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Phytochemical evaluation of Celastrus paniculatus seed oil extracted by a method used by 'Uraanv' tribe of Chhattisgarh

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ABSTRACT

More than 35,000 plant species are being used in various human cultures around the world for medicinal purposes. Medicated oil plays an important role for prevention and cure of diseases in Ayurveda. Celastrus paniculatus seed oil is such wonderful medicinally useful oil. It is commonly known as Black-Oil tree, Intellect tree, Climbing-staff plant and Jyotishmati and "Tree of life" in Ayurveda. It possesses Anti-depressant, Anti-Parkinson, Anti-Alzheimer's, Neuroprotective, IQ improving activity. In tribal district (Jashpur) of Chhattisgarh, Indian people uses traditional method to extract C. paniculatus oil. It is commonly used by the tribes for the treatment of various diseases like headache, muscular spasm and local inflammations. The present study aim is documentation of traditional method of oil extraction used by 'Uraanv' tribe. This paper also dealt with the Physicochemical and qualitative phytochemical evaluation of C. paniculatus seed oil. Oil yield by traditional method was 25%. Carbohydrate, reducing sugar, monosaccharide, protein, amino acid, steroid, flavonoid, alkaloid, fixed oil, phytosterols, saponin, diterpenoid and cardiac glycoside were found in the oil sample. The GC-MS analysis shows the presence of 47 compounds in the oil. These phytochemical might be responsible for the therapeutic effect of C. paniculatsus seed oil.

Key words: Ayurveda, Celastrus paniculatus, Jyotishmati.

INTRODUCTION

Today Ayurveda is recognized worldwide as a system of medicine that provides calm mind with healthy body. This traditional system of medicine of India has an un-enviable position in the field of providing remedies for the ailments, as it provides satisfactory

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answers to all the problems the world is facing today. Nearly 80% of the world populations rely on traditional medicines for primary health. Over the past few decades, the traditional knowledge on the use of medicinally important plant has been widely acknowledged and valued across the world.^[1]

Some medicinal plants have a genuine balancing effect on the complex nervous and mental systems and prevent disorders and unbalanced mental conditions. Enhancing that vital tone is the most urgent need of many persons who suffer from nervous fatigue, asthenia or stress. Ayurveda has quite a sophisticated classification of medicinal plants, as per the dominant pharmacological/ therapeutic activity on mental functions etc. Many interesting leads have emerged on CNS-active medicinal plants. Celastrus paniculatus Willd. (Family - Celastraceae) is wild woody liane, climbing shrub commonly known as

Black-Oil tree, Intellect tree, Climbing-staff plant and *Jyotishmati* and "Tree of life" in Ayurveda. *C. paniculatus* oil performs function of stimulation of *Agni* and nourishment of brain. Thus *Jyotishmati* stimulates the intellect and sharpens the memory by increasing the grasping capacity and nourishes the *Medha*. Hence it is used in the psychiatry disorders.^{[2],[3]} Its oil is used as pomade and also as rubefacient for relieving rheumatic pains of a malicious character and in paralysis. Its black coloured oil is called 'Oleumnigrum'. Oleumnigrum has been tried in beriberi with some benefit.^[4]

Oils aren't get directly and so easily from these sources. They must be extracted in a process having particular purity factors so that they remain usable. Several extraction processes evolved over time and the main two of them are mechanical and chemical extraction. There are several remote areas in Chhattisgarh state of India; especially Jashpur district where people have their own wisdom of medicinal plants growing in the nearby forests, and they specifically use these plants for preparing the extracts and formulations in a very cryptic and traditional ways. Most of it is scientifically unexplored and awaits validation. They extract oil from C. paniculatus Seed. There its local name is "Oonjun" and "Kujuri". Celastrus paniculatus oil is commonly used by the tribes for the treatment of various diseases like headache, muscular spasm and local inflammations.

AIMS AND OBJECTIVES

- Traditional method for extracting oils from seeds by the "Uraanv" community in Jashpur district of Chhattisgarh, India.
- 2. Phytochemical evaluation of *Celastrus paniculatus* seed oil.

