ORIGINAL RESEARCH PAPER

Life Sciences

KEY WORDS: *Clerodendrum serratum* (Linn) *Moon*, Ethanolic Extract, HR-LCMS, Pindolol, kynurenine, Hydroxyhydroquinone, etc.

HR-LCMS ANALYSIS AND PASS (PREDICTION OF ACTIVITY SPECTRA FOR SUBSTANCES) OF ETHANOLIC EXTRACT OF *CLERODENDRUM* SERRATUM (LINN.)MOON (BHARANGI).

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ABSTRACT

Aims: The main aim of the study is to prospect the phytochemical constituents in the ***Clerodendrum serratum* (Linn). Moon** by HR-LCMS Analysis and PASS prediction **Methods:** The leaves of the *Clerodedrum serratum (Linn).Moon* was extracted with Ethanol at room temperature for 24 hours. The bioactive compounds of *Clerodendrum serratum* have been separated and identified using HR-LCMS. **Results:** Preliminary phytochemicals analysis revealed the presence of tannins, quinines, saponins, terpenes, flavonoids, steroids, phenolic compounds and carbohydrates. Total 12 compounds identified were selected for further screening by molecular docking studies. The spectral analysis revealed different compounds Pindolol, Umbelliferon, 1 alpha, 25-dihydroxy-26, 27-dimethyl-20, 21, 22, 22, 23, 23-hexadehydro-24a-homovitamin D3, Hydroxyhydroquinone, Phenylacetic acid, Kynurenine, cholic acid glucuronide, Megastigma -3, 7(E), 9 triene , Alloaromadendrene, Ethambutol ,α santalol and many other compounds were identified as low level. **Conclusion:** The result of this study offers a platform of using *Clerodendrum serratum (Linn) Moon*. As herbal alternatives for various diseases and it can be used as **functional and pharmaceutical agent.**

INTRODUCTION

Clerodendrum serratum (Linn). Moon is a large genus belonging to the family Lamiaceae. The plant is distributed over scrub forests throughout the tropical and sub-tropical parts upto 1500 m particularly in Bengal, Orissa and Peninsular India. Various indigenous systems of medicines like Ayurveda, Siddha and Unani has been reported Ethnomedicinal importance of the plant specially syphilis, typhoid, jaundice and hypertension. Traditionally, it has been used as anti-rheumatic, anti-aesthetic, febrifuge, in cephalagia and ophthalmic. The roots of *C. serratum* are also used as anti-oxidant, anti-bacterial, anti-malarial and anti-fungal. Besides these the antimicrobial utility of this herbal plant have also been reported in its stems and leaves. The present study was carried out the bioactive compounds present in the *C. serratum* (Linn) Moon in the ethanolic extract with the aid of HR-LCMS Techniques which may provide an insight in its use of traditional medicines. PASS predicts pharmacological effects and biochemical mechanism on the basis of the structural formula of the substance.

MATERIAL AND METHODS

Plant Materials Collection and Extraction

The leaves of the *Clerodendrum serratum (Linn)* Moon Were collected from the **Kedarguda forest** near Hadgoan, **Dist. Nanded, Maharastra**, India. The collected leaves washed with running water, shade dried, powdered and extracted with 90% Ethanol using soxhlets apparatus for 6 hours. The extracts were filtered and filtrates were dry in drier. It was used for phytochemical screening and further use.

Phytochemical Screening

Phytochemical analysis was carried out for identification of Quinones, flavonoids, alkaloids, tannins, terpenoids, phenol, carbohydrates, proteins, glycosides steroids, phlobatannins and fatty acids according to the standard methods.

Preparation of Extract

The leaves of *Clerodendrum serratum* (Linn).Moon were dried form. 25 g of the powdered leaves were carried out by hot percolation, using soxhlet apparatus. The extract was then filtered through whatmann filter paper no.41 along with the 2 gm sodium sulphate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with sodium sulphate was wetted with 95% ethanol. The extract contained both polar and non-polar phytocomponents of the plant material used.

