Phytochemical screening and Antioxidant analysis of flavonoids from flowers of *Duranta repens*.

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Abstract : The present study was carried out to determine the phytochemical constituents, thin layer chromatographic profile and UV analysis of Duranta repens flower extracts. The leaves of D.repens were collected, dried, pulverized and extracted with Dichloromethane using maceration method. The extract was concentrated in vacuo with the aid of rotary evaporator to afford a greenish crude dichloromethane extract (DCME). The fractions were subjected to general phytochemical screening and thin-layer chromatography using standard procedures. The fractions were scanned in the wavelength ranging from 200-750nm using Shimadzu 1800 UV –VIS spectrophotometer and characteristics peaks were detected. Qualitative analysis of the phytochemicals revealed the presence of saponins, alkaloids, flavonoids, phenols, steroids and triterpenes. Quantitative analysis showed that the crude flavanoids was the major phytochemical constituent present in highest percentage Thin-layer chromatographic studies using different solvent systems revealed homogenous spots with different Rf values. The UV profile showed different peaks ranging from 220-750nm with different absorptions respectively. These findings provided the evidence that flower of Duranta repens is a potent source for some medicinally important phytochemicals and it justifies its use as a medicinal plant, the antioxidant capacity of of Dr(CH2Cl2)FE was accessed by two different assays: Total Flavanoid and ABTS assay. The flavanoid content increased with increase in the concentration of the extract. The minimum inhibitory concentrations required to scavenge 50% ABTS free radical (IC50) of flower extract was 100µg/ml. This can be further investigated for the isolation and identification of active biochemical compound of medicinal utilities.

IndexTerms **- Duranta repens dichloromethane flower extract (Dr(CH_2Cl_2)FE), TLC, phytochemical screening, ABTS, Flavonoid.**

I. INTRODUCTION

In India, almost all medicinal plants especially in traditional medicine are currently well acknowledged and established as a viable profession. In developing countries like in India synthetic drugs are not only expensive and inadequate for the treatment of diseases but are also often with adulterations and side effects1. Therefore, it is of great interest to carry out a screening of these plants in order to validate their use in folk medicine and to reveal the active principle by isolation and characterization of their constituents. The presence of phytochemical compounds in the plants indicates its medicinal potential.2 The presence of tannins shows plant posses anti-parasitic, antiviral and antibacterial activities. Flavanoids are the phenolic compounds having antioxidant, anti-inflammatory, anti allergic and anticancer activities. Saponins acts as anti-feedants and used as adjuvant in vaccines. Presence of alkaloids shows antimicrobial, anticancer, antiarrhythmic and analgesic activity. Steroids acts as signalling molecules and are important against cardio tonic activity. Phenols are used as antiseptic and active ingredient in some oral analgesics such as carmex and chloraseptic spray. Knowledge of the chemical constituents of plants is desirable because such information may be of great value revealing new sources of economic compounds as tannins, oils, gums, precursor for the synthesis of new chemical substances which can be used in drug5. Discovery of the actual value of a traditional plant as well as discovery of a therapeutic agent solely depends upon the knowledge about the phytochemical composition of the plant 4

The plant used in present study is Duranta repens Linn. (D. repens) belonging to family Verbenaceae, it is native to clean and open forests. It is used as an ornamental plant in tropical nations. Duranta is an upright to hanging bush that occasionally takes the type of a scrambling bush or once in a while a little tree. They mostly occur in tropical and subtropical and few temperate regions. Habit varies from tree lianas to shrubs and herbs. Leaves are simple, opposite or alternate arranged, entire or divided, and exstipulate. Flowers are often involucres of colored bracts.Plants are sometimes thorny. Fruits are fleshy drupes, usually 8-seeded, completely enclosed by the persistent calyx. Fruits are less commonly capsular or schizocarps. Seeds show a presence of oily embryo with little or absence of endosperm. The economic uses include as sources of timbers, essential oils, teas, herbal medicines, fruits, gums, tannins, and ornamentals. Flowers are small mostly blue– purple, or white bracteates with racemes are either terminal or axillary. Calyx tube sub campanulates ribbed and toothed. Corolla tube is cylindrical straight or apically curved and pubescent at the mouth. An Ovary is 4-carpelled with 8 locules, one ovule in each lobule, style terminal with unequally 4- lobed stigma. 5

