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Research Article

Preliminary phytochemical screening and spectroscopic analysis of *Ormocarpum sennoides* DC

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ABSTRACT

Since ancient ages medicinal herbs have gained attention because of its immense potential for treating various disease with less side effects. The present study was carried out to highlight phytochemical and pharmacological aspects of *Ormocarpum sennoides* DC. *Ormocarpum sennoides*, a shrub used in traditional system of Indian medicine has been reported to possess bone fracture healing, and strengthening bone. Phytochemical screening of *Ormocarpum sennoides* DC was analyzed using various solvent such as ethanol, methanol, aqueous leaf extract were tested for presence of bio-active compounds such as alkaloids, glycosides, steroids, phenols, tannis. Further these compounds needs to be isolated to confirm the activities of the individual compound. The unidentified anabolic steroid may act on estrogenic receptors of the bone, used in fracture healing, the high levels of flavonoids content may be responsible for antioxidant potential of *Ormocarpum sennoides*. The HPLC fingerprinting and IR, UV spectroscopy analysis was carried out to help in the identification and authentication of this species and provide referential information for standardization of the plant. The present study was carried out to identify the various phyto constituents present in the different extracts of *Ormocarpum sennoides* DC.

Keywords: Bio-active compound; HPLC; IR-UV spectroscopy; *Ormocarpum sennoides* DC

INTRODUCTION

The phytochemicals are naturally occurring, biologically active chemical compound in plants, which act as a natural defense system for lost plants and provide color, aroma and flavor, they have great potential in treating human disease such as cancer, coronary heart disease, diabetics and infectious diseases (Sakanaka S, *et al*., 2005; Singleton, V.L *et al.*, 1965). The present study is an initiative to analyze the various phytoconstituent's in *Ormocarpum sennoides* DC; the leaves of which have been used in traditional Indian medicine and also among tribal communities in and around Kanchipuram district, Tamilnadu, India for healing bone fracture and strengthening bones.

Ormocarpum sennoides DC belongs to *Fabeacea* family an edible plant found in southern parts of Indian Territory and it's commonly called as Kattumurungai the plant has been documented for treating bone fractures. This herb has been used as a source of medicine for healing bone fracture and bone related deficiencies from ancient Hindu Siddha-Ayurveda time to present day. Traditional herbal medicines are formulated based on plant derived metabolites (Ramar Perumal Samy et al., 2008).

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MATERIALS AND METHODS

Plant Materials

The leaves of *Ormocarpum sennoides* DC leaves collected from forest area of Kanchipuram district, Tamilnadu, India authenticated by Botanical Survey of India/Ministry of Environment and Forest (BSI/SRC/5/2013-14ech/550) , shade dried leaves were ground to get a course powder that was stored in airtight, high density poly ethylene container.

Preparation of Plant Extract

Green leaves were air dried and powdered using mechanical grinder and stored. Leaf powder 500gms was refluked with different solvents ethanol, methanol, aqueous for five days. The total titrate was concentrated to dryness in hot air over at 32^{o}C to render 4 solvent for investigations.

Chemicals

HPLC grade ethanol, methanol, ethylacetate, 1% ammonia solution, aluminium chloride, mercuric chloride, potassium iodide, hydrochloric acid, $NaHCo_3$ ferric chloride, lead acetate chloroform, sulphuric acid.

Phytochemical Screening

Ormocarpum sennoides leaf extract was tested for the presence of various bioactive compounds such as fla-

Table 1: Phytochemical constituents of *Ormocarpum sennoides* leaf extracts

Constituents	Test	Ethanol Extract EtOH	Methanol Extract MeOH	Aqueous Extract H_2O
Alkaloids	Mayer's Wagner's	+	+	+
Flavonoids	Ammonium Test Ammonium Chloride Test	+	+	+
Saponins	Frothing Test	+	-	+
Carbohydrates	Molisch's Test Benedicts Test	+	+	+
Proteins	Biuret's Test	-	-	-
Phenols	Ferric Chloride Test Lead Acetate Test	+	-	+
Steroids	Salkowski's Test	-	-	+
Tannins	Ferric Chloride Test	+	+	+
Glycosides	Lead Acetate Test	+	+	+

vonoids, alkaloids, saponins, carbohydrate, protein, phenols, steroids, tannins, glycosides by standard methods (Table1).