MATERIALS AND METHODS

There are many types of oil mentioned in classic of Ayurveda, which has medicinal uses. Two classical method of oil extraction from *Celastrus paniculatus* is known so far:

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- 1. Extraction of oil from the seed of *C. paniculatus* by using classical *Patala Yantra*.^{[5],[6]}
- 2. By crushing of seeds of *C. paniculatus* through Expeller Machine.^[5]

In Chhattisgarh state of India another mixed type of traditional method is used to extract oil from *C. paniculatus* seed. In which firstly seeds are crushed and there after using water steam they loses the integrity of cell wall and finally compress by wooden *"Tirhi"* to obtain the oil, which is yet not documented by any scholar.

Apparatus used in the traditional method of oil extractions are following:

A. DHEKI

'Dheki' have generally fallen into disuse because of the availability of technologies that require much less physical labour. In earlier times a *Dheki* was an important part of village life in villages of India. Husking machines and automatic rice mills are now used in the country but *Dhekis* are still in use in large numbers in tribal villages. But In the Jashpur district of Chhattisgarh "Uraanv" tribe still uses this apparatus to crush Jyotishmati seed to extract oil.

Dheki consists of a wooden lever, usually about 72 inches long and 6 inches in diameter. It moves on a small bolt passing through it and two cheeks, which are driven into the ground, until the bolt is about 18 inches high. One end of the lever is fastened a cylindrical piece of wood, about 18 inches in length and 6 inches in diameter, the lower end of which is surrounded by an iron hoop. This serves as a pestle, that is raised by the lever, and that falls down by its own weight. The power is increased by the bolt that serves as a fulcrum. It is placed at five-eighths of the whole length of the lever from the pestle. Usually, 2-3 women work with this device; 1-2 alternatively press down the end of the lever with their feet to raise the pestle, and then by removing their feet allow the pestle to fall. One of them removes the beaten grain, and puts a fresh supply into the device which just is a circular hollow in the ground with a piece of wood in the bottom to receive the blow.

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Figure 1: Dheki

B. TIRHI

Tirhi is a manual apparatus used to extract oil from different seeds. It is completely made of wood. Two parallel logs of wood temporarily joined at one end by iron ring. Upper log of wood is 2.3m and lower log is 2m in length. Its width is 22cm. The *Pottali* is kept between these two logs of wood. At the base of *Pottali* a wooden plate is kept. Length of this wooden plate is 54cm. This plate has an apex and a base. Base is 23cm wide is apex is 12cm wide. There is a circular furrow in the centre, which has diameter of 14cm. Total thickness of this plate is 6cm. When the two logs of wood are pressed towards each other, extracted oil flows through the furrow made on the wooden plate and are collected in the container.



Figure 2: Tirhi apparatus



Figure 3: Procedure of Oil extraction by traditional method (a) Crushing of seed, b) crushed seeds, c) Crushed seed tied in Cotton cloth, d) Steaming, e) Steamed seeds Filling in *Pottali*, f) Pressing by *Tirhi*)

Procedure of Oil extraction by traditional method

First of all the dried fruits of *C. paniculatus* were crushed with the help of *Dheki* till the seeds separated. After that separated seeds are kept in *Pottali* made of cotton cloth. Then this *Pottali* is kept for steaming in an earthen pot kept over water filled Tin container. The process of steaming was carried out for 45 minutes. After that steamed seeds are filled in two *Pottali*. Both the *Pottali* are kept in *'Tirhi'* apparatus between the two wooden logs over the wooden plate, for oil extraction. After that two logs of wood were compressed towards each other with the help of another log. After compression oil flows through the furrow and collected in the container.



Figure 4: Wooden plate to be placed between two logs in Tirhi apparatus

C. POTTALI

Pottali is made from the fibre of *Bauhinia vahlii* plant. It diameter is 13cm and height is 6cm.



Figure 5: Pottali

Analysis of C. paniculatus seed oil

Analysis of *C. paniculatus* seed oil was performed on these parameters: Physicochemical analysis, Preliminary phytochemical analysis, Quantitative phytochemical analysis by GC-MS, Quantitative analysis of Inorganic elements in oil, Development of TLC.

Quantitative Analysis of phytochemical by GC-MS^[7]

Analysis of Essential Oil is done by using Gas Chromatography with Mass Spectrometer. The qualitative and quantitative analysis is done to know

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the constituents in the oil and the percentage of components present in the oil respectively, by doing so we can know the purity of that particular oil.

