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Phytochemical screening and *in vitro* antioxidant activity of *Bacopa monniera* stabilized silver and gold nanoparticles

John Sushma N[1],
Department of Biotechnology, Sri Padmavati Mahila University, Tirupati, India

Deva Prasad Raju [2],
Department of Future Studies, Sri Venkateswara University, Tirupati, India

Mahitha B[3],
Department of Biotechnology, Sri Padmavati Mahila University, Tirupati, India

Uma Priya[4],
Department of Biotechnology, Sri Padmavati Mahila University, Tirupati, India

Prashanti Sam[5]
[c] Department of Chemistry, STBC Degree College, Kurnool, India

ABSTRACT

The current study demonstrates the formation of gold and silver nanoparticles using ethonolic extract of neurobeneficial plant, *Bacopa monniera*. The nanoparticles (GNPs and SNPs) were synthesized and coated with varied phytochemicals present in the whole plant ethanolic extract of *Bacopa monniera* (*Bm*GNPs and *Bm*SNPs). The *Bacopa monniera* stabilized nanoparticles (*Bm*GNPs and *Bm*SNPs) are investigated for their phytochemical constituents and antioxidant potential *in vitro*. *Bacopa monniera (Bm)* ethanolic extract, silver nanoparticles (SNPs), gold nanoparticles (GNPs), as well as *Bacopa monniera* stabilized silver *(Bm*SNPs*)* and gold *(Bm*GNPs*)* nanoparticles were studied for their phytochemicals by using standard qualitative methods and also performed DPPH radical scavenging activity, reducing power assay and metal chelating activity for *in vitro* antioxidant potential. The five test samples (*Bm* ethanolic extract, SNPs, GNPs, *Bm* stabilized silver (*Bm*SNPs) and gold (*Bm*GNPs) nanoparticles showed positive results for amino acids, carbohydrates, alkaloids, glycosides, flavonoids, phenols, terpenoids, saponins and steroids. But they showed negative test for tannins and anthraquinones. The results also showed that the *Bm* stabilized silver (*Bm*SNPs) and gold (*Bm*GNPs) nanoparticles have higher DPPH radical scavenging action, strong reducing power and metal chelating activity compared to other groups. The present results indicate that by combining the *Bacopa monniera* plant extract with silver and gold nanoparticles, the antioxidant potential of nanoparticles was enhanced. This study provide scientific rationale for the use of *Bm*SNPs and *Bm*GNPs as an antioxidants.

Keywords: *Bacopa monniera* stabilized nanoparticles, Phytochemicals, Antioxidant activity

Introduction

Free radicals have been implicated in the causation of several diseases like asthma, cancer, cardiovascular diseases, cataract, diabetes, gastrointestinal, liver diseases, muscular degeneration and neurodegenerative disorders (Pourmorad et al., 2006). They are continuously produced during cell metabolism and under normal conditions, they are scavenged and converted to nonreactive species by different intracellular enzymatic and non-enzymatic antioxidant system (Sen et al., 2010). Over production or an ineffective elimination of free radicals may induce oxidative stress and cause damage to all types of biomolecules such as proteins, lipids and nucleic acids (Shao et al., 2008). To attenuate these effects antioxidants act as free radical scavengers, reducing agents, chelating agents for transition metals, quenchers of singlet oxygen molecules and also act as activators of antioxidative defense enzyme system to suppress the radical damages in biological systems (Venkatesh et al., 2009: Murphy et al., 2011). Antioxidants thus play an important role in the protection of human body against damage by free radicals (Peng et al., 2011; Ling et al., 2011). Therefore, inhibition of free radical-induced oxidative damage by supplementation of antioxidants has become an attractive therapeutic strategy for reducing the risk of the diseases.

