Design, Development and Standardization of a Unique Herbal Formulation for the Treatment of Migraine

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ABSTRACT

Migraine is a chronic neurological disorder associated with significant disability and reduced quality of life. Limitations and adverse effects of conventional pharmacotherapy have increased interest in complementary and integrative approaches. Herbal medicines, grounded in traditional systems, offer multi-target therapeutic potential with improved tolerability. A randomised controlled clinical study was conducted involving 60 migraine patients without aura. Participants were allocated to a Herbal Medicine group (HM, n=30) receiving Panchakarma (virechana) followed by a standardized polyherbal formulation for 90 days, and a Control group (CT, n=30) receiving standard symptomatic treatment with NSAIDs. Outcomes were assessed using the Cerebral Headache Questionnaire (CHQQ), Visual Analogue Scale (VAS), Migraine Disability Assessment Scale (MIDAS), Perceived Stress Scale (PSS), heart rate variability (HRV), and surface electromyography (sEMG) of the frontalis muscle. Phytochemical standardisation and antioxidant studies were also performed. Statistical analysis was conducted using repeated-measures ANOVA with Bonferroni post-hoc testing.

Keywords: Migraine, Polyhedral formulation, Panchakarma, Herbal medicine, Clinical evaluation.

INTRODUCTION

Migraine is a debilitating neurovascular disorder characterised by recurrent headaches accompanied by nausea, photophobia, phonophobia, and functional impairment (1). According to the World Health Organisation, migraine ranks among the leading causes of disability worldwide. Although conventional therapies such as NSAIDs and triptans are effective for acute attacks, long-term use is associated with adverse effects and medication-overuse headaches (1).

Traditional systems of medicine emphasise holistic, multi-target approaches using herbal formulations. Medicinal plants such as Salix alba, Piper longum, Withania somnifera, Bacopa monnieri, Glycyrrhiza glabra, and Curcuma longa possess documented analgesic, anti-inflammatory, antioxidant, and neuroprotective properties (2). The present study aimed to scientifically develop and validate a standardized polyherbal formulation for migraine management.

MATERIALS AND METHODS

Preparation of Formulations

The following components were confirmed to be in the uchidum formulation (FS002).

Sesame cake (marg of sesame seeds - Sesamum indicum Family - Pedaliaceace) with the bark of Ficus racemosa Linn (Family - Moracaece)

The following chemicals are found in the parpam formulation (NP003).

Zinc purified.

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Leaf juice from the Compositae species of Eclipta alba.

Aloe vera family (Lilacaceae) leaf pulp

The preparation of the formulations, uchidum formulation (FS002) and parpam formulation (NP003), follows the procedures laid out in the official Siddha texts, Anuboga vaidya navaneetham onpadam padham and the Siddha formulary, 4th edition 1991, respectively (3).

Preliminary Pharmacological Screening

Utilizing fasting pale skinned person bunnies, the details went through pharmacological testing for hypoglycemic movement. Both FS002 and NP003 significantly diminished the fasting blood glucose when contrasted with the pattern readings (4). Blood glucose levels were diminished by FS002 and NP003, and the still up in the air to be measurably huge (P 0.05).

Figure 1: Effect of formulations FS002 and NP003 on Fasting Blood glucose level of Rabbits

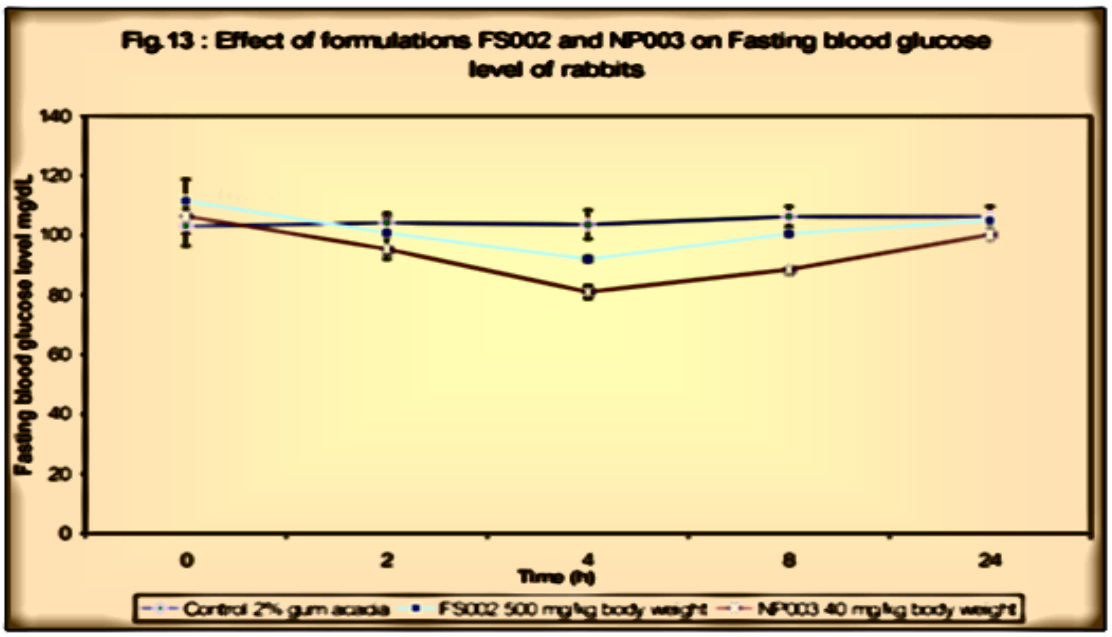
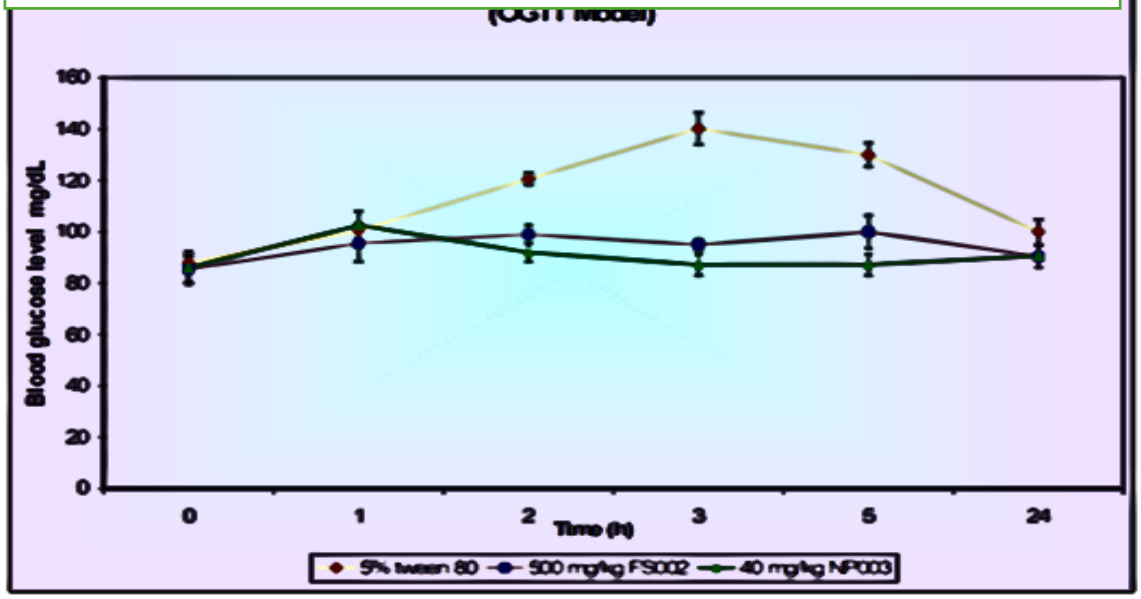


