant, oral deodorant, natural antiseptic, astringent, diuretic, mood elevator, aphrodisiac and nerve tonic (Shrishailappa *et al.,* 2004). In addition, betel nuts and betel leaves relaxes the mind, creates a feeling of wellbeing and improves the vocal chords (Shrishailappa *et al.,* 2004; Bakhru, 2008). Betel leaf is useful for the treatment of various diseases like bad breath, conjunctivitis, boil and abscesses, headache, constipation, hyteria, mastitis, itch, leucorrhoea, ringworm, rheumatism, abrasion, swelling of gum, cut and injuries etc., as folk medicine which the root is known for female contraceptive effect (Chopra *et al.,* 1956; Khanra, 1997). Previous researcher reported that the betel leaves possess antioxidant activity beside antimutagenic and anticarcinogenic properties, particularly against the tobacco carcinogens (Padma *et al.,* 1989; Change *et al*., 2002; Padma *et al.,* 1989; Wu *et al.,* 2004) due to presence of ingredients like hydroxychavicol (Amonkar *et al.,* 1898). Akhilesh *et al*. (2015) suggested Ayurvedic methods of betel leaves chewing are very much safe and good for health. The problems related with betel leaves chewing are mainly due to its use along with tobacco and its product. It has been reported betel leaf and betel nut have a wide spectrum of therapeutic properties. Betel nut (Areca nut) possesses significant analgesic and anti-inflammatory (Bhandare *et al.,* 2010) antidepressant (Dar and Khatoon, 2000) anti HIV activities (Vermani and Garg, 2002) and wound healing (Azeez *et al.,* 2007).

The antioxidant activity provides enormous scope in correcting the imbalances between free radicals and anti-free radicals, which is the major cause of several diseases. Many *in vitro* methods such as, Phosphomolybdenum method, DPPH, Nitric Oxide, Superoxide, Hydroxyl Radical, Hydrogen Peroxide have been developed to evaluate antioxidant activity. In this present study deals

with antioxidant and radical scavenging activity of betel nut and betel leaf extract were discussed.

2. MATERIALS AND METHODS

2.1 Total antioxidant activity

The total antioxidant capacity of the betel nut and betel leaf extract were evaluated by the phosphomolybdenum method according to the procedure described by Prieto *et al*. (1999). The assay based on the reduction of Mo (VI) to Mo (V) by the extracts and subsequent formation of green phosphate/Mo (V) complex at acid pH. A 0.3 ml of betel nut and betel leaf aqueous extract were pipetted into a series of test tubes combined with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated at 95 ºC for 90 min, cooled to room temperature. Then the absorbance of the solution was recorded at 695 nm using UV- Vis spectrophotometer (JASCO V-670) against blank (H_2O). The reducing capacity of the extract was expressed as gram equivalent of ascorbic acid. The calibration curve was prepared by mixing ascorbic (1000, 500, 250, 125, 62.5 and 31.25 µg/mL) with water.

2.2 DPPH radical scavenging activity

About 10 ml of the different concentrations of betel nut and betel leaf extracts were centrifuged at 3000 rpm for 10 min and supernatant collected. The supernatant of the extract (1ml) was added to 3 ml of methanolic solution of DPPH (2, 2-diphenyl-1-picrylhydrazyl) (20 mg/l) in a test tube. The reaction mixture was kept at 25°C for one hour in an incubator. The absorbance of the residual DPPH solution was determined at 517 nm in UV-Visible Spectrophotometer. Vitamin C was used as positive control; the inhibition was calculated in following formula (Mensor *et al*., 2001).

$$I\ (\%) = 100\times (A_0-A_1)/A_o$$

Where A_0 is the absorbance of the control, A_1 is the absorbance of the betel nut and betel leaf extract respectively.

2.3 Nitric oxide scavenging activity

The reaction mixture (3 ml) containing 2 ml of sodium nitroprusside, 0.5 ml of phosphate buffered saline and 0.5 ml of betel nut and betel leaf extracts was incubated at 25°C for 150 min. Control without test compound was kept in an identical manner. After incubation, 0.5 ml of griess reagent was added. The absorbance of the chromophore formed was read at 500 nm and the percentage inhibition was calculated by the following formula (Green and Hill, 1984).