In recent years, it has been investigated that the synthesized nanoparticles from plant species are serving as antioxidants and received therapeutic significance (Pari and Amudha, 2011: Battu et al., 2011: Lawrence and Kapil, 2011). NPs exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. New applications of NPs and nanomaterials are emerging rapidly. Different strategies are used to synthesize NPs. Chemical synthesis lead to the presence of some toxic chemicals absorbed on the surface that may have adverse effect in the medical applications. Nanobiotechnology represents an economic alternative for chemical and physical methods of NPs formation. One of the most important criteria of nanobiotechnology is that of the development of clean, non toxic and eco friendly green chemistry procedures. Metal NPs such as silver, aluminum, gold, zinc, carbon, titanium, palladium, iron and copper have been routinely used for the synthesis of nanoparticles. However, former two metals (silver and gold) are most popular metals in bionanomaterial synthesis i.e, SNPs and GNPs. SNPs and GNPs were known to have antioxidant and antimicrobial properties (Barathmanikanth et al., 2010).

The therapeutic effects of several medicinal plants are usually based on their antioxidant phytochemical compounds. It has been suggested that there is an inverse relationship between dietary intake of antioxidant rich foods and incidence of human diseases (Yildrim et al., 2001). Plant based antioxidants are preferred to the synthetic ones because of their multiple mechanisms of actions and non-toxic nature. These facts have inspired the researchers for widespread screening of plants for possible medicinal and antioxidant properties. Antioxidant properties of *Bacopa monniera* (*Bm)* may offer protection from free radical damage in cardiovascular diseases and certain types of cancers (Russo et al., 2003). Hence, the current study was aimed to investigate the phytochemicals and *in vitro* antioxidant activity of *Bm* stabilized silver and gold nanoparticles.

Materials and Methods

Collection and preparation of *Bacopa monniera (Bm)* extracts

The *Bm* plant was obtained from Thummala gunta fields nearby Tirupati, Andhra Pradesh, India. The whole plant was thoroughly washed with double distilled water and dried under shade in dust-free condition for one week at room temperature. Then, the plant material was ground to fine powder. Finally powdered plant material (10 g) was extracted with 95% of ethanol (100 ml). The solution was left on constant magnetic stirring at room temperature for 24 hrs. The extract was filtered and stored at 4°C for further experiments.

Synthesis of silver nanoparticles (SNPs)

Aqueous solution of silver nitrate (0.025M, $AgNO_3$) was prepared and used for the synthesis of SNPs. 4 ml of plant extract was added to 120 ml of aqueous solution for reduction into Ag^+ ions and kept at room temperature for one hour.

Synthesis of gold nanoparticles (GNPs)

0.001M aqueous solution of $HAuCl_4$ was prepared and used for the synthesis of GNPs. 5 ml of plant extract was added to 120 ml of aqueous solution of 0.001M $HAuCl_4$ for reduction into Au^+ ions and kept at room temperature for one hour.

Characterization of NPs is important to understand and control NPs synthesis and applications. Characterization is performed using a variety of techniques such as UV–Vis spectroscopy, fourier transform infrared spectroscopy (FTIR), X-ray diffractometry (XRD), transmission electron microscopy (TEM) and scanning electron microscopy with EDX (SEM) (Mahitha et al., 2011; 2013).

Preparation of *Bacopa monniera* stabilized nanoparticles

After synthesis and characterization of nanoparticles (silver and gold), the nanoparticles solution was centrifuged at 10,000 rpm for 20 min. The supernatant was separated and then pellet was collected and weighed. For the preparation of *Bacopa monniera* stabilized nanoparticles, 5 mg of nanoparticles (silver and gold) was taken and 5 ml of *Bacopa monniera* extract was added.

Preliminary Phytochemical Screening

Preliminary phytochemical analysis was undertaken using standard qualitative methods as described by Amarasingham et al., (1964), Das and Bhattacharjee, (1970), Later modified by Chhabra et al., (1984) and Kemp, (1986). Preliminary phytochemical analysis was evaluated for *Bm* extract, SNPs, GNPs, *Bm* stabilized silver (*Bm*SNPs) and gold (*Bm*GNPs) nanoparticles.