Figure 2: Effect of Formulations FS002 and NP003 on glucose loaded rate (OGIT



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Surface features

The bark has a smooth exterior. The fractures are either completely undetectable, very shallow, and asymmetrical. The bark's sliced surface appears black on the outside, pale brown inside, and glossy white on the inside. There is no distinct flavour or odour to the bark (5).

Powder Microscopy

Powdered bark shows the following inclusions.

1. Crystals of calcium oxalate . The powder contains crystals that are various sizes and shapes. Most of them are prismatic kind.
2. The powder contains parenchyma cells with starch granules. Small and concentral starch granules can be seen.
3. Phloem fibres: Found in small bundles, these lengthy fibres have strong walls and a limited lumen. They have lignified walls. Pits in the lateral walls are absent. The fibres are inclusion-free.
4. Sclereids: The powder frequently contains brachy-sclereids (stone cells) that are isodiametric or elongated. These cells have broad lumens and thick walls. The trenches are many, basic, and wide.

Organoleptic, microscopic, and physical tests were performed on the herbal raw ingredients used in the chosen formulations.

For the constituents of the formulations, observations on the colour, texture, aroma, taste, shape, and size were recorded as part of the organoleptic evaluation. The plant materials' macroscopy was documented (6).

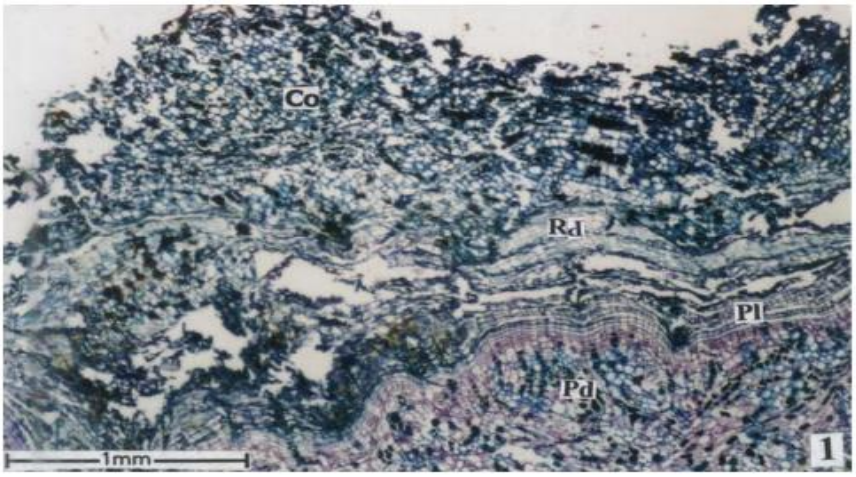

Fig.3 : TS of outer bark of Ficus racemosa L – Periderm zones forming the rhytidome

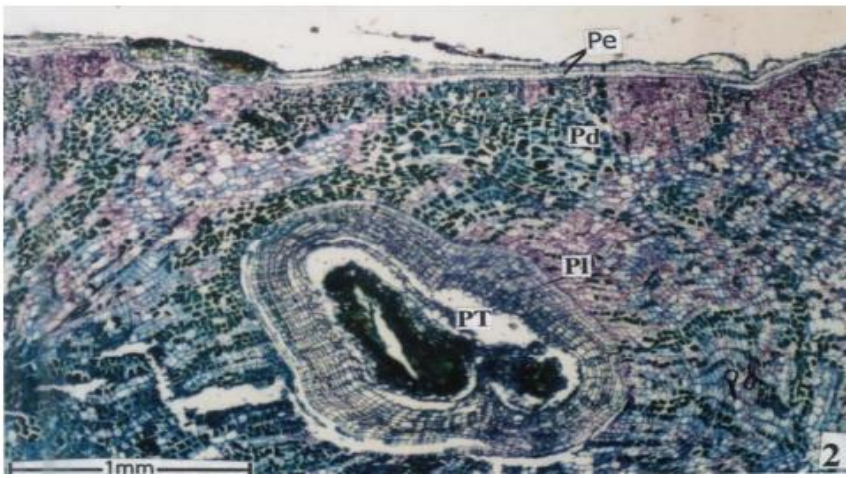

Fig.4: TS showing Periderm and periderm – tube Ficus racemosa l.

