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ANTIOXIDANT AND PHYTOCHEMICAL PROPERTIES OF COMBINATION OF LEAVES OF *AEGLE MARMELOS AND MENTHA PIPERITA*

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Abstract

Therapeutic applications of various Indian medicinal plants of Tamilnadu regions are well documented from its traditional origin in different aspects.In this present investigation we shows the Extraction of *Mentha piperita and Aegle Marmelos* leaves *using ethanol by soxhlet apparatus,* Identification of phytoconstituents from *Mentha piperita* and *Aegle Marmelos* extract by Gas Chromatography and Mass spectroscopy and interpretation of the reslts with Dr.Dukes Phytochemical and Etanobotanical data bases. Evaluation of antioxidant activity and Comparision of various antioxidant parameters between standard and *Mentha piperita and Aegle Marmelos* ethanolic extract. This study suggested that the combination of *Mentha piperita*. L and *Aegle marmelos* leaves extract possess strong free radical scavenging action due to the presence of various phytocompounds.

Key words: Antioxidant activity, *Mentha piperita*, *Aegle marmelos,* free radicals.

INTRODUCTION

Aqueous extract of Aegle marmelos leaves, was evaluated for hypoglycemic and antioxidant effect [1], by using alloxon induced diabetes in male albino rats and proposed AM L may be useful in the long-term management of diabetes. Similarly, The anti hyperlipidaemi c activity of aqueous extract of Aegle marmelos fruits was demonstrated using the streptozotocin- induced diabetic wistar rats. Aegle Marmelos leaf extract on alcohol induced liver injury in albino rats and presented data of excellent hepatoprotective effects [2]. The ethanolic extract of dried fruit pulp of Aegle Marmelos against various intestinal pathogens i.e. Shigella boydii, S. sonnei & S. Flexneri and proposed that certain phytochemicals including Phenols, Tannins and Flavonoids were effective against all. Anti-inflammatory, antipyretic & analgesic propertie s of serial extract of leaves of Aegle Marmelos, and presented that most of the extract caused a significant inhibition of the carrageenan-induced paw oedema and cotton-pellet granuloma in rats [3]. The antifungal activity of ethanolic extract of the Aegle marmelos leaves including antidiarrhoeal, and antimicrobial, activities. The anticancer potential of folk medicine used in Banglad eshi and used extracts of Aegle marmelos for cytotoxic action using brine shrimp lethality assay; sea urchin eggs assay, and MTT assay using tumor cell lines.

Common names for the Peppermint are lamb mint, brandy mint, balm mint, curled mint, amenta, lammint. The Potentially Active Chemical Constituents present in the peppermint are menthol, menthone, menthyl acetate, neomenthol, isomenthone, menthofuran, limonene, pulegone, alpha and beta pinene, and trans-sabinene hydrate Monoterpenes , Caffeic acids. Flavonoids and tannins. Peppermint leaf and oil are used for folk medicine, as flavouring agents, and in cosmetic and pharmaceutical products throughout the world [4]. Herbalists consider peppermint an astringent, antiseptic, antipruritic, antispasmodic, antiemetic, carminative, diaphoretic, mild bitter, analgesic, anticatarrhal, antimicrobial, rubefacient, stimulant, and emmenagogue [5]**.** Peppermint is currently used to treat irritable bowel syndrome, Crohn‟s disease, ulcerative colitis, gallbladder and biliary tract disorders, and liver complaints. Peppermint oil is used to relieve menstrual cramps [6].

In this present study Gas Cromatography and Mas spectroscopy is used to identify phytochemicals present in the *Aegle marmelos with Mentha piperita* leaves extract. Phytocheical identified from *A. marmelos and M. piperita* combined extract is used to investigate the various antioxidant activities.

MATERIALS AND METHODS

Plant material

Leaves of *Mentha piperita* and ***A****egle marmelos* was collected locally in and around Thiruvannamalai, and confirmed and authenticated by Mr.Raja, Botanist Santhimalai research foundation, Tiruvannamalai.

