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INVITRO ANTIOXIDANT STUDY ON COMBINED PLANTS EXTRACTS (*CISSUS QUADRANGULARIS* AND *AEGLE MARMELOS*)

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ABSTRACT

The study was designed to investigate the combined extracts of *Cissus quadrangularis* L. and *Aegle marmelos* were tested for antioxidant activity by DPPH Assay (1,1-diphenyl-2-picrylhydrazyl) method, FTC method, TBA method and qualitative analysis. The stem part of *Cissus Quadrangularis* and the fruit pulp of *Aegle marmelos* was collected and extracted with ethanol and ethyl acetate separately and mixed in equal proportion and it is used for the following tests. Both extracts exhibited significant antioxidant activity in all the above four methods. In DPPH assay, method Both Sample 1 (combined ethanolic extracts) and Sample 2 (combined ethyl acetate extracts) have better antioxidant activity. Sample 2 have more antioxidant activity when compared to Sample 1. In FTC method, Both Sample 1 (combined ethanolic extracts) and Sample 2 (combined ethyl acetate extracts) have better antioxidant activity. Sample 1 has more antioxidant activity when compared to Sample 2. In the TBA method, Both Sample 1 (combined ethanolic extracts) and Sample 2 (combined ethyl acetate extracts) have better antioxidant activity. Sample 1 has more antioxidant activity when compared to Sample 2. In the qualitative analysis of TLC plate method. Both Sample 1(combined ethanolic extracts) and Sample 2 (combined ethyl acetate extracts) have better antioxidant activity.

Keywords: *Cissus quadrangularis*, *Aegle marmelos*, Medicinal plant, Antioxidant activity, DPPH Assay.

INTRODUCTION

Aegle marmelos has been used as a herbal medicine for the management of diabetes mellitus in Ayurvedic, Unani and Siddha systems of medicine in India[1], Bangladesh[2] Sri lanka [3] *Aegle marmelos* (Sanskrit: Bilva) is reported to have a hypoglycemic effect [4]. In diabetes mellitus there is oxidative stress associated with release of free radicals [5]. In recent years; considerable interest has been evinced by the public and the medical professional regarding the use of indigenous drugs in the treatment of diseases. Several members of the family Rutaceae are being used traditionally for a wide

variety of ethnomedical properties. *Aegle marmelose* (L) (Rutaceae) is one among them found in India. *A. marmelose* generally acknowledged as bael or koovalam (Malayalam, India) growing wild throughout deciduous forest of India, climbing to a height of 1,200 m in Western Himalayas and also occurring in Andaman Island. Its fruits and leaves are valued in indigenous medicine. [6] The plant has been employed for a long time in folk therapy. Poultice made of leaves are used for ophthalmia and ulcers. Reactive oxygen species (ROS), such as superoxide anions, hydrogen peroxide, and hydroxyl,

nitric oxide and peroxynitrite radicals, play an important role in oxidative stress related to the pathogenesis of various important diseases [7,8]. A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials. Various herbs and spices have been reported to exhibit antioxidant activity, including *Ocimum sanctum*, *Piper cubeba* Linn. *Allium sativum* Linn. *Terminalia bellerica*, *Camellia sinensis* Linn., *Zingiber officinale* Roscoe and several Indian and Chinese plants. The majority of the antioxidant activity is due to the flavones, isoflavones, flavonoids, anthocyanin, coumarin lignans, catechins and isocatechins [9]. Antioxidant-based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer [10]. The organization estimates that up to 80 per cent of people still rely mainly on traditional remedies such as herbs for their medicines [11]. Medicinal plants constitute one of the main sources of new pharmaceuticals and healthcare products. A whole range of plant-derived dietary supplements, phytochemicals and pro-vitamins that assist in maintaining good health and combating disease are now being described as functional ingredients and Nutraceuticals. The role of medicinal plants in disease prevention or control has been attributed to the antioxidant properties of their constituents, usually associated with a wide range of amphipathic molecules, broadly termed polyphenolic compounds [12]. The number of reports on the isolation of natural antioxidants, mainly of plant origin, has increased immensely during the last decade [13]. Polyphenolic compounds are commonly found in both edible and inedible plants, they have multiple applications in food, cosmetic and pharmaceutical industries [14]. The antioxidant capacity of phenolic compounds is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers or metal chelators. In addition to their roles as antioxidants, these compounds exhibit a wide spectrum of medicinal properties, such as anti-allergic, anti-inflammatory, anti-microbial, anti-thrombotic, Cardio-protective and vasodilatory effects [15].

