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STANDARDIZATION AND QUALITY ASSESSMENT OF TRADITIONAL POLYHERBAL FORMULATION FOR THE TREATMENT OF HEMORRHOIDS (PILES)

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Keywords:

Polyherbal, Hemorrhoids, Microscopic, Phytochemical, Anti-oxidant, Anti-bacterial

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ABSTRACT: Polyherbal formulations are a collection of therapeutic entities that are formulated and prepared on the basis of the healing properties of individual ingredients with respect to the condition of sickness. The main objectives of the present study were to evaluate the pharmacognostic parameters of a traditional polyherbal formulation for the treatment of hemorrhoids (piles). The pharmacognostic parameters *i.e.*, microscopic, physicochemical, phytochemical, antioxidant, anti-bacterial assay and FTIR analysis, was evaluated. The microscopic study of plant materials gives details about anatomical identity. The phytochemical evaluation of crude extract (methanolic and aqueous) of gutika revealed the presence of bioactive compound. In DPPH and H_2O_2 scavenging assay of methanolic extract (IC_{50} 200 µg/ml) has better antioxidant activity than aqueous extract (IC_{50} 250 µg/ml). The study also revealed that the methanolic extract showed the highest total phenolic and flavonoid content followed by aqueous extract. The FTIR spectra of both methanolic and aqueous extracts revealed the presence of polyphenols, terpenes and alkaloids. The antibacterial activity of methanolic extract shows highest zone of inhibition than aqueous extract. The highest zone of inhibition occurred in *E. coli* (16 mm in methanolic and 14 mm in aqueous) and lowest in *E. aerogenes* (11 mm in methanolic and 7 mm in aqueous) at 50 mg/mL concentration. The present study provided quality control standards of the traditional herbal formulation, which may help for the better utility and safe use of this formulation in the treatment of hemorrhoids.

INTRODUCTION: Traditional medicine is the knowledge, skills and practices based on the theories, beliefs, and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose, improve or treat physical and mental illnesses [1-2]. So, the World Health Organization (WHO) has appreciated the importance of medicinal plants for public health care in developing countries and has evolved guidelines to support the member states in their efforts to formulate national policies on traditional medicine and to study their potential usefulness including evaluation, safety and efficacy [3-4].

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A major discovery of the past two decades in the field of gastrointestinal disorder has been the elucidation of understanding the mechanism of anorectal problems. This is commonly known as piles or hemorrhoids [5]. Hemorrhoids are one of the most common gastrointestinal disorders seen by general practitioners. It has been estimated that they can occur at any age and can affect both men and women [6].

The natural evaluation of hemorrhoids is benign in nature, but they tend to get worse over time, and therefore they should be treated as soon as it occurs. The term hemorrhoid (or piles) is used to describe the enlargement of the venous tissues of the anal region, which becomes inflamed or prolapsed [7]. The main objectives of the present study were to evaluate the pharmacognostic parameters of a traditional polyherbal formulation for the treatment of hemorrhoids.

MATERIALS AND METHODS:

Methodology of the Study: The information about polyherbal formulation (*i.e.*, gutika) was selected based on traditional practices. A traditional healer, Mr. Rajen Borah (54 years old) who has been practicing the treatment of hemorrhoids for the last 18 years in the Sripani gaon panchayat, Dhemaji District, particularly tribal inhabitant area lying in the border region of Arunachal Pradesh and 20 km distance from Dhemaji town, was the informant. The formulation comprises of leaves and stems of *Leucas aspara* willd Link, *Eclipta prostate* L., and *Houttunia cordata* Thunb. It is also useful for the treatment of indigestion and dyspepsia. The formulation can be used as a tablet or gutika for oral dosage forms. For preliminary pharmacognostic evaluation, the crude drug (*i.e*., gutika and plant material) was collected from the traditional healer and worked according to WHO guidelines and standard protocols.