Extract preparation

Materials were cleaned with water and dried in the shade until a constant weight was obtained. It was extracted with 95% ethanol in a Soxhlet extractor. Extracts were concentrated; the percentage yield for ethanol extract was 7.9% and for antioxidant studies, since the ethanol extract was not soluble in water, it is suspended in 5% gum acacia.

GC–MS Analysis of ethanol extract of *Mentha piperita* and *Aegle marmelos* linn for the identification of chemical composition

The identification of chemical composition of ethanol extract of *Mentha piperita* and Aegle marmelos was performed using a GC–MS spectrograph (Agilent 6890/Hewlett–Packard 5975) fitted with electron impact (EI) mode. The ethanol extract (2.0 mL) of *Mentha piperita* was injected with a Hamilton syringe to the GC–MS manually for total ion chromatographic analysis in split mode. In quantitative analysis, selected ion monitoring (SIM) mode was employed during the GC MS analysis. SIM plot of the ion current resulting from very small mass range with only compounds of the selected mass were detected and plotted.(Naknean et al,2010).

NON ENZYMATIC ANTIOXIDANT ASSAY

Free radical scavenging assay (DPPH)

The scavenging activity of DPPH free radicals by different plant extracts was determined according to the method reported by Gyamfi [7]. Ascorbic acid was estimated by the method of Roe and Kuether.

Nitric Oxide Radical Inhibition Assay

Nitric oxide radical inhibition can be estimated by the use of Griess Illosvoy reaction [8]. In this investigation Griess Illosvoy reagent was modified by using naphthyl ethylene diamine dihydrochloride (0.1% w/v) instead of 1- napthylamine (5%).

Estimation of lipid hydroperoxide

Tissue lipid hydroperoxide was estimated by the method of Jiang. In this method, oxidation of ferrous ions (Fe2+) under acidic conditions in the presence of xylenol orange leads to the formation of a chromophore with an absorbance maximum at 560nm.

Superoxide anion scavenging activity

Measurement of superoxide anion scavenging activity of *mentaha* was done based on the Nishimiki method [9].

Reducing power

The reducing power of *Mentha* was determined according to the Oyaizu method [10].

Determination of total phenolic compounds

Total soluble phenolic in the aqueous extract of *Mentha* were determined with Folin-Ciocalteu reagent according to the standard method [11] using pyrocatechol as a standard.

Hydroxyl radical scavenging assay

The assay was performed as described by Halliwell method [12] with minor changes.

Statistical analysis

All the invitro experimental results were mean ± S.D of five parallel measurements.

RESULTS AND DISCUSSION

Phytochemical Analysis by Gaschromatography and Mass Spectrospcoy (Gc- Ms)

The ethanol extract of *Mentha piperita and Aegle marmelos* Linn leaves was a complex mixture of many constituents and 12 compounds were identified in this plant by GC–MS. Phytoconstituents such as Decanal, 1-Octanol, 2-butyl-,Eugenol, Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- (Synonyms: à-Curcumene, 1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*,S*)]- (Synonyms: Zingiberene, Cyclohexene, 1-methyl-4-(5-methyl-1- methylene-4-hexenyl)-, (S)- (Synonyms: á-Bisabolene, Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]- (Synonyms: á- Sesquiphellandrene), 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl- (Synonyms: Nerolidol), Tetradecanoic acid, Spiro[4.5]dec-6-en-8-one, 1,7-dimethyl-4-(1- methylethyl)-, n- Hexadecanoic acid and ,Were identified in the ethanol extract of *Mentha piperita and Aegle marmelos* Linn leaves by relating to the orresponding peak area through coupled GC–MS.

