ejpmr, 2016,3(8), 574-578

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EVALUATION OF ANTIOXIDANT ACTIVITY OF KAICHUKATTI CHOORANAM

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Article Received on 13/06/2016 **Article Revised on 03/07/2016** **Article Accepted on 24/07/2016**

ABSTARCT

The antioxidant potential of alcoholic extract of Kaichukatti chooranam (KKC) was evaluated by using DPPH Radical-Scavenging activity, Lipid peroxide inhibition assay and Total antioxidant capacity. The alcoholic extract showed significant antioxidant potential in all these assays. Total antioxidant effect of the extract is 219.47 µg extract is needed to give the effect of 100 µg of ascorbic acid. The results thus obtained suggest significant antioxidant activity of alcoholic extract of Kaichukkati Chooranam.

KEYWORDS: Radical scavenging, Total phenolic, Nutraceuticals, Tamil Nadu. KKC – Kaichukkati Chooranam.

INTRODUCTION

Medicinal plants play a pivotal role in the health care of ancient and modern cultures. Siddha and other Indian system of medicine mainly uses plant based drugs or formulations to treat various human ailments because they contain the components of therapeutic value. Kaichukkati Chooranam (KKC) is a combination of Kaichukkati (*Acacia catechu*) Sathu, Lavangapattai (*Cinnamomum zeylanicum* BL) Pattai, Lavangam (*Syzygium aromaticum* (L.) Merrill & Perry) Mottu, Jadhikkai (*Myristica fragrans* Henlt.) Paruppu.[1]

Free radicals or highly reactive oxygen species are formed by exogenous chemicals or endogenous metabolic processes in the human body. These are capable of oxidizing bio-molecules viz nucleic acids, proteins, lipids and DNA and can initiate different degenerative diseases like neurological disorders, cancer, emphysema, cirrhosis, atherosclerosis, arthritis etc.[2,3] Antioxidants are the compounds which terminate the attack of free radicals and thus reduce the risk of these disorders.[4]

Almost all organisms are protected up to some extent by free radical damage with the help of enzymes such as super-oxide dismutase, catalase and antioxidant compounds viz. ascorbic acid, tocopherol, phenolic acids, polyphenols, flavonoids and glutathione[5] reported that antioxidant supplements or dietary antioxidants protect against the damaging effects of free radicals.

Presently, much attention has been focused on the use of natural antioxidants to protect the human body especially brain tissues from the oxidative damage caused by free radicals. In last two decades, several medicinal plants have shown such effectiveness through the traditional methods of psychoneuropharmacology.[6]

The individual drugs possess various chemical constituents mainly *Acacia catechu* contains catechuic acid, catechutannic acid (25%-33%), acacatechin (10%-12%), catechu red, quercitin, catechin (2%-12%), epicatechin, phlebotanin (25%-33%), gummy matter, quercitrin, quercitin and moisture. Catechu and epicatechin usually accompany other flavonoids. It also contains the active principles like cyanodanol, tannin and polyphenols.[7]

Literature review on *Acacia catechu* shows that it has Anti-pyretic, Anti-diarrhoeal, Hypoglycaemic, Hepatoprotective, Immunomdulatory, Anti-mycotic activities.[8-10]

The present study has been conducted to evaluate the antioxidant activity kaichukatti chooranam which has been traditionally used for diarrhoea, and dysentery.[11]

MATERIALS AND METHODS

COLLECTION OF THE DRUGS

The raw drugs were procured from the local market and authenticated by Dr. Sasikala Ethirajalu, Consultant (Botany), Siddha Central Research Institute, Arumbakkam, Chennai–106. The drugs were dried, powdered separately and mixed.

EXTRACTION

The drug (25 g) was macerated with 75 ml of methanol for 15 minutes and filtered through Whatman No.1 fiter paper. The filtrate was condensed using rotary vacuum evaporator. The extract were stored at 4^{o}C.

DPPH RADICAL SCAVENGING ASSAY

The hydrogen atom or electron donor capacity of the extract was tested by its ability to bleach DPPH radical according to the method of Gulcin.[12] Various Concentrations of the extract (100-500) µg) were added to 1ml of 0.25 mm DPPH solution in ethanol. The tubes were incubated in dark for 30 minutes and the absorbance was read at 517mm.