Literature proposes that D. repens L. has been accounted for a wide variety of medicinal activities. The whole plant is used in the treatment of infertility, pneumonia, and malaria, as diuretic, itches, anthelmintic, neuralgic disorders and also as an insect repellent. D. repens also exhibits activities such as anti-shigellosis, cytotoxic potency, antiviral activity, antioxidant, antibacterial, and antimicrobial against human pathogens. Even though the flower is said to have stimulant properties , the phytochemical constituents present in the flower responsible for the stimulant properties is not mentioned anywhere , Thus the present investigation was aimed to investigate the pharmacognostical features.

MATERIALS AND METHODS:

Standard solutions and Plant material:

All standards chemicals and reagents were of analytical grade and procured from form Sigma (St. Louis, MO, USA). Solvents for extraction and chromatographic analysis were obtained from Merck (Darmstadt, Germany). Analysis of phytochemicals profile was performed on the flowers of Duranta repens were collected from in and around Mysuru, Karnataka state And their identity were confirmed at Department of Botany, PBMMEC. Mysuru, India. They were immediately washed, shade dried, powdered and stored in airtight containers at room temperature for further analysis.

Methods

Preparation of crude extracts:

18gm of shade dried Duranta repens were ground at a high speed with blender and extracted up to clear sample in Dichloromethane with the soxhlet apparatus. The dichloromethane extract was filtered through whaatman no.1 filter paper in a Buchner funnel. The solvent was evaporated in a vacuum evaporator model then crude extracts were stored at - 40C. Crude extracts were diluted with Dichloromethane for further investigation.

Preliminary qualitative phytochemical analysis-

Chemical tests were carried out using the Dichloromethane extracts from the powdered specimens, using standard procedures to identify the constituents Qualitative phytochemical analysis of Duranta repens fruits powder was tested as follows:

1. Detection of alkaloids: Extracts were dissolved in dilute hydrochloric acid and filtered.

Mayer's test: The filtrate was treated with Mayer's reagent (solution of mercuric chloride and potassium iodide). Formation of yellow cream precipitate indicates the presence of alkaloids.

2. Detection of terpenoids:Salkowski's test: extract was treated with chloroform and concentrated sulphuric acid, which forms a layer. Formation of reddish brown color in the inner face indicates the presence of terpenoids.
3. Detecction of phenols:Ferric chloride test: Extract was treated with ferric chloride solution. Formation of bluish black color indicates the presence of phenols.
4. Detection of saponins:Extract was mixed with distilled water and shaken well. Formation of frothing indicates the presence of saponins.
5. Detection of tannins:Extract was mixed with distilled water and heated on water bath, it was filtered and ferric chloride was added to the filterate. Formation of dark green color indicates the presence of tannins.
6. Detection of proteins and amino acids: Ninhydrin test: To the extract, 0.2% ninhydrin reagent was added and boiled for minutes. Formation of pink or purple color indicates the presence of proteins, peptides or amino acids.
7. Detection of oils and resins: Extract was applied on the filter paper. Development of transparent appearance on the filter paper indicates the presence of oils and resins.
8. Flavonoids test: (200 mg plant material in 10 ml ethanol, filtered) 2 ml filtrate + conc. HCl + magnesium ribbon. Pink-tomato red color indicated the presence of flavonoids.

Qualitative measurement of total phytochemicals with spectrophotometer

The qualitative analysis of different phytochemicals was carried out with a spectrophotometer UV-1900(Shimadzu, Japan) on the basis of UV spectra through absorption maxima at individual wavelength of every biocomponent.

Thin layer chromatography (TLC)

Duranta repens flower dichloromethane extract of 10 microliter fractions was further separated by column chromatography on silica gel. The fractions obtained were analyzed by TLC performed on Merck Silica Gel 60 glass plates using different eluents..TLC plate with solvent system of acetone-dichloromethane-ethyl acetate-petroleum ether in the ratio 10:40:3.75:46.25, Dried at room temperature and deravatization of TLC plate was done by UV light.

