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ENSCONCING OF ANTIOXIDANT ACTIVITY ON AQUEOUS EXTRACTS OF *THEOBROMA CACAO* LEAVES

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ABSTRACT

Theobroma cacao is a medicinal plant that has numerous significant properties. Theobroma cacao leaves are used as a medicine for the treatment for wound healing, worm expellers and malaria cure. In this study, the antioxidant activity of aqueous extract of the leaves of Theobroma cacao was investigated. The phytochemicals (alkaloids, flavanoids, steroids, saponins, terpenoids, tannins, triterpenoids, phenols) present in the leaves were determined using standard procedures. The aqueous extracts were prepared by simple maceration and decoction method. The quantitative analysis such as total protein content and total carbohydrate content determination was carried out for both maceration and decoction extracts. The total protein content and carbohydrate content was higher in maceration extract than decoction. The antioxidant activity was determined by using Phosphomolybdenum reduction assay and CUPRAC assay method. The statistical analysis done by ANOVA showed significant antioxidant activity. Comparing with the standard antioxidant ascorbic acid, the maceration extracts showed significant antioxidant activity($p<0.001$).

KEYWORDS: Theobroma, Cacao, phytochemical analysis, antioxiant activity, decoction, maceration, CUPRAC.

INTRODUCTION

Medicinal plants have been identified and used throughout human history. However,in recent years, medicinal plants have been tested extensively and found to have several pharmacological uses such as antifungal, antidiabetic, antiproliferative, anti-inflammatory, anthelmentic activities etc.[1]

Antioxidants act as free radical scavengers by terminating free radical chain reactions and inhibiting other oxidation reactions. Theobroma cacao or cacao is a small, evergreen tree belonging to the family Malvaceae. The leaves are large, simple and alternate about 40cm long and 5-20cm broad. Cacao leaves have many medicinal properties; it is used to stimulate nervous system, lower blood pressure, the treatmentof anaemia, malaria, mental fatigue, fever, as worm expeller and for wound healing.[2]

Hence, this study aims to determine the antioxidant property on the aqueous decoction and maceration extracts of the leaves of ***Theobroma cacao***. The qualitative phytochemical analysis of the extract inhibit the presence of common phyto compounds including alkaloids, flavanoids, steroids, saponins, terpenoids, tannins, triterpenoids, phenol. The quantitative analysis such as total protein content and total carbohydrate determination was carried outfor both decoction and maceration extracts. This study there foreseeks to determineandcompare the photochemical constituents of aqueous extracts of ***Theobroma cacao*** leaves and its antioxidant activity.

MATERIALS AND METHODS

Collection of plant material

The leaf of Theobroma cacao were collected from Athirumada, Kottakkal, Malappuram, Kerala and were authenticated.

Preparation of plant extract

The collected leaves were washed using distilled water and air dried for fourteen days. The dried leaves were powdered using a mechanical blender. The two types of plant extracts are used in this experiment. The two different aqueous extracts were prepared by Decoction and Maceration.

Preparation of extract by Decoction[3,4]

For decoction method, 10g of fine leaf powder was mixed with 100ml of distilled water in a flask. The mixture was boiled for 90min at 90°C, cooled and filtered with Whatmann No:1 filter paper. The filtrate obtained was lyophilised and stored at 4°C.

Preparation of extract by Maceration[5,6]

For maceration method, 10g of fine leaf powder was macerated with 100ml distilled water for 24hours. The mixture was filtered using Whatmann No: 1 filter paper. The filtrate obtained was lyophilised and stored at 4°C.

Phytochemical Analysis

Qualitative phytochemical tests of the aqueous leaves extract of ***Theobroma cacao*** were screened for the presence or absence of phytoconstituents such as alkaloids, flavanoids, saponins, steroids, terpenoids, tannins, triterpenoids, phenols by the standard methods.[7]

Quantitative Analysis

Depending on the above qualitative results, the quantitative assay was carried out for total protein content and total carbohydrate determination.[8]

Total protein content determination

The total protein content was determined by using Barfoed's method. Briefly, to the 100µl of the sample extract and 3ml of the Barfoed's reagents and incubatedin dark for 5 minutes. The absorbance was measured at 595nm. Bovine serum albumin dilutions (0.1mg/ml &0.5 mg/ml) are used asstandard solutions.