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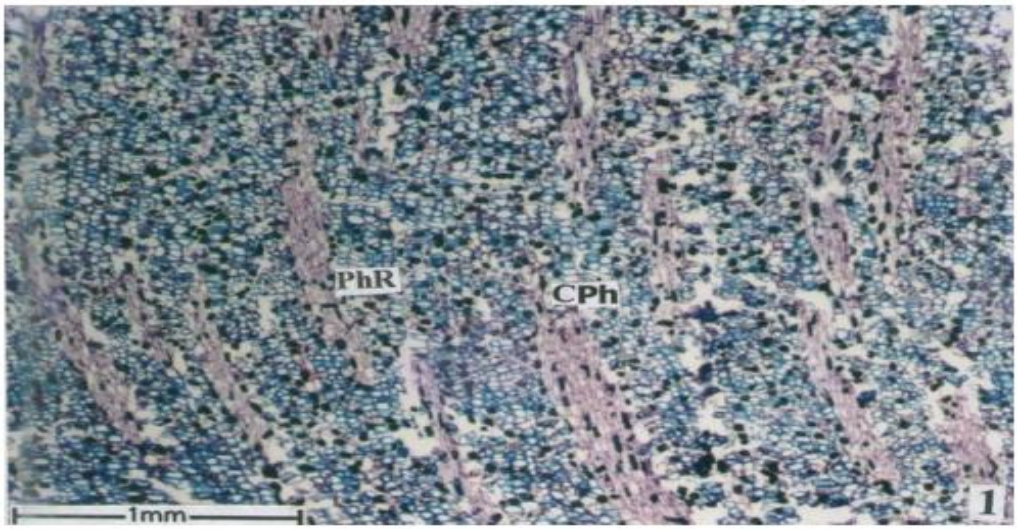

Fig.5: TS of inner bark of Ficus racemosa showing collapsed phloem

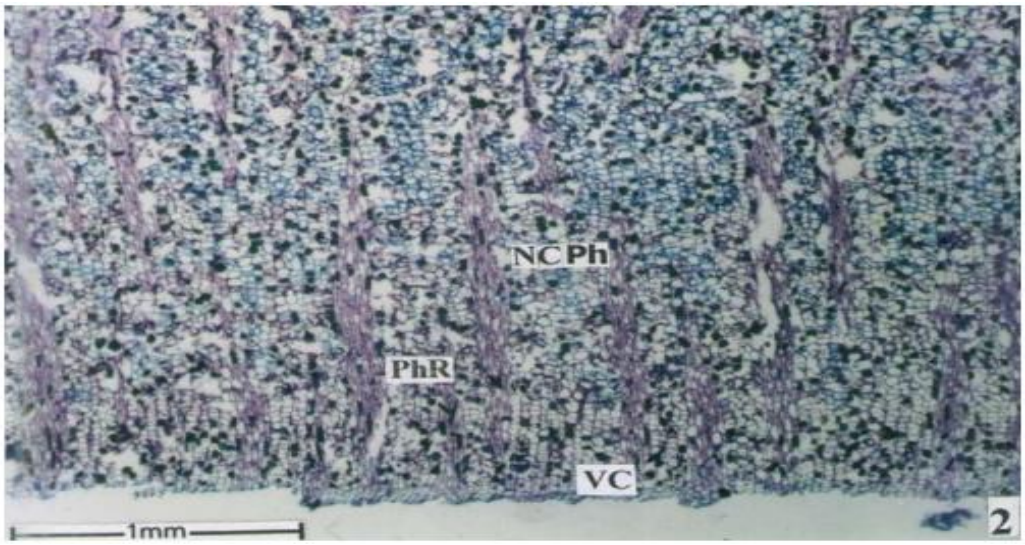

Fig.6: Non-collapsed phloem showing wide rays Ficus racemosa l.