HR-LCMS Analysis

The crude extract was followed by High Resolution Mass Spectroscopy (HR-LCMS) model for the detection of the compounds. It has 1290 Infinity UHPLC System, Aligent Technologies, 1260 Infinity Nano HPLC with 6650 Funnel, Q-TOF Chip cube. The HR-LCMS Analysis was performed in Sophisticated Analytical Instruments Facility (SAIF), Indian Institute of Technology, Bombay. The results obtained were subjected to PASS.

PASS (Prediction Activity Spectrum for Substances)

The PASS (Prediction Activity Spectrum for Substances) software which predict more than 300 pharmacological effects and biochemical mechanisms on the basis of the structural formula of the substance, may be efficiently used to find new targets (mechanism)for the some legends and, conversely, to reveal new legends for some biological targets. By Prediction, either by selecting structural formula of an organic compounds as a file in the Mol file Format or SMILES Code or by entering the structural formula directly in the web 1.000, structures with Pa greater than Pi were the only compounds considered for particular pharmacological activity.(Jamkhede, *et al 2016).*

RESULTS AND DISCUSSION

Phytochemical Analysis

The phytochemical screening of the extract is presented in the Table 1. The analysis divulged the presence of Flavonoid, Terpenoid, Sterols, Carbohydrates, Tannins, Saponins, Alkaloids and Anthraquinone. The compound present in the ethanolic extract *of Clerodendrum serratum* (Linn) Moon, were identified by HR-LCMS analysis.

Table 1: Phytochemical Constituents present in Ethanolic extracts of *Clerodendrum serratum (Linn) Moon*.

Sr. No	Phytochemicals	Ethanolic Extract
1	Flavonoid	+
2	Terpenoids	+
3	Sterols	+
4	Carbohydrates	+
5	Tannins	+
6	Saponins	+
7	Alkaloids	+
8	Anthraquinone	+

Phytocomponents identified in the Ethanolic Extract of *Clerodendrum serratum* (Linn) Moon.

The compounds present in the Ethanolic extract of *Clerodendrum serratum* (Linn) Moon were identified by HR-LCMS Analysis. (Figure 1) The active principle with their retention time (RT), molecular formula, molecular mass is presented in the Table 2. Twelve compounds were identified in Ethanolic extract by HR-LCMS. The major component present in the *Clerodendrum serratum* (Linn) Moon, (Bharangi) were Pindolol, Kynurenine, cholic acid glucuronide , Ethambutol ,Hydroxyhydroquinone , α Santalol, Hydroxy salmeterol, Umbelliferon , Megastigma – 3,7(E),9 triene , Alloaromadendrene , 1 alpha , 25-dihydroxy – 26,27-dimethyl-20, 21,22,22,23,23-hexadehydro-24a-homovitamin D3 and phenylacetic acid.