Estimation of Total Flavonoid Content

Reagents required

- Quercetin (1mg/ml)
- 5% sodium nitrite solution
- 10% aluminium chloride
- 1M sodium hydroxide
- Sample (1mg/ml)

Procedure

Total flavonoid content (TFC) determined using the method described by (Sakanaka, S, *et al.,* 2005). The flavonoid content was determined by aluminium chloride method using Quercetin as standard. Extracts and Quercetin were prepared in ethanol (1 mg/ml). 0.1ml of extract was mixed with 0.9ml of distilled water in test tubes, followed by addition of 75 µL of a 5% sodium nitrite solution. After six minutes, 150µL of a 10% aluminium chloride solution was added and the mixture was allowed to stand for further five minutes after which 0.5 ml of 1M sodium hydroxide was added to the reaction mixture. The 2.5 ml of reaction mixture diluted with distilled water was mixed well. The absorbance was measuredat 425 nm using a spectrophotometer (Fan Gonga, et al., 2009; Lalit Giri, et al., 2009). Determination was performed in three replicates. A calibration curve was generated using various concentrations of Quercetin (20 - 140µg). Blank consist of all the reagents, except for the extract or Quercetin standard solution is substituted with 0.1 ml of ethanol. Results were expressed as mg of Quercetin equivalent/g of dry weight (mg QE/g) of extracts.

Table 2: Total Flavonoid Content Concentration Quercetin

Sample	Concentration of Extract	Total Flavonoid Content (µg/g)
Ormocarpum Sennoides	Ethanol Extract 1mg/ml	120.6±5.2

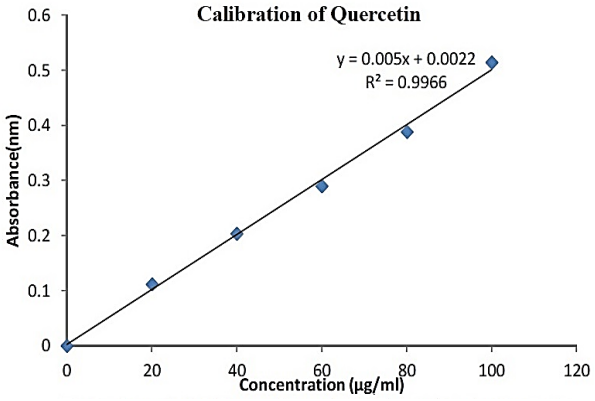

Figure 1: Shows total flavonoid content (Quercetin standard) of *Ormocarpum sennoides* DC

Estimation of Total phenolic content
Reagents required

- Gallic acid (1mg/ml)
- 1N Folin calcteu reagent
- 20% Sodium carbonate solution
- Sample (1mg/ml)

Procedure

Total phenolic content was determined by Folin – Ciocalteu reagent assay (Edwin N. Frankel. 1997; Singleton and Rossi, 1965). The sample (1mg/ml) is mixed with 1ml of the 1N Folin-Ciocalteu reagent. After 3 min, 1ml of the 20% sodium carbonate solution was added. Then the volume was made up to 10ml with distilled water. The mixed solution was allowed to stand for another 90min and the resulting solution was measured at 765nm. Gallic acid (20-100µg/ml) is used as a standard for the calibration curve. The total phenolic content was expressed as µg gallic acid equivalents (GAE) per/mg of sample (µg/mg) , (Atanassova, M et al., 2011).

Table 3: Total Phenolic Content Concentration GAE

Sample	Concentration of Extract	Total Phenolic Content (µg/g)
Ormocarpum Sennoides DC	Ethanol Extract 1mg/ml	265.8+3.0

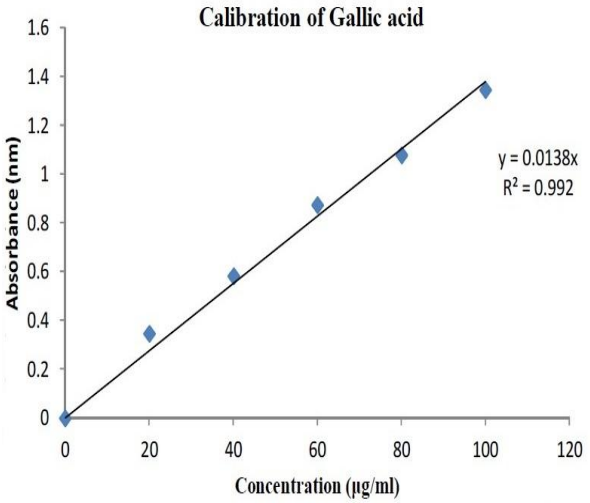

Figure 2: Shows total phenolic content (Gallic acid standard) of *Ormocarpum sennoides* DC