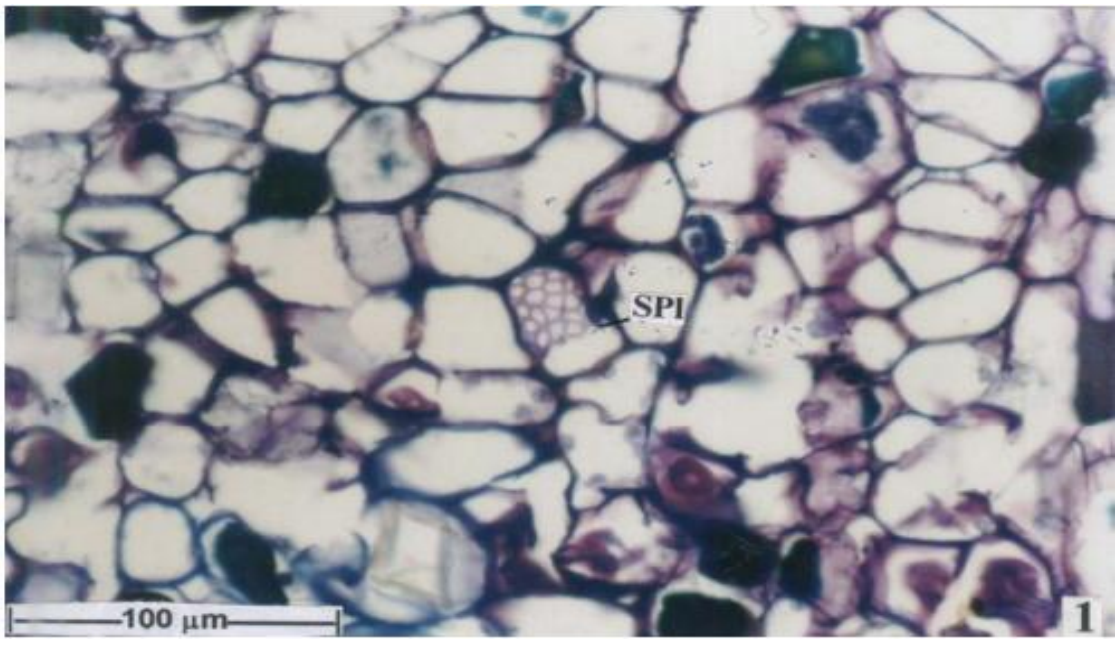

Fig.7: TS of bark showing enlarged sieve plate

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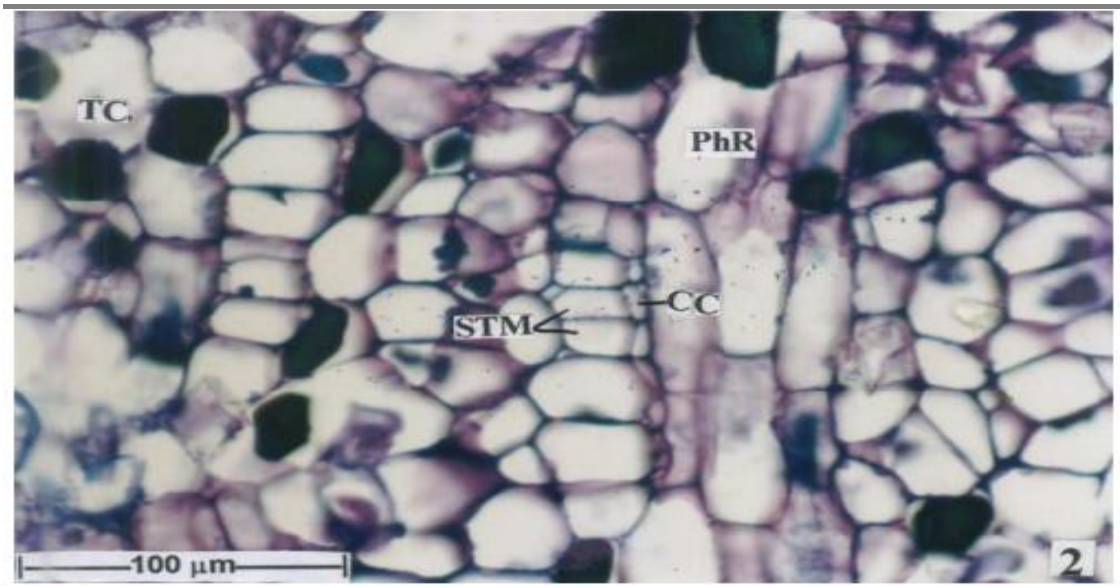

Fig.8: Sieve elements and companion cells enlarged Ficus racemosa l.

