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IN VITRO ANTIOXIDANT AND ANTIDIABETIC ASSESSMENT OF EXTRACTS FROM THE BARK OF *MICHELIA CHAMPACA*, A MEDICINAL PLANT IN BANGLADESH

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ABSTRACT

Michelia champaca (MC), family Magnoliaceae, is a medicinally important plant. Different parts of this plant are used in various ailments in folk medicine. The present study was conducted to demonstrate antioxidant potentiality of *Michelia champaca* bark in two different solvents and evaluate their *in vitro* antidiabetic activity along with screening for their phytochemical composition. The dried coarse powder of *Michelia champaca* bark was exhaustively extracted with ethanol (MCBEE) and water (MCBWE). The antioxidant activity of resulted extracts was determined by several assays: total antioxidant capacity assay, DPPH, ABTS, lipid peroxidation, Nitric oxide and Superoxide scavenging assays. Quantitative analysis of phytochemicals such as total phenolics, flavonoids, flavonols and proanthocyanidins contents were also measured. *In vitro* anti-diabetic activity was determined in terms of DNSA and starch iodine colorimetric assay. All the extractives showed strong antioxidant activities related to the standard. The total antioxidant capacity of extracts in most of the cases was in the following order: MCBEE>MCBWE. In free radical scavenging assay, the percent scavenging value of the extracts were concentration dependent manner and ethanol extract showed the most potent inhibitory activity, which was significantly different ($p< 0.05$) from water extract. At the highest concentration, 51.59 and

41.94% of α-Amylase inhibition were observed in DNSA method by MCBEE and MCBWE respectively, where standard Acarbose exhibited 65.84% inhibition. Total phenolics content and total flavonoids content were strongly correlated ($p< 0.01$) with antioxidant activity. The present study suggests that ethanol extract of *Michelia champaca* bark possess significant *in-vitro* antioxidant and anti-diabetic activity. Bark of *M. champaca* may therefore be a good source for natural antioxidants and a possible pharmaceutical supplement.

KEYWORDS: Phytochemicals, Antioxidants, Antidiabetic, *Michelia champaca*, Bark.

1. INTRODUCTION

From the ancient time plants have been used not only as food, but also as materials for alternative medical therapy because of their nutritional properties and pharmaceutical activities.[1] Experimental and epidemiological evidences ascribe a wide array of positive health effects due to the presence of phytochemicals in plants.[2-3] Medicinal plants can contain a wide variety of free radical scavenging molecules, including phenolics, terpenoids *etc*., which are rich in antioxidant activity.[4-6] Phytochemicals may protect us from severe pathological conditions such as coronary heart diseases, inflammatory disease and cancer, which are caused by exposure to oxidative stress.[7-8] Oxidative stress caused by reactive oxygen species which play a pivotal role in the generation of such chronic diseases.[9-10] However, protecting the body against the consequences of oxidative stress may be achieved by improving the manner by which the available antioxidant in food is maximized. In this regard, scientific studies have suggested that antioxidant compounds from natural sources, such as fruits and vegetables, have higher bioavailability and lower side effects than synthetic antioxidant agents.[11] Natural antioxidants from plants offer an alternative source of dietary ingredients, for example, α-amylase, α-glucosidase inhibitors are considered as one of the effective measures for regulating type II diabetes by controlling glucose uptake and increasing secretion of insulin hormone.[12] The natural products today symbolize safer in contrast to the synthetic drugs that are regarded as unsafe to humans and environment. Hence, researchers are keen to the natural products with the hope of safety and security.[13]

Michelia champaca Linn is a large evergreen tree belongs to the family of Magnoliaceae and locally known as sornochampa or champa. It is native to Bangladesh, China, India, Indonesia, Malaysia, Myanmar, Nepal, Thailand, and Vietnam. It is a medicinal plant used traditionally in the treatment of fever, colic, leprosy, eye disorders, inflammation, cough rheumatism, gonorrhea, cephalagia, gout and a number of diseases including inflammatory conditions.[14-

[15] *Michelia champaca* leaves, flowers, stem bark and seed extract have antioxidant and antimicrobial activity.[15] Methanolic and aqueous extracts of leaves of *Michelia champaca* showed robust antihelmintic, anti-diabetic and diuretic activity.[16-18] Flower buds of *Michelia champaca* is commonly used as herbal preparations for diabetes and kidney diseases.[19-20] The active constituents reported in this plant are alkaloids, saponins, tannins, sterols, flavonoids and triterpenoids.[21]

To the best of our knowledge, few data is available about antioxidant activity of *M. champaca* bark in the literature but detail analysis is still in scarce. Thus, the aim of the present work is to quantify phytochemical composition of *M. champaca* bark extracts and using *in vitro* tests to evaluate its toxicity as well as antioxidant, and antidiabetic properties.

2. MATERIALS AND METHODS

2.1 Chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), potassium ferricyanide, potassium acetate, phosphate buffer, catechin (CA), ferrous ammonium sulphate, butylatedhydroxytoluene (BHT), gallic acid (GA), ascorbic acid (AA), $AlCl_3$, Trichloro acetic acid (TCA), sodium phosphate, ammonium molybdate, tannic acid, quercetin (QU), DMSO, EDTA, thiobarbituric acid (TBA), acetyl acetone, α-amylase enzyme and $FeCl_3$ were purchased from Sigma Chemical Co. (St. Louis, MO,USA); vanillin was obtained from BDH; Folin-Ciocalteus's phenol reagent and sodium carbonate were obtained from Merck (Damstadt, Germany).

2.2 Collection of plant materials

Bark of *Michelia champaca* was collected from Rajshahi University Campus, Rajshahi, Bangladesh in October, 2017 and was identified by an expert taxonomist from the Department of Botany, Rajshahi University, Rajshahi, Bangladesh. Plant materials were then washed with fresh water to remove dirty materials and were shade dried for several days with occasional sun drying. The dried materials were ground into coarse powder by grinding machine, and the materials were stored at room temperature for future use.

2.3 Preparation of extracts

About 200 gm of dried powdered plant materials was taken in two amber colored extraction bottles. Ethanol and water were used for the preparation of the extracts. The powered plant material was soaked in ethanol and water. The sealed bottles were kept for 7 days with

occasional shaking and stirring. The extracts were filtered through Whatman No.1 filter paper. Then the extracts were concentrated with a rotary evaporator under reduced pressure at 45°C to afford crude bark extract. The crude extracts were then stored at 4°C for further analysis.

