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HPTLC FINGERPRINTING AND PHYTOCHEMICAL ANALYSIS OF HEARTWOOD OF TWO DIFFERENT SOURCES OF *KHADIRA* (SENEGALIA CATECHU (L. F.) P.J.H. HURTER & MABB)

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ABSTRACT

Therapeutic value of medicinal formulation highly depends on the secondary metabolites. The Climate influences quality and quantity of phytoconstituents of crude drugs. Here we have selected *Senegalia catechu* (L. f.) P.J.H. Hurter & Mabb belong to Two different geographical area-Western ghat region and the Himalayan region. A Series of Phytochemical and Pharmacognostical studies were conducted and compared. The quantification of the group of phytoconstituents like flavonoid, phenol, individual constituent Rutin was done with different phytochemical tools and noted the differences. It is concluded that biotic and abiotic factors of the geographical region have strong influence on the active principles of the plants.

Keywords: *Khadira*, Phytochemical study, Himalaya, Western Ghat

INTRODUCTION

From the Vedic era itself *Senegalia catechu* (L.f.) p. J.H. Hurter & Mabb has been referred in various literature. Rigveda mentioned the plant in the name of *Khadira* and explained its exotic use as a ritual and medicinal plant[1]. Its heartwood is considered to be very strong and is used as a wedge while

manufacturing chariots. Some references say that heartwood is strong as bone. People use the drug as firewood and vessel. Ayurvedic texts quoted the plant twig as the best among the toothbrush which is astringent in taste. Well known ayurvedic scholar Charaka has described it as the best drug for skin disease[2]. Indian system of medicine uses the plant in various formulations for different ailments. Three varieties are commonly used today, *A. catechu, A. sundra, A. catechunoides*. In Ayurvedic literature, *Kadara* and *Khadira* are described as their varieties. Raja Nighantu which is an Ayurvedic lexicon though mentioned Five verities of the plant viz., *Khadira, Somavalka, Tamrakantaka, Vidkhadira and Arimeda*[3]. The heartwood of the plant is considered as *khadira sara* which possesses' medicinal properties. Growing throughout the forest of India but very common in dry regions of north Karnataka and drier regions of sub-Himalayan tract up to 1200m (Punjab to North eastern states, Madhya Pradesh, Gujarat, Maharashtra, Andhra Pradesh and Tamil Nadu). The plant is a moderate-sized tree that grows up to 40 ft. in height[4]. The biosynthetically derived constituents formed from primary metabolites namely Glycosides, alkaloids, flavonoids, volatile oils etc are mainly used as medicine for human ailments. They are chemical adaptations or may serve as protective chemicals against microbes, insects and other predators. These metabolites are sometimes considered to be waste or secretory products of plant metabolism[5]. Various biotic and abiotic factors can influence the production of secondary metabolites which in turn varies the potency of crude drugs. So, the same species of plant that belongs to the different geographical regions may not have the same amount of phytoconstituents. In Ayurvedic science, it is mentioned that plant belongs to the Himalayan region possess maximum potency[6]. Thus, it is necessary to understand the variation of secondary metabolites in the plants belong to different geographical region.

Himalayan region

The Himalayan region can be divided into three

1. Greater Himalaya-This zone is separated from the lesser Himalayan zone by the 'Main Central Thrust', is characterised by highly fossiliferous sediments and metamorphic with an interspersion of young granites. It varies its width of about 40-60 km. The altitude varies generally between 3000 to 7000 metres. The topography of the region has been rigged by different river[7].
2. Lesser Himalaya -This region is separated from the sub-Himalayan by the Main Boundary Fault. It contains mostly non-fossiliferous granites. Broadly divided into outer sedimentary belt and inner sedimentary belt.
3. Outer Himalaya /Shivalik zone- It is composed of Siwalik sediments mostly of tertiary age and recent alluvium. This zone is usually subdivided into three parts Lower, Middle and Upper.

The climate of the region;

The climate of the region is extremely varied, largely due to variations in Altitude and topography. Applying the principle of lapse rate, the lower valley below 600m has a hot and moist tropical climate. It gradually cools up to the elevation of 2000m, rainfall goes on increasing transversely with height up to an optimum of about 2500m. Intense sun radiation, excessive heat and glare, and sometimes snowstorms, blizzards and extremely frigid condition in the mid-day are experienced at high altitude regions. Rainfall is rather scanty in these regions. The average rainfall observed during the monsoon period in high altitudes is about 10-12cm, while during winter, the precipitation is very heavy and very often. The soil texture ranges from sandy loam to clay loam.