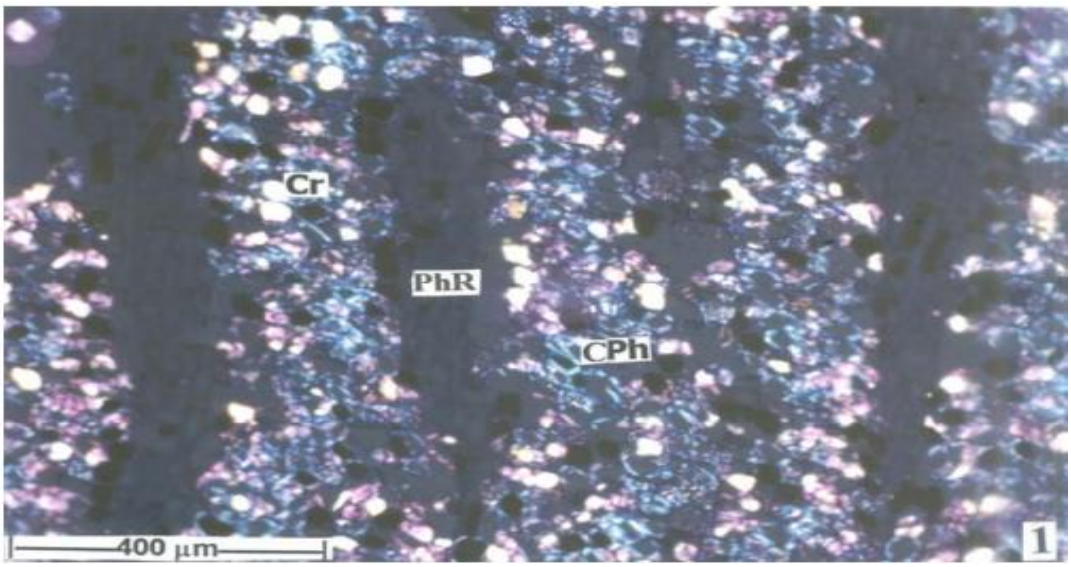

Fig.9: TS of bark showing crystals and starch grains

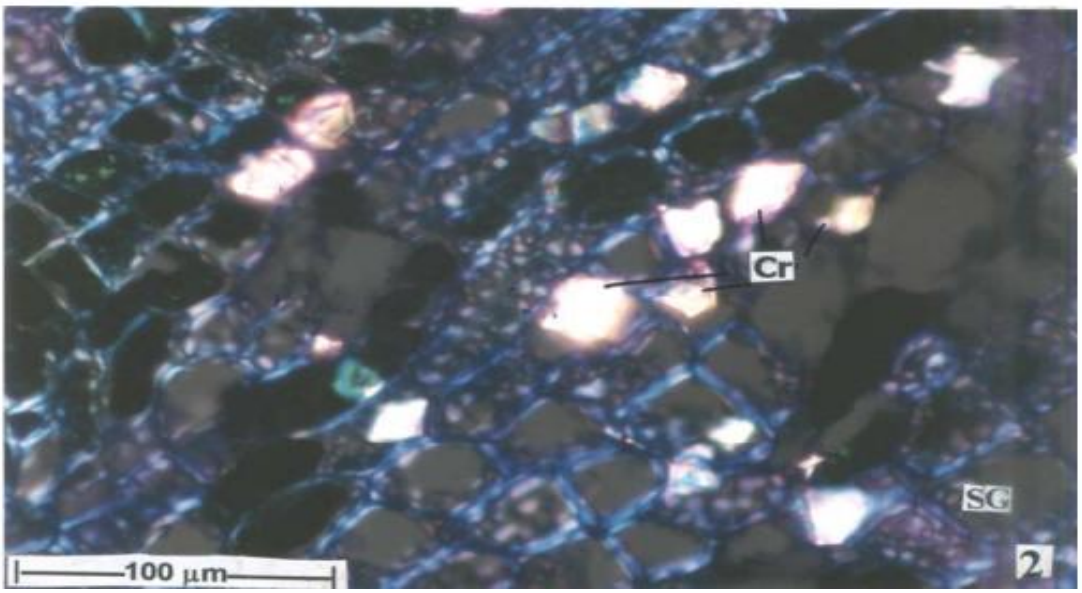

Fig.10: Enlarged crystal

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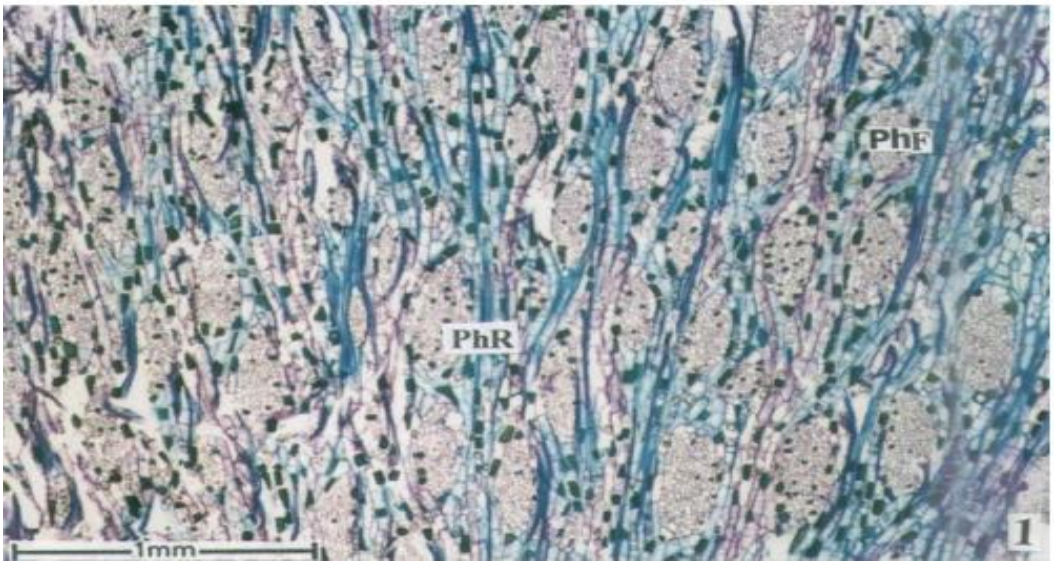

Fig.11: TLS of Non-collapsed phloem

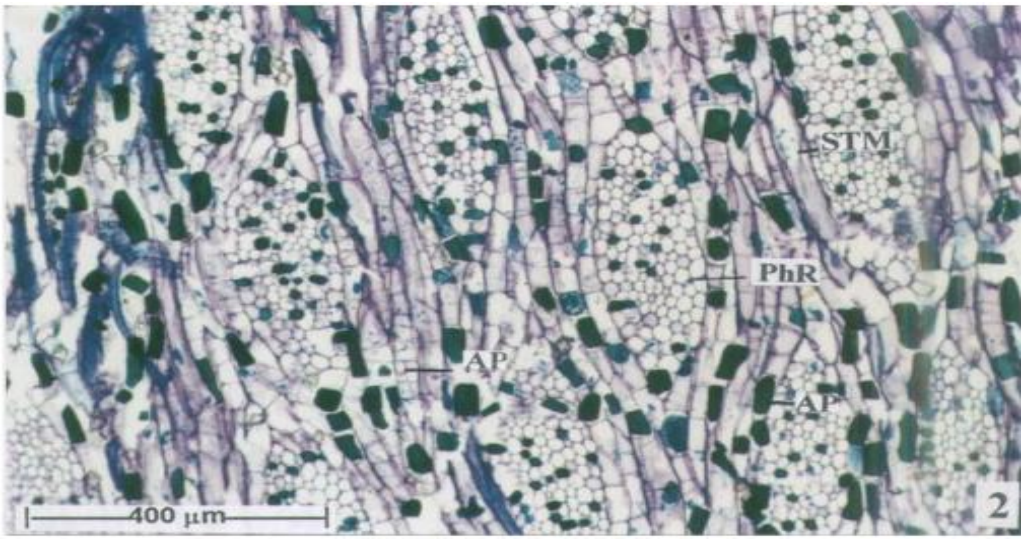

Fig.12: TLS of Non-collapsed phloem – enlarged Ficus racemosa l.