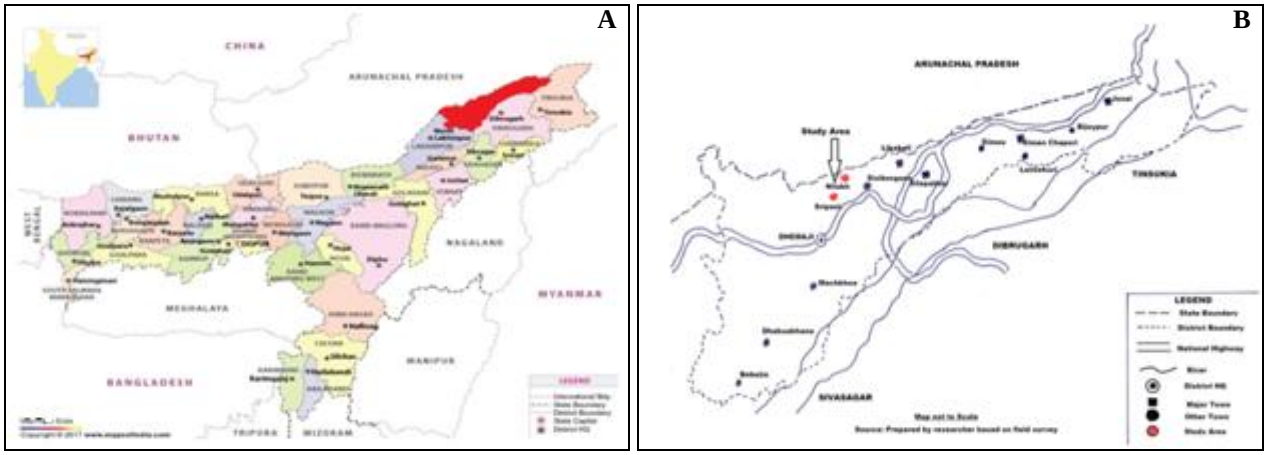

FIG. 1: STUDY AREA: A): DHEMAJI DISTRICT IN ASSAM (RED AREA); B): SURVEY AREA IN DHEMAJI DISTRICT

Plant Species Identification: The information of the plant species used in gutika formulation was collected from the Herbal practitioner. The voucher specimens were submitted to the Department of Herbal Science and Technology, ADP College, Nagaon, for future reference **Table 1**. Further, taxonomic names of plant species were confirmed from online databases, namely: The Plant List (http://www.theplantlist.org) [8].

TABLE 1: ENUMERATION OF PLANT SPECIES USED IN GUTIKA FORMULATION FOR THE TREATMENT OF HEMORRHOID

S. no.	Botanical name/ Herbarium voucher no.	Family	Local name	Habit	Part used
1	*Leucas aspara* willd Link HST-0020	Lamiaceae	Ass.- Durunbon	Herb	Leaf, young stem
2	*Houttuynia cordata* Thunb HST-0020	Saururaceae	Ass.- Mosondoi	Herb	Whole part
3	*Eclipta prostata* L. HST-0020	Asteraceae	Ass.- Kehraj	Herb	Leaf and Stem

N.B. Ass.- Assamese

Microscopic Study of Plant Material: For microscopic evaluation, thin section of different plant parts (*i.e.* T.S and L.S of leaf, stem and roots), the structure of pollen grain, trichome, stomata and crude powder of formulation were studied under Foldscope microscope- an origami-based paper microscope [9].

Collection of Gutika and Preparation of Crude Powder: Freshly prepared gutika was collected from the herbal practitioner and crushed in a mixer grinder to a coarse powder. The dried powder was stored in airtight bottles at room temperature for further analysis.

Physicochemical Evaluation: Physicochemical analysis of the formulation has been done to evaluate the quality and purity of the formulation.

The physicochemical constants such as ash value (*i.e.* total ash, acid insoluble ash, water-soluble ash), extractive value (*i.e.* water and alcohol soluble extractive value), moisture content, pH value and physical characteristics of crude powder *(i.e.* Bulk density, Tape density, Angle of repose, Hausner's ratio, Compressibility / Carr's index *etc.*) were determined by following standard protocol [10-14].

FIG. 2: A): POLYHERBAL FORMULATION (GUTIKA) PREPARED BY TRADITIONAL HEALER, AND B): FORMULATION FOR TREATMENT OF HEMORRHOIDS

Preparation of Plant Extract by Soxhlet Extraction: 10 grams of the crude powder prepared from the sample (gutika) were placed in a thimble. 100 ml of solvent (methanol and water) separately used for extraction. The extraction was carried out at the boiling point of the solvent for a duration of 4 to 5 h. The extracts obtained were transferred to a water bath and evaporated at 40 ºC for obtaining residues. All the residues so obtained were kept in airtight vials and stored in the refrigerator at 4 ºC for further analysis.

Preliminary Phytochemical Screening: The preliminary phytochemical analysis was carried out for both methanolic and aqueous extracts to determine the presence of following bioactive compounds using the standard qualitative procedures [15-16].

Quantitative Estimation of Phytochemicals:

Estimation of Total Phenolic Content: The concentration of phenolic compounds in methanol and water extract of the crude drug was determined by using Folin-Ciocateau (FC) method with a slight modification [17]. Extract solution of 1mg/ml was prepared by dissolving in distilled water. Folin – Ciocalteu reagents was added to the extract solution and incubated for 60 min at room temperature followed by the addition of Na_2CO_3. The same procedure was prepared for the standard solution of Gallic acid (GAE), and the calibration line was constructed. After incubation at room temperature for 60 min, the absorbance was measured at 760 nm.

Total phenolic content in different plant extracts were calculated as Gallic acid equivalent (GAE) –

Total Phenolic Content (T) = C × V/M

Where, T = Total phenolic content (mg/g) of extract as GAE, C = Concentration of GA established from the calibration curve in (mg/ml), V = Volume of the extract solution in ml, M = Weight of extract in g

Estimation of Total Flavonoids Content: The concentrations of total flavonoids in crude extracts were determined according to the Aluminium Chloride method with a slight modification [18]. 1mg/ml plant extract was dissolved in distilled water for the estimation of flavonoids. The plant extract solution was mixed with 5% $NaNO_2$ after 5 minutes, of which 10% $AlCl_3.6H_2O$ was added. To the mixture solution, 1 Molar NaOH was added, and the volume was adjusted to 1 ml. The same procedure was prepared for the standard solution of Quercetin, and the calibration line was constructed. The reaction mixtures were then incubated for 30 min at room temperature. The absorbance was determined using a UV-VIS spectrophotometer at 510 nm. Flavonoid content was calculated from the graph made with standard quercetin and finally expressed in terms of quercetin equivalent (mg/g of extract) as same as phenols content.