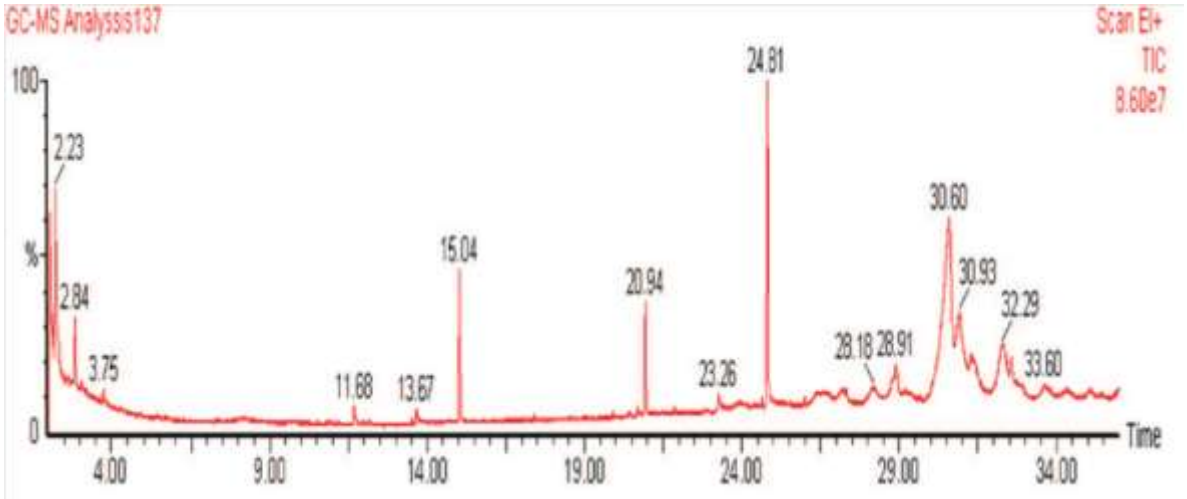

Figure 1. The chromatogram showing different compounds as peaks detected by Gas chromatography–Mass spectrophotometry in ethanolic extract of *Mentha Piperita* (MP) and *Aegle marmelos* Linn Leaves

Free radical scavengigng activity

Free radicals have aroused significant interest among scientists in the past decade. Their broad range of effects in biological systems has drawn the attention of many experimental experimental works. It has been proved that these mechanisms may be important in the pathogenesis of certain diseases and ageing. There are many reports that support the use of antioxidant supplementation in reducing the level of oxidative stress and in slowing or preventing the development of complications associated with diseases [13]. Many synthetic antioxidant components have shown toxic and/or mutagenic effects, which have shifted the attention towards the naturally occurring antioxidants. Numerous plant constituents have proven to show free radical scavenging or antioxidants activity [14]. Flavonoids and other phenolic compounds (hydroxyl cinnamic derivatives, catechines etc) of plant origin have been reported as scavengers and inhibitors of lipid peroxidation [15].

DPPH radical scavenging activity

The hydro alcoholic extract of *Mentha piperita and Aegle marmelos* exhibited a significant dose dependent inhibition of DPPH activity, with a 50% inhibition (IC50) at a concentration of 1.5 µg/ml. The result was mentioned in figure 2. The IC50 value of the extract was found to be lesser than the standard, vitamin C (IC50 3.0 µg/ml).