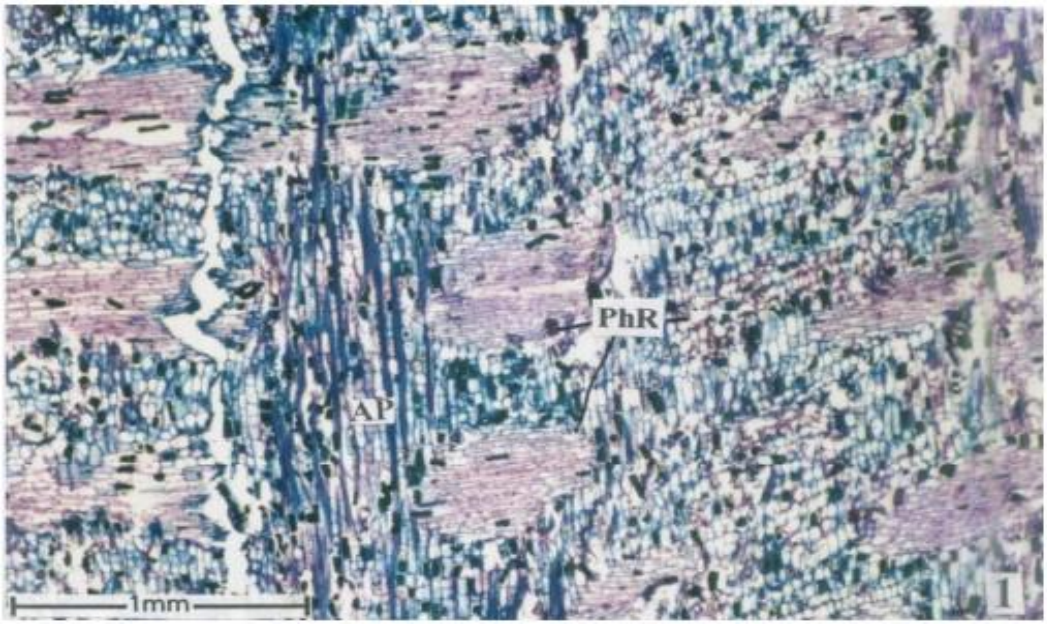

Fig. 13: R.L.S of Non-Collapsed Phloem

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Determination of Foreign Matter in the Formulation, FS002

When the air-dried formulations were tested for the presence of foreign matter, it was discovered that there was 0.01 g/100 g of foreign matter. The results of FS002 were discovered to be within normal bounds. Insect and mould presence were investigated. There are no insects or mould seen in the compositions (7).

Physiochemical evaluation

The following categories of physiochemical evaluation were applied to the chosen formulations and their raw materials:

i. Calculating ash values
ii. Estimating moisture content and extractive value, respectively, in ii
iii. Formulation particle size determination
iv. Using Atomic Absorption Spectrometry, Zinc in NP003 was determined to be present.

Ash value determination

The definition's complete Debris esteem, 14.26 0.35 w/w (FS002)

The definition's complete Debris esteem, NP003 = 34.26 0.46 w/w

Corrosive insoluble debris

The formulation's FS002 acid insoluble ash value is 1.39 0.14 w/w.

The formulation's acid insoluble ash value, NP003 = 10.59 0.54

Water soluble ash

The weight distinction between absolute debris and the buildup following complete debris treatment with water is known as the water-solvent debris.

The formulation's water-soluble ash value, FS002 = 12.2 0.46 w/w

The formulation's water-soluble ash value, NP003 = 1.2 0.13 w/w

Determination of Extractive values

Water soluble extractive

For FS002, the water-solvent extractive worth is 12.5 0.32 w/w.

For NP003, the water-solvent extractive worth is 10.5 0.22 w/w.

Alcohol soluble extractive

For FS002, the extractive value that dissolves in alcohol is 16.7 0.32 w/w.

The extractive value for NP003 in alcohol is 14.5 0.22 w/w.

extractives solvency in n-hexane, chloroform, and methanol

The extractive for FS002 that is solvent in n-hexane is 15.483 0.42 w/w.

The extractive for FS002 that is soluble in chloroform is 14.534 0.32 w/w.

The extractive for FS002 that is soluble in methanol is 18.786 0.32 w/w.

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The extractive for NP003 that is n-hexane soluble is 5.482 0.35 w/w.

The extractive for NP003 that is soluble in chloroform is = 4.634 0.64 w/w.

Extractive for NP003 soluble in ethanol = 16.426 0.46 w/w

Phytochemical Studies

Preliminary phytochemical screening

To lay out the profile of the provided concentrate's synthetic cosmetics, different subjective tests were completed. To distinguish the various phytoconstituents present in the concentrates and its plan, subjective compound tests were run on them.

Ficus racemosa Linn's bark was subjected to qualitative chemical analyses, which identified a number of phytochemicals in hexane, alcohol, aqueous, and petroleum ether extracts. The presence of sterols was demonstrated by the hexane, petroleum ether, and alcoholic extract. While tannins were visible in the alcohol, aqueous, and petroleum ether extracts, saponins were visible in the chloroform, petroleum ether, aqueous, and extracts (8) .

Similar subjective synthetic examinations were led again for plan FS002, uncovering the presence of flavonoids in petrol ether extricate notwithstanding comparative phytochemicals in tantamount solvents.

Table 1: First Phytochemical Tests on Ficus racemosa Bark (9)

Extractive	Reducing sugar	Flavonoid	Alkaloid	Sterols	Tannins	Saponins
Hexane	-ve	-ve	-ve	+ve	-ve	-ve
Alcohol	-ve	-ve	-ve	+ve	+ve	+ve
Chloroform	+ve	-ve	-ve	-ve	-ve	+ve
Water	+ve	-ve	-ve	-ve	+ve	+ve
Pet-ethet 60-80	-ve	-ve	-ve	+ve	+ve	+ve

Table .2: Phytochemical preliminary screening of FS002 formulation (10)

Extractive	Reducing sugar	Flavonoid	Alkaloid	Sterols	Tannins	Saponins	Protein
Hexane	-ve	-ve	-ve	+ve	-ve	-ve	-ve
Alcohol	-ve	-ve	-ve	+ve	+ve	+ve	-ve
Chloroform	+ve	-ve	-ve	-ve	-ve	-ve	+ve
Water	-ve	-ve	-ve	-ve	+ve	+ve	+ve
Pet-ethet 60-80	-ve	+ve	-ve	+ve	+ve	+ve	-ve

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Standardisation of the herbal formulations by HPTLC fingerprinting

HPTLC tests were performed on the Ficus racemosa bark (FR sample) and its preparation, FS002.

When the FR sample was scanned densitometrically at 254, 366, and 600 nm, 4, 8, 4, and 4 peaks were detected. However, when scanned at the aforementioned wave length, its formulations displayed 3, 6, and 8 peaks. Peaks at 254 nm for the formulation (FS002) sample and the FR samples with Rf values of 0.36 and 0.75 overlap (11).

The peaks of the FR sample and FS002 sample differed at 366 nm, although three peaks (Rf values 0.12, 0.21, and 0.50) were shared by both samples.

At 600 nm, the peaks of the FR sample and FS002 differed similarly, however two peaks (Rf values 0.42 and 0.63) were discovered to be common.