Test for Amino acid

Few drops of ninhydrin solution were added to the test samples and heated on water bath. The appearance of violet colour indicated the presence of amino acids in all samples.

Test for Reducing sugars

To the test samples, 1 ml of Fehling solution of 'A' and 'B' were added, formation of brick red precipitate upon heating in water bath indicates the presence of reducing sugars in all the samples.

Tests for Alkaloids

a) **Mayer's reagent**: ($KI+HgCl_2$ solution) was added to 1 ml of test samples. A creamy precipitate was obtained in all samples indicating presence of alkaloids.
b) **Dragondorff's reagent**: (Solution of potassium bismuthiodide) was added to 2 ml of each test sample, reddish brown precipitate appeared in all the samples indicating the presence of alkaloids.

Test for Flavonoids

To few ml of ethyl acetate 0.5 ml of the test sample was added and heated in boiling water bath. Then the solution was filtered. 4 ml of filtrate was shaken with 1 ml of 1% aluminum chloride solution and observed for the formation of yellow colour in the presence of 1 ml dilute ammonia solution, indicates the presence of flavonoids.

Test for Phenolic compounds

To the test sample, few drops of Fehling solution was added. Appearance of blue/green colour shows the presence of phenolic compounds. The test was positive in all samples.

Test for Terpenoids

The test sample was extracted with chloroform and the extract was concentrated to 1/5th of its volume. To 1 ml of this solution few drops of acetic anhydride and concentrated H_2SO_4 were added. Appearance of blue to violet colour shows the presence of terpenoids. The test was positive in all samples.

Test for Saponins

1 ml test samples were separately mixed with 20 ml of distilled water and then agitated in a graduated cylinder for 15 minutes. Foam formation indicates the presence of saponins.

Test for Steroids

1 ml of conc. H_2SO_4 was added to the test samples and allowed to stand for 5 minutes, after shaking. lower layer turning into golden yellow colour indicates the presence of steroids.

Test for Carboxylic Acid

1 ml test samples were individually treated with a few ml of sodium bicarbonate solution. Effervescence (due to liberation of CO_2) indicates the presence of carboxylic acid.

Test for Tannins

a) **Lead acetate test**: 5 drops of lead acetate solution was added to the 2-3 ml of test samples. Yellowish white precipitate was not appeared. This test was negative in all the extracts.
b) **Gelatin test**: 5 drops of 1% solution of gelatin was added to the 2 ml of test sample. There is no precipitate formation or turbidity. The test was negative in all samples.

Test for Anthraquinones

Borntrager test: Test sample was boiled with 5 ml of dil.H_2SO_4 for 2 min. The extract was filtered while hot and the filtrate was cooled and shaken with equal volume of benzene. The benzene layer was allowed to separate completely. To half of its volume 10% ammonia solution was added and shaken gently and the layer was allowed to separate. The purple colour was present in ammonical layer. The test was positive in all samples.

Test for Reducing Power

The reducing power of the individual plant extracts as well as their mixture was determined according to the method of Oyaizu, 1986. The experiment was carried out in triplicate. Pre-determined concentration of 100µg/ml of individual test sample was mixed into the mixture of 2.5 ml of 0.2 M phosphate buffer (pH 7.4) and 2.5 ml of 1% potassium ferricyanide. The mixture was then incubated at 50º C for 20 minutes. After incubation, 2.5 ml of trichloroacetic acid was added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml, 0.1%) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

$$\text{Reducing power ability (\%)} = \frac{(\text{OD of the control - OD of the sample})}{(\text{OD of control})} \times 100$$