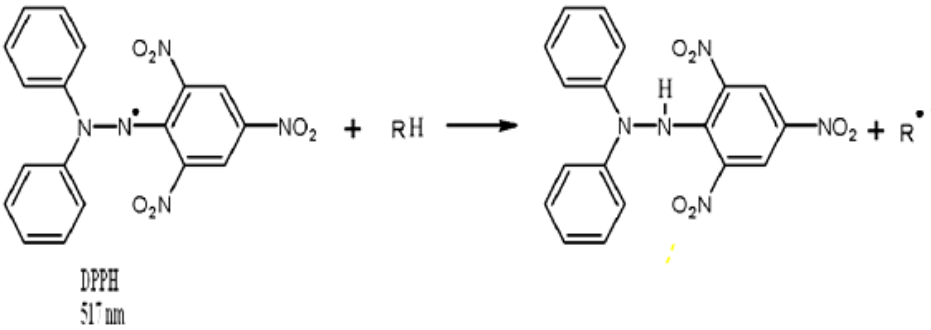
Each assay was carried out in triplicate. The percentage scavenging was calculated as the ration of absorption of the sample relative scavenging activity was calculated using the following formula: DPPH radical scavenging activity (%) = (1-antioxidant OD/control OD) x 100. Antiradical OD was defined as EC50 and it was calculated using non-linear regression.[13]

DETERMINATION OF DPPH RADICAL SCAVENGING ACTIVITY AIM

To determine the free radical scavenging activity by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method (Alothman et al., 2009).

PRINCIPLE

A simple method that has been developed to determine the antioxidant activity of foods utilizes the stable DPPH radical. The structure of DPPH and its reduction by an antioxidant is shown in following mechanism.



The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in color. The color turns from purple to yellow as the molar absorptive of DPPH radical at 517 nm reduces as the odd electron of DPPH radical. Under goes pairing with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The color developed is measured at 517 nm.

REAGENTS

DPPH SOLUTION (100MM)

DPPH solution was prepared by dissolving 33 mg of DPPH in 100 ml of methanol. From this stock solution, 10 ml was taken and diluted to 100 ml using methanol to obtain 100 ml DPPH solution and kept in amber colored bottle to protect from sunlight.

DETERMINATION OF TOTAL ANTIOXIDANT CAPACITY OF THE EXTRACT

The total antioxidant activity of the extract was determined by reduction of Mo (VI) to Mo (V) by the extract and the subsequent formation of a green colored Mo (V)/Phosphate complex in acidic PH. 100µg of each extract in 0.3 ml of distilled water was added to 3 ml of molybdate reagent containing 0.6 mm sulphuric acid, 28mm sodium phosphate and 4 mm ammonium molybdate. The tubes were incubated at 95^{o}C for 90 minutes. Then the mixture was cooled to room temperature and the absorbance was measured at 695 mm. The results were expressed as equivalents of ascorbic acid and BHT.[14]

LIPID PEROXIDE INHIBITION ASSAY

The scavenging of lipid peroxidation was carried out on liposomes in which prepared extract were added during or after liposome formation. Briefly, four types of samples were prepared, two controls (C1, C2) and two tubes for samples (ST1, ST2). Ten mg of phospholipids from egg yolk were suspended in 10 mL of CH_2Cl_2/CH_3OH 50:50 and 100 µL of the samples (EAP1–6) were added to the solution in the tubes (ST2). The solvent was evaporated to obtain a phospholipid film which was suspended with 2.0 mL of PBS buffer (50 mM, pH 7.4), producing liposomes. One hundred and ninety µL of each solution (C1, C2, ST1 & ST2) were taken. To the tube ST1 10 µL of the aqueous solutions of EAP1–6 were added while 10 µL of milliQ water were added to tube C1, C2 and ST2. After addition of 30 µL of H_2O_2 (1 mM) to tube C1, ST1 and ST2 and 30 µL of H_2O to tube C2, the samples were allowed to stand 3 h at room light.

Lipid peroxidation was assayed according to Yagi.[15] One hundred µL of each liposome suspension was taken and 4 mL of 1/12 N H_2SO_4 was added. After stirring, 500 µL of phosphotungstic acid solution (10%) was added. The resulting solution was left 5 min in darkness. Centrifugation at 1600 rpm was done over 10 min. The supernatant was removed and the residue was suspended in 2 mL H_2SO_4, then 300 µL of phosphotungstic acid was added. Another centrifugation at 1600 rpm was carried out over 10 min. The supernatant was discarded and the resulting sediment was dissolved in 4 mL of distilled water and 1 mL of TBA reagent (335 mg of thiobarbituric acid in 50 mL water/50 mL of acetic acid