***In-vitro* Antioxidant Activity:**

DPPH Method: The ability of the plant extract to scavenge DPPH free radicals was assessed by the standard method with slight modification [19-20]. The stock solution of crude extract and standard (Gallic acid monohydrate), was prepared in methanol to achieve the concentration of 1mg/ml. Dilutions of the stock solution was made to obtain the concentrations of 50, 100, 150, 200, 250, 300 and 350 µg/ml.

Diluted solution (0.5 ml each extract) was mixed with an aliquot of 2.5 ml of 0.1 mM DPPH solution. After 90 min incubation in darkroom temperature (23 °C) absorbance of the mixture was recorded at 517 nm using a UV-VIS spectrophotometer. A blank sample was prepared by using all reagents except plant extract. The data were presented as mean values ± standard deviation. % of scavenging activity was calculated as follows

Scavenging % = The absorbance of control – Absorbance of samples / Absorbance of control × 100

All the tests were performed in triplicates.

Hydrogen Peroxide (H_2O_2) Radical Scavenging Activity: Hydrogen peroxide is a colorless, odorless, syrupy liquid in the anhydrous state. Pure hydrogen peroxide is unstable, but a powerful oxidizing agent. The scavenging ability of extracts (methanolic and aqueous extracts) to hydrogen peroxide was measured by standard method [21]. A solution of hydrogen peroxide (40 mM, *i.e.,* 3.66 ml) prepared in 96.34 ml phosphate buffer at a pH of 7.4. The stock solution of both plant extract (*i.e.,* methanolic and aqueous) and standard (gallic acid) was prepared by dissolving 1 mg extract and gallic acid in 1 ml methanol in the concentration of 1 mg/ml respectively.

The solution of plant extract and standard was prepared at the following different concentrations (50, 100, 150, 200, 250 and 300µg/ml) by adding methanol and adjust 400µl total volume. Then different dilution concentration was added to 0.6 ml hydrogen peroxide solution. The sample mixture and standard solution were than stand for 10 min. A blank solution was prepared to contain the 0.6 ml H_2O_2 solution and 0.4 ml methanol. Thereafter, the absorbance of sample mixtures and standard were determined at 230 nm by UV-VIS spectrophotometer. The hydrogen peroxide radical scavenging activity of the extract was reported as % inhibition and calculated by using the following equation.

% scavenging = The absorbance of control – Absorbance of sample / Absorbance of control × 100

All the tests were performed in triplicates.

Detection of Functional Group by Fourier Transform - Infra-Red Spectrophotometer (FTIR): Fourier Transform- Infrared Spectroscopy (FTIR) is an analytical technique used to identify organic (and in some cases inorganic) compounds. This technique measures the absorption of infrared radiation by the sample material versus wavelength. FTIR is one of the most widely used analytical techniques for standardization and evaluation of the plant-based product.

FTIR has proven a valuable tool for the identification and monitoring of the functional groups of chemical compounds in plants. Because of the plants contain different types of bioactive compounds that are used for a different therapeutic purpose or used as a precursor for the synthesis of useful drugs. Both methanolic and aqueous extracts prepared from gutika formulation were analyzed using the FTIR spectrophotometer at Biotechnology Sciences Division, CSIR-NEIST, Jorhat.

Microbiological Evaluation: The extracts (methanol and water) were evaluated for antibacterial assay against two-gram positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* and three-gram negative bacteria *Escherichia coli*, *Enterobacter aerogenes,* and *Klebsiella pneumonia*. The organisms were purchased from MTCC, Chandigarh, (Punjab), and India and maintained in nutrient agar slants at 4 ºC.

The antibacterial evaluation of different bacterial strains was tested by Agar Well Diffusion method [22-23]. The diluted bacterial culture was swabbed over the nutrient agar plates by sterile bud. Three wells of 6 mm diameter were made by sterile cork-borer in agar plate aseptically. The wells were then loaded with 40, 60 and 100 µl of extract (50 mg/1ml in DMSO) and allowed to dry and incubated at 37 ºC for 18 h. The inhibition zones were recorded in the test samples as well as standard. The assay was repeated thrice.

Determination of Microbial Contamination: A crude powdered drug was prepared from gutika formulation for determination of the presence of *E. coli* by Agrawal and Paridhavi [24]. 1 gm of the fine powdered crude drug was mixed with 100ml lactose broth media. Then the mixture solution was incubated for 4 h at 35-37 ºC. After incubation, 1ml sample taken and serial dilutions are made with 9 ml of nutrient broth media of concentrations 100

mg/ml, 10 mg/ml, 0.1 mg/ml and 0.01 mg/ml. These samples are incubated at 37 ºC for 18 h. Then 1 ml of each was inoculated on MacConkey agar media and further incubated for 24 h at 35-37 ºC. After incubation, colony characteristic of the MacConkey agar plate was observed under the Digital colony counter. The growth of red, generally non-mucoid colonies, indicates the presence of *E. coli*.

RESULTS AND DISCUSSION:

Microscopic Study of Plant Material:

Leucas aspara willd Link:

Microscopic Observation of Leaf: Transverse section of leaf midrib is broadly convex on the lower side and slightly grooved or flat on the upper side, centrally located conjoint and collateral meristele with a parenchymatous pericycle layer on the lower side.