HPLC Analysis

HPLC analysis of ethanolic extract of *Ormocarpum sennoides* carried out with Shimadzu HPLC 2020 (Shimadzu, Japan) equipped with binary solvent delivery system column compartment and photodiode array detector (PDA) was used for analysis. The chromotographic separation was performed on phenomenex C18 column (i.e., 250mmx4.6mm, 5µm) and the column over temperature was set at 30°C. All instrumentation data were collected and synchronized by lab solution software (version7.1) from Shimadzu sample preparation. Sample were dissolved in methanol and filtered through 0.2 micron filter paper and analysed.

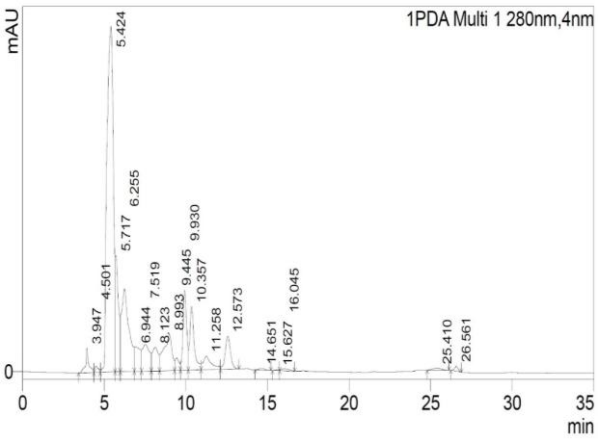

Figure 3: HPLC analysis of *Ormocarpum sennoides* DC

Spectroscopy Analysis

UV Visible Spectroscopy analysis

Ethanol extract of *Ormocarpum sennoides* was analyzed in UV visible range between 200-800nm using UV labs India, 3200 UV double beam spectrophotometer and characteristic peaks were detected. The peak values of UV were recorded, the analysis was repeated twice for spectrum confirmation (Sahaya Sathish, S, et al., 2012).

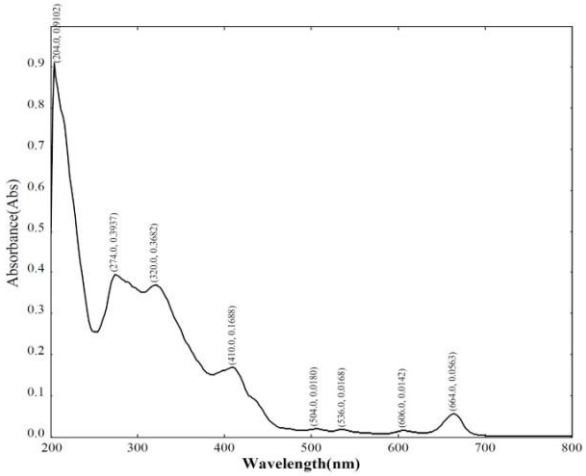

Figure 4: Shows UV Analysis of *Ormocarpum sennoides* DC

IR Spectroscopy analysis

Infrared spectroscopy is based on the principle that chemical substance shows selective absorption in infrared region.

After absorption of Infrared radiations, the molecules vibrate, giving rise to absorption spectrum, it is an excellent method for the quantitative analysis because except optical isomers, the spectrum of compound is unique. The IR spectrum of ethanol extract of *Ormocarpum sennoides* leaves were scanned on FT-IR-8400 over the frequency range from 4000-400 cm^{-1}.