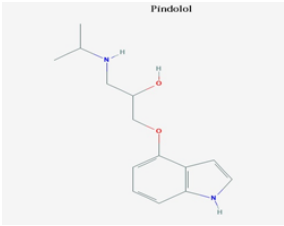

Fig.1 Structure of Pindolol

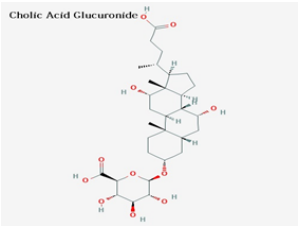

Fig.2 Structure of Cholic acid Glucuronide

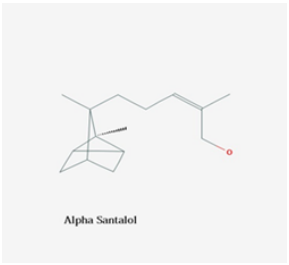

Fig.3 Structure of Alpha Santalol

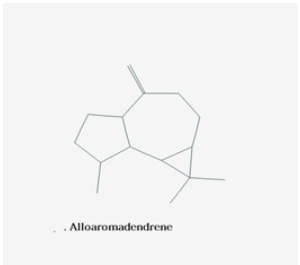

Fig.4 Structure of Alloaromadendrene

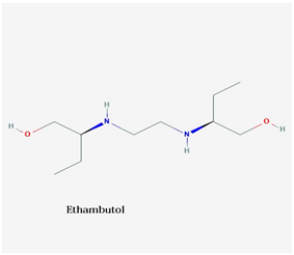

Fig.5 Structure of Ethambutol

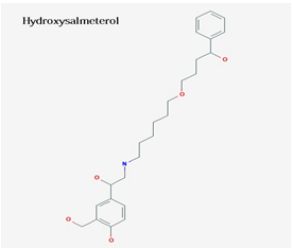

Fig.6 Structure of Hydroxysalmeterol

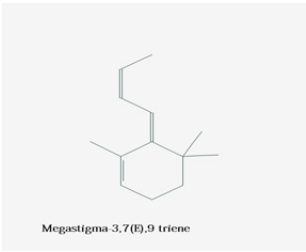

Fig.7 Structure of Megastigma-3, 7(E), 9 triene

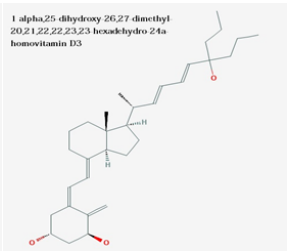

Fig.8 Structure of 1 alpha, 25 dihydroxy 26, 27 diethyl 20, 21, 22,22,23,23 Hexadehydro 24a homovitamin D3

Fig.9 Structure of Kynurenin

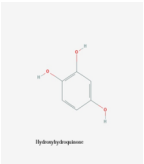

Fig.10 Structure of Hydroxyhydroquinone

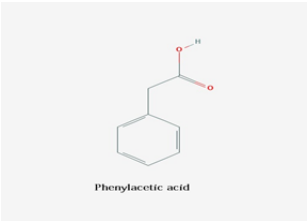

Fig.11 Structure of Phenylacetic acid

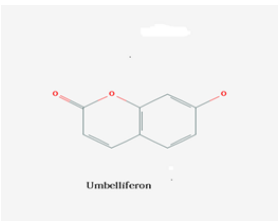

Fig.12 Structure of Umbelliferon

Activity of phyto-components identified in *Clerodendrum serratum* by HR-LCMS –

The Phytocomponent identified in *Clerodendrum serratum* (Bharangi) are responsible for various pharmacological actions like Bronchodilatory activity, anti-inflammatory, antineoplastic, antimetastatic property, bacteriostatic, antimicrobial, antitubercular, antibiotic ,anticancer,memory enhancer , skin cancer prevention, analgesic, fungicide etc. (Table 3). *Clerodendrum serratum* (Linn) Moon (Bharangi) has medicinal value the presence of these vital constituents.

Table 2: Phytocomponents identified in the Ethanolic Extracts of *Clerodendrum serratum (Linn)* Moon.

Sr. No.	RT	Name of the compounds	Molecular formula	Mass
1.	0.902	Pindolol	C14H20N2O2	*248.1491*
2.	5.507	Hydroxysalmeterol	C25H37NO5	431.2715
3.		1 alpha,25-dihydroxy-26,27-dimethyl-20,21,22,22,23,23-hexadehydro-24a-homovitamin D3	C30H44O3	452.335
4.	6.466	Umbelliferon	C9H6O3	162.0312
5.	14.828	Hydroxyhydroquinone	C6H6O3	126.034
6.	8.702	Phenylacetic acid	C8H8O2	136.0548
7.	*7.217*	Kynurenine	*C10H12N2O3*	*208.0867*
8.	9.35	Cholic acid glucuronide	C30H48O11	584.3313
9.	27.009	Ethambutol	C10H24N2O2	204.1851
10.	11.94	Megastigma-3,7 (e),9-triene	*C13H20*	176.1565
11.	7.85	Alloaromadendrene	C15H24	204.1878
12.	10.74	α Santalol	*C15H24O*	220.1827