2.4 Qualitative phytochemical analysis

The freshly prepared extract of *M. champaca* bark was qualitatively tested for the presence of chemical constituents. Qualitative phytochemical tests for the identification of alkaloids, flavonoids, steroids, carbohydrates, saponins, reducing sugar, tannins and terpenoids were carried out for the extract by the method described previously.[22]

2.5 Quantitative analysis of phytochemicals

2.5.1 Determination of total phenolics

Total phenolics content of the extracts was determined by Folin-Ciocalteu method as described by Wolfe *et al*., with slight modification.[23] A 300µl of the extracts/standard dissolved in ethanol was mixed with 2 ml Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 2 ml (75 g/l) of sodium carbonate. The tubes were vortexed for 15 seconds and allowed to stand for 20 minutes at 25°C for color development. Absorbance was then measured at 760 nm using UV-spectrophotometer. Samples of extracts/standard were evaluated at a final concentration of 1 mg/ml. Total phenolic contents were expressed in terms of gallic acid equivalent, GAE (standard curve equation: $y = 0.00202x+0.0036$, $R^2 = 0.9994$), mg of GA/g of dry extract.

2.5.2 Determination of total flavonols

Total flavonols was estimated as Quercetin equivalents, expressed as mg of Quercetin per gram of dry extract by the method of Miliauskas *et al*.,[24] The Quercetin calibration curve was prepared by mixing 2 ml of Quercetin solution with 2 ml (20 gm/l) $AlCl_3$ and 6 ml (50 gm/l) sodium acetate. The absorption at 440 nm was read after 2.5 hours at 25ºC. The same procedure was carried out with 2 ml of plant extracts (10 gm/l) instead of Quercetin solution. Samples of extracts/standard were evaluated at a final concentration of 1 mg/ml. Total content of flavonols was expressed in terms of Quercetin equivalent, QUE (standard curve equation: $y = 0.0008x + 0.00337$, $R^2 = 0.9942$), mg of QU/g of dry extract.

2.5.3 Determination of total flavonoids

Total flavonoids were estimated using the method described by Ordonez *et al*., with some modification.[25] To 0.5 ml of samples/standard, 1.5 ml of methanol, 100 µl of 10% aluminum chloride, 100µl of 1M potassium acetate solution and 2.8 ml of distilled water was added. After one hour 30 minutes of incubation at room temperature, the absorbance was measured at 420 nm. Samples of extracts/standard were evaluated at a final concentration of 1 mg/ml. Total flavonoid contents were expressed in terms of catechin equivalent, CAE (standard curve equation: $y = 0.0041x+0.0335$, $R^2 = 0.9884$), mg of CA/g of dry extract.

2.5.4 Determination of total proanthocyanidins

Determination of proanthocyanidins was based on the procedure reported by Sun *et al*.,[26] A volume of 0.5 ml of 0.1 mg/ml of extracts/standard solution was mixed with 3 ml of 4% vanillin-methanol solution and 1.5 ml hydrochloric acid; the mixture was allowed to stand for 15 minutes. The absorbance was measured at 500 nm. Samples/standard was evaluated at a final concentration of 0.1 mg/ml. Total content of proanthocyanidins was expressed in terms of catechin equivalent, CAE (standard curve equation: $y = 0.0157x + 0.014$, $R^2 = 0.9985$), mg of CA/g of dry extract.

2.6 Determination of antioxidant activity

2.6.1 Determination of total antioxidant capacity

The antioxidant activity of the extract was evaluated by the phosphomolybdenum method in accordance with the procedure described by Prieto *et al*.,[27] The assay was based on the reduction of Mo (VI) to Mo (V) by the extracts/standard and subsequent formation of a green phosphate/Mo (V) complex at an acidic pH. A 0.3 ml of samples/standard at different concentrations was mixed with 3 ml of reaction mixture containing 0.8 M sulphuric acid, 14 mM sodium phosphate and 0.4% ammonium molybdate into the test tubes. The test tubes were incubated at 95°C for 10 minutes to complete the reaction. The absorbance was measured at 695 nm using a spectrophotometer against blank after cooling at room temperature. Ascorbic acid was used as standard. A typical blank solution contained 3 ml of reaction mixture and the appropriate volume of the same solvent used for the samples/standard were incubated at 95°C for 10 minutes and the absorbance was measured at 695 nm. Increased absorbance of the reaction mixture indicated increase total antioxidant capacity. Here Ascorbic acid was used as reference standard.

2.6.2 Determination of ferrous reducing antioxidant capacity

The ferrous reducing antioxidant capacity of samples/ standard was evaluated by the method described by Oyaizu *et al*.,[28] A 0.25 ml of extracts and ascorbic acid as standard of different concentration were mixed with potassium buffer (0.625ml, 0.2 M, pH 6.7) and 0.625 ml of 1% potassium ferricyanide [$K_3Fe(CN)_6$]. The mixture was incubated at 50°C for 20 minutes. After incubation, 0.625 ml of 10% trichloroacetic acid solution was added to each tube and the mixture was centrifuged at 3000 rpm for 10 minutes. Clear supernatant (1.8 ml) was mixed with equal amount of distilled water, 0.36 ml of ferric chloride (0.1% w/v) solution was added and absorbance was recorded at 700 nm. Reducing capacity of the extracts was linearly proportional to the concentration of a sample. Here Ascorbic acid was used as reference standard.

2.6.3 DPPH free radical scavenging assay

DPPH free radical scavenging activity was determined spectrophotometrically as described by Cheel *et al*., with some modification.[29] Ascorbic acid was used as reference control. In brief, 1ml of different extract solution in methanol and ascorbic acid were mixed with 3 ml of DPPH solution (0.1 mM) in methanol. The mixture was allowed to stand for 30 minutes to perform complete reaction. Finally, the absorbance of each extract was measured at 517 nm by using UV spectrophotometer. Free radical scavenging activity of each sample was calculated by using the following formula:

$$\text{DPPH Radical scavenging rate (\%)} = [\{A_0 - A\}/ A_0] \times 100$$

Where A_0 (control) was the absorbance of DPPH blank solution, and A was the final absorbance of the tested sample after 30 minutes of incubation. The concentration which caused a half-maximal reduced DPPH radical level (IC_{50}) was determined. Percent of inhibition was plotted against concentration, and IC_{50} was calculated from the nonlinear regression curve using graph pad prism software.