Total carbohydrate determination

For estimating the carbohydrate content, take 1ml of sample solution and add 1ml of 5% phenol and then add 5ml of concentrated sulphuric acid mix well and leave for 10mins. Measure the absorbance at 488nm against blank. Then compare with standard solution of glucose. To prepare blank 1ml of distilled water added to 1ml of 5% phenol followed by 5ml of concentrated sulphuric acid.

ANTIOXIDANT ACTIVITY

Phosphomolybdenum Reduction Assay

The aqueous extracts of ***Theobroma cacao*** were evaluated by the Phosphomolybdenum reduction assay. The assay is based on the reduction of Mo(IV) to Mo(V) and subsequent formation of green phosphate/Mo(V) complex at acid p^H.[9]

Procedure

Aqueous extract of ***Theobroma cacao*** leaves in different concentration ranging from 10µg/ml to 50µg/ml were added to each test tube individually containing 3ml of distilled water and 1ml of molybdate reagent solution. These tubes were kept incubated at 95°C for 90min.

After incubation, the tubes were normalised to room temperature for 20 to 30minutes and the absorbance of reaction mixture was measured at 695nm. Mean values from two independant

samples were calculated for each extract. Ascorbic acid was used as the positive reference standard.

CUPRAC Assay

This method involves mixing the antioxidant solution with aqueous copper (II) chloride, alcoholic neocuproine and ammonium acetate aqueous buffer at p^H7 and subsequently measuring the developed absorbance at 450nm after 30min.[10,11]

Procedure

1ml 10Mm cupric chloride, 1ml 7.5millimole neocuproine and 1ml 1M ammonium acetate buffer of p^H7 solutions were added to the test tubes containing 2ml of distilled water. Aqueous extract of ***Theobroma cacao*** leaves in different concentration ranging from 10µg/ml to 50µg/ml were added to each test tube separately. These mixtures were incubated for half an hour at room temperature and measured against blank at 450nm. Ascorbic acid was used as positive reference standard.

RESULTS AND DISCUSSION

Qualitative Analysis

The qualitative analysis of leaf crude extract of ***Theobroma cacao*** has been analysed in this study and there is a wide range of phytochemical compound present in these extract is tabulated in table 01.

Table 01: Qualitative analysis of aqueous leaves extracts of Theobroma cacao.

Phytochemical constituents	Decoction	Maceration
Alkaloid	++	++
Phenol	++	++
Flavanoid	++	++
Steroids	+	+
Saponin	+	+
Terpenoids	+	+
Triterpenoids	+	+
Tannin	+	+

(++ Strong Positive result, + Positive result)

Quantitative Analysis

Total Protein Content

The total protein content of aqueous extract of leaf and standard(Bovine serum albumin) was determined by Barfoed's method and the result was tabulated in table 02.

Table 02: Total protein content of aqueous leaves extract of Theobroma cacao and standard.

Sample	Absorbance 595nm
Decoction	0.238
Maceration	0.317
Standard	0.183

The total protein content of decoction and maceration aqueous extracts of ***Theobroma cacao*** leaves are higher when compared to standard.

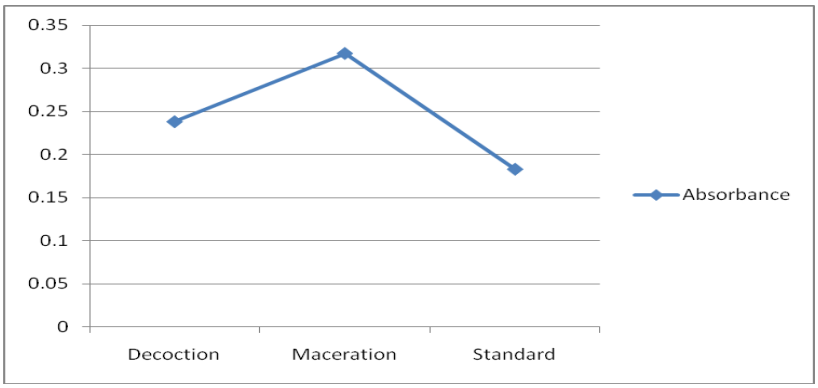

Figure 01: Total protein content of aqueous extracts of leaf and standard-Bovine serum albumin.