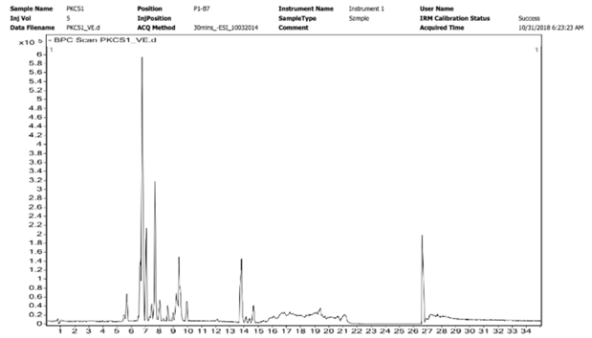

Figure 1: HR-LCMS Analysis of *Clerodendrum serratum* (Linn). Moon of Ethanolic Extract.

The **Pindolol** compound is sesquiterpene in nature.. **Hydroxysalmeterol** phenol in nature. **1 Alpha, 25-dihydroxy-26, 27-dimethyl-20, 21, 22, 22, 23, 23-hexadehydro-24a-homovitamin D3** is sterol in nature. **Umbelliferon** is hydroxycoumarin in nature. **Phenyl acetic acid** is naturally occurring auxin found in vascular plants, it play an important

role in human metabolism. **Kynurenin** is a ketone and acts as human metabolites. It also plays a key role in the process of regulation of immune system**. Cholic acid glucuronide** is steroid in nature and human metabolites. **Ethambutol** acts as an antibiotic and used in the antimicrobial activity. It is bacteriostatic and eliminates certain bacteria that cause tuberculosis (TB). **Megastigma-3, 7 (E), 9-triene** is alkene compound is used as anticancer and antitumor agent. Alloaromadendrene is a sesquiterpene in nature and anti-inflammatory agent. α **Santalol** is used as an analgesic, antibacterial, anti-inflammatory agent and sedative. **Hydroxyhydroquinone** is a novel compound for the antimalarial activity. It is also used as anti-inflammatory, antineoplastic, antimetastastic activity. It is Quinone compounds.

Table NO.3 Nature of the Compounds present in the *Clerodendrum serratum* (Linn) Moon.

Sr. No.	Compound	Nature of the Compound
1.	Pindolol	Sesquiterpene Alcohol
2.	Hydroxysalmeterol	Phenol
3.	1 alpha,25-dihydroxy-26,27-dimethyl- 20,21,22,22,23,23-hexadehydro-24a-homovitamin D3	Sterol
4.	Umbelliferon	Hydroxycoumarin
5.	Hydroxyhydroquinone	Quinone
6.	Phenylacetic acid	Acidic in nature
7.	Kynurenin	Ketone
8.	Cholic acid glucuronide	Steroid
9.	Ethambutol	Antibiotic
10.	Megastigma-3,7 (E),9-triene	Alkene compound
11.	Alloaromadendrene	Sesquiterpene
12.	α Santalol	Sesquiterpene

Table No.4. Activities of phytocomponents identified in *Clerodendrum serratum* (Linn) Moon by PASS.