2.6.4 ABTS radical scavenging assay

The ABTS method was used according to Re *et al*., with some modifications.[30] ABTS was dissolved in distilled water at a final concentration of 7 mM and mixed with 88 µl of 140 mmol/l potassium persulfate solution. The mixture was left to stand for 12 hours in the dark before use. For each experiment, $ABTS^{\cdot}+$ solution was diluted with water to an absorbance of 0.70 ± 0.02 at 734 nm. Then, 1ml of various concentrations of sample was mixed with 3.0 ml

of $ABTS^{\cdot+}$ solution. Ascorbic acid was used as positive control. The scavenging rate was calculated using the formula:

$$\text{ABTS Radical scavenging rate (\%)} = [\{A_0 - A\}/ A_0] \times 100$$

Where A_0 (control) was the absorbance of ABTS blank solution, and A was the final absorbance of the tested sample after 6 minutes of incubation. The concentration which caused a half-maximal reduced ABTS radical level (IC_{50}) was determined. Percent of inhibition was plotted against concentration, and IC_{50} was calculated from the nonlinear regression curve using graph pad prism software.

2.6.5 Nitric oxide radical scavenging activity

$NO^{\cdot}$ reacts with oxygen to produce stable products (nitrate and nitrite), the quantities of which can be determined using Griess reagent procedure described by Marcocci *et al.*,[31] Two (2) ml of 10 mM sodium nitroprusside dissolved in 0.5 ml phosphate buffer saline (pH 7.4) is mixed with 0.5 ml of sample at various concentrations. The mixture is then incubated at 25ºC. After 150 minutes of incubation, the incubated mixtures were then mixed with 0.5 ml of Griess reagent [(1.0 ml sulfanilic acid reagent (0.33% in 20% glacial acetic acid at room temperature for 5 minutes with 1 ml of naphthylethylenediamine dichloride (0.1% w/v)]. The mixture is then incubated at room temperature for 30 minutes and its absorbance was measured at 546 nm. Catechin was used as reference standard. The amount of nitric oxide radical inhibition is calculated following this equation:

$$\text{Percent (\%) inhibition of NO radical scavenging} = [\{A_0 - A\}/ A_0] \times 100$$

Where A_0 is the absorbance of the blank control (NO radical solution without test sample) and A is the absorbance of the test sample. Percent of inhibition was plotted against concentration, and IC_{50} was calculated from the nonlinear regression curve using graph pad prism software.

2.6.6 Superoxide scavenging assay

The assay was done following the method of Rao *et al.*,[32] A 0.1ml each of the plant extracts and standard were added to the reaction mixture containing 50mM phosphate buffer (pH 7.6), 20µg/ml riboflavin, 12 mM EDTA and 0.1 mM NBT. The reaction was initiated by illuminating the reaction mixture for 5 minutes and the absorbance was measured at 590 nm. Qurecitin was used as reference standard. The percent (%) of nitric oxide radical inhibition is calculated following this equation:

$$\text{Superoxide scavenging assay (\%)} = [\{A_0 - A\}/ A_0] \times 100$$

Where A_0 is the absorbance of the control, and A is the absorbance of the extractives/standard. Then percent (%) of inhibition was plotted against concentration, and from the graph $IC_{50}$was calculated

2.6.7 Lipid peroxidation inhibition assay

Malondialdehyde content was estimated according to the methods described by Rani *et al*.,[33] 15% w/v Trichloroacetic acid, 0.375%w/v thiobarbituric acid and 0.25N Hydrochloric acid were mixed to form the stock Thio-barbituric acid (TBA)-Tricholoro acetic acid (TCA)-HCl reagent. This solution was mildly heated to assist the dissolution of TBA. Male Albino rats weighing about 180-200g was used for the study. After decapitation, the liver was removed carefully. The tissue was immediately weighed and homogenated with cold 1.15%w/v KCl to make 10%v/v homogenate. The homogenate (0.5ml) was added to 1 ml of various concentrations of the extracts. Then the mixture was incubated for 30 minutes. The per-oxidation was terminated by the addition of 2 ml of TBA-TCA-HCl reagent. The solution was heated for 15 minutes in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation at 1000 rpm for 10 minutes. The absorbance of the supernatant was measured at 535 nm. Quercetin was used as reference standard. The percent of inhibition of various concentrations was calculated by using the formula.

$$\text{Lipid peroxidation assay (\%)} = [\{A_0 - A\}/ A_0] \times 100$$

Where A_0 is the absorbance of the control, and A is the absorbance of the extractives/standard. Then percent of inhibition was plotted against concentration, and from the graph IC_{50} was calculated.

2.7 *In vitro* anti-diabetic activity (α-Amylase inhibition test)

2.7.1 3, 5-Dinitrosalicylic acid method (DNSA)

The inhibition assay was performed according to Miller *et al*., using DNSA method.[34] A 500 µl of extracts at different concentration were added to 500 µl of 0.02 M sodium phosphate buffer (pH 6.9 containing 6 mM sodium chloride) containing 0.5 mg/ml of α-amylase solution and were incubated at 37°C for 10 minutes, followed by addition of 500 µl of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) all the test tubes. The reaction was stopped with 1.0 ml of 3, 5 DNSA reagent. The test tubes were then incubated in a boiling water bath for 5 minutes and cooled to room temperature. The reaction mixture was

then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm. The control samples were also prepared accordingly without any plant extracts and were compared with the test samples containing various concentrations of extracts prepared. The results were expressed as percent of inhibition calculated using the formula:

$$\alpha\text{-amylase inhibition activity} = [1 - \{(A_1-A_2)/A_0\}] \times 100$$

Where A_1 is the absorbance of test sample, A_2 is the absorbance of product control (sample without α-Amylase solution) and A_0 is the absorbance of negative control (α-Amylase without extract).

2.7.2 Starch iodine color assay

The inhibition assay was performed according to Islam *et al*., using starch iodine color assay.[35] Different concentration (200 to 1000 µg/ml) of 500 µl extracts and acarbose were added to 500 µl of 0.02 M sodium phosphate buffer (6 mM sodium chloride at pH 6.9) containing 0.5 mg/ml of α-amylase solution. Then the reaction mixture was incubated at 37°C for 10 minutes. This was followed by the addition of 500 µl soluble starch (1%, w/v) and was incubated at 37°C for 15 minutes. The enzymatic reaction was stopped by adding 1 M HCl (20 µl), followed by the addition of 100 µl of iodine reagent (5 mM I_2 and 5 mM KI). The color change was noted and the absorbance was recorded at 620 nm.

The results were expressed as percent (%) of inhibition calculated using the formula: α-amylase inhibition activity = $[1 - \{(A_1- A_2)/A_0\}] \times 100$

Where A1 is the absorbance of test sample, A_2 is the absorbance of product control (sample without α-amylase solution) and A_0 is the absorbance of negative control (α-amylase without extract).