Scavenging activity (% inhibition) = $A_e/A_c \times 100$

Where,

A_c = Absorbance of control

A_e = Absorbance in the presence of betel nut and betel leaf extract

2.4 Superoxide scavenging activity

The reaction mixture consisted of 2.55 ml phosphate buffer (0.067 M, 0.05 ml riboflavin, 0.1 ml of (NBT) nitro blue tetrazolium and 0.2 ml sodium chloride). The control tubes were also set up where dimethyl sulfoxide (DMSO) was added instead of sample of betel nut and betel leaf extracts. All the tubes were vortexed and measured the initial optical density at 560 nm. After that, these tubes were placed in an area where they received uniform illumination for 30 min; again the optical density was measured at

560 nm. The difference in optical density before and after illumination is the generation of superoxide by the test sample and calculated by comparing with the optical density of the control (Misra and Fridovich, 1972).

The scavenging activity of super oxide by of betel nut and betel leaf extracts and the standard compound was calculated using the formula,

Scavenging activity (% inhibition) = $(A_c - A_e)/A_c \times 100$

Where,

A_c = Absorbance of control

A_e = Absorbance in the presence of betel nut and betel leaf

2.5 Hydroxyl radical scavenging activity

The ability of betel nut and betel leaf extracts to scavenging hydroxyl radicals was determined according to the method of Elizabeth and Rao (1990). The hydroxyl radical attacks deoxyribose, which results in thiobarbituric acid reacting substance (TBARS) formation. The reaction mixture contained deoxyribose (2.8 mM), ferric chloride (0.1 mM), ethylenediaminetetraacetic acid (0.1 mM), hydrogen peroxide (1 mM), ascorbic acid (0.1 mM), potassium hydrogen phosphate/potassium hydroxide buffer (20 mM, pH 7.4), various concentrations of the betel nut and betel leaf extract was made up to a final volume of 1 mL. The reaction mixture was incubated for 1 h at 37°C. Deoxyribose degradation was measured as TBARS and percentage inhibition was calculated. Deoxyribose degradation was measured as (TBARS) Thiobarbituric acid reactive substances by adding 0.5 ml of (TBA) and 0.5 ml of (HCl) Hydrochloric acid, boiled in a water bath for 20 min, cooled and measured the absorbance at 532nm. The scavenging activity of hydroxyl ion by of betel nut and betel leaf extracts and the standard compound was calculated using the formula,

Scavenging activity (% inhibition) = $(A_c - A_e)/A_c \times 100$

Where,

A_c = Absorbance of control

A_e = Absorbance in the presence of betel nut and betel leaf extract

2.6 Hydrogen peroxide scavenging activity

The ability of betel nut and betel leaf extractsto scavenge Hydrogen Peroxide (H_2O_2) was determined according to the method of Ruch *et al.*, (1989). A solution of hydrogen peroxide (40mM) was prepared in phosphate buffer (pH 7.4). The betel nut and betel leaf extracts (100 µg/mL) in distilled water were added to a hydrogen peroxide solution (0.6 mL, 40 mM). Absorbance of Hydrogen Peroxide at 230 nm was determined 10 min later against a blank solution containing the phosphate buffer without hydrogen peroxide. The scavenging activity of (H_2O_2) hydrogen peroxide by betel nut and betel leaf extract and the standard compound was calculated using the formula,

Scavenging activity (% inhibition) = $(A_c - A_e)/A_c \times 100$

Where,

A_c = Absorbance of control

A_e = Absorbance in the presence of plant extract

3. RESULTS AND DISCUSSION

3.1 Total antioxidant activity

The phosphomolybdenum method usually detects antioxidants such as ascorbic acid, some phenolics, α-tocopherol, and carotenoids (Prieto *et al*., 1999). Ascorbic acid, glutathione, cysteine, tocopherols, polyphenols and aromatic amines have the ability to donate hydrogen and electrons and can thus be detected by the different assay models (Tevfik, 2010). TAC of the phosphomolybdenum model evaluates both water-soluble and fat-soluble antioxidant capacity (Total antioxidant capacity). The total antioxidant capacity of betel nut and betel leaf extracts expressed as equivalents of ascorbic acid. The result of the total antioxidant activity is shown in figure 1.

