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COMPARATIVE PHYTOPHARMACOLOGICAL EVALUATION OF LEAVES AND STEM BARK OF *Bridelia airy-shawii*

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Abstract:

Bridelia airy-shawii is well known and used plants among the Karnataka region for its anti-inflammatory potential; however the exact mechanism of action, constituents responsible for therapeutic potential and part of plant possessing optimum or greater extent of medicinal prospective are still unknown. There is also environmental concern as stem bark are promoted on larger scale to mitigate the inflammatory conditions but its process of scratching can turn out fatal for plant as delicate heartwood is exposed to brutal environmental consequences. Leaves are also used as remedy in some distal part of Maharashtra like Satpuda region. Hence the present work is taken to elaborate the phytochemical and pharmacological comparison between stem bark and leaves of plant. Previous primary screening suggest leaves and stem bark are varying to very little extent in their chemical set up and hence it is more fruitful and interesting to recognize and implement pharmacological potential of plant.

Key words: *Bridelia airy-shawii, Phytochemistry, anti-inflammatory, tannins, bark and leaves*

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INTRODUCTION:

In this era of medical pluralism, no single system of medicine is perfect and most beneficial all the time; hence an appropriate blend of these medicinal systems that can be incorporated practically is essential to serve the humanity. Conventional system of medicine is capable of providing symptomatic relief at much faster rate whereas Ayurveda and other alternative medicinal systems takes longer time but are uprooting the pathological reason and producing affirmative changes in etiological rationale. However it seems intricate to convert the herbal moieties as thriving dosage form with better shelf life and enhanced bioavailability. Hence a new approach to include these moieties as part of mitigation along with conventional system of medicine or as separate entity appears essential. Ancient approach to use plant and other natural sources as quath or decoction is simpler and succeed by using powder of plant part, it emerge as straightforward and effortless mode of utilizing herbs but it put extra stack on whole physiological process to tolerate additional load of secondary molecules which are present in plant parts. Also it increases the dose regimen and schedule of the medicine. To make it more specific and to avoid later consequences, individual molecule responsible for medicinal potency is being discovered and efforts are taken to formulate it as successful dosage form. Nevertheless isolation of individual molecule is tedious, time consuming and costlier process also its common observation that these isolated molecule do possess vicinity of some of the synergistic molecules which are neutral as such and do not possess any medicinal potency individually but when these molecules surround the active molecule enhance its pharmacological influence to greater extent. So in nutshell it's better to focus on active fraction with maximum pharmacological potential instead of individual molecule and it generates the concept of bio-activity guided fractionation. It creates win-win situation as the fraction with most efficiency gets separated, reducing further purification process and saving the cost of operation. However when it comes to generalizing the concept for masses ancient approaches are firm options. The scenario demands the concept of medicinal farming, harvesting and small scale industry development based on agricultural products. Methods should be invented considering sustainable development and environmental concerns. It is possible and likely needful to render this attitude towards including the herbs and products of natural origin in mainstream of healthcare.

Arthritis is common complication all over the world; around 350 million people are suffering from arthritis. There are about 100 types of arthritis and major complication among all sorts is inflammation. Inflammation is as such protective phenomenon but may prove fatal if left untreated. Conventional system of medicine relies on symptomatic relief and most cases are handled with NSAID's as per standard protocol. But long term exposure to these moieties can cause variety of side effects. Herbals as they are known for their liberty from side effects are great choice to cure inflammation, many herbs are known to cure inflammation at best, Turmeric is one of the popular condiment and household cuisine ingredient among Indian culture well known and utilized for its anti-inflammatory properties. Bridelia airy-shawii is similar sort of plant commonly referred as Asana in Marathi and possessing potent ethno-botanical claims for mitigation of inflammation.

Inflammation is studied on acute and chronic scale, with unique animal models; it segregates intensity and overall execution of inflammatory cascade and allows one to understand mechanism of action and moderation of episode. The protocol for mitigation also varies depending on the intensity of inflammation. Herbal sources must be seen with integrity to cure and mitigate inflammation with better narrative.

MATERIAL AND METHODS:

Collection, Authentication and Storage of plant material: The fresh bark and leaves of Bridelia airy-shawii were collected in the month of July (2012) from Ranipur (Toranmal) of Nandurbar District (MS), India. It was authenticated by Dr. D. A. Patil, Taxonomist, H.O.D. of Botany, Dr. P. R. Ghogrey College, Dhule (MS).