2.8 Brine Shrimp lethality bioassay

Brine shrimp lethality bioassay was carried out according to the method described by Islam *et al*., for the determination of cytotoxic property of the sample extracts.[36] The eggs of Brine Shrimp (*Artemia salina* Leach) were hatched in a tank with 48 hours oxygen supply facility at 37°C. Samples of different concentrations were prepared. 10 living naupliis were taken to each of the test tube containing different concentrations of test sample with Pasteur pipette. Then the extracts at different concentration were added to the test tube containing ten living brine shrimp nauplii in 5ml simulated sea water. After 24 hours, the test tubes were inspected

using a magnifying glass and the number of survived nauplii in each vial were counted. From this data, the percent (%) of lethality of the brine shrimp nauplii was calculated for each concentration.

2.9 Statistical analysis

All analyses were carried out in triplicates and data were expressed as mean ± standard deviation. Statistical analyses were performed by ANOVA. Duncan's test was used to determine the significance of differences between the groups. Differences at $p< 0.05$ were considered significant. Free graph pad prism software was used to determination of IC_{50} value. Correlation coefficients were determined by using SPSS software of version 15. LC_{50} of compounds were determined using probit analysis application software.

3. RESULTS

3.1 Preliminary phytochemical analysis

The extracts of *M. champaca* showed the presence of several phytochemicals including alkaloids, flavonoids, tannins and steroids *etc*. Phytochemical compositions of two extracts were given in Table 1.

Table 1: Phytochemical constituents of extracts of MCB.

Name of tests		Name of sample extract	
		MCBEE	MCBWE
Alkaloids	Dragendorff's test	+	-
	Hager's test	+	-
	Wagner's test	+	-
	Mayer's test	+	-
Carbohydrate	Benedict's test	+	+
	Fehling's test	+	+
	Molisch's test	+	+
Flavonoid test		+	+
Glycosides		+	+
Resins		-	-
Saponins		-	+
Triterpenoids		-	-
Steroids	Liebermann-Burchard's test	+	+
	Salkowski reaction	-	+
Tannins		+	-
Coumarins		-	-
Anthracenosides		-	+
Cardiac glycosides		-	-

(+) sign indicate the presence of phytochemical and (-) sign indicate the absence of phytochemical. MCBEE; Micheliachampaca bark ethanol extract and MCBWE; Micheliachampaca bark water extract.

3.2 Total phenolics, flavonoids, flavonols and proanthocyanidins content

Table 2 showed the results of total phenolics content of two extracts of *M. champaca* bark. Total phenols and total flavonoids contents of the extracts were expressed in gallic acid and catechin equivalents respectively. Ethanol extract has highest phenolics and flavonoids contents, on the other hand, water extract contains more flavonols and proanthocyanidins than ethanol extract. Total flavonols and total proanthocyanidins content of the extracts were expressed in catechin and quercetin equivalents respectively. Total phenolics and flavonoids content of the extracts correlate with their antioxidant activity.

Table 2: Polyphenols content of two extracts of *M. champaca* bark.

Polyphenols content	MCBEE	MCBWE
Phenolics [1]	113.16±5.39[b]	68.50±3.80[c]
Flavonoids [2]	179.55±4.41[a]	41.89±3.97[c]
Flavonols [3]	80.38±5.04[b]	87.17±3.36[b]
Proanthocyanidins [2]	0.47±0.69[c]	8.55±0.52[b]

Each value is the average of three analyses ± standard deviation 1, 2 and 3 expressed in terms of GAE, CAE and QUE respectively (mg of GA, CA and QU/g of dry extract, respectively). Means with superscript with different letters in the rows are significantly ($p<0.05$) different from each other

3.3 ANTIOXIDANT ACTIVITY

3.3.1 Total antioxidant capacity

The total antioxidant capacity of MCB extracts was shown in Fig. 1A. MCB extracts showed lower antioxidant activity compared to standard ascorbic acid at all the concentrations. The absorbance of MCB extracts found to be increased with the increasing concentration. In lower concentration, absorbance of the MCB extracts was approximately same but in higher concentration starting from about 150 µg/ml, absorbance became higher. Ethanol extract of MCB showed higher antioxidant capacity at the concentration of 200 µg/ml than water extract.

3.3.2 Ferrous reducing antioxidant capacity

In ferrous reducing antioxidant assay, antioxidant sample causes the reduction of the Fe^{3+}/ferricyanide complex to the Fe^{2+}/ferrous form, so the reducing power of the sample can be monitored by measuring the formation of Perl's Prussian blue. The ferrous reducing antioxidant capacity of MCB extracts is shown in following (Fig. 1B). A higher absorbance indicates a higher reducing power, but sample showed moderate reducing activity than

standard ascorbic acid. The reducing activity increased with the increasing concentration of the MCB sample.

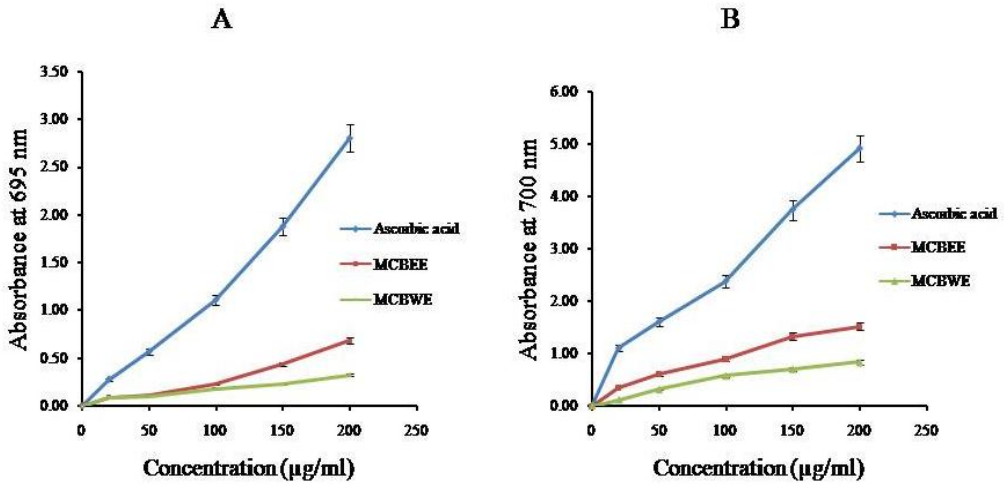

Fig. 1: Total antioxidant and ferrous reducing capacity of MCB extracts and Ascorbic acid.

3.3.3 DPPH radical scavenging activity

The effect of antioxidants on DPPH radicals is thought to be due to their hydrogen donating ability**.** The reduction capability of antioxidants was determined by the decrease in absorbance of DPPH radical than control. Fig. 2A showed DPPH free radical scavenging activity of MCB extracts. The different concentration of sample and standard exhibited antioxidant activities in a concentration dependent manner. The scavenging activity of MCB against DPPH was moderate when compared with standard vitamin C. The IC_{50} of MCBEE and MCBWE were 94.99±5.244 and 186.8±3.038 µg/ml whereas IC_{50} value of AA was 26.83±3.363 µg/ml.