99%). The resulting mixture was heated in water bath at 95 °C for 60 min. The solution was cooled and extracted with 5.0 mL of butanol. The fluorescence of the butanolic phase was measured with an excitation wavelength at 515 nm; an emission wavelength at 553 nm, the slide was 10 nm with the cut off at 515 nm. The quantity of lipid peroxidation was expressed as MDA equivalent from a linear curve drawn with several concentrations of the ascorbic acid(AA) standard.[16]

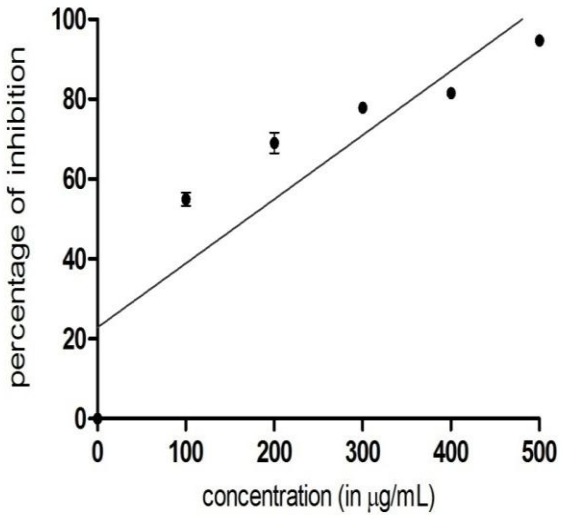
RESULTS AND DISCUSSION

Pharmaceuticals research conducted over the past three decades has shown that the natural products are a potential source for novel molecules for drug development. Targeted collection of plant materials based on traditional uses had a higher degree of positive results than those selected randomly. The results obtained during present study are discussed below.

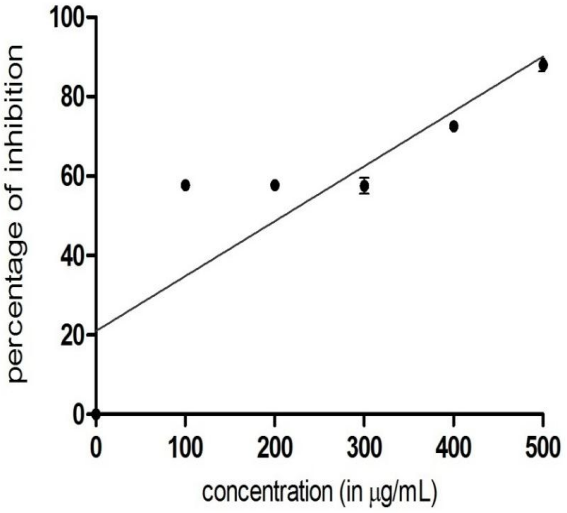
DPPH RADICAL SCAVENGING ASSAY

The DPPH assay constitutes a screening method currently used to provide basic information on the antiradical activity of the extract. When a solution of DPPH is mixed with a substance that can donate a hydrogen atom, the reduced form of the radical is generated accompanied by loss of color and reduction in the absorbance. In the present study, the methanol extract of Kaichukkati Chooranam a strong antiradical activity with least IC 50 value (78.21 µg; r –0.757; $p < 0.0001$). Their IC_{50} value for the positive control ascorbic acid was (21.17; r2 - 0.941; $p < 0.0001$). In this study extract showed higher IC_{50} value able than the positive control; however, the extract were able to scavenge the DPPH free radicals suggesting that it may have a role in preventing free radical mediated chain reactions.

DETERMINATION OF DPPH RADICAL SCAVENGING ACTIVITY (STANDARD – ASCORBIC ACID)



DETERMINATION OF DPPH RADICAL SCAVENGING ACTIVITY (KKC)



LIPID PEROXIDE INHIBITION ASSAY

Lipids can undergo peroxidation in the presence of ROS or RNS by a chain reaction and the biological membranes and sub-cellular organelle are susceptible to lipid peroxidation. Experimental evidences showed that the formation of lipoperoxides is involved in the membrane damage, enzyme damage, and nucleic acid mutagenicity.[17] It is known to play a crucial role in the pathology of several diseases, such as cancer, cardoivascular and neurodegenerative diseases, as well as in aging.[18] Many evidences were available in the literature showing that the atherosclerosis is initiated from lipid peroxidation of low-density lipoprotein.[19]