Optical density- OD

Evaluation of DPPH scavenging activity

Radical scavenging activity of plant extracts against stable 2, 2-diphenyl 2- picryl hydrazyl hydrate (DPPH) was determined by the slightly modified method of Brand-Williams et al., 1995. DPPH reacts with an antioxidant compound, which can donate hydrogen, and reduce DPPH. The change in colour (from deep violet to light yellow) was measured at 517 nm on a UV-Vis spectrophotometer. 3 ml of this solution was mixed with 100 µg/ml concentration of individual sample. The samples were kept in the dark for 15 minutes at room temperature and the decrease in absorbance was measured. The experiment was carried out in triplicate. Radical scavenging activity was calculated by the following formula.

$$\text{\% DPPH radical scavenging activity} = \frac{(\text{OD of the control- OD of the sample})}{(\text{OD of control})} \times 100$$

The activity was expressed as 50% inhibitory concentration (IC 50) based on the percentage of DPPH radicals scavenged. Lower the IC 50 value, higher is the antioxidant activity.

Test for Metal chelating activity

The chelating of ferrous ions by test samples was estimated by the method described by **Dinis *et al.* (1994)** with slight modification. 100 µg/ml of test samples were added to 1 ml of 2 mM $FeCl_2$ separately. The reaction was initiated by the addition of 5 mM ferrozine (1 ml). After 10min, absorbance was measured at 562 nm.

$$\text{Chelating activity (\%)} = \frac{(\text{OD of the Control - OD of the Sample})}{(\text{OD of the Control})} \times 100$$

Results

Synthesis and characterization of silver and gold nanoparticles

The synthesis of silver nanoparticles was first confirmed by colour change and then it was analysed by UV-Vis spectroscopy. The UV-Vis spectra showed the appearance of a single and strong band absorption peaks centered at about 436 nm and 433 nm respectively, thus indicating the nanoparticles were isotropic and uniform in shape and size. From the typical TEM micrographs of the synthesized SNPs it was observed that most of the silver nanoparticles were spherical in shape, most of them are ranged from 5 to 30 nm in size, among these many particles are 10 nm in size. The energy dispersive X-ray analysis (EDX) reveals strong signal in the silver region and confirms the formation of SNPs. The FTIR spectra of SNPs shows the presence of alcohols, phenols, aromatic amines, carboxylic acids, ethers and esters (Data was not shown) (Mahitha et al., 2011).

Reduction of the gold nanoparticles during exposure to the plant extract could be detected by the colour change. Formation of nanoparticles was confirmed by UV-vis spectrophotometer which shows the strong surface plasmon resonance absorption peak at 557 nm indicating the formation of GNPs at room temperature. The typical

TEM micrographs of the synthesized GNPs were spherical in shape. There is a variation in particle size and the average size estimated was 21 nm, most of the particles are ranged from 15 to 35 nm in size. The energy dispersive X-ray analysis (EDX) reveals strong signal in the gold region and confirms the formation of GNPs. FTIR absorption spectrum of GNPs shows the presence of alkynes, primary amines, aromatic carboxylic acids, esters, ethers, primary and secondary amines, hydroxyl functional group in alcohols and phenolic compounds (Data was not shown) **(**Mahitha et al., 2013).

Qualitative chemical tests

The phytochemical investigation of *Bacopa monniera* ethanolic extract, SNPs, GNPs, *Bm* stabilized silver (*Bm*SNPs) and gold (*Bm*GNPs) nanoparticles was carried out for the presence and absence of primary and secondary metabolites. All the five test samples (*Bm* extract, SNPs, GNPs, *Bm* *Bm*SNPs and *Bm*GNPs) showed positive results for amino acids, carbohydrates, alkaloids, glycosides, flavonoids, phenols, terpenoids, saponins and steroids. But they showed negative result for tannins and anthraquinones (Table.1).