3.3.4 ABTS free radical scavenging activity

The reduction capability of ABTS was determined by the decrease in absorbance than control which is induced by antioxidants. The scavenging capacities of MCB extracts for the ABTS radical were measured and compared with ascorbic acid. As shown in Fig. 2B, the scavenging effect of extracts increased with increasing concentration. The IC_{50} value of the two extracts were 46.07±3.331and 44.39±3.622 µg/ml respectably where the IC_{50} value of reference standard was 7.40±4.043 µg/ml.

3.3.5 Lipid peroxidation inhibition activity

Lipid peroxidation inhibition activity of two extracts of MCB was shown in Fig. 2C. It was clear that every extract has moderate lipid peroxidation inhibition activity as compare to reference standard at 1000 µg/ml concentration. The IC_{50} values were as following MCBEE (524.1±4.318) and MCBWE (410.2±3.817). Reference standard Quercetin had IC_{50} value 251.7±4.130 µg/ml.

3.3.6 Superoxide Anion Radical Scavenging Activity

MCB extracts, as well as Quercetin showed moderate superoxide radical scavenging activities at high concentration (1000 µg/ml). The scavenging capacity of the extracts followed the order of MCBEE>MCBWE where QU having significantly higher activity than all of the extracts (Fig. 2D).

3.3.7 NO radical scavenging activity

The reduction capability against NO was determined by the decrease in absorbance than control. The different concentration of MCB extracts exhibited antioxidant activity against NO radical in a concentration dependent manner (Fig. 2E). Water extract caused a mild inhibition of nitric oxide radical with an IC_{50} of 1584±1.415 µg/ml and ethanol extract caused a moderate dose-dependent inhibition of nitric oxide with an IC_{50} of 1113±2.440 µg/ml. The scavenging activity of catechin showed IC_{50} values of 287.4±1.553 µg/ml.

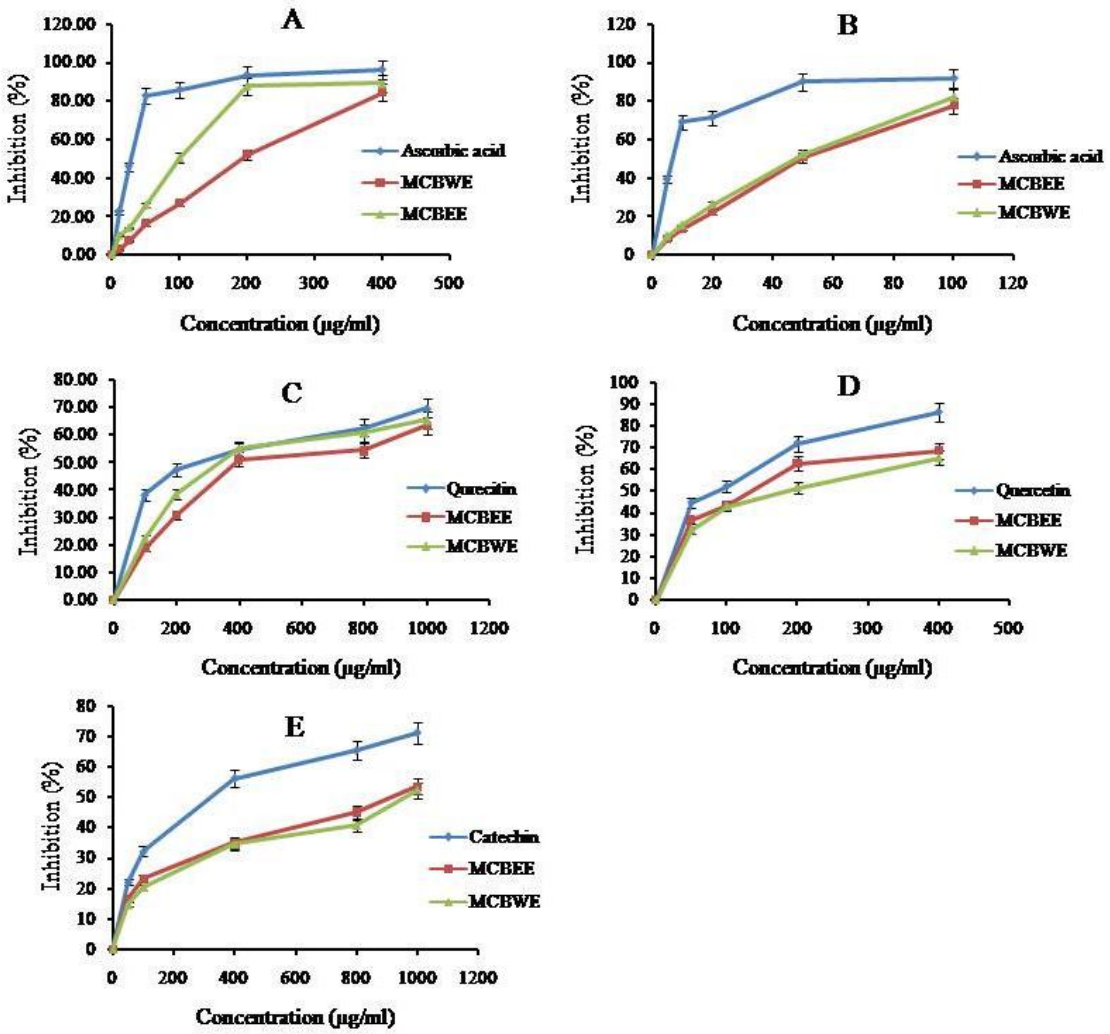

Fig 2: Inhibition percentage of MCB extracts and standard compound against various free radicals.

IC_{50} values of MCBEE, MCBWE and standard compounds in different free radical assays were given in Table 3.

Table 3: Free radical scavenging activity of MCB extracts.

Extracts and Standards		IC_{50} (µg/ml) value			
	DPPH	ABTS	SO	NO	LP
MCBEE	94.99±5.24	46.07±3.33	116.4±3.97	1113±2.44	524.1±4.31
MCBWE	186.8±3.03	44.39±3.62	162.0±2.29	1584±1.41	410.2±3.81
AA	26.83±3.36	7.40±4.043	-	-	-
QUERCETIN	-	-	69.35±3.99		251.7±4.13
CATECHIN	-	-	-	287.4±1.55	-

Result were expressed as mean ± SD (n=3). Where each value were significantly different (P<0.05) from each otherin an each Colum. SO: Supreoxide; NO: Nitric oxide; LP: Lipid peroxidation.