Total Carbohydrate Content

The total carbohydrate content of aqueous extract of leaf and standard(glucose)was determined and result was tabulated in table 03.

Table 03: Total carbohydrate content of aqueous leaves extract of Theobroma cacao and standard used.

Sample	Absorbance
Decoction	2.653
Maceration	2.779
Standard	2.256

The total carbohydrate content of decoction and maceration aqueous extracts shows higher when compared to standard.

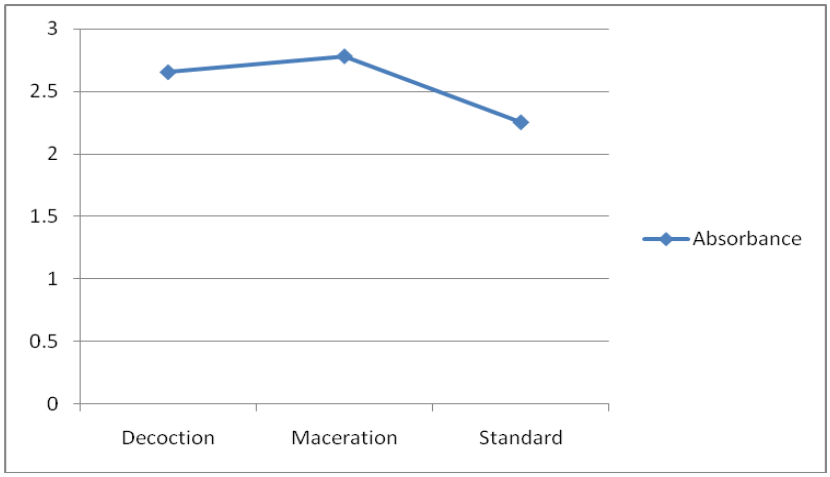

Figure 02: Total carbohydrate content of aqueous extract of leaf of Theobroma cacao and standard-glucose solution.

ANTIOXIDANT ACTIVITY

Phosphomolybdenum Reduction Assay

The total antioxidant capacity of aqueous extracts of leaf and standard(Ascorbic acid) was determined by Phosphomolybdenum reduction assay. The results obtained were tabulated in table 04.

Table 04: Antioxidant activity of aqueous leaves extract of Theobroma cacao.

SL.NO	Concentrationμg/ml	Decoction	Maceration	Standard
1	10	0.013	0.014	0.012
2	20	0.015	0.017	0.014
3	30	0.019	0.021	0.017
4	40	0.025	0.026	0.021
5	50	0.026	0.027	0.024

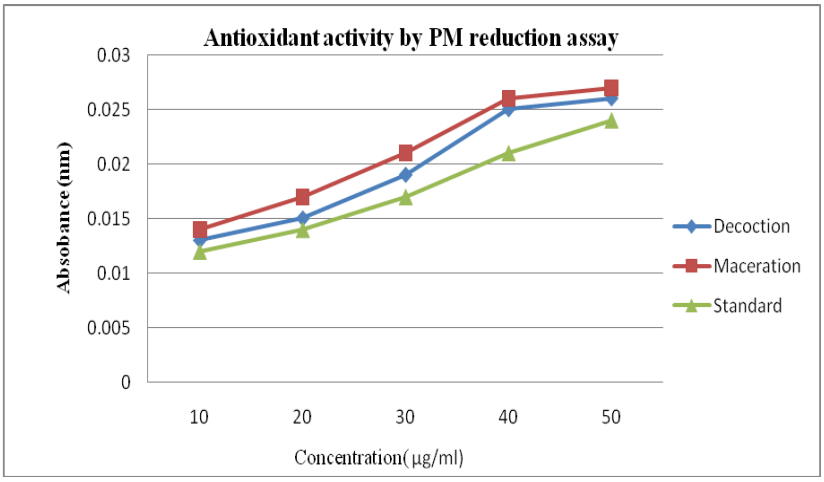

Figure 03: Antioxidant activity of aqueous extract of leaf of *Theobroma cacao* and standard-Ascorbic acid by PM reduction assay.

IC 50 value for the aqueous leaves extracts of Theobroma cacao were determined and its value is tabulated in table05.

