Comparative Study of *Madhuca Longifolia* Flower Extract Between Northwest Corner and Eastern Part of Maharashtra

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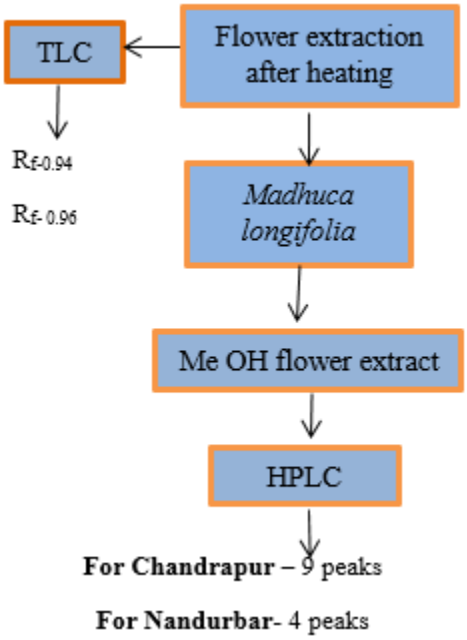
Abstract- ***The species of Madhuca longifolia scientifically classified into family sapotaceae and kingdom Plantae. The Mahua is the most highly valued tree among tribal communities, and every part is utilized in central India. It is known by different vernacular names such as Mahua, Mohwa in Marathi, and Mahuda in Gujarati. The flowers are fermented to make country wine, which is also distilled to make liquor. Tribal use indigenous technology for fermenting mahua flowers to make quality fermented beverages. About 5-6 bottles of liquor can be 2-3kg of dried flowers by mixing and heating palm sugar, urea, and water with the flower. Mahua liquor is grouped under the category of country of country liquor, and hence the Government of India has imposed legal restriction on transaction of mahua flowers away from their area of production. In Madhya Pradesh, mahua liquor is substantial revenue-earner, for the state and for tribal a like. Nearly 70%-80% of mahua flower collected from the forest by tribal people are used for the production of liquor. It is a very popular a alcoholi beverage in the tribal regions of India. To explore the bioactive components present if flower extract of Madhuca Longifolia using TLC, HPLC. Decoction is the process that involves continuous hot extraction using specified volume of water as a solvent, done on dried flower Madhuca longifolia. The dried flower extracted with methanol the filtered and used for HPLC and for TLC hot water extraction filtered and analyzed. This analysis resulted as, in TLC the fluorescent band (under 254 nm) for sample Northwest corner (Nandurbar) R_F 0.94 and Eastern part (Chandrapur) sample R_F 0.96 in mobile phase chloroform: Ethyl acetate: water (10:15:5). In HPLC 20µl of both extract get injected into the HPLC column at 280nm sample of Nandurbar shows four peaks and sample Chandrapur shows nine peaks. The results of the present study enhance the traditional use of Madhuca longifolia which possesses several known and unknown bioactive compounds, in which some compounds are helpful to cure disease and some being reason of the disease.***

Graphical abstract: -



Indexed Terms- ***M. Longifolia, TLC, HPLC, Bioactive Component, Phenolic, Flavonoids***

I. INTRODUCTION

The term medicinal plant includes various types of plants used in herbalism ("herbology" or "herbal medicine"). It is the use of plants for medicinal purposes, and the study of such uses. Nature has blessed us extensively with wide range of diverse plants used for various purpose lie decoration, flowering, fruiting, and medicinal, etc. India is known for wide diversity of such plants, which are utilized

traditionally and have significance of being commercialized such as *mahua, rhododendron, kacchar, maringo,* etc. *Mahua (Madhuca longifolia)* belongs to family sapotaceae and finds origin in different region of India, Sri Lanka, Myanmar and Nepal.[1] It is multipurpose tree which fulfills three fundamental needs of tribal i.e. Food, Fodder, and Fuel.[2] Flower of plants are edible and have high nutritive value majority high amount of sugars and subsequently having good amount of vitamins, proteins, minerals and Fats.[2]

According to WHO (2004) approximately 65% of the world's population integrate the medicinal plant for treatment. *Mahua* is one of the naturally occurring plants which possess numerous health benefits. Tribal use *mahua* flower for curing of skin, to make traditional liquor, curing diseases.[3] *Mahua* is common tree deciduous forest of India, quite prominent in states of Andhra Pradesh, Bihar, Gujarat, Madhya Pradesh, Orissa, Rajasthan, Uttar Pradesh and West Bengal. *Mahua* flowers are in dense fascicles near end of the branches having 1.5 cm long fleshy cream coloured corolla tube and are scented. Flowering period of *mahua* is from the month of March to May. Flower induction start from the top portion to lower branches and also from illuminated part of shaded part of tree. Flowers mature in about 32-35 days. One to two good flowering is expected every three years, that is, it has an alternate bearing habit. This research study state the comparison of dry *mahua* flower from different two regions of Maharashtra, possesses same compound and bioactive components or not.