MATERIALS AND METHODS

Collection and Preparation of the Extract

Cissus quadrangularis and *Aegle marmelos* were collected from in and around the area of Nandyal, Andhra Pradesh. The above mentioned plants were examined, identified and authenticated by Dr. Prasad Rao, Professor, Department of Botany, P.S.C & K.V.S.C. Govt. Degree College, Nandyal. The stem part of *Cissus quadrangularis* and the fruit pulp of *Aegle marmelos* were air dried and pulverized into powder. About 25gm of the powdered sample of each medicinal plant were weighed into 100 ml of ethanol and ethyl acetate extract in a Soxhlet apparatus separately and the process is carried out for 7 days at 40-

50°C. The filtrate was evaporated to dryness at 40° c in a rotary evaporator. And the above process was repeated for several times, until the sufficient amount of extract is produced. The concentrated extract of each plant was stored at 4° c until when required for use.

INVITRO ANTIOXIDANT ACTIVITY

DPPH Method

The free radical scavenging activity of different plant extracts was done according to the method reported by [16]. Fifty micro liters of the plant extract in methanol, yielding 100µg/ml respectively in each reaction was mixed with 1ml of 0.1mM DPPH in methanol solution and 450µl of 50mM Tris-HCl buffer (pH 7.4). Methanol (50µl) only was used as a control of the experiment. After 30 min of incubation at room temperature the reduction of the DPPH free radical was measured reading the absorbance at 517nm. L-Ascorbic acid and BHT used as controls.

The percent inhibition was calculated from the following equation:

$$\% \text{ Inhibition} = [\text{Absorbance of control} - \text{Absorbance of test sample}] / \text{Absorbance of Control} \times 100$$

FTC Method

The standard method as described by [17] was used. A mixture of 4.0 mg plant extract in 4ml absolute ethanol, 4.1 ml of 2.5% linolenic acid in absolute ethanol, 8.0 ml of 0.05M phosphate buffer (pH 7.0) and 3.9 ml of water were placed in a vial with a screw cap and then placed in an oven at 40 °C in the dark. To 0.1 ml of this solution was added 9.7 ml of 75% ethanol and 0.1 ml of 30% ammonium thiocyanate. Precisely 3 min after addition of 0.1 ml of 0.02M ferrous chloride in 3.5% HCl to the reaction mixture, the absorbance of red colour was measured at 500nm each 24 hr until the day after absorbance of control reached maximum. α -tocopherol were used as positive controls while the mixture without plant sample was used as the negative control.

Thiobarbituric acid (TBA) method

The method of [18] was referred. Two ml of 20% trichloroacetic acid and 2 ml of 0.67% 2-thiobarbituric acid was added to 1 ml of the sample solution, as prepared by FTC method. The mixture was placed in a boiling water bath and, after cooling, was centrifuged at 3000 rpm for 20 min. Absorbance of supernatant was measured at 552 nm. Antioxidant activity was based on the absorbance on the final day of FTC method.

Qualitative analysis

The ethanol extract was applied on a TLC plate as a spot (100 µg/ml) for chromatographic separation of the extract using the mobile phase methanol: chloroform (95:5, v/v). It was allowed to develop the chromatogram for 30 minutes. After completion of the chromatogram the whole plate was sprayed with DPPH (0.15 % w/v) solution

using an atomizer. The color changes (yellowish color development on a pinkish background of the TLC plate)

were noted as an indicator of the presence of antioxidant substances.

Table 1: Absorbance of samples in DPPH Method

Sample	Absorbance
Sample 1	0.23
Sample 2	0.21
Negative control (Water)	0.53
Methanol	0.51
Standard (Vit E)	0.05

Table 2. Absorbance of samples in FTC Method

Sample	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Sample 1	0.77	0.91	1.05	0.69	0.69	0.69
Sample 2	0.76	0.92	1.06	0.69	0.68	0.68
-ve control	0.20	0.39	0.40	0.41	0.44	0.44
+ve control	0.20	0.23	1.04	1.06	1.06	1.07