Table 3: HPTLC studies of the Ficus racemosa Linn bark and its formulation (tabulated Rf values) (12)

S.No	Track	Sample applied	Volume applied(μl)	RF Values @254nm	RF Values @366nm	RF Values after Anisaldehyde treatment@600nm
1	T1	FR	10	0.37,0.53,0.65,0.76	0.13,0.22, 0.31,0.45 0.51,0.64 0.77,0.84	0.43,0.58,0.65, 0.78
2	T2	FS 002	10	0.36,0.73,0.77	0.13,0.22, 0.50,0.61, 0.82,0.86	0.23,0.36,0.43, 0.50,0.55,0.56, 0.64,0.74

Microbial Standardization of the Herbal Formulation

Materials from restorative plants commonly contain a few microscopic organisms and molds, a considerable lot of which come from soil. The normally happening microflora of spices is comprised of a wide assortment of microscopic organisms and parasites, however oxygen consuming spore-framing microbes prevail (13). Current strategies for creation, dealing with, and gathering might bring about microbial expansion and further contamination. Escherchia coli and shape recognition might be marks of creation quality.

Determination of Enterobacteriaceae and presence of E. coli

There was no discernible increase in any of the three samples. As a result, the formulation passes the microbiological standardisation test. However, after 90 days of storage, the FS002 formulation exhibited the presence of E. coli and other enterobacteria, while the herbo-mineral NP003 displayed no detectable microbial presence (14).

Determination of total viable aerobic count Plate count method

As indicated by the test convention, the absolute suitable count of the example being scrutinized was determined. At the end of the fifth day, colonies are counted, and the findings are tabulated. A formulation batch is deemed unsuccessful and unfit for human consumption if its count is greater than 104.

After 30, 60, and 90 days of preparation, bacterial counts for the formulations FS002 and NP003 were examined. After days, the formulations NP003 and FS002 displayed a colony count of 103 and 102, respectively. The formulations FS002 and NP003 revealed a colony count of > 104 and 103 respectively after 90 days, compared to 103 and 102 after 60 days for the two formulations, respectively. After 90 days, the sample FS002 failed the test for total viable count.

The standard types of Escherchia coli (ATCC No. 25922) were utilized to approve tests for specific microorganisms. Candida albicans (ATCC No. 10231), Aspergillus niger (No. 16400), and Bacillus sublitis (No. 6633) are three instances of molds.

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Table 4: Determination of Enterobacteriaceae and presence of E.coli in FS002 (15)

Formulation	Presence or absence of E. coli per g of FS002		
	BI	BII	BIII
Freshly prepared	-	-	-
After 30 days:	-	-	-
After 60 days:	-	-	-
After 90 days:	+	+	+

Table 5: Determination of Enterobacteriaceae and presence of E.coli in NP003

Formulation	Presence or absence of E. coli per g of FS002		
	SI	SII	SIII
Freshly prepared	-	-	-
After 30 days:	-	-	-
After 60 days:	-	-	-
After 90 days:	-	-	-

Table 6: Total viable bacterial count of the different batches of the formulation FS002

Formulation	Viable bacterial count per g of FS002		
	BI	BII	BIII
Freshly prepared	10^2	10^2	10^2
After 30 days:	10^3	10^3	10^3
After 60 days:	10^3	10^3	10^3
After 90 days:	$>10^4$	$>10^4$	$>10^4$

Table 7: Results of total viable count of different batches of formulation FS002

Formulation	BI	BII	BIII
Freshly prepared	+	+	+
After 30 days:	+	+	+
After 60 days:	+	+	+
After 90 days:	-	-	-

Table 8: Total viable bacterial count of the different batches of the formulation NP003

Formulation	Viable bacterial count per g of NP002		
	SI	SII	SIII
Freshly prepared	10^2	10^2	10^2
After 30 days:	10^2	10^2	10^2
After 60 days:	10^3	10^3	10^3
After 90 days:	10^3	10^3	10^3

Table 9: Results of total viable count of different samples of formulation, NP003

Formulation	SI	SII	SIII
Freshly prepared	+	+	+
After 30 days:	+	+	+
After 60 days:	+	+	+
After 90 days:	+	+	+

Table 10: Ficus racemosa ethanol extract and FS002 formulations reduced blood sugar levels in rats (15).

Group I-VI	Body weight (G)		Liver glycogen (mg/g wet tissue)	Serum Glucose (mg/dl)			
	Initial	Final		Day			
				1	5	10	15
Normal (control)	209.7±5.5	215.3 ± 3.1	49.1±1.9	90.1±0.6	88.4±4.3	86.5±3.7	89.1±2.9
0.5ml of gingelly oil	210.7±2.5	202.5±5.5	49.1±3.3	279.5±4.9	269.8±8.7	265.3±4.9	275.5±5.3
FG (200 mg/kg) body weight	209.5±4.7	204.6±4.9	4.4.1±2.5[a]	286.1±8.3	225.1±10.1 *	193.1±4.9 **	170.5±4.1 **
EE1(200 mg/kg) body weight	208.7±3.5	205.7±5.5	40.7±3.3[a]	287.1±4.9	217.1±9.4 *	163.1±7.6 **	141.1±4.4 **
Standard glibenclaimed (500 mcg/kg) body weight	207.7±3.5	206.5±5.5	40.4±2.3 [a]	281.5±4.3	190.0±7.7 *	166.1±6.9 **	129.1±8.8 **
TaNP003(80mg /kg)	207.5±3.5	205.7±4.5	25.7±3.3$_a$	277.1±3.9	197.1±9.4 **	197.1±9.4 **	141.1±4.4 **
NP003(200mg/ kg)	215.7±2.5	210.5±5.5	23.1±3.3$_a$	299.5±5.9	181.8±8.7 **	181.8±8.7 **	141.5±5.9 **
Standard glibenclaimed (500 mcg/kg) body weight	206.7±3.5	203.5±5.5	40.4±2.3 $_a$	271.5±4.3	170.1±5.7 **	172.1±6.7 **	125.1±5.8 **

Results: The HM group demonstrated a significant reduction in migraine frequency, duration, and severity compared to the control group ($p < 0.001$). VAS, MIDAS, and PSS scores were significantly reduced after 90

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days of treatment. HRV analysis revealed improved autonomic balance with increased parasympathetic activity (17). SEMG recordings showed reduced frontalis muscle tension. The formulation exhibited notable antioxidant and anti-inflammatory activity. No serious adverse effects were reported.