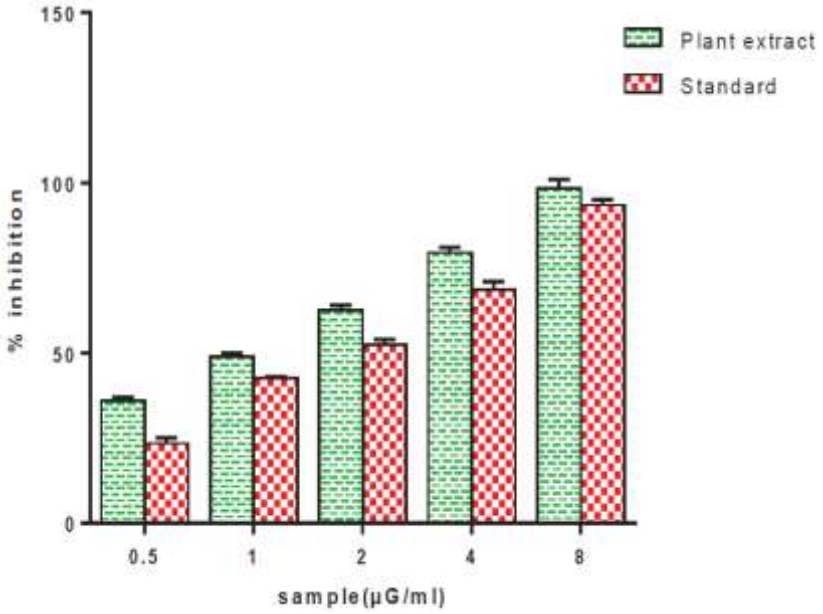

Figure 2. Scavenging effect of Mentha Piperita & Aegle marmelos Extract and standard vitamin Con 1, 1 '-Dipheny l-2 -picrylhydrazyl (D P P H) radical. Results are mean ± S.D of five parallel measurements.

Nitric oxide radical inhibition assay

The scavenging of nitric oxide by plant extract was increased in a dosedependent manner as illustrated in figure 3. At concentration of 116.0 µg/ml of extract 50% of nitric oxide generated by incubation was scavenged. This IC50 value of extract found to be lesser than the standard, rutin (IC50 160.0 µg/ml).

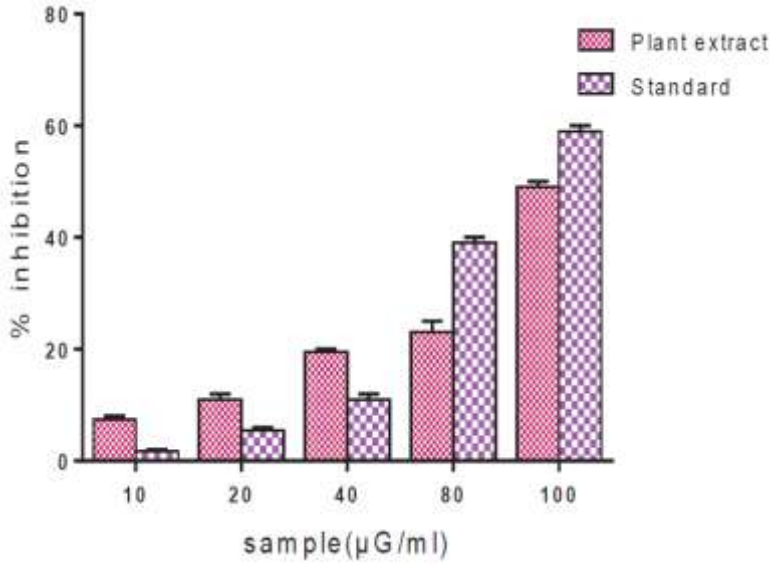

Figure 3. Scavenging effect of Mentha Piperita & Aegle marmelos Extract and standard rut in on Nitric oxide radical. Results are mean ± S.D of five parallel measurements.

Superoxide anion scavenging activity

The superoxide anion derived from dissolved oxygen by Phenazine methosulphate/NADH coupling reaction reduces nitro blue tetrazolium. The decrease the absorbance at 560 nm with the plant extract thus indicates the consumption of superoxide anion in the reaction mixture. As mentioned in figure 4, the plant extract as well as curcumin showed the scavenging activity; IC50 values, 4.7 µg/ ml and 5.84 µg/ml, respectively.