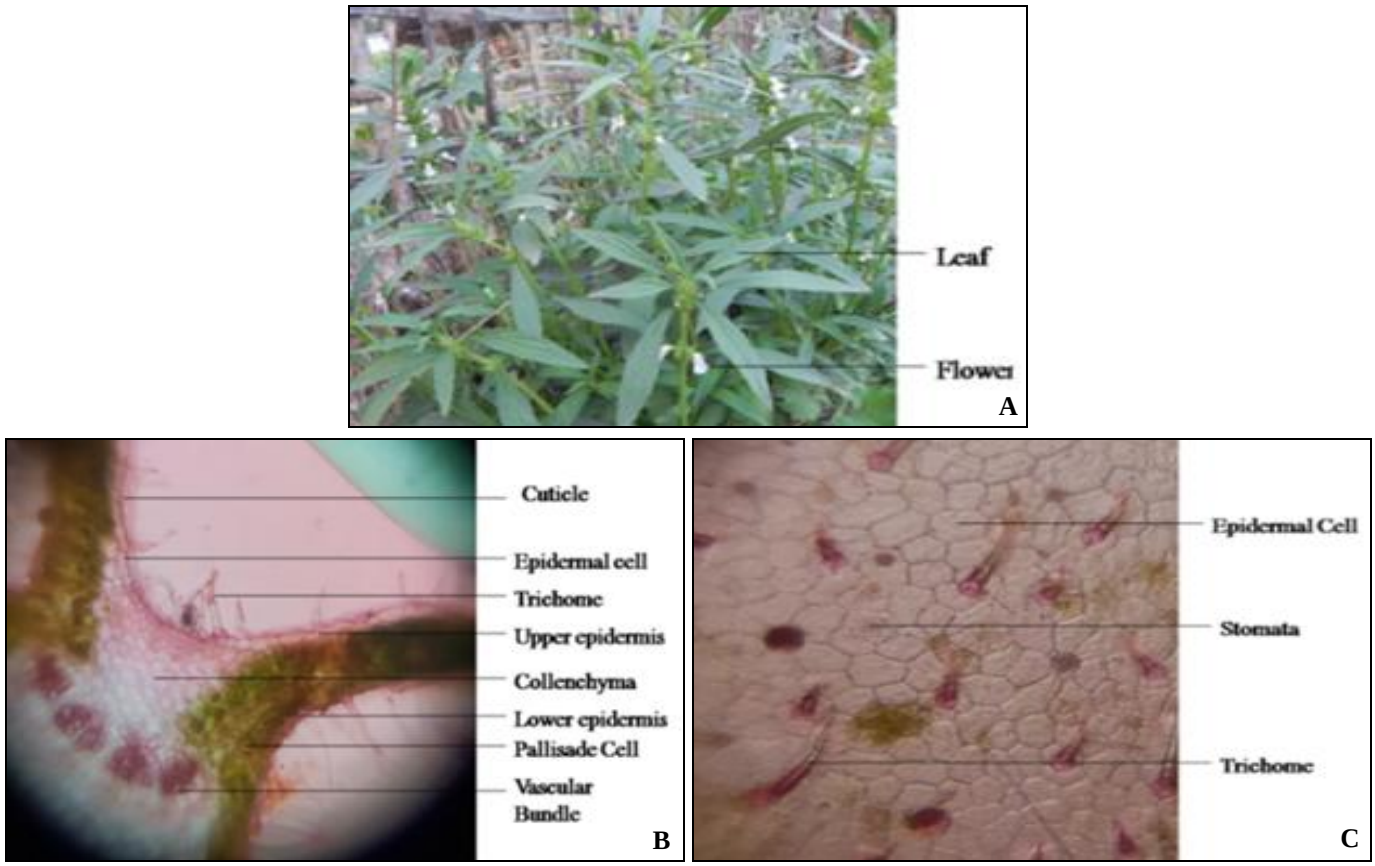

FIG. 3: *LEUCAS ASPERA* (A) PLANT, (B) T. S. OF LEAF WITH MIDRIB, (C) STOMATA WITH TRICHOME (L. E.)

The epidermis of lamina covered by cuticle, with simple, multi-cellular and glandular trichomes. The stomata of both upper and lower epidermis were paracytic in nature. Glandular and unicellular trichome was observed on the leaf surface.