Botanical aspects of Madhuca longifolia

Family – Sapotaceae

Botanical name – Madhuca longifolia

Maharashtra name – moha or mohwa

- Description and distribution

M. Longifolia trees are normally 15-16 m high, with clustered leaves at the end of branches. The bars are brownish to yellowish grey in color. Elliptic flower are small, cream coloured produced in cluster. These are cultivated in large deciduous dry tropical and such tropical climatic condition

- Sample collection: -

The flower of M. longifolia (Mahua) was collected from two different places from Maharashtra for comparison. Samples were collected from northwest corner Nandurbar and eastern part Chandrapur of Maharashtra.

II. EXPERIMENTAL

- Chemical reagents: -

Methanol of HPLC grade, formic acid, and hydrochloric acid, chloroform, ethyl acetate, water was procured from CFS department (Yashvantrao Chavan institute of science Satara).

- Sample Solution Preparation for HPLC: -

The dried powdered flower of *M. longifolia* was macerated with petroleum ether for 24hr. The powder was filtered and dried at room temperature. The dried powder was re fluxed on the water bath with 50 mL of methanol for 5-6 hr. The extract was concentrated under reduced pressure at 50-60 degree Celsius. The solution was kept tightly in the refrigerator.

- Chromatographic condition: -

HPLC analysis was carried out on injection valve with a 20µl, a UV variable wavelength detector (set at 280 nm) sensitivity was 0.001, 5 µm RP-18 column (30℃). The HPLC solvents were phosphate buffer (v/v) as an aqueous solvent (A) and CH3CN as an organic solvent

(B). The analytes were eluted gradientlly at a flow rate of 1.2 ml/min. Chromatograms were generated on software. The HPLC instrument was operated at room temperature (23 ± 2℃). Each diluted extract 20 µl was injected in to the HPLC three times and the average peak area was reported.

- Sample preparation for TLC: -

For TLC Dicoctiona extraction method used for analysis. This is a process that involves continous hot extractiom using specified volume of water as a solvent. A dried and powdered *M. longifolia* flower placed into clean container. Water is then poured and stirred. Heat is then applied throughout the process to hasten the extraction. The process is lasted for a short duration usually about 15min. The ration of solvent to crude drug is usually 4:1 or 16:1. It is used for extraction of water soluble and heat stable plant material. After 15-20 min, extract of *M longifolia* extracted through filter paper into the clean container.

- TLC conditions: -

TLC plate consists of 10×2 cm, precoated with silica gel 60 F254 TLC plates of uniform thickness 0.2 mm with aluminium sheet support. The spotting apparatus was small capillary tube having diameter 0.3cm. The stationary phase is Silica Gel 60 F254, and mobile phase condition is chloroform: ethyl acetate: water (10:15:5). When sample run ¼ th distance of stationary phase then take out the plate outside the mobile phase and dry at room temperature.

- TLC Analysis: -

About 5µl test solution was applied on TLC plate and the plate was developed in chloroform; ethyl acetate; water (10:15:5) solvent system to distance of 1.5 cm. The plates were dried at room temperature in air. The plate was observed at 254 nm using UV light chamber. The R_f value and colour of the resolved bands were noted.

III. RESULTS AND DISCUSSION

- TLC Results: -

Sample of Nandurbar was applied on TLC plate (5µl) and plate was developed in chloroform, ethyl acetate and water. It shows R_f value 0.94 at 254 nm using UV light chamber in fig.1

Fig.1

Sample of chandrapur was applied on TLC plate (5µl) and plate was developed in chloroform, ethyl aetate and water. It shows R_f valvue 0.96 at 254 nm using UV light chamber in fig.2

Fig.2

World is endowed with plentiful of medicinal plants. Different extracts of *madhuca longifolia* flowers have been found possessing pharmacological activity.

TLC result shows that both samples have different retenion factor as they possess same struture but different components.