Determination of total flavonoid content:

The AlCl3 method was used for quantification of the total flavonoid content of the methanolic plant extracts. 20 µl of the sample extract was added to a solution of 2% AlCl3.6H2O. The mixture was vigorously shaken and diluted with double distilled water to make the total volume 10 ml. The absorbance of the reaction mixture was measured after 10 min incubation at 440 nm. Flavonoid contents were expressed as quercetin equivalents in mg per gram dry material.

ABTS radical scavenging activity:

Free radical scavenging activity of plant samples was determined by ABTS radical cation decolorization assay . ABTS•+ cation radical was produced by the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate (1:1), stored in the dark at room temperature for 12-16 h before use. ABTS•+ solution was then diluted with methanol to obtain an absorbance of 0.700 at 734 nm. ABTS stock solution; 7.4mM ABTS and 2.6mM potassium persulfate were mixed in equal ratio that is (1:1) and incubated overnight in dark. 0.2 ml of flowers extract of ten different concentrations (0.625, 1.25, 2.5, 05, 10, 20, 40, 60, 80, 100 µg/mL) and 2.8ml of ABTS reagent were mixed together and incubated at room temperature for one hour and O.D was taken at 734nm. Tannic acid was used as a standard. For calculating the capability to scavenge the ABTS free radical, the following formula was used ABTS scavenging effect(%inhibition) = [(Ao−A1)/Ao] ×100.

RESULTS

The qualitative study carried out on Duranta repens dichloromethane flower extract revealed the presence of medicinally active constituents such as flavonoids, terpenoids, alkaloids , phenolds ,saponons ,tannins (Table 1). However, oils- fats and proteins and amino acids are absent .

Table.1 Phytochemical composition of *Duranta repens* dichloromethane flower extract

Phytochemical Analysis	*Duranta repens* dichloromethane flower extract
Detection of alkaloids	++
Detection of Terpenoids	++
Detection of Phenols	++
Detection of Saponins	+
Detection of Tannins	+
Detection of proteins and amino acids	-
Detection of Oils & Fats	-
Detection of Flavonoids	+++

+++ Highly present, ++ moderately, + low, - absent

Qualitative measurement of total phytochemicals with spectrophotometer:

Preliminary spectrophotometric screening represents a primary analysis of Duranta repens flower dichloromethane extract. It finds that DrDcm flower extract have maximum absorbance (1.57) in this screened range of wavelength (200nm-1000nm) Spectrophotometric pattern completely matched with the qualitative pattern of phytochemical analysis.