3.4 *In vitro* antidiabetic activity

α-amylase inhibitory activity of *M. champaca* Bark extracts illustrated in Figure 3. It was observed that each extract showed significant α-amylase inhibitory activity, which was in a dose-dependent manner. In 3, 5 DNSA method, as much as 39.5% inhibition was exhibited by MCBEE at the highest concentration which was relatively lower than standard acarbose (Fig. 3A). Consistently, in starch iodine color assay, at the concentration of 1000 µg/ml, ethanol extract had the higher inhibitory activity (51.59±1.39%) than water extract. Nevertheless, compared with the commercial drug acarbose, no precedence of extracts possessed higher inhibitory activities than acarbose in this test. At 1000 µg/ml concentration (Fig. 3B) 51.59±1.39 and 41.94±1.70% of α-amylase inhibition were observed in Starch iodine color assay method by MCBEE and MCBWE respectively, where standard Acarbose exhibited 64.703±1.46% inhibition.

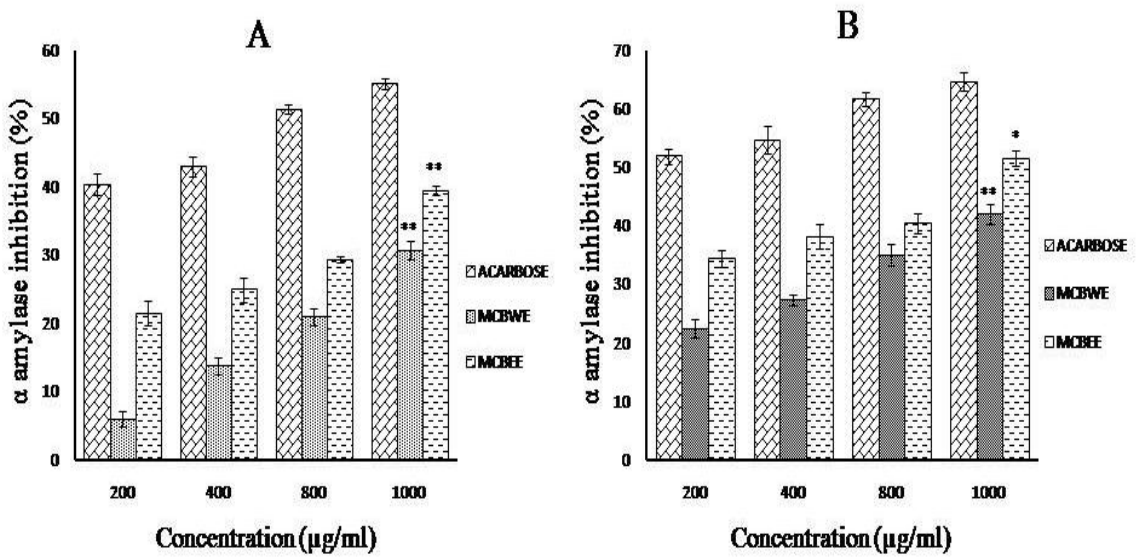

Fig. 4: α-amylase inhibition percentage of MCB extracts and ascorbic acid.

3.5 Brine Shrimp cytotoxic lethality bioassay

The median lethal concentration (LC_{50}) in brine shrimp lethality bioassay was found to be 269.82, 140.28 and 6.25 µg/ml for MCBEE, MCBWE and gallic Acid respectively, which was obtained from probit statistical analysis. The data and result of the test compound and LC_{50} of standard gallic acid were given in Table 4. Cytotoxic effect of MCB by brine shrimp lethality showed high value of LC_{50}, which indicates the low cytotoxic effect of MCB.

Table 4: Cytotoxic ability of MCB extracts and standard gallic acid.

Conc. (µg/ml)	Log conc.	% of mortality			LC_{50} (µg/ml)		
		MCBEE	MCBWE	Gallic acid	MCBEE	MCBWE	Gallic acid
10	.99	10	20	60	269.82	140.28	6.25
25	1.39	20	30	80			
50	1.69	20	40	90			
100	1.99	30	50	100			
200	2.30	50	50	100			

Cytotoxic effect of MCB in brine shrimp lethality showed high value of LC_{50} indicates the low cytotoxic effect of MCB extracts.

Table 5: Correlations between the biological activities and total phenolics and flavonoids contents of different extracts of *Michelia champaca* Bark.

Correlations R^2				
Free radical Scavenging assays	TPC		TFC	
	MCBEE	MCBWE	MCBEE	MCBWE
DPPH	0.860*	0.999**	0.903*	0.995**
ABTS	0.991**	0.992**	0.984*	0.988*
NO	0.944*	0.948*	0.930*	0.931*
SO	0.906	0.983	0.920	0.982*
LP	0.870	0.834	0.850	0.807
α-amylase inhibition	0.998**	0.982*	1.0**	0.980*

*The crude extracts from the MCB were used in the correlation. **Correlation is significant at the 0.01 level, *Correlation is significant at the 0.05 level. TPC; Total phenolics content, TFC; Total flavonoids content; SO: superoxide; NO: Nitric oxide; LP: Lipid peroxidation*

4. DISCUSSION

We used two solvents to prepare extracts from the bark of *M. champaca* to determine phytochemical contents, antioxidant and antidiabetic activities. The findings of our study varied between two extracts. It was suggested that the differences in polyphenol contents and biological activities of plant extract depend on the type of solvent used.[33,37] Phytoconstituents such as phenolics, flavonoids, flavonols and proanthocyanidins may exert their antioxidant activity by directly scavenging reactive oxygen species, including hydroxyl, peroxyl and superoxide radicals to reduce the risks of several human health problems.[38-39] Present study showed that each extracts of MCB bark contain a significant amount of total phenolics, flavonoids, flavonols and proanthocyanidins (Table 2). These polyphenols have attracted particular attention due to reduce free radical induced tissue injury. Six in vitro assay models were used to evaluate the antioxidant activity of MCB extracts. The total antioxidant potentials of MCB extracts and standard ascorbic acid were estimated from their

ability to reduce the reduction of Mo (VI) to Mo (V) and subsequent formation of a green phosphate/Mo (V) complex at acidic pH. MCB extracts showed a concentration-dependent total antioxidant activity. However, the antioxidant activity of MCB was significantly lower ($p < 0.05$) than standard antioxidant.