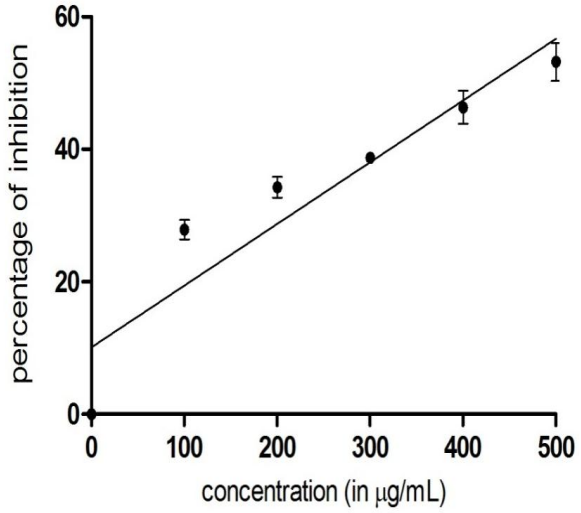
Spectroscopic estimation of thiobarbituroic acid (TBA) reactive substance is a convenient and one of the routine methods used to estimate lipoperoxides; though it has some limitations. TAB reacts with malondialdehyde, an end product of lipid peroxidation and gives an red adduct, which can be measured spectroscopically.[20] Ascorbic acid changes into dehydroascorbic acid in the body; which forms synergistic antioxidant-combination with tocopherols. The eficicacy of ascorbic acid towards the inhibition of lipoperoxide formation was already reported in the literature.[21]

And it has been used as one of the standard compound. In this study, the untreated cells showed the generation of more lipoperoxides, while treatment with ascorbic acid significantly lowered it. The treatment with KKC also showed significant reduction and the IC_{50} value was near to that of the standard compound, ascorbic acid. Polyphenols are one of the most abundant antioxidants in the diet and the consumption of polyphenols is related with the prevention of cardiovascular diseases and diabetes mellitus.[22]

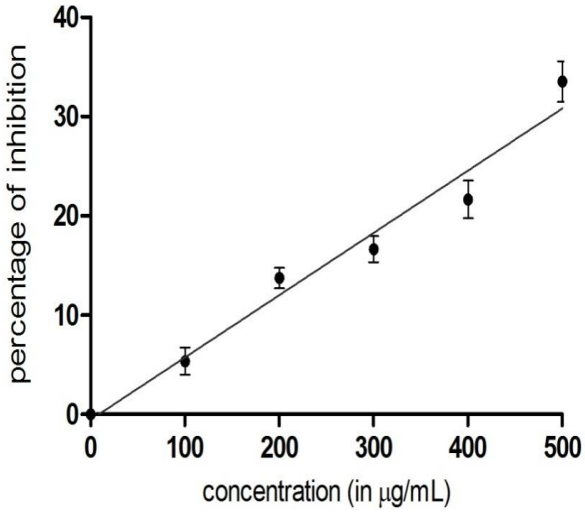
The catechu, one of the important ingredients of KKC is rich in polyphenols. Preclinical experiments have shown

that chronic diarrhea causes in imbalance in the intestinal antioxidant defense system[23], thereby makes the intestine more susceptible to oxidative damage. The polyphenols present in KKC may improve the antioxidant defense of the intestine; and it might be the possible reason for its traditional usage against diarrhea, further detailed investigations are needed to establish the relationship between the improvement of intestinal antioxidant defense by KKC and its anti-diarrheal effect.

LIPID PEROXIDE INHIBITION ASSAY: (STANDARD –ASCORBIC ACID)



LIPID PEROXIDE INHIBITION ASSAY: (KKC)



TOTAL ANTIOXIDANT EFFECT OF THE EXTRACT

Total antioxidant activities of the extract were expressed as equivalents of ascorbic acid BHT graph. The phosphomolybdenum method is quantitative since the antioxidant activity is expressed as the number of equivalents of ascorbic acid.[16] In the present study, 219.47 µg extract is needed to give the effect of 100 µg of ascorbic acid.

CONCLUSION

In this study, the aqueous extract of Kaichukkati Chooranam found to posses antioxidant activity. The result of antioxidant evaluation based on Determination of DPPH radical scavenging activity, Determination of total antioxidant capacity and Lipid peroxide inhibition assay revealed that aqueous extract of Kaichukkati Chooranam possesses significant antioxidant activity. Hence, further evaluation has to be carried out to isolate the specific bioactive compound.