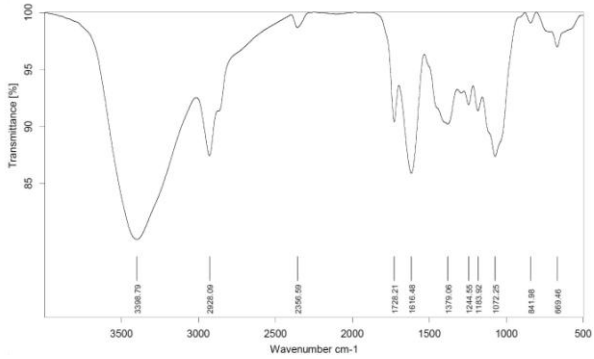

Figure 5: Shows IR Analysis of *Ormocarpum sennoides* DC

RESULT AND DISCUSSION

Phytochemical screening

The preliminary phytochemical screening of the chemical constituents of different solvent from the *Ormocarpum sennoides* plants showed that the leaves generally contain flavonoids, alkaloids, steroid, saponins, tannins, carbohydrates, phenol and glycosides, these constituents may be responsible for antioxidant potentiality of *Ormocarpum sennoides*.

Effect of TPC and flavonoid content

The quantitative determination of the total phenolic content expressed as µg gallic acid equivalent (GAE) per/mg of sample (µ/mg) TPC (Total Phenolic Content)

Table 4: HPLC analysis of ethanol extract of ormocarpum sennoides DC at 254nm

Peak#	Ret. Time	Area	Height	Area %
1	3.947	89816	6609	1.556
2	4.501	28269	1745	0.490
3	5.424	2506108	92840	43.420
4	5.717	423415	31266	7.336
5	6.255	719701	22328	12.469
6	6.944	163676	6580	2.836
7	7.519	223391	7215	3.870
8	8.123	163118	6392	2.826
9	8.993	339276	9711	5.878
10	9.445	58039	3313	1.006
11	9.930	334011	21484	5.787
12	10.357	316699	17069	5.487
13	11.258	129379	3572	2.242
14	12.573	197342	8780	3.419
15	14.651	13378	493	0.232
16	15.627	4690	245	0.081
17	16.045	19772	589	0.343
18	25.410	21297	559	0.369
19	26.561	20372	1430	0.353
Total		5771750	242219	100.00

of ethanol extracts showed the content values of 265.8±3.0 µg/g and total flavonoid content of the extracts was expressed a percentage of Quercetin equivalent/g of dry weight (mg Qe/g) of extracts the total flavonoids estimation of ethanol extracts showed the content values 120.6±5.2 µg/g. The above results showed that ethanol extract contains move tannins and flavonoid content.

Flavonoids are the potent water soluble antioxidants and free radical scavengers, which present oxidative cell damage therefore have strong anticancer activity (Edwin N. Frankel. 1997). Presence of phenolic compounds in the plant indicates that this plant may be used as an antimicrobial agent (Vijayalakshmi, R, et al., 2012).

Ultraviolet visible absorption (UV)

Ethanol extract of *Ormocarpum sennoides* was analyzed in UV visible range between 200-800nm using UV visible spectrophotometric and the characteristic peaks were detected the peak values of UV and IR were recorded the analysis was repeated twice for the spectrum confirmation. The UV visible spectra of ethanol extract was selected at wavelength 400-800nm due to sharpness of the peaks and proper baseline. The profile showed the peaks between 200nm and 400nm with the absorption between 0.17abs and 0.9abs respectively (Figure 1).

Infrared spectroscopy (IR)

Infrared spectroscopy is an analytical technique based on the principle that chemical substance shows selective absorption in infrared region. (Sumitra Chanda et al., 2013).

After absorption of IR radiations, the molecules vibrate, giving rise to absorption spectrum, it is an excellent method for the quantitative analysis because except optical isomers, the spectrum of compound is unique. The IR spectrum of ethanol extract of *Ormocarpum sennoides* leaves were scanned on FR-IR-8400 over the frequency range from 4000 - 400 cm^{-1}. IR spectra of ethanol extract of *Ormocarpum sennoides* is shown in Fig.2. 4000-400 cm^{-1} (2.5-25µm) was used to study the fundamental vibrations and associated vibrational spectrum. Interpretation of ethanol extract are given in Table 1.

HPLC analysis

HPLC analysis of ethanol extract of *Ormocarpum sennoides* at 254nm shows presence of various constituents as evidenced by the chromatogram obtained at various retention time (high is 5.424) are found in the leaves of *Ormocarpum sennoides*.

CONCLUSION

Further exploration to isolate lead compounds from the extracts of *Ormocarpum sennoides* would provide an insight to develop a potent formulation for treatment of bone disease like osteoporosis, fracture healing and strengthening the bone.

CONFLICT OF INTEREST

We have no conflict of interest.

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