The bark and leaves collected were shade dried and separately extracted with methanol. Both methanolic extracts are further subjected to fractionation to produce various fractions; it was passed through column to yield tannin rich fractions. These fractions were further studied for anti-inflammatory potential on acute and chronic scale.

Method for acute inflammation- carrageenan induced paw oedema: Four groups of animals namely control, standard and two test group with methanolic extract and isolated fraction were used in this model. Each group contains six animals. The test and standard drugs were administered by dissolving in 2% gum acacia. The dose of test drug was selected by

guidance of toxicity studies.

Group I	orally 2 ml of 2% gum acacia -	control
Group II	orally 100 mg/kg of Aspirin	standard
Group III	orally 500mg/kg of methanolic extract of *B. airyshawii*	Test I
Group IV	orally 1000mg/kg of methanolic extract of *B. airyshawii*	Test II

After an hour, 0.1 ml of 1% carrageenan in normal saline injected in sub plantar region of the right hind Paw of each rat in each group. The paw volume was measured immediately after injection and recorded as 0 hrs, and again at interval of 3 hrs by digital plethysmometer. The increase in paw volume is expression of oedema where as the difference in initial and later paw volume indicates the percentage inhibition of oedema. The percentage inhibition for each group was calculated with reference to control group.

Percentage of inhibition of paw oedema = $(1-V_t/V_c) \times 100$
Where V_t - average paw volume of standard and test group
V_c - average paw volume of control group

Method for chronic inflammation – cotton pellet granuloma: In this model four groups were used in which inflammation was produced using cotton pellets. Sterile cotton pellets (50 ±1 mg) were placed subcutaneously by making incision bilaterally in the axialla under ether anesthesia. All procedures were performed strictly in aseptic condition. After a week, on seventh day animals were sacrificed and pellets were removed along with granulation tissue and dried at 60^0C for 24 hrs. The net dry weight of granuloma estimated and used as indication of anti-inflammatory potency.

Group I orally 2 ml of 2% gum acacia - control
Group II orally 100 mg/kg of Aspirin - standard
Group III orally 500mg/kg of methanolic extract of *B. airyshawii* Test I
Group IV orally 1000mg/kg of methanolic extract of *B. airyshawii* Test II

Statistical analysis: Paw volume and cotton pellet granuloma data was analysed by One Way Analysis of Varience (ANOVA). $P < 0.05$ was considered significant.

Isolation: carrageenan induced paw oedema model works on shorter time frame and hence it is easy to compare pharmacological efficiency of fractions being separated by column chromatography. It allows bioactivity guided fractionation due to less time taken for execution.

Column was developed for column chromatography. The base of column was prepared by fixing cotton plug at the bottom. About 180gm of activated silica (in hot air oven at 110^0 C for 1 hour) blended with solvent to prepare suspension. The activated silica gel (60-120) suspension in the solvent was poured slowly in the column from the top and allowed to settle. From the bottom outlet excess solvent was removed. By gentle tapping air bubble from the column was removed to prepare uniform adsorbent bed. The chromatogram was allowed to develop over night; to prevent drying of column open end was plugged with adsorbent cotton and aluminum foil. The methanolic extract was mixed with silica gel in a glass mortar to form free flowing mixture. This mixture was added in the column along with solvent; a quantity of solvent about 2 cm was allowed to remain at the top. Gently pour mobile phase in the column and allowed to drain through bed to give different fractions. Solvent was distilled off and concentrated residue was monitored with TLC. Fractions were collected and combined based on TLC pattern. Fractions with abundant quantity and less interference in TLC were subsequently studied for anti-inflammatory potential with carrageenan induced paw oedema for optimum activity. .

Spectral elucidation of compounds by using modern analytical methods UV analysis The UV spectra of major Fraction were recorded at 200–800 nm. (Shimadzu UV/Vis 2401 spectrophotometer)

FT-IR analysis The IR spectrum of compounds was recorded on FT-IR 8400S using solid plate Technique with KBr. the spectra were recorded for each sample from 4000 to 400 cm- 1 by averaging thirty scan for each sample with a spectral resolution of 1 cm- 1. Although the whole spectral range (4000–400 cm- 1) was stored for each sample, to avoid interference only selected intervals were considered.