The reducing power of a compound could be used as an indicator of its potential antioxidant capacity, and the ability to reduce Fe^{3+} to Fe^{2+} is often assayed as an indicator of electron-donating activity.[28,40] All the extracts as well as AA presented linearly concentration-dependent increase in absorbance, and their reducing power followed the order of AA>MCBEE>MCBWE (Figure 1B). The ability to scavenge DPPH is extensively used as an easy, rapid, and sensitive way to evaluate free radical-scavenging capacities of natural antioxidants. The DPPH radical scavenging activity of all the extracts from MCB increased with the increase in concentration (Figure 2A). The IC_{50} of MCBEE was significantly lesser ($p < 0.05$) than MCBWE. It has been found that phenolics, flavonoids and tocopherols reduce the DPPH radicals by their hydrogen donating ability.[41-42] ABTS is more reactive than DPPH and unlike the reactions with DPPH which involves H atom transfer, the reaction with ABTS involves an electron transfer process.[43] The ABTS scavenging activity suggested that the phytoconstituents within the extracts of *M. champaca* might donate electron/hydrogen while minimizing the oxidative stress. Reactive oxygen species induce membrane damage by peroxidising lipid moiety, specially the polyunsaturated fatty acids with a chain reaction known as lipid peroxidation.[44] Two extracts of MCB bark have appreciable lipid peroxidation inhibition activity (Figure 2C). Therefore it is suggested that MCB extracts might prevent lipid peroxidation induced cancer generation. Superoxide radicals are the most common ROS in vivo and involve in many pathological conditions.[45] They can give rise to stronger ROS such as singlet oxygen and hydroxyl radicals, and initiate peroxidation of lipids. The two extracts as well as QU, showed moderate superoxide radical scavenging activity in a concentration dependent (Figure 2D).

Nitric oxide (NO) has a role in physiologic processes and it participates in pathogenic pathways underlying a large group of disorders including inflammation, inflammatory bowel disorder, primary headaches and stroke.[46] *M. champaca* bark extracts showed NO scavenging activity suggesting the ability to ameliorate inflammatory response associated with NO. Diabetes mellitus (DM) is a complex disease characterized by a metabolic disorder due to absolute or relative insulin deficiency, which leads to imbalanced carbohydrate, lipid,

and protein metabolism.[47] The polyphenolic compounds in plants inhibit the activities of carbohydrate hydrolyzing enzymes, such as α-amylase because of their ability to bind with proteins.[48] In this study, the potential of MCB bark as an antidiabetic supplement was investigated using DNSA and starch iodine method to determine α-amylase inhibitory activity of the crude MCB extracts. The ethanol extract showed higher inhibitory effects than water extract. The high level of α-amylase inhibitory activity of the MCB extracts can potentially reduce postprandial hyperglycemia by delaying carbohydrate digestion. The toxicity of all the crude extracts to brine shrimp was determined. The mortality rate of brine shrimp was found to be increased with the increase in concentration of the test sample. The high value of LC_{50}, indicates the cytotoxic effect of MCB extracts. The results of this study indicate the presence of potent bioactive principles in this crude extracts which might be very useful as antiproliferative, antitumor, pesticidal and other bioactive agents. Taken together, this antioxidant activity of MCB extracts can be attributed to the presence of various phytochemicals in MCB bark. Therefore, a significant correlation between phytochemical contents and anti-oxidant properties was found in our study (Table 5). To analyze this correlation the Pearson correlation coefficient for TPC and TFC was employed. As shown in Table 5, antioxidant activity and in vitro α-amylase inhibitory activity showed a good correlation with TPC and TFC, whereas the coefficient of correlation between TPC, TFC and lipid peroxidation activity was not significant. This finding indicates that polyphenolic compounds are major contributors to the antioxidant activity of MCB extracts. In addition, the absence of correlation between phytochemical content and lipid peroxidation activity suggests that non-polyphenolic compounds that possess strong lipid peroxidation scavenging activities are the active compounds in MCB extracts. This feature causes a nonlinear relationship between polyphenolic content and lipid peroxidation activity.

5. CONCLUSION

The present study demonstrates the phytochemical profiling, *in vitro* antioxidant, antidiabetic properties of *M. champaca* bark extracts. The results serve as important insight into the pharmacological properties of *M. champaca* bark and provide valuable evidence for the potential of this part of the plant as good source of natural antioxidant. Further works are needed to better understand the mechanism of its preventive or protective effects. In addition, it will be required to investigate the isolation of the bioactive compounds, mechanisms of action, *in vivo* test and safety and long-term side effects of bioactive compounds. Results may

be useful for further studies on *Michelia champaca* for its applications in pharmaceutical industries.

Conflict of interest: The authors declare no conflict of interest.

REFERENCES

1. Kim JY, Kwon O. Culinary plants and their potential impact on metabolic overload. Ann. N. Y. Acad. Sci., 2011; 1229(1): 133-9.
2. Tapsell LC, Hemphill I, Cobiac L, Sullivan DR, Fenech M, Patch CS, Roodenrys S, Keogh JB, Clifton PM, Williams PG, Fazio VA. Health benefits of herbs and spices: the past, the present, the future. Med. J. Aust, 2006; 185: S4–24.
3. Zhang A, Sun H, Wang X. Recent advances in natural products from plants for treatment of liver diseases. Eur J Med Chem., 2013; 63: 570-7.
4. Shahidi F, Naczk M. Food Phenolics: Sources, Chemistry, Effects and Applications. Technomic Pub. Co. Basel, 1995; 331.
5. Yanishlieva NY, Marinova EY, Pokorný J. Natural antioxidants from herbs and spices. Eur J Lipid Sci Tech., 2006; 108(9): 776-793.
6. Pirbalouti AG, Setayesh M, Siahpoosh A, Mashayekhi H. Antioxidant activity, total phenolic and flavonoids contents of three herbs used as condiments and additives in pickles products. Herba Polonica, 2013; 59(3): 51-62.
7. Caillet S, Salmiéri S, Lacroix M. Evaluation of free radical-scavenging properties of commercial grape phenol extracts by a fast colorimetric method. Food Chem., 2006; 95(1): 1-8.
8. Liu RH. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. Am J Clin Nutr., 2003; 78(3): 517S-20S.
9. Braca A, Sortino C, Politi M, Morelli I, Mendez J: Antioxidant activity of flavonoids from Licanialicaniaeflora. J Ethnopharmacol, 2002; 79: 379–381.
10. Maxwell SR: Prospects for the use of antioxidant therapies. Drugs, 1995; 49: 345–361.
11. Atawodi SE. Antioxidant potential of African medicinal plants. Afr J Biotechnol, 2005; 4(2): 128-33.
12. Kim GN, Shin JG, Jang HD. Antioxidant and antidiabetic activity of Dangyuja (Citrus grandis Osbeck) extract treated with Aspergillus saitoi. Food Chem., 2009; 117(1): 35-41.
13. Chatterjee GK, Pal SP. Search for anti inflammatory agents from Indian medicinal plants a review. Indian Drugs, 1984; 21: 413.