SUMMARY AND CONCLUSION

The study suggests that people who experience migraine headaches may gain from a plan that incorporates both conventional drugs and herbal therapy. This comprehensive approach enhances patient quality of life and symptom management while reducing migraine-related impairment. The gainful impacts could be credited to various variables, including diminished apparent tension, worked on autonomic equilibrium, and decreased muscle strain.

The discoveries of this study demonstrate that there is a promising future for the synergistic coordination of at least two clinical frameworks. Consolidating the upsides of a few treatment strategies can assist medical care specialists with upgrading the clinical results for headache cerebral pain victims. By perceiving the worth of both natural treatment and traditional medication, this coordinated strategy features the significance of a comprehensive and all encompassing treatment plan.

More review and exploration are expected to approve these discoveries and grasp the instruments hidden the synergistic impacts of joining different clinical frameworks. Nonetheless, the consequences of this study give areas of strength for a to thinking about integrative ways to deal with headache treatment, opening up additional opportunities for working on quiet consideration and results.

The discoveries of this study give consoling proof to the adequacy of natural treatment related to traditional drugs in the treatment of headache cerebral pains. Consolidating at least two clinical frameworks can have synergistic impacts that diminish side effects, work on personal satisfaction, and decrease headache related incapacity for patients. The review features the meaning of tending to a scope of headache pathophysiology issues, including pressure decrease, autonomic equilibrium, and muscle strain. These discoveries show the potential for a complete and comprehensive treatment methodology that utilizes the upsides of a few treatment modalities. Extra exploration and assessment in this field will work with the advancement of proof based suggestions for coordinating natural treatment with regular medication, which will ultimately improve clinical results and patient consideration.

Initial phytochemical examination of petroleum ether, ethyl acetate derivatives, hard drinks, and aqueous concentrates of knotweed, canthium dichocum, Ochna obtussata and Argireia nervosa ridged pieces revealed alkaloids, phenols, tannins, flavonoids, terpenoids, the presence of various optional plant metabolites such as steroids, glycosides and proteins were revealed.

Absolute phenolic and flavonoid contents of plants were measured using the Folin-Ciocalteu and AlCl3 strategies. The absolute phenolic contents of shards of Polygonum, Canthium dichocum, Ochuna obtusata and Argireia nervosa are considered to be 13.16, 8.06, 4.13, 8.53%, 1.5, 1.53, 0.26%, gasoline ether and ethyl acetate derivatives, divided into heavy drinkers, concentrates. The aeronautical pieces of Polygonum glabrum, Canthium dicoccum, Ochna obtusata, and Argyreia nervosa were removed with oil ether, ethyl acetic acid derivation, liquor, and water, and their particular complete flavonoid contents were viewed as 0.116, 0.266, 0.15, and 0.166 mg QE, 1.10, 1.525, 1.316, and 1.23 mg QE, and 1.

The ethanol concentrate of the picked plants showed the most significant levels of phytoconstituents, a more noteworthy rate yield, and a higher centralization of phenolic and flavonoid content in light of the fundamental phytochemical study. Hence, the ethanol concentrate of the four plants was picked for extra exploration. A selection of ethanolic extracts from the flying parts of Polygonum glabrum, Canthium dicoccum, Ochna obtusata, and Argyreia nervosa were used to generate the polynatural divisions (PHF1-PHF5).

To isolate and identify individual components or combinations of components of the polynatural fractions (PHF1–PHF5) containing varying amounts of ethanol concentrates from the aerial fragments of knotweed, cantium dichocum, and Ochna obtusata, enrichment was performed. Subjective thin-layer chromatography was

performed on the material. The poly home grown parts PHF1-PHF5 were surveyed for their all out phenolic and flavonoid content. PHF2 and PHF4 among the concentrated on parts had more prominent centralizations of complete phenolic content (16.26 percent by weight and 12.52 percent by weight) and all out flavonoid content (2.453 mg Q and 1.652 mg Q, individually).

Aerial parts of plants Polygonum glabrum, Canthium dicoccum, Ochna obtusata and Argyreia nervosa with petroleum ether (PE), ethyl acetate derivatives (EA), Dranquard (Et) and concentrates (Aq) at 10-100 g/ml used in different fixations. The picked plants' all's ethanol removes showed a significant concealment of free extremists among the tried concentrates. Thus, utilizing the DPPH extremist and nitric oxide revolutionary searching strategy, poly home grown parts PHF1-PHF5 containing ethanol concentrate of the picked plants in different proportions were created.

With an IC50 worth of 54 g/ml, 56 g/ml, 48 g/ml, 52 g/ml, and 49 g/ml, individually, the poly natural parts PHF1-PHF5 showed extensive free revolutionary searching action at higher measurements of around 89%, 98%, 99, 82, and 94%. With an IC50 worth of 51 g/ml, 58 g/ml, 50 g/ml, 54 g/ml, and 53 g/ml, separately, the poly natural portions PHF1-PHF5 shown impressive free revolutionary rummaging movement at higher measurements of around 92%, 96%, 92, 82, and 84%.

The poly natural parts PHF1-PHF5 were in this manner tried for hostile to joint movement involving in-vitro models to recognize the most proficient poly home grown portions, which contained ethanol concentrate of the picked plants in fluctuating sums.

Rodents managed with the improved poly natural case PHCF4 (300 mg/kg b.w.) showed a significant decrease in the volume of rodent paw oedema (4.23 0.0 mm) when contrasted with the benchmark group in the Total Freund's Adjuvant-prompted joint model. After enlistment with Freund's adjuvant, the normal prescription indomethacin decisively diminished the paw thickness, estimating 5.9 0.6 mm.

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