REFERENCES

1. Anonymous, The Siddha Formulary of India, Government of India, Tamil Version, 2: 22-23.
2. Halliwell B., Gutteridge J.M., Oxygen toxicity, oxygen radicals, transition metals and diseases. Biochem J., 1984; 219: 1-4.
3. Maxwell S.R., Prospect for the use of antioxidant therapies. Drugs, 1995; 49: 45–361.
4. Rice-Evans C.A., Miller N.J., Paganga G., Structure antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med., 1996; 20: 933–56.
5. Prior R.L., Cao G., Variability in dietary antioxidant related natural product supplements: The need for methods of standardization. J Am Nutraceutical Assoc, 1999; 2: 46–56.
6. Dhawan B.N., Centrally acting agents from Indian plants. In: Koslow S.H., Murthy R.S., Coelho G.V., editors. Decade of the Brain: India/USA Research in Mental Health and Neurosciences. Rockville: National Institute of Mental Health, 1995; 197–202.
7. Rangari V., Pharmacognosy and Phytochemistry. Part I, 1st ed. Nashik: Career Publications, 362-363.
8. Ray D.K., Thakchom I.S., Anti-pyretic, antidiarrhoeal, hypoglyceamic and hepatoprotective activities of *acacia catechu* Wild. In albino rats. Indian J Pharmacol 2006; 38: 408-413.
9. Ismail S., Asad M. Immunomodulatory activity of *Acacia catechu*. Indian J Pharmacol 2009; 53(1): 25-33.
10. Bornett B.P., Jia Q., Zhau Y., levy R.M., A medicinal extract of *Scutellaria baicalensis* and *Acacia catechu* acts as a dual inhibitor of cyclooxigenase and 5- lipoxygenase to reduce inflammation. J Med Food, 2007; 10(3): 442-451.
11. Saini M., Saini R,, Roy S., Kumar A., Comparative Pharmacgnostical and Antimicrobials Studies of acacia species (Mimosaceae). Journal of Medicinal plants Research, 2008; 2(12): 378-386.
12. Gulcin I., Comparison of in vitro antioxidant antiradical activities of l–tyrosine and l-DOPA, Amino acids, 2007; 32: 431-438.
13. Slusarczyk S., Hajnos M., Skalicka-Wozniak K., Matkowski A., Antioxidant activity of polyphenols from Lycopus lucidus Turcz., Food Chem, 2009; 113: 134-138.
14. Kubola J, Sirimornpun S, Phenolic contents and antioxidant effects of bitter guard (*Monordica*

charantia L.,) leaf, stem and fruit fraction extract *in vitro*. *Food chem*, 2008:110; 881-890.
15. Yagi K.A., simple fluorometric assay for lipoperoxide in blood plasma. Biochem. Med., 1976; 15: 212–216.
16. Prieto P, Pineda M and Aguilar M, Spectrophotometric quantization of antioxidant capacity through the formation of a phosphor-molybdenum complex: Specific application to the determination of vitamin E, Analytical Biochem, 1999; 269(2): 337-341.
17. Fujita, T., Formation and removal of Reactive Oxygen Species, lipid peroxides and free radicals, and their biological effects. Yakugaku Zasshi, 2002; 122(3): 203-218.
18. Yoshida, Y., Umeno. A., Akazawa, Y., Shichiri, M., Murotomi, K., Horie, M. Chemistry of lipid peroxidation products and and their use as biomarkers in early detection of disease. J Oleo Sci., 2015; 64(4): 347-56.
19. Kalyanaraman, B., Teaching the basics of redox biology to medical and graduate students: Oxidants, antioxidants and disease mechanisms. Redox Biol., 2013; 1(1): 244-257.
20. Garcia, Y.J., Rodriguez-Malaver, A.J. Penaloza, N. Lipid peroxidation measurement by thiobarbituric acid assay in rat cerebeller slices. J Neurosci Methods.
21. Schneider, A.K., Smith, E.E. and Hunter, F.E., Corrrelation of oxygen consumption with swelling and lipid peroxide formation when mitochondria are treated with the swelling-inducing agents Fe, glutathione, phosphate, or ascortate, Biochemistry, 1964; 3: 1470.
22. Pandey, K.B., Rizvi, S.I., Plant polyphenols as dietary antioxidants in human health and disease. Oxid Med Cell Longev. 2009; 2(5): 270-278.
23. Nieto, N., Lopez-Pedrosa, J.M., Mesa, M.D., Torres, M.I., Fernandez, M.I., Rios, A., Suarez, M.D., Gil, A. Chronic diarrhea impairs intestinal antioxidant defense system in rats at weaning. Dig Dis Sci., 2000; 45(10): 2044-50.