Table 3: Absorbance of samples in Thiobarbituric acid (TBA) method

Sample	Absorbance
Sample 1	0.15
Sample 2	0.36
-ve control	0.08
+ve control	0.12

Figure 1: Absorbance of samples in DPPH Method

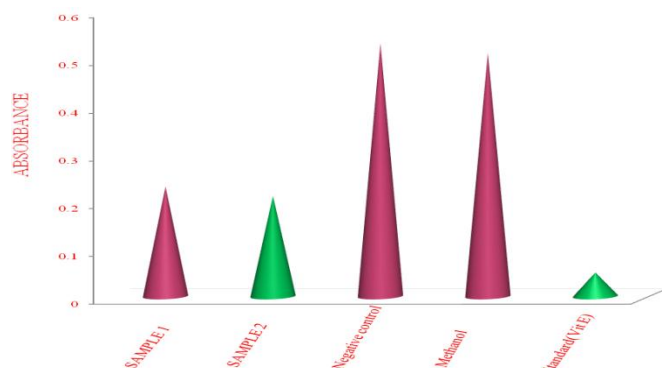


Figure 2: Absorbance of samples in Thiobarbituric acid (TBA) method

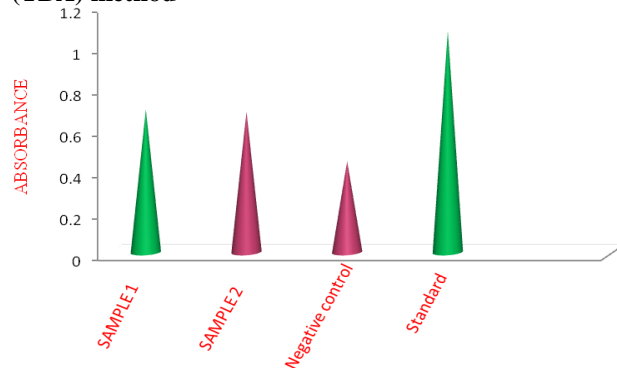


Figure 3: Qualitative analysis of combine extracts by TLC

SAMPLE:1(Combined ethanolic extract)



SAMPLE :2 (Combined ethyl acetate extract)



RESULTS AND DISCUSSION

It is well-known that free radicals are one of the causes of several diseases, such as Parkinson's disease, coronary heart disease, and cancer [19,20,21]. This study demonstrated that ethanolic and ethyl acetate extracts of *Aegle marmelos* and *Cissus quadrangularis*, have excellent antioxidant activities. It is interesting and worthy to further investigate the potential effectiveness of the combined extracts of *Aegle marmelos* and *Cissus quadrangularis* or usage of in preventing diseases caused by the overproduction of radicals. The significance of natural antioxidants from the combined extracts of *Aegle marmelos* and *Cissus quadrangularis* will be further characterized, and they will be evaluated for their bioavailability and potential toxicity *in vivo*. These points will be addressed in the coming series of studies of the combined extracts of *Aegle marmelos* and *Cissus quadrangularis*. For the health conscious consumer, the words "free radicals and antioxidants" have become very important. Antioxidants help the organisms to deal with oxidative stress, caused by free radical damage. It is possible to reduce the risk of chronic diseases and prevent their progression by either enhancing the body's natural antioxidant defense or by supplementing with proven dietary antioxidants [22]. Cancer chemoprevention by using antioxidant approaches has been suggested to offer a good potential in providing important fundamental benefits to public health, and is now considered by many

clinicians and researchers as a key strategy for inhibiting, delaying, or even reversal of the process of carcinogenesis [23,24]. DPPH is a stable radical that has been used to evaluate the antioxidant activity of plant and microbial extracts [25]. FTC is used to measure the production of peroxide compound at the initial stage of oxidation while TBA test is used to measure the secondary product of oxidation such as aldehyde and ketone [26].

CONCLUSION

Both extracts exhibited significant antioxidant activity in all the above four methods. In DPPH assay, method Both Sample 1 (combined ethanolic extracts) and Sample 2 (combined ethyl acetate extracts) have better antioxidant activity. Sample 2 have more antioxidant activity when compared to Sample 1. In FTC method, Both Sample 1 (combined ethanolic extracts) and Sample 2 (combined ethyl acetate extracts) have better antioxidant activity. Sample 1 has more antioxidant activity when compared to Sample 2. In the TBA method, Both Sample 1 (combined ethanolic extracts) and Sample 2 (combined ethyl acetate extracts) have better antioxidant activity. Sample 1 has more antioxidant activity when compared to Sample 2. In the qualitative analysis of TLC plate method. Both Sample 1(combined ethanolic extracts) and Sample 2 (combined ethyl acetate extracts) have better antioxidant activity.

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