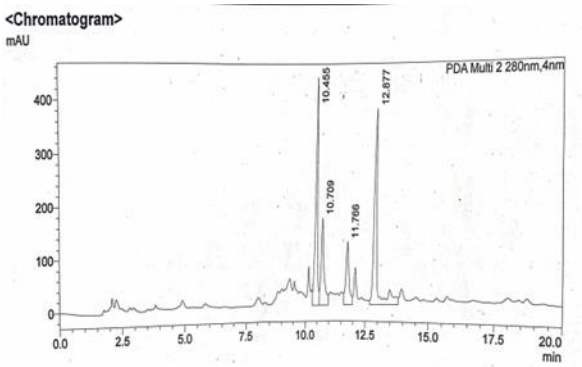
- HPLC Results: -

HPLC analysis was done by injection valve with a 20µl, a UV variable wave lenghth detector (280 nm), 5µm RP-18 column (30℃).

Mahua extract sample from Chandrapur was injected (20µl) in column and chromatogram shows total nine peaks of sample in *fig.4*. it shows phenolics compound like gallic acid (122.59 mg), caffeic acid (10.76 mg), tannic acid (12.7), vanillin (20.05), p-coumaric acid (4.18mg), as well as the flavonoids (+)- catechins, (35.31 mg) and (-)- epi-catechin (39.51mg), (+)- quercetin (11.7mg) vanillin (20.05).

Mahua extract sample from nandurbar was injected (20µl) in column and chromatogram shows total four peaks of analyzed sample in *fig.3.* it shows phenolics compound like vanilin (33.78), p-hydroxy benzoic acid(p-HBA), stigmasterol. The HPLC of these phenolic compounds obtained using the methods describe above would serve the purpose of established benchmark for future plant research. The qualitative and quantitative analysis of the actual phenolic and flavonoid compounds present in any comparison with such standard chromatograms. The use of multiple methods involving different mobile gradient phases would increase the validity and reliability of the obtained results.

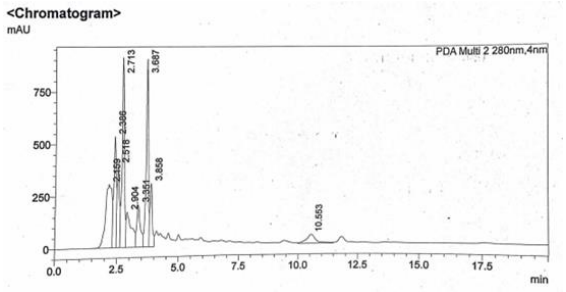
HPLC analysis of Nandurbar sample



Peak	Ret. Time	Area	Height	Conc.
1	10.455	2720156	440680	31.567
2	10.709	1514923	172623	17.580
3	11.766	1148615	124694	13.329
4	12.877	3233517	379383	37.524

Peaks	Ret. Time	Area	Height	Conc.
1	2.159	5451915	306436	17.177
2	2.386	3616947	536140	11.395
3	2.518	2591292	398382	8.164
4	2.713	6198401	912571	19.528
5	2.904	2612605	170878	8.231
6	3.351	2103782	204925	6.628
7	3.687	5839803	903517	18.399
8	3.858	2119818	307439	6.679
9	10.553	1205764	43915	3.799

HPLC analysis of Chandrapur sample



CONCLUSION

Although *mahua* has been part of supplemental diet in India, information on the flavonoids and phenolic compounds contain same or different amount compare to both sample. In concluding this investigation on *mahua*, it could be said that extracted sample of Chandrapur compared to extracted sample of Nandurbar according to HPLC chromatogram peaks only one common phenolic compound is present and in extracted sample of Chandrapur shows total eight different phenolic and flavonoids compare to extracted sample from Nandurbar. In Nandurbar sample it shows total three different phenolic and flavonoid compounds compare to Chandrapur sample. Variations may occur when one or more number of

constituents present in herbal medicines is compared. Chromatographic techniques are the methods from which the chemical fingerprints are obtained. It also used as a tool for authentication and identification of *mahua*. The present study is used as a pathway to determine the comparison of phenolic and flavonoids compounds in *Madhuca longifolia* from two different regions of Maharashtra.

ACKNOWLEDGEMENT

The study supported by the Shivani patole assistant professor in department of forensic science, Pramod Gaikwad department of chemistry, Yashvantrao chavan institute of science Common Faculty Center, Dr. B.T. Jadhav principle of Yashvantrao chavan institute of science Satara, Dr. A.R. Mali Head of department of forensic science, Vikram Hankare In-charge department of forensic science.

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