Western ghat region

The Western ghat spreads to 1600 km which is parallel to the western coast of India. It passes through the Indian states ranging from Gujarat to Kerala. Asper the UNESCO it is older than the Himalayas. It influences the Indian climate by intercepting in the southwest monsoon. It is endorsed with tropical monsoon forests. The average elevation is 900-1600m. The western ghats cause different orographic rain by facing the rain-bearing moist winds to rise along the western slopes of the ghats. The height of the western ghat increase from north to south progressively.[8]

The climate of the region;

Western ghat climate varies according to the distance from the equator and altitude gradation. The average annual temperature is around 15°C. The continuous Western Ghats chain acts as a barrier to the moisture-laden clouds during the monsoon period, which runs from June to September. The rain-bearing clouds that are moving eastward are forced to rise, depositing the majority of their rain on the windward side. Rainfall averages 3,000–4,000 mm (120–160 in) in this area, with localised extremes exceeding 9,000 mm (350 in).

Botanical description of the plant

The plant is a moderate-sized tree that grows up to 40 ft. in height. The bark is dark coloured and shoots are dark brown or purple. Leaves are bipinnately compounds having 10-15 cm in length. The main rachis is pubescent and glands present between many of the pairs of pinnae and a large conspicuous gland at or near the middle of the petiole. Stipular spines are short, hooked and arise from a broad triangular base. Pinnae have 10-30 pairs,4-5 cm long, nearly sessile, their rachises are also pubescent in appearance. Leaflets are 30-50 pairs,0.5 cm wide, linear, subacute, sessile, often ciliate. Flowers are pale yellow sessile with corolla longer than calyx. Pod fruit has a length of 5-7.5 cm length and 1-1.5 cm width. They are flat, thin, brown and shining with a triangular beak at the apex and narrowed at the base. Seeds are 3-10 in number[9].

Abbreviations

HSC-Himalayan sample of *Senegalia catechu* (L.f.) p. J.H. Hurter & Mabb

WSC-western ghat sample of *Senegalia catechu* (L.f.) p.J.H. Hurter & Mabb

Material and method

Collection of samples;

The samples for the study got collected from Haridwar (Himalayan region) and Aryankavu (Western ghat region). The samples collected were identified as *Senegalia catechu* (L.f.) p.J.H. Hurter & Mabb from the Botanical Survey of India (Dehradun) with an assession no 2020-396,2019-265 respectively for the samples of Himalaya and Westernghat.

Pharmacognostical study;

All the collected genuine samples were dried and studied macroscopically with the naked eye, magnifying lens and measuring tape with the help of Pharmacognostical parameters i.e., size, shape, surface, colour, odour and taste, findings were recorded. For powder microscopy, dried heartwood samples of specimens were taken, stained with saffron and carefully examined under the microscope.

Phytochemical analysis;

The collected specimens were dried and powdered, The Cold extractive techniques were followed, and resultant extracts were used for the further phytochemical study. The physiochemical parameters like moisture content, Extractive values, Total ash values and loss on drying were determined using standard methods[10]. Preliminary phytochemical analysis was done using different reagents as per the standard norms. Tests for the phytoconstituents were carried out by using different extracts of solvents like Aqueous, Alcohol, N-hexane and Chloroform.

Quantification of Total Phenol, Total Flavonoid and Rutin;

Estimation of total Flavonoids and Phenols of the alcoholic extracts was carried out using UV Spectroscopy.

1) Phenol

The total phenolic content of the test samples was determined by using the Folin–Ciocâlteu (FC) reagent. The test samples(100µl) were mixed with 400µl of distilled water and 150µl of FC reagent (1:1) was added and stored in the dark, a quick spin was given to mix the samples properly and was left for 10 minutes at room temperature, then 100µl of 20% of saturated sodium carbonate was added and incubated for an hour in the dark for the reaction to take place. The absorbance of blue colour from different samples which were taken as 1mg/ml and standard readings ranging(10-60µl) using a spectrophotometer at 750nm. The results were derived from the calibration curve concerning the standard.

2) Flavonoid

Flavonoids have antioxidant properties, the flavonoid content in plant extracts was determined using Aluminium Chloride by UV spectrometry method. Test

samples of 100µl were mixed with 400 µl of methanol a quick spin was given to mix it properly. Then 100 µl of 10% of Aluminium Chloride was added and kept for 10 minutes at room temperature, 100 µl of 1 M of Sodium Acetate was added and incubated in the dark for the reaction to take place. The absorbance of greenish-yellow colour from different test samples which were taken in a concentration of 1mg/ml and standard (Rutin) ranging from (10-60 µl) were made using a spectrometer at 415nm. The results were derived from the calibration curve concerning the standard. All reagents used were of the GPR range.