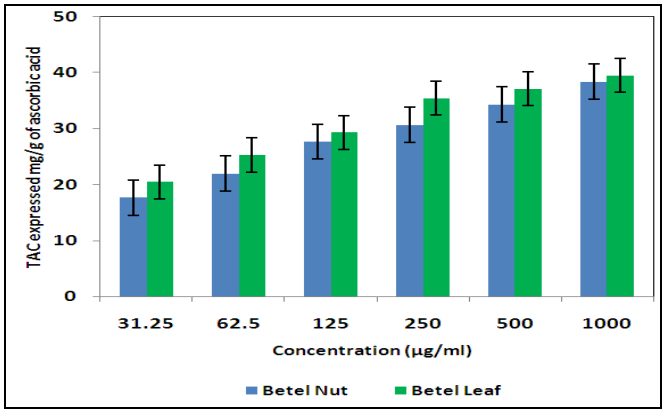

Fig. 1: Total antioxidant activity of betel nut and betel leaf extract

It is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate/Mo (V) complex at acid pH.

Antioxidant capacity of betel nut and betel leaf extract shown an increase in antioxidant capacity with an increase in concentration. Among the betel nut and betel leaf extract, total antioxidant capacity was found too modest highest in betel leaf extract. The antioxidative capacity is mainly due to phenolic components, such as phenolic acids, and phenolic diterpenes (Shahidi *et al*., 1992).

3.2 DPPH radical scavenging activity

Antioxidant on interface with DPPH radical transfer hydrogen or electron atom to DPPH and thus neutralizing its free radical nature and convert it to 1-1-diphenyl-2-picryl hydrazine and the degree of staining indicates the scavenging activity of the medicine. The reducing capacity of DPPH radical is calculated by the decrease in its absorbance at 517 nm induced by antioxidants. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Indumathy and Ajithadas, 2013). Therefore, DPPH is usually used as a substance to evaluate the antioxidant activity. The percentage of DPPH radical scavenging activity of extracts of betel nut and betel leaf are presented in Table 1.

Table 1: DPPH Radical Scavenging Activity

Concentration (µg/ml)	Ascorbic acid Inhibition (%)	Betel Nut Inhibition (%)	Betel Leaf Inhibition (%)
50	54.65±0.03	7.66±0.00	10.68±0.01
100	58.67±0.02	11.93±0.01	14.32±0.02
250	66.08±0.01	17.96±0.03	23.37±0.05
500	72.74±0.00	27.51±0.04	29.52±0.05

1000	84.42±0.02	37.94±0.05	47.11±0.00

Values are mean ± S.D of three replicates

The aqueous extract of betel nut and betel leaf exhibited a maximum DPPH scavenging activity of 37.94% and 47.11% whereas, ascorbic acid (standard) exhibited 84.42% at concentration of 1000 µg/ml. The present study revealed that the aqueous extract of betel nut and betel leaf showed significant DPPH radical scavenging activity.

3.3 Nitric oxide scavenging activity

Nitric oxide generated from sodium nitroprusside is measured by the Greiss reagent reduction. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide. The percentages of nitric oxide radical scavenging activity of betel nut and betel leaf aqueous extract are presented in Table 2. The absorbance of the chromophore formed was read at 500 nm and the percentage inhibition was calculated. The aqueous extract of betel nut and betel leaf revealed a maximum nitric oxide scavenging activity of 35.93 and 37.06%. The standard exhibited 76.12% at the maximum concentration of 1000 µg/ml. Indumathy and Ajithadas (2013) study reported that n-hexane, chloroform, ethyl acetate and methanol extracts of *Lepidium sativum* exhibited a maximum nitric oxide radical scavenging activity at 1000 µg/ml. The present experiment revealed that the betel nut and betel leaf aqueous extract exhibit considerable nitric oxide scavenging activity.

