PHYTOCHEMICAL PROPERTIES OF CONVENTIONAL AQUEOUS EXTRACT AND MICROWAVE ASSISTED EXTRACT OF *CESTRUM NECTURM* LEAVES

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Cestrum nocturnum Linn. belongs to Solanaceae. It is commonly known as Raat rani, lady of night or night Jessamine have great medicinal value. The plant was extracted using conventional extraction as well as microwave-assisted extraction. The phytochemicals such as carbohydrate, proteins, amino acid, glycoside, phenolic compounds and tannins have been qualitatively determined. The physicochemical properties such as relative density, viscosity, surface tension and refractive index were determined. All features of microwave assisted extract were found to be higher than aqueous extract. UV-Vis and IR data were evaluated. The extracts were screened for biological activity and aqueous extract is found to be active against *E. Coli, B. Subtilis, S. Typhi, S. Aureus, tuberculosis and malaria*

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Introduction

Plants are the vital source to combat the severe diseases in the world.[1] World health organization (WHO) reported that the more than 80 % of the world population used the remedies based on plants for their primary health care need.[2,3] The plants are the source for the new drugs, in which the majority are still unexplored. Among the 25 000 000 to 50 000 000 plant species - several percentages of the plants are investigated for their phytochemical and biological screening.[4] India is known for the thousands of species for its medicinal value and the use of the different parts of the plant to cure specific alignment.[5]

Cestrum nocturnum Linn. belongs to Solanaceae. It is commonly known as Raat rani, lady of night or night Jessamine.[6] It contains simple glossy leaves, vine-like stems, greenish-creamy white tubular flowers. The species name 'nocturnum' refers to the species which have the habit of opening its small, heavily-scented flowers at night.[7,8] Hemant Kumar Nagar et al. reported the wound healing activity of *Cestrum nocturnum* (L.) ointment,[9] antidiabetic[10] and antibacterial[11,12] activities.

The present study is done for the investigation of the phytochemicals are occurred in *Cestrum nocturnum* and to determine the physical parameters of its aqueous-extract by different methods. The comparative study was done for conventional extraction (CE) and microwave-assisted extraction (MAE).

Experimental

The plant leaves were collected from the nearby field of Aurangabad city. The leaves were washed gently and dried under shade and were ground. The extraction and analytical methods of phytochemical and physico-chemical parameters like relative density, viscosity, surface tension and refractive index along with phytochemical qualitative tests were carried out as described in our earlier reports.[13-16] The UV-visible spectra were recorded in the range from 190 to 800 nm by using double beam spectrophotometer of Model Elico-159 and λmax values were determined. The FT-IR instrument IRT3000 (JASCO) used to get IR spectra. Antibacterial activity is investigated by cup plate method in which 70 µL of standard test solution was added in each cups or wels and these cups were prepared by using sterile metal borer. The media used was sterile nutrient agar and sterilization was performed in autoclave at 121 ^{0}C for 20 min. For the present study Streptomycine is used as a standard against bacterial culture.

Results and discussion

The leaves of *Cestrum nocturnum* were analyzed to determine their phytochemical, physicochemical and biological properties.

The powder sample is treated with different chemicals and changes in color were registered. The results are shown in Table 1. The solutions gave different colors, for example, concentrated hydrochloric acid gave red while 1 M hydrochloric acid gave creamy color. It can be attributed due to differences in reactivity and color changes according to the pH (indicator property) of different materials are present. The ash content showed the presence of inorganic compounds. The total ash content in the leaves of *Cestrum nocturnum* was found to be 13.4 %. The water-soluble part of the ash was found to be 50 %, while 22 % of the ash does not dissolve even in 1 M hydrochloric acid either.

The summarized properties of the ash made from leaves of *Cestrum nocturnum* are shown in Table 2.

Table 1. Fluorescent test for the leave powder.

Sr. No.	Solutions	Observation
1	The powder as such (P)	Dark green
2	P + n-butanol	Whitish green
3	P + conc. HCl	Red
4	P + conc. HNO_3	Dark orange
5	P + conc. H_2SO_4	Blackish brown
6	P + Ethanol	Whitish green
7	P + Ammonia	Cream
8	P + Glacial acetic acid	Fluorescent green
9	P + 1N HCl	Cream
10	P +1N NaOH	Yellowish green
11	P + 5% HCl	Cream
12	P + 5% NaOH	Yellowish
13	P + benzene	Fluorescent green

Table 2. Ash analysis and densities of leave powder

Sr. No.	Ash	Result
1.	Total ash	13.4 %
2.	Water soluble	50 %
3.	Acid-insoluble	22 %
4.	Bulk density	0.3885 g mL^{-1}
5.	Tab density	0.5018 g mL^{-1}
6.	Housner ratio	1.2916
7.	Carr's index	22.58 %

The leaves of *Cestrum nocturnum* was extracted using the same solvent but different techniques. The difference between conventional extraction (CE) and microwave-assisted extraction (MAE) was registered. The percentages of both extractions were almost the same, but the MAE is more convenient because it gives nearly about the same percentage within half an hour only. The results are shown in Table 3.

Table 3. Extractive value of Cestrum nocturnum leaves

Sr. No.	Solvent	Percentage
1.	Conventional hydrodistillation	24.79
2.	Microwave-assisted hydrodistillation	24.52

Physicochemical properties like density, viscosity, surface tension and refractive index were measured for different concentration of the leave extracts of *Cestrum nocturnum* (Table 4 and 5).