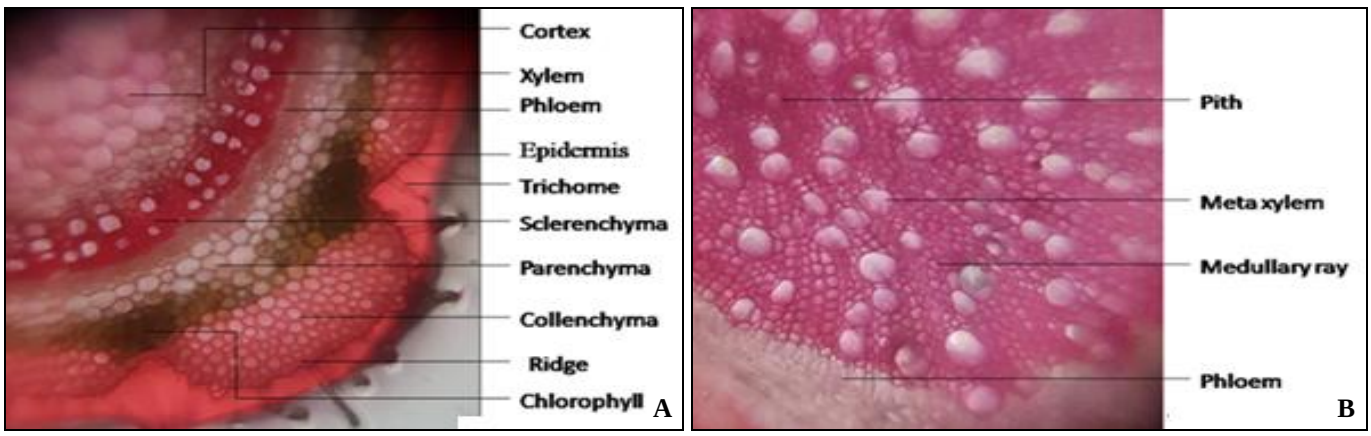

FIG. 4: *L. ASPERA*, (A) T. S. OF STEM, (B) T.S. OF ROOT

Microscopic Observation of Stem: The T. S. of the young stem is quadrangular with four collenchyma ridges, covered with thick cuticle and multi-cellular trichome. It shows a narrow parenchymatous cortex and distinct endodermis. The ring of vascular bundles connected with the sclerenchymatous band, very narrow parenchymatous phloem and radially arranged xylem tissue.

Microscopic Observation of Root: T. S. of the root is circular in outline with thick epidermis. Cortex cells are tangentially elongated and irregular. Xylem vessels, tracheids, medullary tube and secondary phloem were distinct.

***Houttuynia cordata* Thunb:**

Microscopic Observation of Leaf: The transverse section of the leaf showed the epidermal layer (upper and lower), which is made up of a single layer of parenchymatous cells covered with a cuticle layer. The leaf is of isobilateral nature bearing a single-layered of wide palisade cells in both upper and lower epidermis continued over the midrib region.