Table 2: Nitric Oxide Scavenging Activity

Concentration (µg)	Ascorbic acid Inhibition (%)	Betel Nut Inhibition (%)	Betel Leaf Inhibition (%)
50	48.13±0.03	1.76±0.02	2.76±0.01
100	57.76±0.02	6.66±0.03	12.31±0.03
250	61.54±0.01	14.07±0.05	20.23±0.05
500	69.25±0.00	24.5±0.02	27.89±0.02
1000	76.12±0.02	35.93±0.01	37.06±0.03

Values are mean ± S.D of three replicates

3.4 Superoxide scavenging activity

Super oxide radical known to be very harmful to the cellular components. Superoxide free radical is formed by alkaline DMSO which reacts with nitro blue tetrazolium (NBT) to produce coloured diformazan (Indumathy and Ajithadas, 2013). The percentage of superoxide scavenging activity of betel nut and betel leaf extract are shown in table 3. Betel nut and betel leaf aqueous extract showed maximum superoxide scavenging activity of 33.42 and 35.93%. On other hand, the standard ascorbic acid exhibited 72.74 at 1000 µg/ml. The results depict that the aqueous extracts of betel nut and betel leaf showed moderate superoxide scavenging activity than that of standard ascorbic acid.

Table 3: Superoxide Scavenging Activity

Concentration (µg/ml)	Ascorbic acid Inhibition (%)	Betel Nut Inhibition (%)	Betel Leaf Inhibition (%)
50	43.17±0.03	6.91±0.02	9.17±0.00
100	49.01±0.02	7.29±0.03	14.95±0.01
250	58.02±0.01	19.35±0.01	25.75±0.02
500	66.15±0.00	23.12±0.03	31.28±0.03
1000	72.74±0.00	33.42±0.04	35.93±0.04

Values are mean ± S.D of three replicates

3.5 Hydroxyl radical scavenging activity

Hydrogen peroxide is a central reactive oxygen species because of its ability to infiltrate biological membranes. Though, it may be toxic if converted to hydroxyl radical in the cell (Gulcin *et al*., 2009). Scavenging of H_2O_2 by the plant extracts may be attributed to their phenolics, which donate electron to H_2O_2, thus reducing it to water (Vadivukkarasi and Pavithra, 2014). The percentage of superoxide scavenging activity of betel nut and betel leaf extract are exposed in Table 4.

Table 4: Hydroxyl Radical Scavenging Activity

Concentration (µg)	Ascorbic acid Inhibition (%)	Betel Nut Inhibition (%)	Betel Leaf Inhibition (%)
50	48.13±0.03	1.38±0.01	4.27±0.01
100	56.71±0.02	10.43±0.02	14.32±0.01
250	64.07±0.01	14.2±0.03	23.12±0.02
500	71.25±0.00	25.02±0.00	28.02±0.02
1000	77.34±0.02	32.04±0.00	38.07±0.03

Values are mean ± S.D of three replicates

The aqueous extract of betel nut and betel leaf showed maximum hydroxyl radical scavenging activity of 32.04 and 38.07% respectively, whereas standard exhibited 77.34%. The obtained results described that the aqueous extracts of betel nut and betel leaf showed reasonable hydroxyl radical scavenging activity than that of standard.

3.6 Hydrogen peroxide scavenging activity

The ability of betel nut and betel leaf extract to scavenging H_2O_2 was measured spectrophotometrically. The percentage of superoxide scavenging ability of betel nut and betel leaf extract are represented in Table 5.

The betel nut and betel leaf extract showed maximum H_2O_2 scavenging activity of 34.55 and 35.18%. Standard ascorbic acid exhibited maximum 85.74% at 1000 µg/ml. The hydrogen peroxide scavenging activity was concentration depended behavior and was comparable to that of the standard ascorbic acid.

The composition of hydrogen peroxide into water may take place according to the antioxidant compounds present in the extract are superior electron donors, they may accelerate the conversion of H_2O_2 to H_2O (Ranju *et al.*, 2011).