Gas Chromatography-Mass Spectrometry (GC-MS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. Applications of GC-MS include drug detection, fire investigation, environmental analysis, explosives investigation and identification of unknown samples. Additionally, it can identify trace elements in materials that were previously thought to have disintegrated beyond identification.

Method

Instrument Used	Thermo Scientific GC Trace 1310 Equipped with Thermo Scientific MS TSQ 8000
Method type	Acquisition - General
MS transfer line temperature	300°C.
lon source temperature	230°C
Ionization mode	EI
Temperature Program	Initial 60°C hold for 2 mins Ramp at 10°C to 240°C Ramp at 10°C to 300°C, hold for 2 mins
Flow Rate	1 ml/min
Carrier Gas	Helium
Column Used	Agilent DB 5MS (30 meter X 0.25 mm)

Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.

Qualitative analysis of Inorganic elements

Prepare ash of drug material. Add 50% v/v HCl or 50% v/v HNO₃ to ash. Keep for 1hour or longer.

Development of TLC

Materials: Capillary tubes, Sprit lamp, TLC Chamber, Aluminium sheet, Silica gel F254.

Extract: Alcoholic Extract of drug Sample

Stationary Phase: Aluminium sheet Silica gel F254 (Merck KGaA Company)

Mobile Phase: Benzene: Chloroform: Ethyl acetate (8:1:1)

TLC Method

The tank was prepared by lining the wall with sheets of filter paper, sufficient amount of mobile phase was poured into the tank to form a layer of solvent 5 to 10 mm deep, and the tank was closed and allowed to stand for 1 hour at room temperature. From the vertical sides of the plate, a narrow strip of the coating substance about 5 mm wide was removed. The solutions being examined was applied in the form of circular spots about 2 to 6 mm in diameter on a line parallel with, and 20 mm from, one end of the plate, and not nearer than 20 mm to the sides; the spots were 15 mm apart. The sides of the plate were marked 15 cm from the starting line. The solvent was allowed to evaporate and the plate was placed in the tank, ensuring that it was as nearly vertical as possible and that the spots were above the level of the mobile phase. The tank was closed and allowed to stand at room temperature, until the mobile phase has ascended to the marked line. The plate was removed and dried and visualized as directed in the monograph.

RESULTS AND DISCUSSION

In this Traditional method 4 kg seeds of *Jyotishmati* yielded 1 litre of oil, which is 25%.

Organoleptic characters

Odour, Taste, Touch and Color of the oil are respectively Unpleasant, Bitter, Smooth and Light to Dark brown.

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Physicochemical Analysis of oil

Table 1: Physicochemical characters of Celastruspaniculatus seed oil

SN	Physicochemical parameters	Result
1.	Colour	Brown
2.	Refractive Index at 25º C	1.4711
3.	Weight/ml	0.956
4.	Specific gravity	0.944
5.	lodine Value	13.66
6.	Saponification Value	189.33
7.	Acid Value	1.122
8.	Peroxide Value	0.4
9.	Rancidity Test	Negative
10.	Mineral oil	Absent

Phytochemical screening tests for alcoholic extracts of *Celastrus paniculatus* seed oil showed the active phytochemical classes as cardiac glycosides, alkaloids, reducing sugars, phenols and flavonoids as presented in Table 2.

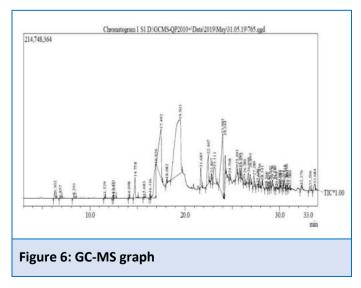
Table 2: Phytochemical screening C. paniculatus seed oil

SN	Parameters	Sample I
1.	Carbohydrtae	Positive
2.	Reducing sugar	Positive
3.	Monosaccharides	Positive
4.	Pentose sugar	Negative
5.	Hexose sugar	Negative
6.	Non reducing sugar	Negative