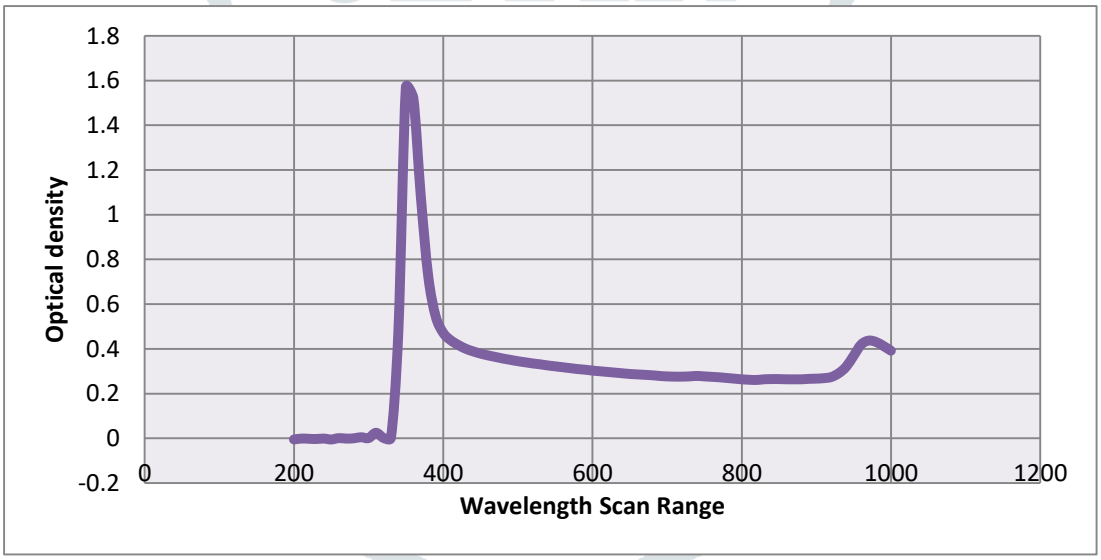

Fig 1 : Qualitative measurement of total phytochemicals with spectrophotometer

Thin layer chromatography (TLC):

The thin layer chromatograms (TLC) of chemicals separated from Dr(CH_2Cl_2)FE extracts reveals separation had single pink spot having Rf values as 0.16-quercetin, and 0.159 confirming the presence of metabolite flavonoids

Fig 4 : Thin layer chromatogram

Determination of total flavonoid content:

Flavonoid make up one of the most pervasive groups of plant phenolics. Due to their importance in plants and human health. The present study indicates the flavonoid concentration increases with increase in the concentration of the extract thus indicating their potentials as therapeutic agents, and also for predicting and controlling the quality of medicinal herbs.

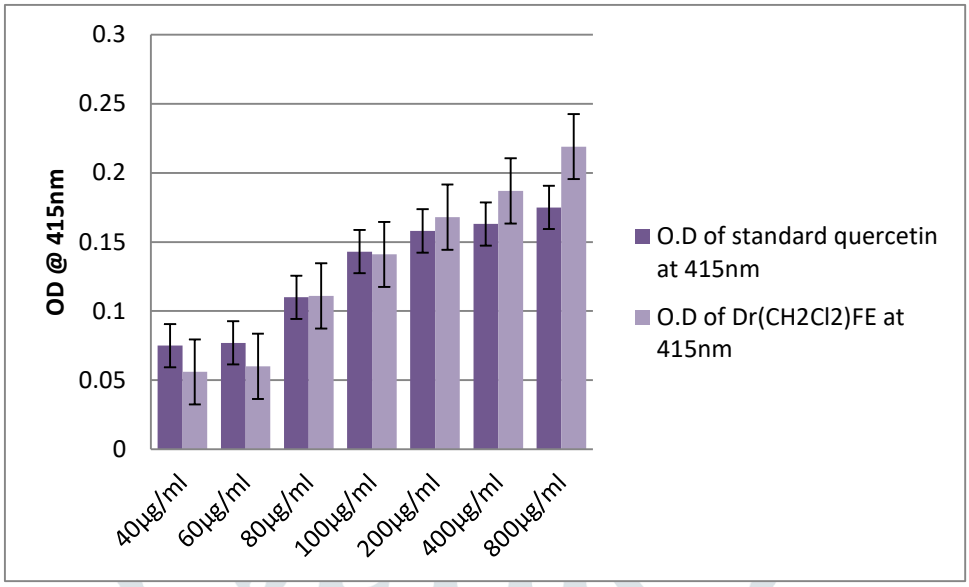

Fig 3 : Total Flavonoid Content

ABTS radical scavenging activity:

In this assay a stable blue green colored ABTS radical cation was generated by reacting ABTS with potassium persulphate before adding antioxidants.