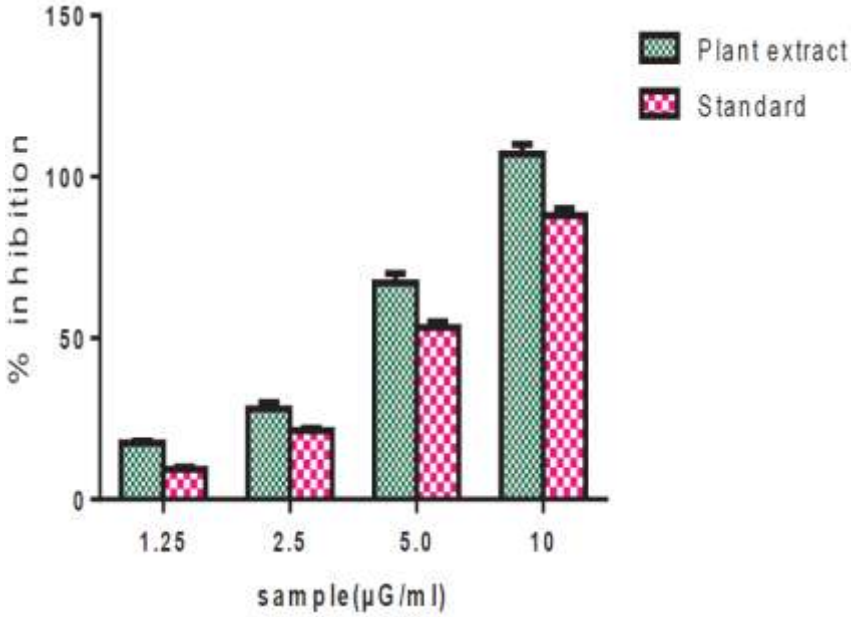

Figure 4. Scavenging effect of *Mentha Piperita & Aegle marmelos* Extract and standard curcum in on Scavenging of superoxide anion radical formation

Lipid peroxidation assay

Activity of plant extract against non-enzymatic lipid peroxidation in rat liver microsomes has been shown in figure 5. Addition of Fe 2+/ascorbate to the liver microsomes cause increase in lipid peroxidation. The extract showed inhibition of peroxidation effect in all concentrations which showed 50% inhibition effect at 104.0 µg/ml. The extract inhibition value was found to be lesser than the standard, vitamin E (IC50 120.5 µg/ml).

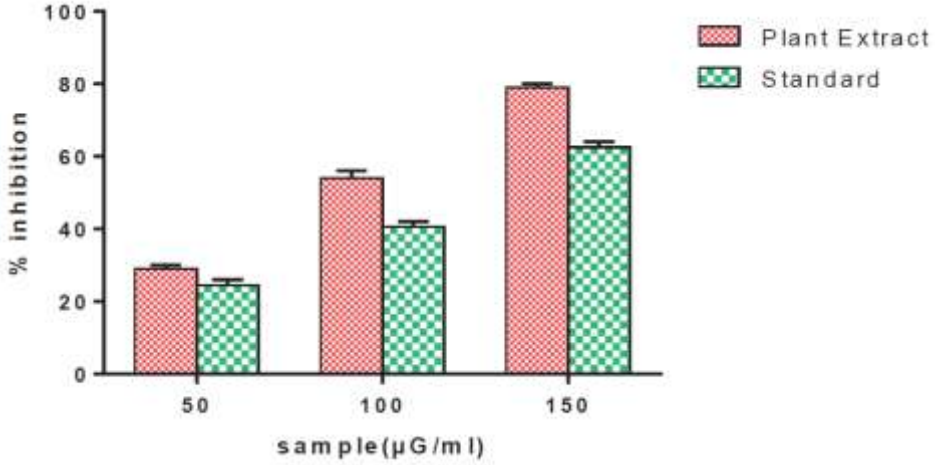

Figure 5 : Scavenging effect of *Mentha Piperita & Aegle marmelos* Extract and vitamin E on lipid peroxidation of liver microsome induced by Fe2+/ascorbate.

Hydroxyl radical scavenging assay

To attack the substrate deoxyribose hydroxyl radicals were generated by reaction of Ferric-EDTA together with H2O2 and ascorbic acid. When the plant extract were incubated with the above reaction mixture, it could prevent the damage against sugar. The results are shown in figure 6, the concentrations of 50% inhibition were found to be 27.0 µg/ml and 32.5 µg/ml for the extract and standard of vitamin E, respectively. The extract inhibition value was found to be lesser than the standard.