The phytochemical analysis of the plant leaves of both techniques gives the same results (Table 6). This proves that there is no breaking of the compound takes place due to high radiation is the microwave or no breaking takes place due to constant heat in conventional distillation. The test is showing a positive result for alkaloids, carbohydrate, glycoside, saponins, proteins or amino acid phenolic compounds or tannins.

Table 4. Physicochemical properties of CE of leave extract.

Sr. No.	Concentration, ppm	Relative density	Viscosity, Pa s	Surface tension, N m^{-1}	Refractive index
1.	5	0.99758	0.8338	74.8828	0.99970
2.	10	0.99859	0.8346	77.9530	0.99871
3.	20	0.99677	0.8331	74.8230	1.00070
4.	40	0.99677	0.8331	77.8131	1.00070
5.	60	0.99720	0.8335	77.8473	1.00029
6.	80	0.99697	0.8333	74.8380	1.00022
7.	100	0.99556	0.8321	74.7326	1.00176

Table 5. Physicochemical properties of MAE of leave extract.

Sr. No.	Concentration, ppm	Relative density	Viscosity, Pa s	Surface tension, N m^{-1}	Refractive index
1.	5	0.9986	0.8371	50.1188	0.9989
2.	10	1.0037	0.8762	51.6972	0.9936
3.	20	1.0053	0.8779	54.6630	0.9923
4.	40	1.0033	0.8762	54.5551	0.9943
5.	60	1.0054	0.8779	54.6684	0.9922
6.	80	1.0000	0.8733	54.3770	0.9976
7.	100	0.9979	0.8366	59.1979	0.9996

Table 6. Phytochemical analysis of the leave extract of *Cestrum nocuturnum*

Sr. No.	Reagent	CE	MAE
1.	Detection of Alkaloids		
A.	Mayer's test	-ve	-ve
B.	Wagner's test	+ve	+ve
C.	Hager's test	+ve	+ve
2.	Detection of carbohydrate		
A.	Molish test	+ve	+ve
B.	Fehling's test	+ve	+ve
C.	Benedic test	-ve	-ve
D.	Barfoad's test	+ve	+ve
3.	Detection of Glycosides		
A.	Borntrager's test	-ve	-ve
B.	Legal's test	-ve	-ve
4.	Saponins	+ve	+ve
5.	Detection of proteins and amino acid		
A.	Millon's test	+ve	-ve
B.	Nitric acid test	-ve	+ve
C.	Biuret test	+ve	+ve
D.	Ninhydrine test	-ve	-ve
6.	Detection of phenolic compound and tannins		
A.	Ferric chloride test	-ve	-ve
B.	Gelatin test	+ve	+ve
C.	Lead acetate test	+ve	+ve
D.	Alkaline reagent test	+ve	+ve

Spectroscopic results

The IR spectrum of an extract of *Cestrum nocturnum* was also recorded. (ESI Fig.1, Table 7)

Table 7. IR bands of *Cestrum necturnum* plant leaves extract

Band (cm^{-1})	Intensity	Functional group
3329	Very broad	OH (NH) bonds
2942	Sharp	Aliphatic C-H bonds
1609	Sharp	δ(OH)
1417	Broad	C-H bending vibrations
1075	Broad	C-O-C asymmetrical stretching
819	Broad	C-H bands
776	Broad	C-H bands
623	Very broad	aromatic hydrocarbon bands

Though it contains a mixture of compounds but still in order to find out various functional groups and a general fingerprint of samples. The IR bands observed are shown in Table 7. UV spectra of the solutions at ~50 ppm concentration were recorded for both extracts with water as a reference. The spectral data are shown in ESI Fig 2 and Fig 3.

The λ_{max} values were found to be 197 and 248 nm for conventional aqueous extract and microwave assisted aqueous extract, respectively.

Anti-bacterial activity

Both the conventional extraction and microwave-assisted extraction products were tested against E. coli, B. Subtilis and S. Aureus, but antimicrobial activity was found only in the case of CE extract (Table 8). Unfortunately, it does not show any activity against tuberculosis and malaria, while the microwave assisted extract does not show any activity against the listed bacteria trains and tuberculosis or malaria. This may be due to the degradation of compounds which are responsible for the antimicrobial effect.

Table 8. Antibacterial, antituberculosis and antimalarial properties of leaves extract

Sr. No.	Micro-organism	Dead zone diameter, mm		
		MAE	CE	Standard drug
1.	*E. coli*	0	18	12
2.	*B. Subtilis*	0	18	12
3.	*S. Typhi*	0	10	12
4.	*S. Aureus*	0	18	12
5.	T. B	0	0	12
6.	Malaria	0	0	12

Conclusions

The present study revealed that microwave extraction gives the same percent of extraction as the conventional extraction, but the latter is more time-consuming. Both the extract provides the same result for the phytochemicals, including alkaloids, carbohydrates, glycosides, saponins, proteins amino acids, phenolic compounds or tannins content. The conventional extracts showed antibacterial activity against *E. coli*, *B. Subtilis* and *S. Aureus*, while the microwave-assisted extract has no antimicrobial effect due to the degradation of biologically active components.

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