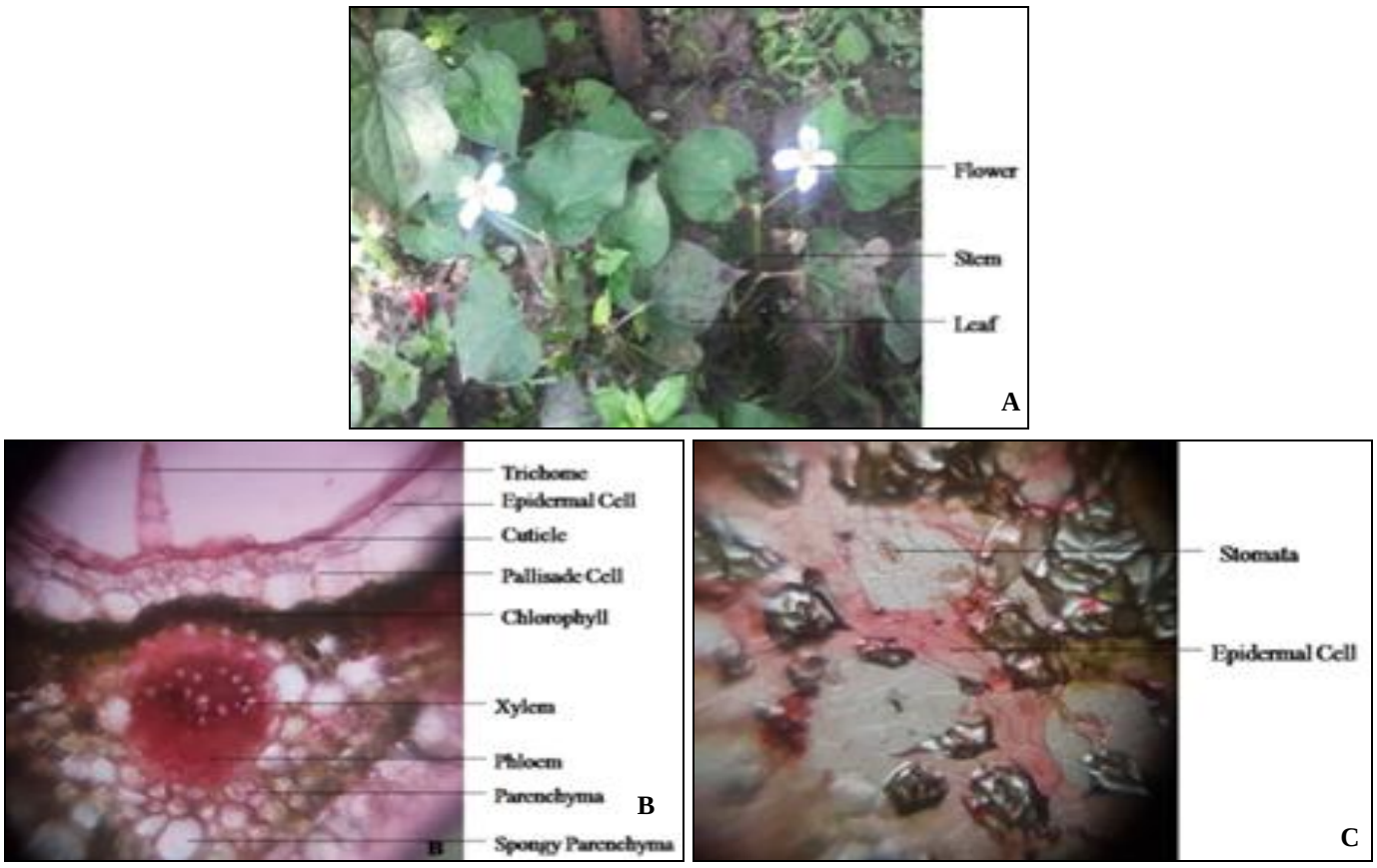

FIG. 5: *HOUTTUYNIA CORDATA*, (A) PLANT, (B) LEAF MIDRIB, (C) STOMATA (L.E.)

Trichome present on the upper epidermis is multi-cellular in nature. The central region of the midrib contains the vascular bundle bearing phloem and lignified xylem vessels with spiral thickenings; in addition, the leaf at several places of the lamina bear the presence of a ranunculaceous type of stomata on the lower surface of the leaf.

Microscopic Observation of Stem: The T.S. of the stem shows an outer layer of the epidermis, followed by a single layer of palisade cell, 5-6 layers of collenchymascells, sclerenchyma, pericycle and inner cortex. A complete ring of sclerenchyma fibers is present outside the vascular bundles with a width of one to three cell layers. Deposition of starch grain and calcium oxalate crystal found in the inner- cortex region.

***Eclipta prostata* L:**

Microscopic Observation of Leaf: The transverse section of the leaf shows the presence of upper and lower epidermis, mesophyll and midrib region. The midrib contains vascular Bundles. Both upper, as well as lower epidermis of leaf T.S. has unicellular glandular trichomes.

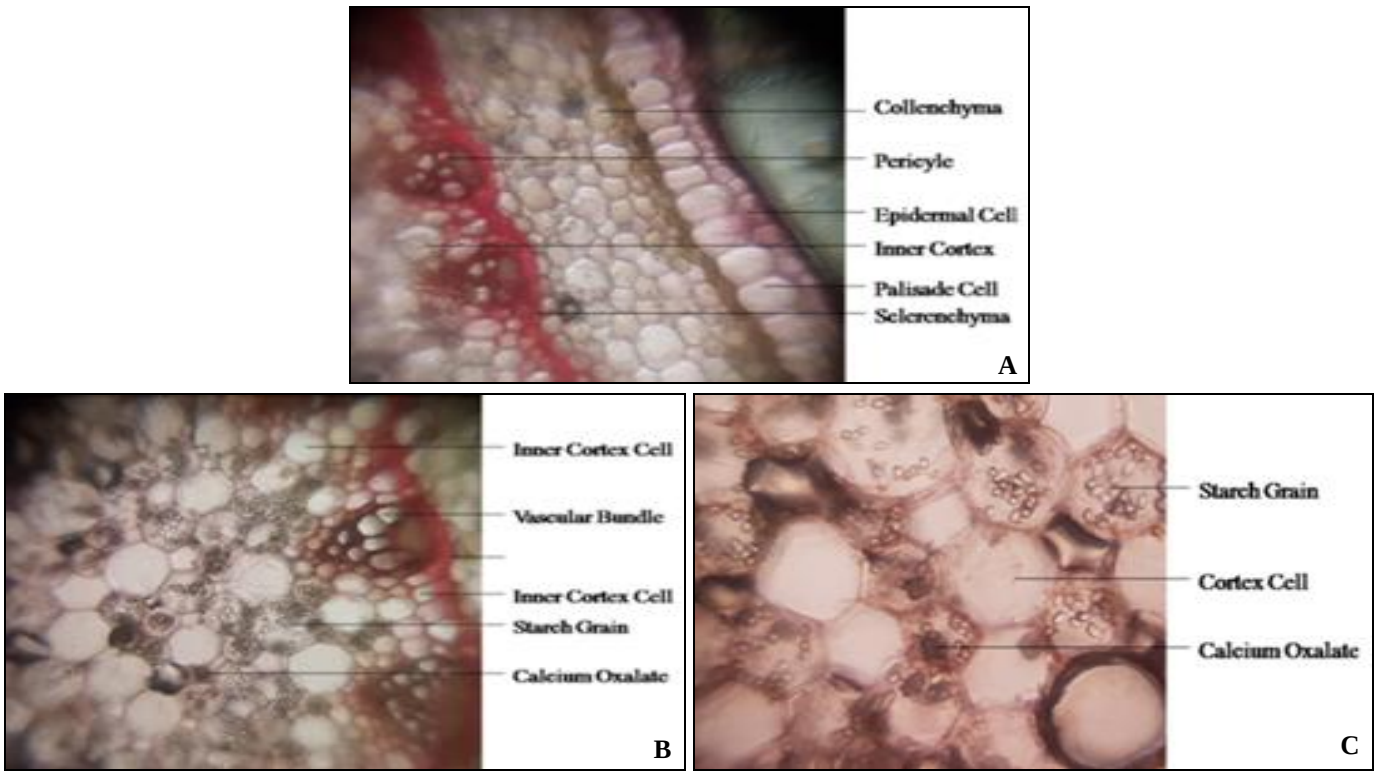

FIG. 6: *H. CORDATA*, (D) T. S. OF STEM, (E) INNER CORTEX REGION OF STEM, (F) CALCIUM OXALATE AND STARCH GRAIN IN INNER CORTEX REGION (ENLARGED VIEW)

