

GC-MS analysis and phytochemical studies of methanolic fruits extract of *Garcinia cambogia* Hort. Ex Boerl and *Ziziphus trinervia* Roth

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Abstract:

The investigation was carried out to determine the possible phytochemical components from the methanolic extracts of *Garcinia cambogia* (fruits) and *Ziziphus trinervia* (fruits). Among the phytochemical screening of these two plant extracts showed that the plant was rich in alkaloids, flavonoids, phenols, saponins and quinones. This study was extended by analyzing the potent bioactive compounds in the methanolic extract of *Garcinia cambogia* (fruits) and *Ziziphus trinervia* (fruits) using GC-MS. The analysis revealed that *Garcinia cambogia* fruits extracts 40 compounds were identified in the fruit methanol extract. Another one plant *Ziziphus trinervia* methanol fruit extract showed 3 compounds were identified. Medicinal potential of these compounds needs further research on microbial aspects to develop safe drug.

Keywords: *Garcinia cambogia*, *Ziziphus trinervia*, Phytochemicals, GC-MS, Bio active compounds.

Note: All the figures and tables are listed in supplementary article

Introduction

Medicinal plants are widely used by the traditional medicinal practitioners to cure different diseases due to their world-wide availability and fewer side effects. The herbal medicines occupy distinct position right from the primitive period to present day. Medicines that are used today are not definitely the same as those that were used in ancient times or even in the recent past. India has a wealth of medicinal plants most of which have been traditionally used in Ayurveda, Unani systems of medicine and by tribal healers for generation. The medicinal value of this plant lies in the bioactive phytochemical constituents that produce definite physiological effect on human body (Krishnaiah *et al.*, 2014). These natural compounds signify the base of modern drugs as we use today. Plants have many phytochemicals with various bioactivities, including antioxidant, anti-inflammatory and anticancer. Studies have reported that extracts from natural products, such as fruits, vegetables and medicinal herbs, have positive effects against cancer, compared with chemotherapy or recent hormonal treatments (Wu *et al.*, 2002). Therefore, many plants have been examined to identify new and effective antioxidant compounds, as well as to

elucidate the mechanisms of action (Swamy *et al.*, 2000).

The plant *Garcinia cambogia* belongs to the family Guttiferae {Clusiaceae}. It is a wild sub-tropical and tropical plant. The plants are shrubs or trees with yellow or greenish juice. The fruits of the plant are commercially important for its valuable chemical components like hydroxyl citric acid, tarteric acid, camogin, euxanthone, gucinol, reducing sugars and fats. The plant is commercially important as their fruit extracts are used for various treatments such as astringent, demulcent, rheumatism, bowel complaints and purgative (Obolskiy *et al.*, 2009).

Zizyphus trinervia, is a small tree that grows up to 30 ft in height, having olive brown wood and commonly found in the forests of Gujarat, Western Ghats of Tamil Nadu and Kerala at low elevations. Plants belonging to the genus *Zizyphus* (Rhamnaceae). Leaves are used in cachexia and venereal diseases (Gritto,). Leaves are used to depurative; employed to purify blood and as an alternative in chronic venereal affections. (Khare, 2007).

Materials and methods

Plant materials

Plants were collected from natural population growing in the Courtallum and Sadhuragiri forest area, Tamil Nadu, India, during October 2015. The plant sample was carried to the Botany Research Laboratory; Voucher specimen of the plant was deposited in the Botany research laboratory V.H.N.S.N. College (Autonomous) for further references.

Preparation of fruit extracts

The fruits were cleaned and cut into small pieces before being dried in a hot air-blowing oven at 50°C. All samples, after drying, water contents below 10%. They were ground to a fine powder in a mechanical blender and kept at room temperature prior to extraction. In the basis of polar solvent of methanol. 20g of the dried fruit sample was taken in a conical flask and 100ml of methanol was added. The conical flask was kept on mechanical shaker for 24 hours, after that the extract was filtered through Whatman No: 1 and the pellet were allowed for drying. The dried extract was recovered and stored in Refrigerator -4°C for further analysis. The dried plants were used for the analysis of phytochemical test.

Phytochemical screening

The collected plant fruits extracts were subjected to qualitative and GC-MS analysis for identification of various classes of active chemical constituents were carried out using standard methods.

Test for Alkaloids (Mayers Test)

To 1 ml of fruits extract, 6 drops of Mayers reagent was added. The formation of yellowish creamish precipitate indicated the presence of alkaloids (Edeoga *et al.*, 2005; Harbone, 1973).

Test for Tannins (Braymers Test)

1ml of the fruits extract was added mixed with 2ml of water. To this 2 drops of 5% ferric chloride solution was added. Appearance of dirty green precipitate indicated the presence of tannins (Edeoga *et al.*, 2005; Harbone, 1973).

Test for steroids (Salkowski Test)

To 2ml of the extract, 2ml of chloroform was added and followed by concentrated sulphuric acid. Formation of reddish brown ring at the junction showed the presence of steroids (Yadav *et al.*, 2014).