Table 1. Qualitative chemical tests for the presence/absence of plant metabolites

Secondary metabolites	***Bacopa monniera* extract** *(Bm)*	**Silver nano particles** (SNPs)	**Gold nano particles** (GNPs)	***Bacopa monniera* stabilized silver nanoparticles** (*Bm*SNPs)	***Bacopa monniera* stabilized gold nanoparticles** (*Bm*GNPs)
Amino acids	+	+	+	+	+
Carbohydrates	+	+	+	+	+
Alkaloids	+	+	+	+	+
Glycosides	+	+	+	+	+
Flavonoids	+	+	+	+	+
Phenolic compounds	+	+	+	+	+
Terpenoids	+	+	+	+	+
Saponins	+	+	+	+	+
Steroids	+	+	+	+	+
Tannins	–	–	–	–	–
Anthraquinones	–	–	–	–	–

(+) - present, (-) - absent

DPPH radical scavenging activity

The table 2 depicts the results of DPPH radical scavenging activity. The DPPH radical scavenging activity was recorded in terms of percent inhibition. 1It was observed that SNPs and GNPs have minimum DPPH scavenging activity when compared to *Bm. Bm*SNPs and *Bm*GNPs have maximum DPPH scavenging activity when compared to *Bm,* and were statistically significant (*p<0.001)*. *Bm*SNPs and *Bm*GNPs have shown better DPPH scavenging activity when compared to *Bm,* SNPs and GNPs.

Table 2. DPPH scavenging activity of *Bm* extract, SNPs, GNPs, *Bm*SNPs and *Bm*GNPs.

Sample	Concentration µg/ml	Percent Inhibition
Bm	100	38.45 ± 0.27
SNPs	100	36.97 ± 0.34
GNPs	100	37.82 ± 0.40
*Bm*SNPs	100	63.02 ± 0.47*
*Bm*GNPs	100	64.75 ± 0.37*

Values are mean + SD of three six individual observations.
*Bm*SNPs and *Bm*GNPs were statically significant (P< 0.001) compared with *Bm*, SNPs and GNPs

Reducing power assay

The reducing power of *Bm,* SNPs, GNPs, *Bm*SNPs and *Bm*GNPs was mentioned in table 3. *Bm*SNPs and *Bm*GNPs shown maximum reducing power, and were statistically significant (*p<0.001)* when compared to *Bm*. SNPs and GNPs have minimum reducing power when compared to *Bm*. *Bm*SNPs and *Bm*GNPs have maximum reducing power amongst individual extracts. The reducing power was recorded in terms of absorbance, higher absorbance indicates more reducing power.

Table 3. Reducing power assay of *Bm* extract, SNPs, GNPs, *Bm*SNPs and *Bm*GNPs.

Sample	Concentration µg/ml	Absorbance
Bm	100	0.201 ± 0.018
SNPs	100	0.189 ± 0.015
GNPs	100	0.190 ± 0.016
*Bm*SNPs	100	0.401 ± 0.026*
*Bm*GNPs	100	0.410 ± 0.038*

Values are mean + SD of three six individual observations.
*Bm*SNPs and *Bm*GNPs were statically significant (P< 0.001) compared with *Bm*, SNPs and GNPs

Metal chelating activity

The percentage of metal chelating activity was determined in terms of percent inhibition as shown in table 4. *Bm*SNPs and *Bm*GNPs have shown maximum metal chelating activity and were statistically significant (*p< 0.001)* compared to *Bm*, SNPs, and GNPs have minimum metal chelating activity when compared to *Bm*.

Table 4. Metal chelating activity of *Bm* extract, SNPs, GNPs, *Bm*SNPs and *Bm*GNPs.

Sample	Concentration µg/ml	Percent Inhibition
Bm	100	52.16 ± 0.41
SNPs	100	49.81 ± 0.38
GNPs	100	48.12 ± 0.25
*Bm*SNPs	100	64.40 ± 0.29*
*Bm*GNPs	100	62.41 ± 0.48*

Values are mean + SD of three six individual observations.
*Bm*SNPs and *Bm*GNPs were statically significant (P< 0.001) compared with *Bm*, SNPs and GNPs

Discussion

Free radicals and other reactive species are thought to play an important role in many human diseases. Radical scavenging activities are very important due to the deleterious role of free radicals in biological systems. Many secondary metabolites such as flavonoids, phenolic compounds etc serve as sources of antioxidants and do scavenging activity.