RP-HPLC analysis HPLC analysis were performed in an Agilent 1200 series apparatus at different wavelengths (220, 254, 280, 316, 354 nm) equipped with and DAD-UV as detector. The oven temperature was maintained at 300^0C, and the separation was carried out isocratically in a Eclipse plus XDB C-18 150 mm x 4.6 mm (I.D.), with particle size of 5µm, using Methanol: 0.1% H3PO4 in water of different concentrations, as mobile phase adapted from at a flow rate of 1.0 ml min−1 and 20µl were used for injection.

RESULTS AND DISCUSSION:

Carrageenan induced rat paw edema is model to investigate anti-inflammatory potential mainly of acute scale, methanolic extract of leaves and stem bark both showed excellent anti-inflammatory latent when compared with Aspirin as standard. 1000 mg/kg dose of methanolic extract shows better activity over 500 mg/kg dose suggesting gradual increase activity along with dose. However bark does show superior performance after 3 hours with 500mg/kg methanolic extract and for 1000mg/kg dose after 6 hours whereas leaf extract proves better at 500mg/kg after 6 hours and 1000mg/kg at 3 hours interval, so leaves and stem bark are equally effective at coarse scale.

Table 1: Effect of methanolic extract of B.airy-shawii in carrageenan induced rat paw edema

Group			Bark				Leaves			
	Drug	Dose	Mean increase in paw volume in ml at		Percent inhibition		Mean increase in paw volume in ml at		Percent inhibition	
			3h	6h	3h	6h	3h	6h	3h	6h
I	2% gum acacia	2 ml	0.45± 0.02	0.39± 0.06	--	--	0.45± 0.02	0.39± 0.06	--	--
II	Aspirin	100 mg/kg	0.22 ± 0.01	0.17 ± 0.02	51.12	56.41	0.22 ± 0.01	0.17 ± 0.02	51.12	56.41
III	B.airyshawii metanolic extract	500mg/kg	0.39 ± 0.04	0.34 ± 0.08	13.34	12.83	0.40±0.15	0.33 ± 0.05	11.12	15.40
IV	B.airyshawii metanolic extract	1000mg/kg	0.35 ± 0.01	0.29 ± 0.02	22.21	25.64	0.34 ± 0.40	0.30 ±0.60	24.45	23.07

To determine chronic inflammatory response cotton pellet granuloma is best suited model as it works on longer time frame as well as considers internal mitigation cascade, it depicts from the data that though both leaves and stem bark methanolic extract show considerable potency, bark extract yield better outcome over leaf extract. Nevertheless potency was much higher and closer to standard used Aspirin, so it can be concluded both leaves and stem bark methanolic extract are showing better activity on chronic scale. Leaves extract seems to possess significant potential as anti-inflammatory moiety though a bit less than bark but moderately analogous impact can be expected from both leaves and stem extracts.

Table 2: Effect of methanolic extract of B.airy-shawii on cotton pellet granuloma in rats

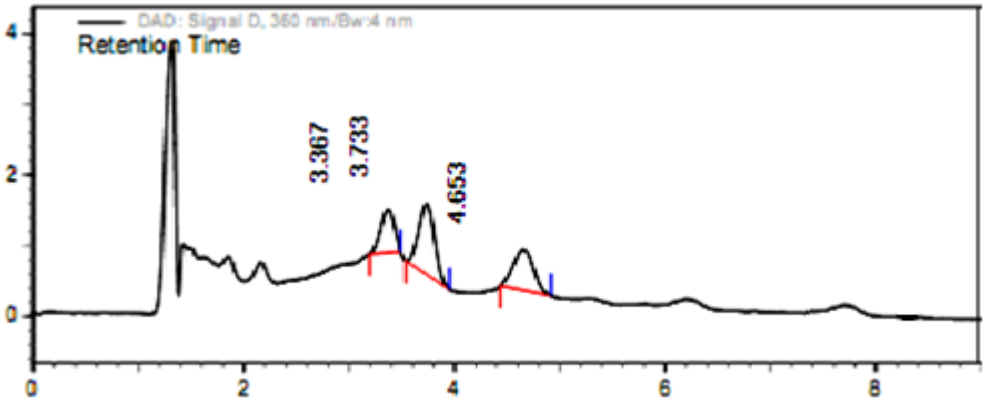
Group	Dose	Dry weight of granuloma (mg) Bark extract	% inhibition of granuloma formation	Dry weight of granuloma (mg) Leaf extract	% inhibition of granuloma formation
I gum accacia	2 ml	85.33 + 10.30	--	85.33 + 10.30	--
II Aspirin	100mg/kg	39.75 + 9.2	53.42	39.75 + 9.2	53.42
III B. airyshawii metahnolic	500mg/kg	49.90 +8.1	41.53	51.25 + 6.80	39.94
IV B. airyshawii metahnolic	1000 mg/kg	45.50 +	46.67	47.30 + 8.90	44.57