Test for terpenoids

2ml of the extract was added with 2ml acetic acid. Then concentrated sulphuric acid was added. Deep red color development showed the presence of terpenoids (Yadav *et al.*, 2014).

Test for Coumarins

Take 2ml of the extract and added 3ml of 10% sodium hydroxide. Formation of yellow coloration indicates the presence of coumarins ((Yadav *et al.*, 2014).

Test for Catachins

2ml of alcoholic extract solution was treated with few drops of Ehrlich reagent and few drops of concentrated HCL. The pink color formation indicates the presence of catachin (Yadav *et al.*, 2014).

Test for phenols

1ml of the extract was treated with 3% ferric chloride. The appearance of deep blue color, then it shows the presence of phenol (Kokate, 2000; Harborne, 1999).

Test for flavonoids

1ml of the extract was added with 1ml of sulphuric acid. Orange color formation confirmed the presence of flavonoids (Kokate, 2000; Harborne, 1999).

Test for Quinones

1ml of the extract was treated with 5ml of HCL. Formation of yellow color precipitate indicated the presence of quinine (Kokate, 2000; Harborne, 1999).

GC-MS Analysis

GC-MS analysis of these extracts were performed using a Perkin-Elmer GC Clarus 500 system and Gas chromatograph interfaced to a Mass spectrometer GC-MS)

equipped with a Elite-I, fused silica capillary column (30mmX0.25mm 1D X 1 iMdf, composed of 100% Dimethyl poly siloxane). For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1ml/min and an injection volume of 2 μ l was employed (split ratio of 10:1); Injector temperature 250 $^{\circ}$ C; Ion-source temperature 280 $^{\circ}$ C. The oven temperature was programmed from 110 $^{\circ}$ C (isothermal for 2 min), with an increase 10 $^{\circ}$ C/min to 200 $^{\circ}$ C, then 5 $^{\circ}$ C/min to 280 $^{\circ}$ C, ending with a 9 min isothermal at 280 $^{\circ}$ C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 minutes. The relative % amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was a Turbomass. Interpretation on mass spectrum GC-MS was conducted using the database of national Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The Name, Molecular weight and

structure of the components of the test materials were ascertained.

Identification of compounds

The characteristics identify of the compounds in the extracts were assigned by the comparison of their retention time and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures. NIST 11 Library source were used for matching the identified components from the plant material.

RESULTS AND DISCUSSIONS

The present study was carried out on the plant samples revealed the presence of medicinally important bioactive compounds. The plant *Garcinia cambogia* shows Alkaloids, phenols, saponins, steroids, terpenoids, and tannins from different solvent extract (Table 1) whereas *Ziziphus trinervia* comprises of alkaloids, Phenols, flavonoids and quinone from methanol solvent extracts. (Table 1). In another study Krishnamoorthy *et al.*, 2014 revealed the presence of flavonoids, phenols, Tannin saponin, Terpenoids and steroids. As flavonoids having antioxidant property, it protects tissues against oxygen free radicals, thus has a role in prevention of atherosclerosis, cancer, chronic inflammation and may inhibit aging. The polyphenols possess anti-parasitic activity,

and monoterpenes have been reported to constitute anti-plasmodic, anti-neoplastic and anti-viral activities (Sharma, 2006).

+ = indicates presence of phytochemicals,

- = indicates absence of phytochemicals

The GC-MS analysis was employed to define the chemical compound present in the methanolic fruit extracts of *Garcinia cambogia* and *Ziziphus trinervia*. The GC-MS analysis revealed the presence of forty compounds from the methanolic fruit extract of *Garcinia cambogia*. The major constituents were Ethane, (chloromethoxy)- (19.30%); Cyclohexanecarboxylic acid, 2-ethylcyclohexyl ester- (18.74%); Cycloheptasiloxane, tetradecamethyl (5.44%); 1-Octadecanesulphonyl chloride (3.44%) and Hexadecanoic acid, methyl ester (3.34%) (Table 2), along with other minor constituents were also present. The GC-MS chromatogram (Fig. 1) shows the peak area separation. The GC-MS analysis of methanolic fruit extract of *Ziziphus trinervia* indicates three compounds. The compounds are Benzenemethanol .alpha. .alpha.-dimethyl- (31.06%); n-Hexadecanoic acid (21.30%) and 2, 3, 4, 6-Tetrafluorophenyl isothiocyanide (44.64%) (Table 3 & Figure 2).

The presence of various bioactive compounds in *G. cambogia* and *Z. trinervia* justifies the use of the fruit for various treatments by traditional practitioners. A detailed study of the various compounds present in *G. cambogia* and *Z. trinervia* and their pharmaceutical importance requires to be carried out such that a drug with multiple effects can be made available in near future.

Conclusion

From the present study, it can be concluded that most of the biologically active phytochemicals were present in the methanolic extract of *Garcinia cambogia* and *Ziziphus trinervia* fruit. In other words, the results confirmed the presence of therapeutically potent compound in fruit extract of *Garcinia cambogia* and *Ziziphus trinervia*.

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