Table 4.5: Hydrogen Peroxide Scavenging Activity

Concentration (µg/ml)	Ascorbic acid Inhibition (%)	Betel Nut Inhibition (%)	Betel Leaf Inhibition (%)
50	55.15±0.03	5.4±0.01	3.14±0.01
100	62.87±0.02	12.94±0.02	11.93±0.02
250	72.02±0.01	20.35±0.03	17.84±0.05
500	79.12±0.00	25±0.00	26.38±0.06
1000	84.74±0.02	34.55±0.00	35.18±0.04

Values are mean ± S.D of three replicates

4. CONCLUSION

The findings of the present study indicated that the aqueous extract of betel nut and betel leaf showed reasonable antioxidant activity by inhibiting DPPH, nitric oxide, superoxide, hydroxyl and hydrogen peroxide savening activities. In addition aqueous extract betel leaf found to contain a noticeable amount of total antioxidant capacity compared with betel nut extract by phosphomolybdenum method. The antioxidant activity of these extracts may be attributed to the polyphenolic compounds with potential application to reduce oxidative stress with health benefits.

REFERENCES

Akhilesh, S., S. Anupama, A.S. Baghel and V. Mahes, (2015). Ayruvedic Tambula Savana- A Healthy Traditional Practice. ***Int. J. Herbal Medicine,*** 3(1): 40-44.

Azeez, S., S. Amudhan, S, Adigam N. Rao, N. Rao and L. A. Udupa, (2007). Wound healingprofile of Areca catechu extracts on different wound models in wistarrats. ***Kuwait Med. J.,***39:48-52.

Bakhru, H.K. (2008). *Herbs that Heal: Natural remedies for good Health*, published by, Orient paperbacks, New Delhi. pp. 40-43.

Bhandare, A.M., A.D. Kshirsagar, N. S. Vyawahare, A. A. Hadambar and V.S Thorve, (2010). Potential analgesic, anti-inflammatory and antioxidant activities ofhydroalcoholic extract of Areca catechu L. nut. ***Food Chem. Toxicol.,*** 48:3412-3417.

Change, M.C., B.J. Uang, H.L. Wu, J.J, Lee, L.J. Hahn and J.H. Jeng, (2002). Indcing the cell cycle arrest and apoptosis of oral KB carcincoma cells by hydroxychavicol: Roles of glutathione and reactive oxygen species. ***British J. Pharma.,*** 135: 619-630.

Chopra, R.N., S.L. Nayar and I.C. Chopra, (1956). *Glossary of India Medicinal Plants*, CSIR, New Delhi, pp.194.

Dar, A. and S. Khatoon, (2000). Behavioral and biochemical studies ofdichloromethane fraction from the Areca catechu nut. ***Pharmacol.Biochem. Behav.***, 65:1-6.

Elizabeth, K. and M. N. A. Rao, (1990). Oxygen radical scavenging activity of curcumin. ***Int. J. Pharm.,*** 58: 237-240.

Goncalves, C., T. Dinis and M.T. Batista, (2005). Antioxidant properties of proanthocyanidins of *Uncaria tomenosa* bark decoction: A mechanisms for anti-inflammatory activity. ***Phytochemistry***, 66: 89-98.

Green, M.J. and H. A.O. Hill HAO, (1984). Chemistry of dioxygen Methods. ***Enzymol.,*** 105.

Gulcin, I., M. Oktay, E. Kirecci and O. I Kufrevioglu, (2003). Screening of antioxidant and Antimicrobial activities of anise (*Pimpinella anisum* L) seed extracts**.** ***Food Chem.,*** 83:371-382.

Khanra, S. (1997). Pannvittiksilpakendra (In Bengali), *Betel Leaf based Industry*, NabannaBharati, 30(2):169.

Manmohan, S., A. Paul, H. P. Singh, S. K. Dubey and R. K. Songara, (2011). Synthesis and Evaluation of Antioxidant Activity of Semicarbazone Derivatives. ***Int. J. Pharmaceutical Sci. and Drug Res.,*** 3(2): 150-154.