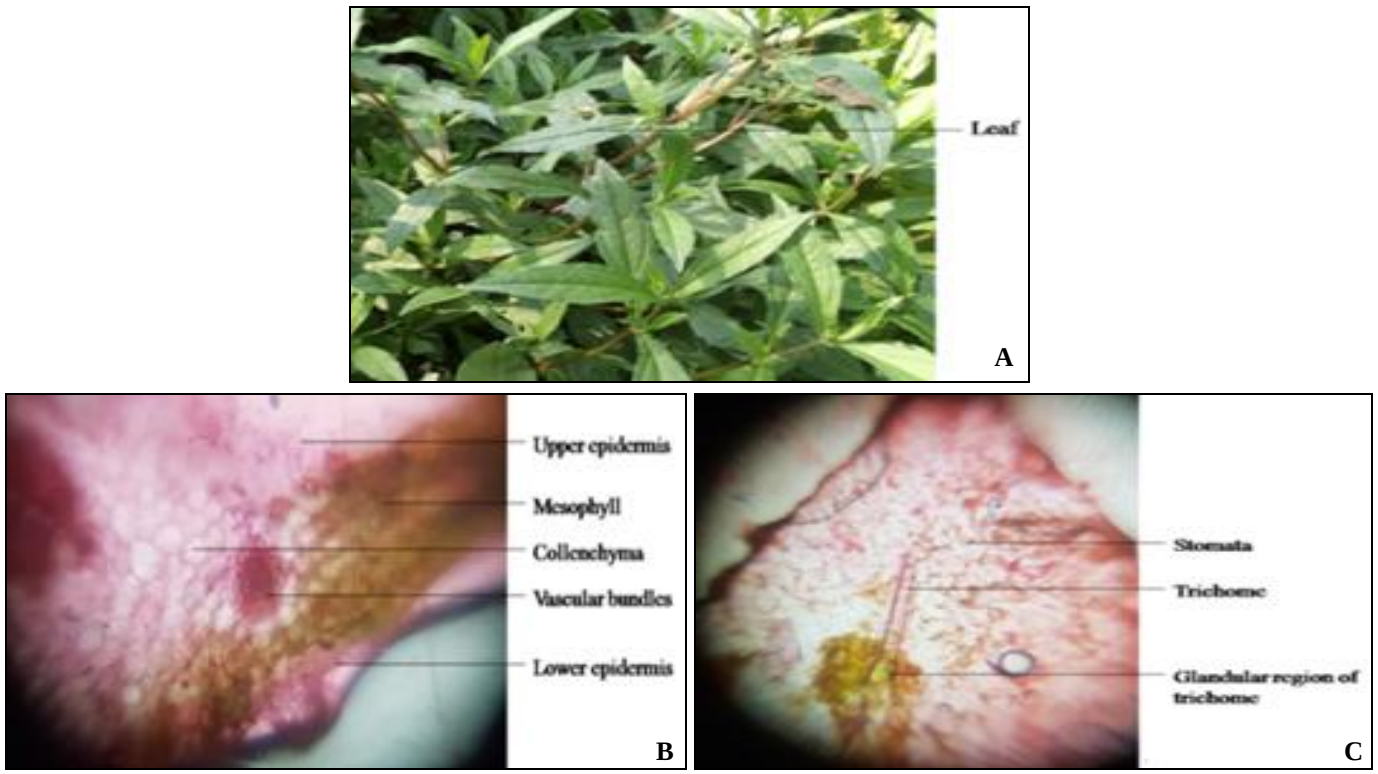

FIG. 7: *ECLIPTA PROSTATA*: (A) PLANT, (B) T.S. OF LEAF, (C) STOMATA AND UNICELLULAR TRICHOME (L.E)

Microscopic Observation of Stem: The transverse section of stem shows an outermost layer of epidermis, cortex and vascular bundle covered with per cyclic fibers and pith. The epidermis is two to three-layered and formed of quadrangular cells. The cortex shows the presence of layers of spongy parenchyma cells. Below the cortex, lignified per cyclic fibers are present which cover the vascular bundle. Below this, a well developed vascular bundle is present.

Microscopic Observation of Root: The T.S. of outermost layers of root covered by thick cuticle, followed by cork cells and tangentially elongated aerenchyma cells with air cavities. The parenchymatous cortex cells are irregular shaped. The xylem composed of vessels, fiber-tracheids and medullary ray rays.