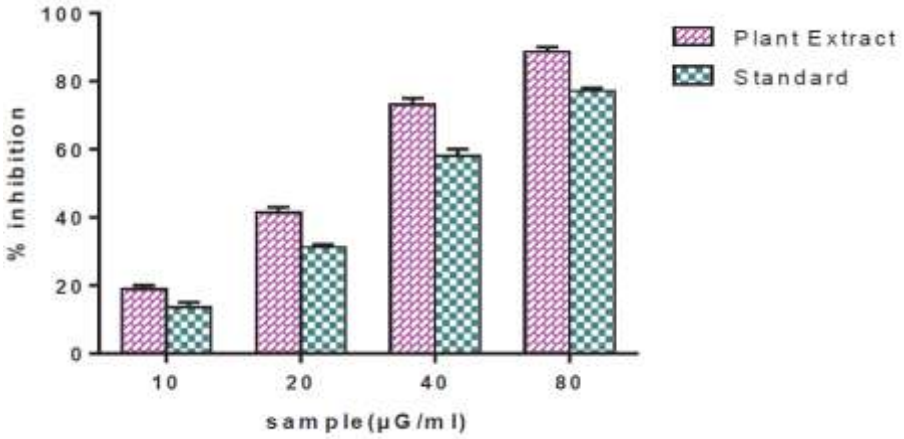

Figure 6. Hydroxyl radical scavenging assay, effect of *Mentha Piperita & Aegle marmelos* Extract and vitamin E on deoxy ribose degradation assay.

Reducing power

Figure 7 shows the reductive capabilities of the plant extract compared to butylated hydroxy toluene. The reducing power of extract of *Mentha piperita and Aegle marmelos* was very potent and the power of the extract was increased with quantity of sample. The plant extract could reduce the most Fe3+ ions, which had a lesser reductive activity than the standard of butylated hydroxy toluene.

Determination of total phenolic compounds

The total phenolic contents of hydro alcoholic extract of *Mentha piperita and Aegle marmelos* was 0.0589 µg pyrocatechol equivalent / mg.

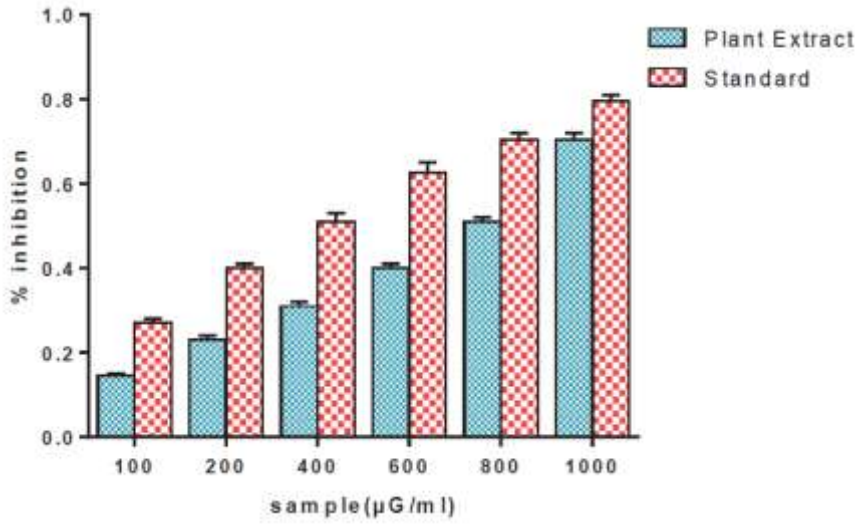

Fig.7: The reductive ability of *Mentha Piperita & Aegle marmelos* Extract butylated hydroxy toluene. Results are mean ± S .D o f five parallel measurements.

CONCLUSION

This study suggested that the combination of *Mentha piperita*. L and *Aeglemarmelos* leaves extract possess strong antioxidant activity due to the presence of phytocompounds like n-Hexadecanoic acid, tetradecampoic acid , Benzene, 1-(1,5- dimethyl-4-hexenyl)-4-methyl-à-Curcumene which might be helpful in preventing or slowing the progress of various oxidative stress- related diseases. Further investigation on the isolation and identification of antioxidant component(s) in the plant may lead to chemical entities with potential for clinical use.

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