Shorter time frame of carrageenan induced paw edema allows estimating anti-inflammatory potential of various fractions isolated from column chromatography as soon as they propagated. Fraction III depicts maximum inhibition among all isolated fractions; from solvent system and TLC patterns it seems to be tannin rich fraction. The inhibition was even more than standard Gallic acid and hence further purification of fraction III was avoided on the principles of bioactivity guided fractionation. Methanolic extract can be considered as mixture of variety of molecules. Some molecules may possess anti-inflammatory; some are inert whereas other category contains pool of synergistic molecules that enhance the biological potential instead absence of individual medicinal efficacy. To exactly locate the fraction with maximum potential is difficult task as results are ambiguous and always complicated. It can be best overcome by centering the secondary molecule category with proper blend. Fraction III was result of combination of 3 spot TLC pattern fractions discharged from column. Fraction III was further elucidated by sophisticated techniques like UV spectra, IR fingerprinting and HPLC. To pipe n conclude molecular existence in fraction III.

Table 3: Effect of isolated major fractions of B.airy-shawii methanolic extract in carrageenan induced rat paw edema

Group	Drug	Dose	Bark			
			Mean increase in paw volume in ml at		Percent inhibition	
			3h	6h	3h	6h
I	2% gum acacia	2 ml	0.45± 0.02	0.39± 0.06	--	--
II	Aspirin	100 mg/kg	0.22 ± 0.01	0.17 ± 0.02	51.12	56.41
III	Fraction I	500mg/kg	0.44 ± 0.04	0.36 ± 0.08	2.23	7.69
IV	Fraction II	500mg/kg	0.36 ± 0.01	0.28 ± 0.02	20.01	28.20
V	Fraction III	500mg/kg	0.25 ± 0.09	0.19 ± 0.05	44.45	51.28
VI	Fraction IV	500mg/kg	0.37 ± 0.01	0.23 ± 0.07	17.78	41.02
VII	Fraction V	500mg/kg	0.39 ± 0.06	0.29 ± 0.04	13.34	25.64
VIII	Fraction VI	500mg/kg	0.41 ± 0.08	0.31 ± 0.01	8.89	20.51
IX	Gallic acid	500mg/kg	0.27 ± 0.07	0.21 ± 0.08	40.01	46.15

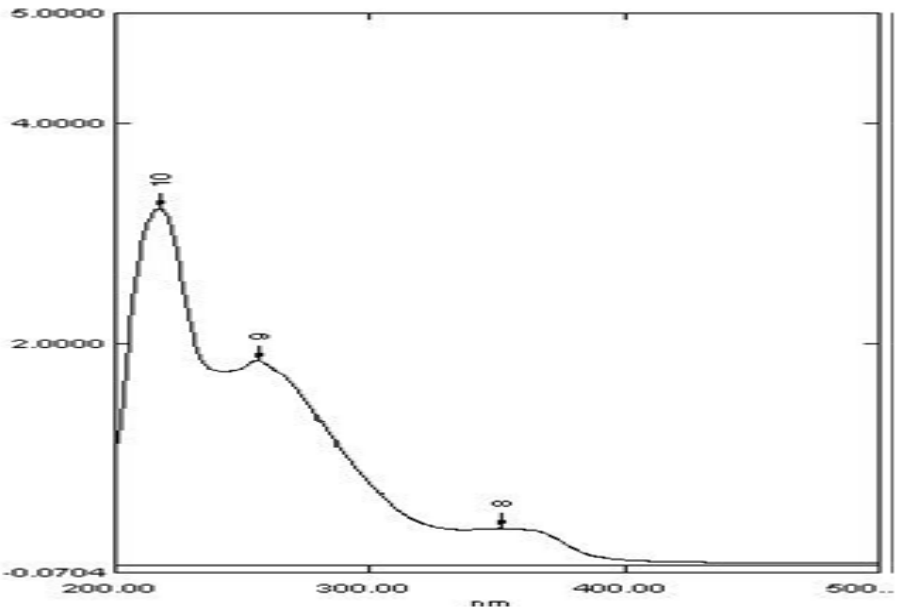
All 3 spectral elucidations suggest fraction III may be combination of tannins like Gallic acid, Ellagic acid and some unknown tannins that do possess anti-inflammatory potential.