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7.	Proteins	Positive
8.	Amino acids	Positive
9.	Steroids	Positive
10.	Flavonoids	Positive
11.	Alkaloids	Positive
12.	Tannins	Negative
13.	Terpenoids	Positive
14.	Volatile oil	Negative
15.	Fixed oil	Positive
16.	Phytosterols	Positive
17.	Phenols	Negative
18.	Diterpenoids	Positive
19.	Cardiac glycosides	Positive
20.	Anthraquinone glycosides	Negative
21.	Cumarin glycosides	Negative
22.	Saponin glycosides	Positive

Results of GC-MS Analysis



The graph generated by Gas chromatography shows the composition of the oil and the other graph shown

by Mass Spectrometer gives the percentage of each component. Some of the graphs obtained after analysis are shown below in Figure No. 6.The gas chromatogram shows the presence of 47 peaks in this oil, which means this oil sample have compounds.Chemical constituents of CP oil revealed in GC-MS are shown in Table No. 3.

Table 3: Chemical constituents of CP oil revealed inGC-MS

SN	Peak name	Retention Time (min. sec)	% Peak area
1.	1,2,3-Propanetriol, monoacetate	6.302	0.58
2.	1,2,3-Propanetriol, 1-acetate	6.857	0.07
3.	1,2,3-Propanetriol, monoacetate	8.291	0.25
4.	Benzene, 1-(1,5-dimethyl-4- hexenyl)-4-methyl-	11.529	0.09
5.	Dodecanoic acid	12.421	0.30
6.	Benzeneacetaldehyde, 2- methoxyalpha.,5- dimethyl-	12.513	0.10
7.	Naphthalene, 1,2,3,4- tetrahydro-5-nitro-	14.098	0.07
8.	Tetradecanoic acid	14.758	2.34
9.	Pentadecanoic acid	15.683	0.09
10.	Hexadecanoic acid, methyl ester	16.316	0.19
11.	l-(+)-Ascorbic acid 2,6- dihexadecanoate	16.936	6.83
12.	n-Hexadecanoic acid	17.492	13.77
13.	9-Octadecenoic acid, methyl ester, (E)-	18.082	0.32
14.	cis-9-Hexadecenal	19.503	35.53
15.	Hexadecanoic acid, 2- hydroxy-1- (hydroxymethyl) ethyl ester	21.685	1.62
16.	2-(Palmitoyloxy)-1- [(palmitoyloxy)methyl] ethyl	22.467	3.95

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	icosanoate		
17.	5(4H)-Oxazolone, 4- [[4- (dimethylamino) phenyl] methylene] -2-phenyl-	22.807	0.62
18.	Propyleneglycolmonoleate	23.113	3.63
19.	1,6,10,14,18,22- Tetracosahexaen-3-ol, 2,6,10,15,19,23- hexamethyl- , (all-E)-	23.985	12.63
20.	Pregn-4-ene-3,20-dione, 11- hydroxy-,	24.244	1.98
21.	Pregn-4-ene-3,20-dione, 11- hydroxy-, (11.alpha.)-	24.708	0.46
22.	1,5-Pentanedione, 3-(1- methylethyl)-2-(2-methyl- 1- propenyl)-1,5-diphenyl-	25.491	1.19
23.	3,4,5-Tribenzoyl-2-methyl-2- phenyltetrahydrofuran	25.795	0.87
24.	N-Methylbis(5- methylfurfuryl)amine	25.972	0.85
25.	D-Glucitol, cyclic 3,4- (phenylboronate) 1,2,5,6- tetrabenzoate	26.286	1.16
26.	2-Benzoyl-3,4-diacetyl-d- galactosan	26.699	1.23
27.	2-Benzoyl-3,4-diacetyl-d- galactosan	26.895	1.17
28.	2-Benzoyl-3,4-diacetyl-d- galactosan	27.289	1.19
29.	Ethanone, 1-[1-(furan-2- carbonyl)-3,5-dimethyl -1H- pyrazol-4-yl]-	27.689	0.40
30.	Maymyrsine	27.856	0.64
31.	Ethanone, 1-[1-(furan-2- carbonyl)-3,5-dimethyl-1-H- pyrazol-4-yl]-	28.141	0.32
32.	1,2,3,4-Butanetetrol, 2,3- diacetate 1,4-dibenzoate, (R*,S*)-	28.566	0.24
33.	Ethanedione, di-2-furanyl-	28.829	0.07