3)Chromatographic evaluation and HPTLC for Quantification of Rutin

The quantification of Rutin in the alcoholic extract of both samples was carried out by using HPTLC. Mobile phases of chloroform/ethyl acetate /formic acid (5:4:1) were used for the identification of rutin[11]. The standard sample and extract samples were dissolved in the methanol (10 mg in 1ml) and were filtered through Whatman filter paper no.1 before spotting on the plates, exposed to ammonia and visualized under a UV lamp. Standard and plant extracts were dissolved in methanol. Quantification was done by application of the spots on the HPTLC plate which were coated with silica gel (plate size of 6.0 x 10.0cm). The plates were developed at 60F 254nm under UV visible wavelength. The details of the instrument used in studies are CAMAG LINOMAT 5 with 5 application parameters, inert gas (nitrogen) as spray gas and methanol as sample solvent. The syringe size was 25µl with 3 tracks. The calibration parameter used was calibration mode multilevel, with CV statistics mode and the evaluation mode was based on peak areas. The study was carried out in the Centre for medicinal plant research, Arya Vaidya Shala Kottakkal.

Chemicals used;

Standard biomarkers, Rutin and Gallic acid are from Sigma Aldrich Private Limited, Mumbai and all the chemicals used throughout the research were procured from local suppliers.

RESULT AND DISCUSSION

Pharmacognostical study

1) Macroscopical study of Samples;

The heartwood of HSC is light brownish-red in colour while WSC is dark Brownish. Both were attached with whitish sapwood and fractured hard (Table 1).

2) Microscopical study of two sample;

Powder microscopy procedure was followed, were examined through microscope and found structures like Fibres with scleroid, a fragment of border pitted vessels, starch grains with hilum along with prismatic crystals, yellowish dark brown colouring matter, lignified fibres, fibbers passing through medullary rays and tannin with oil containing bodies in both samples.

Physio chemical study

Physiochemical parameters such as water-soluble extractives, alcohol-soluble extractive, n-hexane soluble extractive, chloroform-soluble extractive, ether soluble extractive, total ash value and moisture content of the samples were calculated and depicted (Table 2). Preliminary phytochemical analysis of alcoholic extract was done and result tabulated in the (table 3).

Phytochemical study

The quantification of flavonoid showed the absorbance values of the samples at 415nm on the standard Rutin. Thus, corresponding total flavonoid content of the samples was reported as Rutin equivalents. The Sample HSC Alcohol extract showed the highest flavonoid content (20.05 µg mg-1) while the sample WSC Alcohol extract showed (16 µg mg-1) Rutin equivalent. In the quantification of total Phenol content procedure, Absorbance values of the samples at 750 nm were marked on the standard Gallic acid curve. The corresponding total phenol contents of the samples were reported as Gallic acid equivalent in µg mg-1 of the dry mass of the sample extract. Sample HSC Alcohol extract showed the highest phenolic content (18 µg GAE mg-1) followed by the samples WSC alcoholic extract (17.8 µg GAE mg-1).

The HPTLC analysis of the plant extract for Rutin (figure1) showed yellow and greenish, yellow-coloured zones at 254 nm. The Rf value and peak area for standard Rutin were found to be 1.59 and 6462.8 with an area percentage of 47.70 The coinciding Rf and area

with a percentage of area for WSC and HSC are 1.58,6065.8(22.19%) and 1.58,3760.5(7.5%) respectively (Tables 4,5,6), (Figure 1).

The safety and quality of crude medicinal plants have been cited in Ayurveda based on major variables such as *Desha* (habitat), *Kala* (time), *Guna* (properties), etc. Western Ghat with its diverse climate has a great diversity of soils. The factors like temperature, rainfall, sunlight determine the nature of the soil. While Himalayan region (Haridwar) is a fertile region with mountain meadow, alluvial soil and red loamy soils and monsoon rains coupled with the Ganga River water, severely contaminated with effluents and waste from domestic and commercial sources. With gradually increasing inhabitants, the city has faced a severe shortage of water and its groundwater levels have been depleted. Western ghat region, most of its seasonal rainfall is from the southeast monsoon and south-west monsoon winds, from June to mid–September with an average annual rainfall of about 300 cm. Himalayan region monsoon is by the Southwest winds and annual rainfall of 210 cm. It was thought-provoking to detect that the phytochemicals in the samples continued the same qualitatively but varied quantitatively. The total phenol and Flavonoid were found to be more in HSC than WSC. The antioxidant property is influenced by the flavonoid content it has. Rutin is a Phyto constituent known for its antioxidant and ant-inflammatory properties. The HPTLC result showed the HSC sample got more amount of Rutin content than WSC. The therapeutic effect of the crude drug can be influenced by the quantity of phytoconstituents.