In recent years more than 100 disorders have been reported as the ROS-mediated disorders due to change in lifestyle, food habits, pollution and stress. Antioxidants fight against free radicals and protect us from various diseases. They exert their action either by scavenging the reactive oxygen species or protecting the antioxidant defense mechanisms (Umamaheswari et al., 2008). At stress conditions there is a need for additional supplementation of antioxidants. There is a wide range of natural and synthetic antioxidants but due to their macro-size they are rejected by blood brain barriers. Alternate to the synthetic antioxidants, nanoparticles are used as antioxidants due to the presence of phenols and saponins. Based on the DPPH, reducing power assay and metal chelating activity it was proven that *Bacopa monniera* stabilized nanoparticles are more potential compared to *Bacopa monniera* extract alone. The electron donation ability of natural products can be measured by 2,2 - diphenyl-1- picrylhydrazyl radical (DPPH) purple-coloured bleaching solution (Nunes, 2012). The method is based on scavenging of DPPH through the addition of a radical species or antioxidant that decolourizes the DPPH solution. The degree of colour change is proportional to the concentration and potency of the antioxidants. A large decrease in the absorbance of the reaction mixture indicates significant free radical scavenging activity of the compound under test (Krishnaiah et al., 2011).

The presence of transition metal ions in a biological system could catalyze the Haber-Weiss and Fenton type reactions, resulting the generation of free radicals. However, these transition metal ions could be chelated by antioxidants, which results in the suppression of free radical generation and inhibition of peroxidation processes of biological molecules (Anusha et al., 2014) . The high metal ion scavenging activity of the *Bm*SNPs and *Bm*GNPs is probably due to the chelating agents, which form sigma bonds with the metal and effective as secondary antioxidants because they reduce the redox potential, there by the oxidized form of the metal converted into the metal ion (Chew et al., 2009).

In this study, it is evident that the *Bacopa monniera* stabilized nanoparticles i.e *Bm*SNPs and *Bm*GNPs possesses effective antioxidant activity compared to only *Bacopa monniera* plant extract, silver nanoparticles and gold nanoparticles. This feature perhaps may be due to the presence of respective phytochemicals like phenols and flavonoids etc in this species. The antioxidant activity of nanoparticles mainly depends on the route of synthesis and also based on the selected plant because the plant which was used for synthesis should possess the phytochemicals. *Bm* is reported to contain tetracyclic triterpenoid saponins, bacosides A and B, hersaponin, alkaloids viz. herpestine and brahmine and flavonoids. Saponins are natural products, which have been shown to possess antioxidant property (Yoshiki, 1998). During synthesis of nanoparticles, these compounds are involved in the reaction process they may bind tightly to these silver and gold ions and leads to the formation of nanoparticles. Due to the presence of alkaloids, carbohydrates, phenols, saponins etc silver and gold nanoparticles showed antioxidant activity equal to the plant *Bm.* Our results are in agreement with the previous reports that SNPs synthesized from plants showed antioxidant activity (Velavan et al., 2012).

By the addition of *Bm* extract to the silver and gold nanoparticles the antioxidant activity was enhanced because both the nanoparticles and *Bm* has the phenols and flavonoids. Phenolic compounds are considered as secondary metabolites and these phytochemical compounds derived from phenylalanine and tyrosine occur ubiquitously in plants and are diversified (Naczk and Shahidi 2004). Flavonoids have been shown to be highly effective scavengers of oxidizing molecules, including singlet oxygen, and various free radicals (Bravo, 1998) implicated in several diseases.

Hence the present study shows that the *Bm*SNPs and *Bm*GNPs have excellent antioxidant properties compared to *Bm*, SNPs and GNPs.