Compound→ Activity↓	Fig.1	Fig.2	Fig.3	Fig.4	Fig.5	Fig. 6	Fig. 7
Antibacterial	×	✓	×	×	✓	×	✓
Antiinflammatory	×	✓	✓	✓	×	✓	✓
Antiviral	✓	✓	✓	✓	✓	✓	✓
Anticancer	✓	✓	✓	✓	✓	✓	✓
Antifungal	✓	✓	×	✓	✓	✓	✓
Antiseptic	✓	✓	✓	×	✓	✓	×
antioxidant	✓	✓	✓	×	✓	✓	✓
Antidiabetic	✓	✓	×	×	✓	✓	×
Antiprotozoal	✓	✓	✓	✓	×	✓	✓
Antiulcerative	✓	✓	×	✓	✓	×	✓
Anticarcinogenic	✓	✓	✓	×	×	✓	✓
Antileprosy	×	×	×	×	✓	×	✓

Compound→ Activity↓	Fig.8	Fig.9	Fig.10	Fig.11	Fig.12
Antibacterial	✓	✓	✓	✓	✓
Antiinflammatory	✓	✓	✓	✓	✓
Antiviral	✓	✓	✓	✓	✓
Anticancer	✓	✓	✓	✓	✓
Antifungal	✓	✓	✓	✓	✓
Antiseptic	✓	✓	✓	✓	✓
Antioxidant	✓	✓	✓	✓	✓
Antidiabetic	✓	✓	✓	✓	✓
Antiprotozoal	✓	✓	✓	✓	✓
Antiulcerative			✓	✓	
Anticarcinogenic	✓	✓	✓	✓	✓
Antileprosy	✓	✓	✓	✓	✓

Activities of phytocomponents identified in Clerode ndrum serratum (Linn) Moon by PASS

The compound Pindolol has anticancer, antiviral, antifungal, antiseptic, antioxidant, antidiabetic, antiprotozoal, antiulcerative and anticarcinogenic activity**.** Cholic acid Glucuronide has multiutility compounds, it is used as antifungal, anticancer, antiviral, antiseptic, antioxidant, antidiabetic, antiulcerative, anticarcinogenic, antibacterial and anti-inflammatory. Alpha santalol is also used in different activities such as antiinflammatory, antiviral, anticancer, antioxidant, antiseptic, antiprotozoal and anticarcinogenic in functions. The compound alloaromadendrene shows anti-inflammatory, antiviral, anticancer, antifungal, antiulcerative and antiprotozoal activities. The compound Ethambutol play an important role in antibacterial, antiviral, anticancer, antifungal, antiseptic, antidiabetic, antioxidant, antiulcerative and antileprosy. Hydroxysalmeterol has different activities such as antiinflammatory, antifungal,anticancer, antiviral, antiseptic, antioxidant, antidiabetic and anticarcinogenic.The compound Megastigma 3,7 (E) 9 triene shows antibacterial, anti-inflammatory, antiviral, anticancer, antifungal, antioxidant, antiprotozoal, antiulcerative, anticarcinogenic and antileprosy. The compound 1 alpha,25-dihydroxy, 26,27 dimethyl-20,21,22,22,23,23 hexadehydro 24a homovitamin D3 is Sterol in nature, it has antibacterial, anti-inflammatory, anticancer, antiviral, antifungal, antioxidant, antidiabetic, antiprotozoal and anticarcinogenic. Kynurenine shows all activies except antidiabetic and antiulcerative. **Hydroxyhydroquinone** is one of the compound shows all the activities. Phenyl acetic acid shows anticancer, anti-inflammatory, antiviral, antiseptic, antidiabetic, antiprotozoal and anticarcinogenic in nature. Umbelliferon shows different activities such as antibacterial, anti-inflammatory, antiviral, anticancer, antiseptic, antidiabetic, antiprotozoal and antioxidant.

DISCUSSION

The present work has been performed to prospect the various Phytochemicals, HR-LCMS and PASS parameters which could serve as important and has commercial interest in both research institutes and Pharmaceuticals companies for the manufacturing of the innovative drugs. This primary information will facilitate in conducting further studies on discovery of bioactive constituents, resolve of their efficacy by *in vivo* studies and demonstration of their safety and efficacy in clinical trials.

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Conflict Of Interest

There are no conflicts of interest.

Abbreviation Used

HR-LCMS: High Resolution Mass Spectroscopy; PASS: Prediction Activity Spectrum for Substances.

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