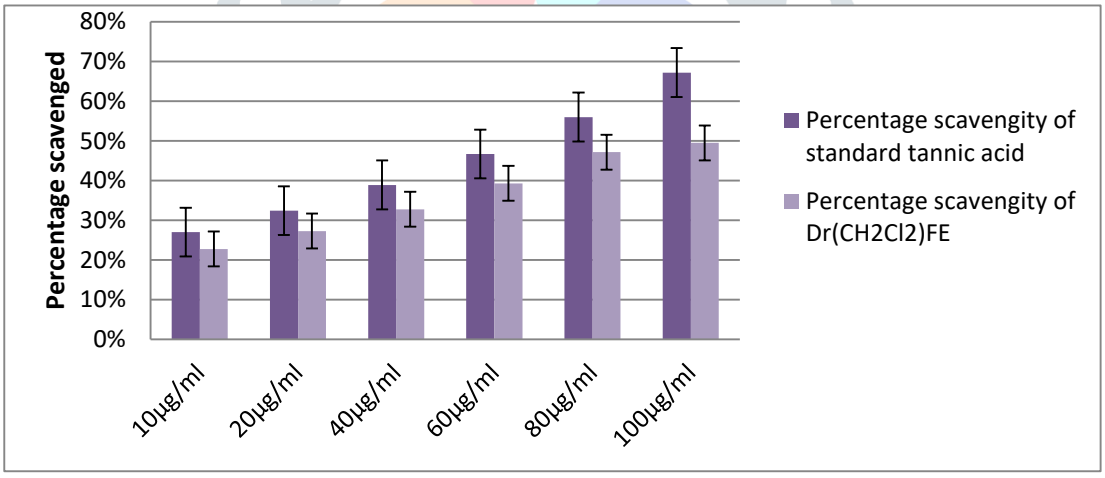

Fig 2 : ABTS radical scavenging activity

Adding antioxidants after stable radical formation results in decolorization and decrease in absorbance at 600nm and this decrease is proportional to antioxidant concentration. The minimum inhibitory concentrations required to scavenge 50% ABTS free radical (IC50) of flower extract was 100µg/ml.

DISCUSSION

Nature has been a source of medicinal agents for thousands of years and since the beginning of the man. Indian medicinal plant-based industry is growing 7-15% annually for pharmaceuticals, phytochemicals, neutraceuticals, cosmetics and other valuable products. The crude extracts of Dr(CH2Cl2)FE studied were found to contain one or more of the following phytochemical compounds flavonoids, terpenoids, alkaloids , phenolds ,saponons ,tannins (Table 1). However, oils- fats and proteins and amino acids are absent .Herbal drug technology includes various steps that are involved in converting botanical materials into medicines. Chemical profiling establishes a characteristic chemical pattern for a plant material, its fractions or extracts. Thin layer chromatography analysis is a valuable method in the identification and quality assurance of vegetable drugs. The chromatographic conditions used herein allowed good resolution and Rf-values to different compounds present at the Dr(CH2Cl2)FE extracts. Based on the TLC chemotypic profile, this study represents that Dr(CH2Cl2)FE has a maximum content of phytochemicals eg.

Flavonoids, which correlates with the The UV analysis showed wavelength range from 200nm-1000nm. The ultraviolet spectroscopy is very useful method for identification of unsaturated bonds present in plant components, which can be used to distinguish between conjugated and nonconjugated system. Using the principle of absorption maxima, the structure of compounds can be deduced . The result of the UV analysis of the fractions gave absorption peaks at 350nm It can be inferred that the compounds present in the fractions have chromophores and hence, absorption takes place to allow transition.

Antioxidant activity of the antioxidants is concerning with those compounds capable of protecting the organism system against the potential harmful effect of oxidative stress . In this study, the antioxidant capacity of of Dr(CH2Cl2)FE was accessed by two different assays: Total Flavanoid and ABTS assay. Flavonoid which contain hydroxyls, are responsible for the radical scavenging effect of plants. ABTS assay is better to assess the antiradical capacity of both hydrophilic and lipophilic antioxidant because it can be used in both organic and aqueous solvent system as compared to other antioxidant assay.The present results indicate the Antioxidant activity of the extracts as reducing agents, terminating the free radical chain reaction by removing the same, absorb light in the ultraviolet region (100-400 nm), and chelate transition metals, thus inhibit oxidation reactions by itself being oxidized and also forbid the production of oxidative damage. Although It is well-known that flavonoids can neutralize different types of oxidizing species including hydroxyl radical, superoxide anion or peroxy radicals. They may also work as quenchers of singlet oxygen.

CONCLUSION

Present study provides basic information on phytochemical composition. Considering that at this moment the active component is flavanoid and in view of the large pharmaceutical utilization of Dr(CH2Cl2)FE extracts it can be used in traditional medicine to treat ailments. Further detailed isolation and characterization of active constituents is under progress.

CONFLICT OF INTEREST STATEMENT: None

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