Conclusions

From the phytochemical studies, the presence of amino acids, carbohydrates, alkaloids, glycosides, flavonoids, phenols, terpenoids saponins and steroids was confirmed. Based on the DPPH activity, reducing power assay and metal chelating activity it was proven that *Bacopa monniera* stabilized silver and gold nanoparticles are more potential compared to only *Bacopa monniera* extract, silver and gold nanoparticles. From this study it was evident that *Bacopa monniera* stabilized silver and gold nanoparticles can be used as therapeutic agents for the prevention of free-radical-mediated diseases.

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Declaration of interest

The authors declare that there is no declaration of interest

References

Amarasingham, R.P., Bisset, N.G., Millard, P.H., Woods M.C, 1964, Phytochemical survey of Malaya part-III, Alkaloids and Saponins, Economic Botany, 18, pp 270-278,.

Anusha, K., Shwetha, B., Sindhu, S., Sekar Babu H, 2014, Screening the metal chelating efficacy of *Trichodesma indicum*, Research Journal of Pharmacuetical Biologiacl Chemical Sciences, 5(2), pp 1259-1262.

Barathmanikanth, S., Kalishwaralal, K., Sriram, M., Pandian, S.R., Youn, H.S, Eom, S., Gurunathan S, 2010, Anti-oxidant effect of gold nanoparticles restrains hyperglycemic conditions in diabetic mice, Journal of Nanobiotechnology, 8, pp 1-16.

Battu, G.R., Ethadi, S.R., Veda Priya, G., Swathi Priya, K., Chandrika, K., Venkateswara Rao A, 2011, Evaluation of antioxidant and anti inflammatory activity of *Euphorbia heyneana* Spreng, Asian Pacific Journal Tropical Biomedicine,1 (2), pp 191-194.

Brand-Williams, W., Cuvelier, M.E., Berset C, 1995, Use of free radical method to evaluate antioxidant activity, Lebens Wissens Technology, 28, pp 25-30.

Bravo L, 1998, Polyphenols: chemistry, dietary sources, metabolism and nutritional significance Nutrition Reviews, 56, pp 317-333.

Chew, Y.L., Goh, J.K., Lim Y.Y, 2009, Assessment of *in vitro* antioxidant capacity and polyphenolic composition of selected medicinal herbs from leguminosae family in Peninsular Malaysia, Food Chemistry, 116, pp 13-18.

Chhabra, S.C., Uiso, F.C., Mshiu E.N, 1984, Phytochemical screening of Tanzanian medicinal plants, Journal of Ethnopharmacology, 11, pp 157-179.

Das, A.K and Bhattacharjee A, 1970, A systematic approach to phytochemical screening, Tropical Science, 12, pp 54-58.

Dinis, T.C.P., Madeira, V.M.C., Almeida L.M, 1994, Action of phenolic derivatives (acetoaminophen, salycilate, and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers, Archives of *Biochemistry* and *Biophysics,* 315, pp 161-169.

Kemp, W, (1986).Qualitative organic analysis (spectrochemical techniques)" McGraw Hill, London.

Krishnaiah, D., Sarbatly, R., Nithyanandam R.R, 2011, A review of the antioxidant potential of medicinal plant species, Food Bioproducts Processing, 89, pp 217-233.

Lawrence, R., Kapil L, 2011, Antioxidant activity of garlic essential oil (*Allium sativum*) grown in north Indian plains, Asian Pacific Journal of Tropical Biomedicine,1(1), pp 1-3.

Ling, L.U., Tan, K.B., Lin, H., Chiu G.N.C, 2011The role of reactive oxygen species and autophagy in safingol induced cell death, Cell Death Diseases, 2, pp 1-12.

Mahitha, B., Deva Prasad Raju, B., Dillip, G.R., Madhukar Reddy, C., Manoj, L., Priyanka, S., Jayantha, Rao K., John Sushma N, 2011, Biosynthesis, characterization and antimicrobial studies of AgNPs extract from *Bacopa monniera* whole plant, Digest Journal of Nanomaterials and Biostructures, 6, pp 135-142.