CONCLUSION

All of this indicates that geographical properties played a critical role in differentiating the phytochemical composition of the samples, which was also responsible for the observed differences in biological activity. In various climatic and edaphic environments, plants develop abundant phytochemicals as secondary metabolites, which can be used to make a potent drug.

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Table 1

Parts	Colour	Odour	Touch	RASA	SHAPE	SIZE
WSC	Dark brownish red	Odourless	Coarse	Kashaya (Astringent)	Chips (Plano-convex)	3-5 cm
HSC	Light brownish red	Odourless	Coarse	Kashaya (Astringent)	Chips (Plano-convex)	3-5 cm

Table 2

	WSC (%)	HSC (%)
Water-soluble extractive	3.4	3.7
Alcohol soluble extractive	1.58	1.44
Chloroform soluble extractive	0.12	0.14
N-Hexane soluble extractive	0.43	0.26
Moisture content	9.65	8.32
Total ash value		

Table 3

	WSC	HSC
Saponin	+	+
Phenol	+	+
Alkaloid	+	+
Paleobotanic	-	-
Flavonoid	+	+
Tannin	+	+
Glycoside	+	+

Table 4

Peak	Rf	Area	
1	0.09	1305.2	
2	0.23	271.6	
3	0.89	185.9	
4	1.16	352.3	
5	1.42	414.3	
6	1.59	6462.8	Rutin
7	1.77	912.0	

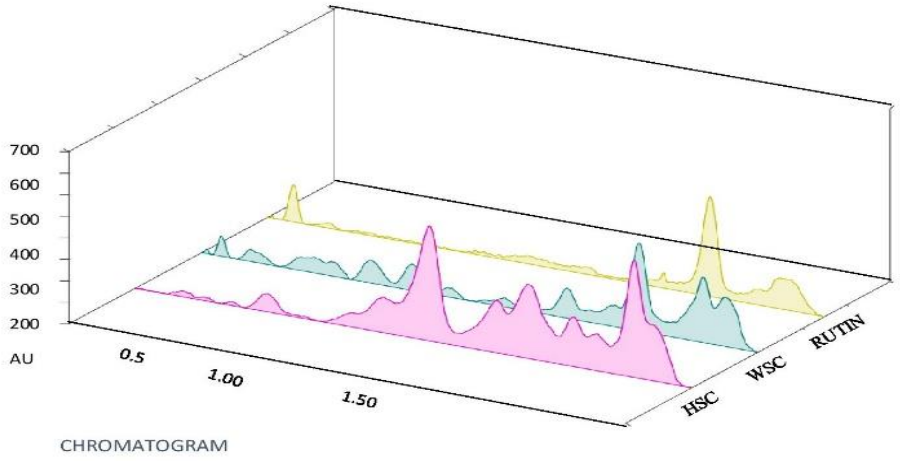
Table 5 (WSC)

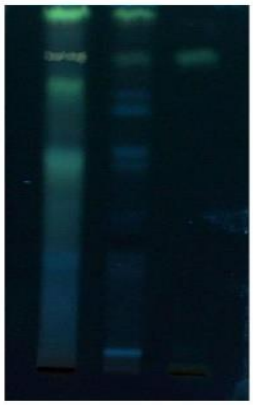
Peak	Rf	Area	Area%
1	0.17	353.6	0.71
2	0.48	1370.9	2.74
3	0.89	3966.7	7.94
4	1.06	11463.9	22.94
5	1.30	5251.4	10.51
6	1.41	8763.5	17.54
7	1.58	3760.5	7.52
8	1.66	2527.9	5.06

Table 6 (HSC)

Peak	Rf	Area	Area%
1	.02	67.8	0.25
2	0.07	595.7	2.18
3	0.18	881.3	3.22
4	0.41	1544.9	5.65
5	0.48	804.4	2.94
6	0.61	1756	6.43
7	0.76	1951.8	7.14
8	0.89	562.9	2.06
9	1.09	671.6	2.46
10	1.31	1970	7.21
11	1.49	1297.4	4.75
12	1.58	6065.8	22.19
13	1.80	5317.9	19.46

Figure 1





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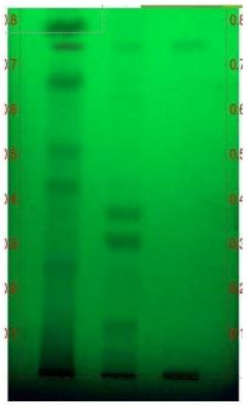

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