Mensor, L.L., F.S. Meneze, G.G. Leitao, A.S. Reis, J.C. Dos santor, C.S. Coube and S.G. Leitao, (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. ***Phytother. Res.***, 15: 127-130.

Misra, H.P. and I. Fridovich, (1972). The Role of Superoxide Anion in the Autoxidation of Epinephrine and a Simple Assay for Superoxide Dismutase. ***J. Biol. Chem.,*** 247: 3170.

Padma, P.R, A.J. Amonkar and S.V. Bhide, (1989). Antimutagenic effects of betel leaf extract against the mutagenicity of two tobacco-specific N-nitrosamines. ***Mutagenisis,*** 4: 154-156.

Padma, P.R., V.S. Lalitha, A.J. Amonakar and S.V. Bhide, (1989). Anticarcinogenic effect of betel leaf extract against tobacco carcinogens. ***Cancer Lett,*** 45(3): 195-202.

Prieto, P., M. Pineda and M. Aguilar, (1999). Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E. ***Anal. Biochem.*** 269 (2): 337-341

Ranju, P., G. Kundlik, S. Nidhi, M. Misbah Hussain and N. Thirumoorthy, (2011). Antioxidant and free radical scavenging activity of ethanolic extract of *Morinda citrifolia*. ***Annals Biol. Res.***, 2(1): 127-131.

Ruch, R.J., S. J. Cheng and J.E. Klaunig, (1989). Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. ***Carcinogenesis.*** 10: 1003-1008.

Sachin, U.R., R. P. Priyanka and R. S. Mane, (2010). Use of Natural Antioxidants to Scavenge Free Radicals: A Major Cause of Diseases, ***Int. J. PharmTech Res.,*** 2(2): 1074-1081.

Setiadi, D.H., G. A. Chass, L. L. Torday, A. Varro and J. G. Papp JG, (2003). Vitamin E models. Can the antioxidants and pro-antioxidant dichotomy of α- tocopherol be related to ionic ring closing and radical ring opening redox reactions? ***J. molecular structure***, 620:93-106.

Shahidi, F., P. K. Janitha and P. D. Wanasundara PD (1992). Phenolic antioxidants. ***CRC Critical Rev. Food Science and Nutrition***. 32 (1): 67-103.

Sharma, M.L., A.K.S. Rawat, R.K. Khanna, A.R. Chowdhury and R.M. Raina, (1996). Flavour characteristics of betel leaves. ***Euro Cosmetics***. 5:22-24.

Shrishailappa, B., R.R. Sujaya and B. Suresh, (2004). In Vitro antioxidant preperties of India traditional pann and its ingredents. ***Indian J. Traditional knowledge.*** 3(2): 187-191.

Sulekha. M., Y. Satish, Y. Sunita and N. Rajesh Kumar, (2009). Antioxidants: A Review. ***J. Chemical and Pharmaceutical Res.,*** 1(1): 102-104.

Tevfik Ozen. (2010). Antioxidant activity of wild edible plants in the Black Sea Region of Turkey. ***Grasas Y Aceites***, 61 (1): 86-94.

Vadivukkarasi Sasikumar and Pavithra Kalaisezhiyen, (2014). Evaluation of Free Radical Scavenging Activity of Various Leaf Extracts from *Kedrostis foetidissima* (Jacq.) Cogn., ***Biochem. Anal. Biochem.,***3(2): 1000150-1000157.

Vermani, K. and S. Garg, (2002). Herbal medicines for sexually transmitted diseasesand AIDS. ***J. Ethnopharmacol.,*** 80:49-66.

Wu, M.T., D.C. Wu, H.K. Hsu, E.L. Kao and J.M. Lee, (2004). Constituents of areca chewing related to espohageal cancer risk in Taiwanese men. ***Disease of the Esophagus***, 17(3): 257-259.

Zhou, L. and R. J. Elias, (2013). Antioxidant and pro-oxidant activity of (-)-

epigallocatechin-3-gallate in food emulsions: Influence of pH and phenolic concentration. ***Food Chem.,*** 138(2-3):1503-1509.