Mahitha, B., Deva Prasad Raju, B., Madhavi, T., Durga Mahalakshmi, Ch.N., John Sushma N, 2013, Evaluation of antibacterial efficacy of phytofabricated gold nanoparticles using *Bacopa monniera* plant extract, Indian Journal of Advances in Chemical Sciences, 1, pp 94-98.

Murphy, M.P., Holmgren, A., Goran Larsson, N., Halliwel B, 2011, Unraveling the biological roles of reactive oxygen species, Cell Metabolism, 13, pp 361-366.

Naczk, M and Shahidi F, 2004, Extraction and analysis of phenolics in food, Journal of Chromatography A, 1054, pp 95-111.

Nunes, P.X., Silva S.F., Guedes, R.J., Almeida S, 2012, Biological oxidations and antioxidant activity of natural products, phytochemicals as nutraceuticals -global approaches to their role in nutrition and health, Edited by Rao, V. pp 1-20,.

Oyaizu M., 1986, Studies on product of browning reaction prepared from glucose amine, Japanese Journal of Nutrition, 44, pp 307-315..

Pari, L and Amudha K, 2011, Antioxidant effect of *naringin* on nickel induced toxicity in rats: an *in vivo* and *in vitro* study, International Journal of Pharmacy Science Research, 2(1), pp 151-158.

Peng, K.T., Hsu, W.H., Shih, H.N., Hsieh, C.W., Huang, T.W., Hsu R.W.W, 2011, The role of reactive oxygen species scavenging enzymes in the development of septic loosening after total hip replacement, Journal of Bone Joint Surgery, 93, pp 1201-1209.

Pourmorad, F., Hosseinimehr, S.J., and Shahabimajd N, 2006, Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants, African Journal of Biotechnology, 5, pp 1142-1145,.

Russo, A., Izzo, A.A., Borrelli, F., Renis, M., Vanella A, 2003, Free radical scavenging capacity and protective effect on DNA damage of *Bacopa monniera*, Phytotherapy Research, 17, pp 870-875.

Sen, S., Chakraborty, R., Sridhar, C., Reddy Y.S.R, 2010, Free radicals, antioxidants, diseases and phytomedicines: current status and future prospect, International Journal of Pharmaceutical Sciences Reviews and Research, 3(1), pp 91-100.

Shao, H.B., Chu, L.Y., Lu, Z.H., Kang C.M, 2008, Primary antioxidant free radical scavenging and redox signaling pathways in higher plant cells, International Journal of Biological Sciences, 4, pp 8-14.

Umamaheswari, M., and Chatterjee T.K, 2008, *In vitro* antioxidant activities of the fractions of *Coccinia Grandis* leaf extract, *African Journal* of *Traditional*, Complementary and Alternative medicines, 5, pp 61-73.

Velavan, S., Arivoli, P., Mahadevan K, 2012, Biological reduction of silver nanoparticles using *Cassia auriculata* flower extract and evaluation of their *in vitro* antioxidant activities, Nanoscience and Nanotechnology an International Journal, 2(4), pp 30-35.

Venkatesh, S., Deecaraman, M., Kumar, R., Shamsi, M.B., Dada R, 2009, Role of reactive oxygen species in the pathogenesis of mitochondrial DNA (mt DNA) mutations in male infertility, Indian Journal of Medical Research,129, pp 127-137.

Yildrim, A., Oktay, M., Bilaloglu V, 2001, The antioxidant activity of leaves of *Cydonia vulgaris*", Turkish Journal of Medical Sciences, 31, pp 23-27.

Yoshiki, K., Kudou, S., Okubo K, 1998, Relationship between chemical structures and biological activities of triterpenoid saponins from soybean, Bioscience Biotechnology and Biochemistry, 62, pp 2291-2299.