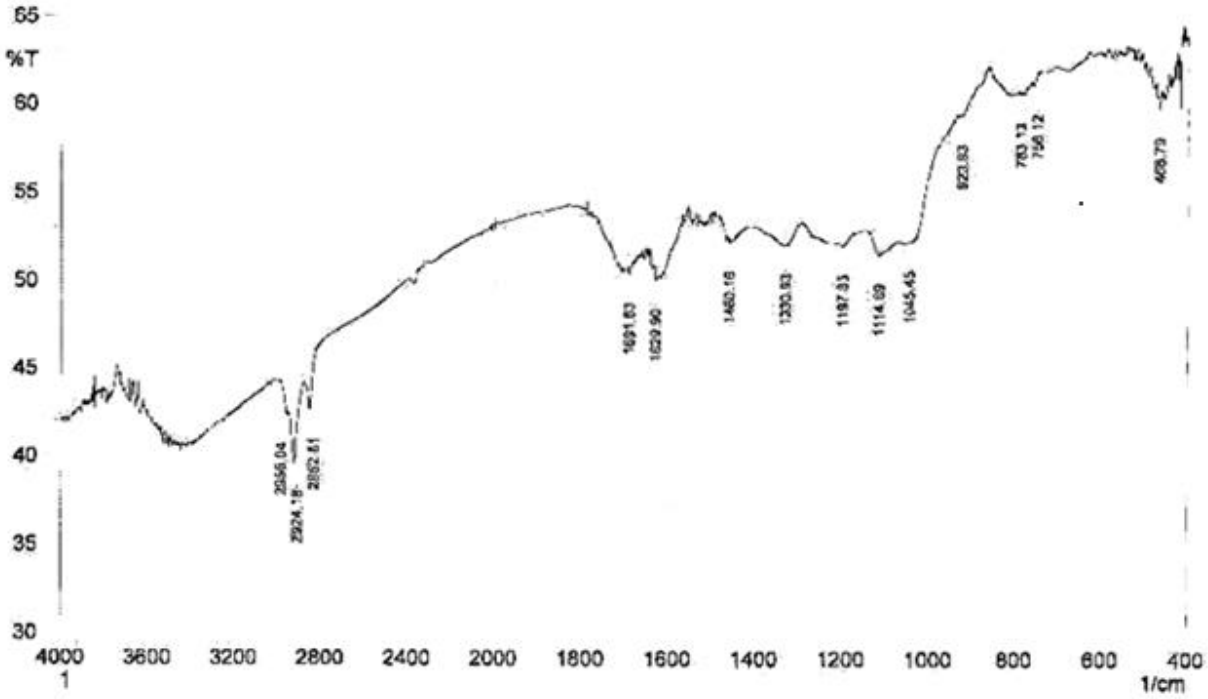
Chromatogram of fractiom III at 360 nm

Table 4: UV details of Fraction III

Sr. No.	Peak	λ max	Absorbance
1	1	351.5	0.3306
2	2	256.0	1.8634
3	3	218.0	3.2466



UV spectra of **fractiom III**



IR Spectra for fraction III

UV spectra with 3 major peaks with λ max 351.5nm, 256 and 218nm indicate 3 major molecules; higher absorbance depicts molecular hindrance on large scale, further fraction III at 360 nm .HPLC chromatogram similarly depicts 3 peaks giving confirmation to 3 molecular combinations in Fraction III. IR spectra of fraction III when overlaid on Gallic acid standard spectra shows similar pattern and functional group distribution. IR interpretation suggests presence of Gallic acid in fraction III. Fraction III may be further purified to yield individual tannins like Gallic acid but if it

compromise with therapeutic potential then it's rather beneficial to summarize fraction III. It is future scope of this work that a blend with maximum efficiency and least molecular hindrance to be isolated instead of individual molecule.

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