Table 05: IC_{50} value of aqueous leaves extracts of *Theobroma cacao.*

SL.NO	SAMPLE	IC_{50}(µg/ml)
1	Standard	43.689
2	Decoction	31.359
3	Maceration	30.17

Since IC50 value of the decoction and maceration aqueous extract of ***Theobroma cacao*** is lesser than the standard, it indicates the antioxidant capacity of the decoction and maceration aqueous extract are higher than the standard.

Cuprac Assay

The antioxidant activity of aqueous extract of leaf of ***Theobroma cacao*** and standard (Ascorbic acid) was determined by CUPRAC assay and their results were tabulated in table 06.

Table 06: Antioxidant activity of aqueous extract of Theobroma cacao and standard.

Sl No.	Concentrationµg/ml	Decoction	Maceration	Standard
1	10	0.022	0.01	0.017
2	20	0.052	0.024	0.018
3	30	0.088	0.043	0.02
4	40	0.13	0.05	0.031
5	50	0.136	0.063	0.053

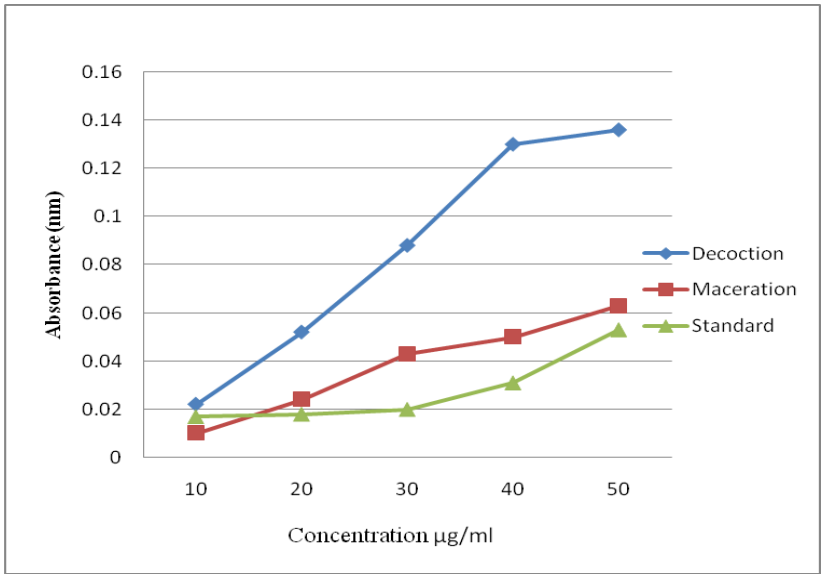

Figure 04: Antioxidant activity on aqueous extract of leaf of Theobroma cacao and standard-ascorbic acid by Cuprac Assay method.

IC 50 values for the aqueous leaves extracts of Theobroma cacao were determined and its value is tabulated in table 07.

Table 07: Results of IC_{50} value of aqueous leaves extract of *Theobroma cacao*.

Sl.No	Sample	IC_{50}(µg/ml)
1	Standard	52.189
2	Decoction	20.741
3	Maceration	37.997

Since IC50 value of the decoction and maceration aqueous extract of Theobroma cacao are lesser than the standard used, it indicate the antioxidant activity of the aqueous extracts are higher than the standard.

CONCLUSION

The research work was performed to explore the antioxidant activities of the aqueous leaves extract of ***Theobroma cacao***. On the basis of results obtained from two different antioxidant assays, both the aqueous extracts show significant antioxidant activity. Aqueous extract of ***Theobroma cacao*** leaves show the reducing capacity and reduction capacity of free oxidative metallic ions such ferric and cupric ions by PM assays and CUPRAC method respectively. The statistical analysis done by ANOVA showed significant antioxidant activity. Comparing with the standard antioxidant ascorbic acid, the maceration extract showed significant antioxidant activity ($p<0.01$). Over viewing the reducing capacity; the use of ***Theobroma cacao*** might contribute a certain level of health protection against oxidative damages with the established antioxidant activity of these extracts. From the present study, it was revealed that the aqueous extracts of ***Theobroma cacao*** leaves firmly possess strong antioxidant effects and can be a potential bioantioxidant.

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