14. Sobhagini N, Soumit KB, Malaya KM. Ethno-medico-botanical survey of Kalahandi district Orissa. Indian J Trad Knowl, 2004; 3(1): 72-79.
15. Kumar RV, Kumar S, Shashidhara S, Anitha S, Manjula M. Antioxidant and antimicrobial activities of various extracts of Michelia champaca Linn flowers. World Appl Sci J., 2011; 12(4): 413-8.
16. Jarald EE, Joshi SB, Jain DC. Antidiabetic activity of flower buds of Michelia champaca Linn. Indian J Pharmacol, 2008; 40(6): 256.
17. Lahlou S, Tahraoui A, Israili Z, Lyoussi B. Diuretic activity of the aqueous extracts of Carum carvi and Tanacetum vulgare in normal rats. J Ethnopharmacol, 2007; 110(3): 458-63.
18. Dama G, Bidkar J, Deore S, Jori M, Joshi P. Helmintholytic activity of the methanolic and aqueous extracts of leaves of Michelia champaca. Res J Pharmacol & Pharmacody, 2011; 3(1): 25-6.
19. Rajagopalan PM. Madurai: Siddha MaruthuvaGurukulam. 2000; Siddha medicine.
20. Nadkarni M. Indian Materia Medica. 1st ed., Mumbai; Popular Book Depot., 1954.
21. Khan MR, Kihara M, Omoloso AD. Antimicrobial activity of Michelia champaca. Fitoterapia, 2002; 73: 744–8.
22. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr. J. Biotechnol, 2005; 4(7): 685-8.
23. Wolfe K, Wu X, Liu RH. Antioxidant activity of apple peels. J Agr Food Chem., 2003; 51: 609–614.
24. Miliauskas G, Venskutonis PR, Van Beek TA. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food chem., 2004; 85(2): 231-7.
25. Ordonez AAL, Gomez JD, Vattuone MA, Isla MI. Antioxidant activities of Sechiumedule (Jacq.) Swart extracts. Food Chem., 2006; 97: 452–458.
26. Sun JS, Tsuang YW, Chen IJ, Huang WC, Hang YS, Lu FJ. An ultra-weak chemiluminescence study on oxidative stress in rabbits following acute thermal injury. Burns, 1998; 24: 225–231.
27. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal Biochem, 1999; 269: 337-341.
28. Oyaizu M. Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. Japn. J. Nut., 1986; 44: 307–315.

29. Cheel J, Theoduloz C, Rodriguez J, Schmeda-Hirschmann G. Free radical scavengers and antioxidants from Lemongrass (Cymbopogoncitratus (DC.) Stapf.). J Agric Food Chem., 2005; 53(7): 2511-2517.
30. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med, 1999; 26(9-10): 1231-7.31.
31. Marcocci L, Maguire JJ, Droy-Lefaix MT, Packer L. The nitric oxide-scavenging properties of Ginkgo biloba extract EGb 761. Biochem Biophys Res Commun, 1994; 201(2): 748-55.32.
32. Rao MR, Ravi A, Narayanan S, Prabhu K, Kalaiselvi VS, Dinakar S, Rajan G, Kotteeswaran N. Antioxidant study and GC MS analysis of an ayurvedic medicine 'Talisapatradi choornam'. Int J Pharm Sci Rev Res., 2016; 36(1): 158-66.
33. Rani P, Unni KM, Karthikeyan J. Evaluation of antioxidant properties of berries. Indian J Clin Biochem, 2004; 19(2): 103-10.
34. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem., 1959; 31(3): 426-8.
35. Islam MD, Akter SF, Islam MA, Uddin MS. Exploration of Antidiabetic Activity of Stephania japonica Leaf Extract in Alloxan-Induced Swiss Albino Diabetic Mice. J Pharm Res Int., 2019; 20:1-2.
36. Islam F, Khatun H, Khatun M, Ali SM, Khanam JA. Growth inhibition and apoptosis of Ehrlich ascites carcinoma cells by the methanol extract of Eucalyptus camaldulensis. Pharm Biol., 2014; 52(3): 281-90.
37. Khanna S, Shiv Prasad HN, Devi K. In vitro antioxidant screening models. Rev Pharmacol Chem., 2004; 38: 180-183.
38. Huang YS, Ho SC. Polymethoxy flavones are responsible for the anti-inflammatory activity of citrus fruit peel. Food Chem., 2010; 119(3): 868-73.
39. Chanet A, Milenkovic D, Manach C, Mazur A, Morand C. Citrus flavanones: what is their role in cardiovascular protection? J Agric Food Chem., 2012; 60(36): 8809-22.
40. Mai W, Chen D. Antioxidant activity of Rhizoma cibotii in vitro. Adv Pharm Bull, 2012; 2(1): 107.
41. Duan X, Wu G, Jiang Y. Evaluation of the antioxidant properties of litchi fruit phenolics in relation to pericarp browning prevention. Mole, 2007; 12: 759–771.
42. Li H, Wang X, Li Y, Li P, Wang H. Polyphenolic compounds and antioxidant properties of selected China wines. Food Chem., 2009; 112: 454–460.

43. M Alvarez-Suarez J, Giampieri F, Battino M. Honey as a source of dietary antioxidants: structures, bioavailability and evidence of protective effects against human chronic diseases. Curr Med Chem., 2013; 20(5): 621-38.
44. Scully C. Oral cancer: new insights into pathogenesis. Dent update, 1993; 20(3): 95-100.
45. Dröge W. Free radicals in the physiological control of cell function. Physiol. Rev., 2002; 82: 47–95.
46. Moncada S, Palmer RM, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev., 1991; 43(2): 109-42.
47. Loizzo MR, Pugliese A, Bonesi M, Tenuta MC, Menichini F, Xiao J, Tundis R. Edible flowers: a rich source of phytochemicals with antioxidant and hypoglycemic properties. J Agric Food Chem., 2015; 64(12): 2467-74.
48. Mai TT, Thu NN, Tien PG, Van Chuyen N. Alpha-glucosidase inhibitory and antioxidant activities of Vietnamese edible plants and their relationships with polyphenol contents. J Nutr Sci Vitaminol, 2007; 53(3): 267-76.
49. Meyer BN, Ferringm NR, Puam JE, Lacobsen LB, Nichols DE, MeLaughlin JL. Brine shrimp: a convenient general bioassay for active constituents. Planta Medica, 1982; 45: 31-32.