34.	.gammaSitosterol	29.002	0.61
35.	Ethanedione, di-2-furanyl-	29.226	0.46
36.	2-Furoic acid, 4-nitrophenyl ester	29.480	0.12
37.	12-Oleanen-3-yl acetate, (3.alpha.) –	29.659	0.16
38.	4,4,6a,6b,8a,11,12,14b- Octamethyl- 1,4,4a,5,6,6a,6b,7,8, 8a,9,10,11,12,12a,14,14a,14 b-oc	29.983	0.14
39.	Cyclopenta[d]anthracene- 8,11-diol, 3-isopropyl- 1,2,3,3a,4,5,6,6a,7,12- decahydro-, diben	30.085	0.08
40.	2-Furoic acid, 2,6- dimethylnon-1-en-3-yn- 5-yl ester	30.334	0.19
41.	2(1H)-Pyridone, 6- (acetoxymethyl)-5- (benzoyloxy)-5,6-dihydro-	30.518	0.78
42.	2-Furancarboxylic acid, 2- isopropoxyphenyl ester	30.706	0.29
43.	.betaSitosterol acetate	30.809	0.31
44.	Stigmast-4-en-3-one	31.060	0.28
45.	Cyclopenta[d]anthracene- 8,11-diol, 3-isopropyl- 1,2,3,3a,4,5,6,6a,7,12- decahydro-, diben	32.276	0.33
46.	2H-3,9a-Methano-1- benzoxepin-4,5,6,7,9,10- hexol, 5a- [(acetyloxy)methyl]octahydr o-2,2	33.206	0.57
47.	3-Benzoyl-2-t-butyl-4- methyl-oxazolidin-5-one	33.684	0.91

Qualitative analysis for inorganic elements

Result of Qualitative analysis for inorganicelements in two Samples of *Celastrus paniculatus* seed oil is shown in Table No.4.

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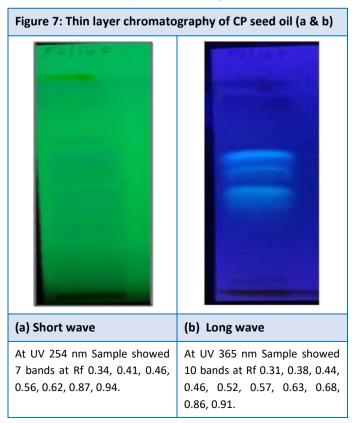
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Table 4: Analysis for inorganicelements of CP seed oil

SN	Elements	Sample I
1.	Calcium	Negative
2.	Magnesium	Negative
3.	Potassium	Positive
4.	Iron	Positive
5.	Sulphate	Negative
6.	Phosphate	Positive
7.	Chloride	Positive
8.	Carbonates	Negative
9.	Nitrates	Negative
10.	Sodium	Positive

TLC

Thin Layer Chromatography of alcoholic extract of the *C.paniculatus*seed oil extracted by traditional method and mechanical method were developed with the solvent, Benzene:Chloroform: Ethyl acetate (8:1:1 v/v)and results are presented in figure 7.



CONCLUSION

Ayurveda, a holistic health care system prescribes usage of different medicated oils for application on the body, with or without massage for providing health benefits and to treat specific indications. Medicated oils prepared using process as mentioned in Ayurveda are used for external and internal administrations to treat various disorders. Traditional knowledge is the base of treatments. Method followed by 'Uraanv' tribes of Chhattisgarh state of India is very unique. So this paper will be helpful for the documentation and conservation of this traditional system of oil extraction.Oil yield by traditional method was 25%. Carbohydrate, reducing sugar, monosaccharide, protein, aminoacid, steroid, flavonoid, alkaloid, fixed oil, phytosterols, saponin, diterpenoid and cardiac glycoside were found in the oil sample. These phytochemical might be responsible for the therapeutic effect of C. paniculatsus seed oil. This study has revealed the chemical composition of Celastrus paniculatus oil, can serve as standard reference for identification and initial step for further studies like absorption study and so on. It can be concluded that the bio-analytical approaches used in the present study is useful in the quality control of Celastrus paniculatus oil. As Celastrus paniculatus oil contains a wide range of phytochemical components, it is needed to validate its therapeutic utility through preclinical and clinical studies.

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