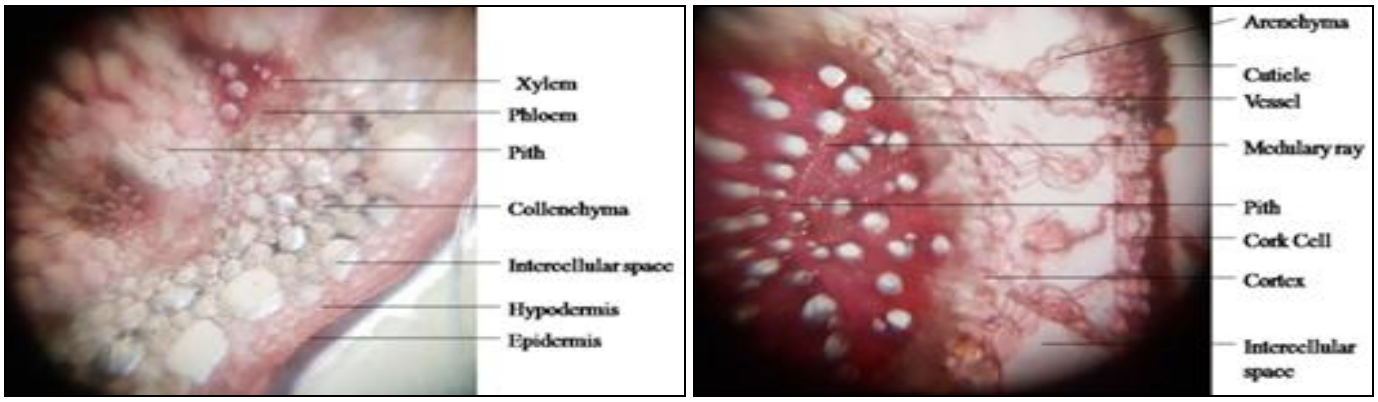

FIG. 8: *E. PROSTATA*- T.S. OF STEM (LEFT), T.S. OF ROOT (RIGHT)

Physicochemical Study of Polyherbal Formulation: The physicochemical parameters **Table 2** of formulation (*i.e.* Gutika) showed significant results with total ash value (11.23% w/w), water-soluble ash (6.13% w/w), acid insoluble ash (5.04%), water-soluble extractive value (31.84% w/v), alcohol soluble extractive value (20.04% w/v), moisture content (11.23% w/w) and pH (6.04 ± 0.04) as determined by WHO guidelines for quality control and evaluation of herbal drug formulations.

TABLE 2: PHYSICOCHEMICAL PARAMETERS OF CRUDE POWDER PREPARED FROM POLYHERBAL FORMULATION

S. no.	Parameter		Result
1	Ash value	Total ash	11.23% (W/W)
		Water-soluble ash	6.13% (W/W)
		Acid insoluble ash	5.04% (W/W)
2	Extractive value	Water soluble extractive value	31.84% (W/V)
		Alcohol soluble extractive value	20.4% (W/V)
3	Moisture content		11.23% (W/W)
4	pH		6.04 ± 0.04
5	Physical characteristic of a crude drug	Bulk density (g/ml)	0.312 ± 0.012
		Taped density (g/ml)	0.454 ± 0.013
		Angle of Repose (Ɵº)	38.7º ± 0.12
		Compressibility index (%)	31.28 ± 0.021
		Hausner's ratio	1.46 ± 0.013

Pre formulations studies have a significant part to play in anticipating formulation problems and identifying logical path in liquid, semisolid and solid dosage form drug formulation. The physical characteristic of crude powder like bulk density (0.312 ± 0.012 g/ml), tapped density (0.454 ± 0.013 g/ml), angle of repose (Ɵº) (38.7º ± 0.12), compressibility index (%) (31.28 ± 0.021), and Hausner's ratio (1.46 ± 0.013) were recorded. These parameters play an important role in preformulation study of tablet preparation.

Preliminary Phytochemical investigation: The preliminary phytochemical analysis of two (methanolic and aqueous) crude extracts of formulation shows the presence of alkaloids, glycosides, flavonoids, phenols, saponins and carbohydrates **Table 3**.

TABLE 3: PRELIMINARY PHYTOCHEMICAL SCREENING OF CRUDE EXTRACT PREPARED FROM FORMULATION

S. no.	Plant constituent	Chemical test	Extract	
			Methanolic	Aqueous
1	Alkaloids	Dragendroff's test	+	+
		Mayer's test	-	-
2	Carbohydrates	Fehling's test	+	+
		Benedict's test	+	+
3	Flavonoids	Alkaline reagent test	+	+
4	Glycosides	Keller killani test	-	-
		Conc. H_2SO_4 test	+	+
5	Phenols & Tannins	$FeCl_2$ test	+	+
		Lead acetate test	+	+
6	Saponins	Foam test	-	+
7	Proteins & Amino acids	Xanthoprotein test	-	-

Note: '+' indicate present and '-'indicate absent

Quantitative Phytochemical (Phenols and Flavonoids) Estimation: Phenols and flavonoids are the secondary plant metabolites that have strong antioxidant activities that act as potent radical terminators by donating hydrogen to the free radical. Total phenolic content in methanolic and aqueous extracts calculated from the calibration curve (R^2 = 0.882), was 165.95 ± 4.48 mg and 109.18 ± 4.94 mg gallic acid equivalents/g respectively. On the other hand, total flavonoids content (R^2 = 0.973) was 125 ± 3.55 mg and 69.44 ± 2.89 mg quercetin equivalents/g **Table 4**.

TABLE 4: TOTAL PHENOLS AND FLAVONOIDS CONTENT

Compounds	Extracts	
	Methanol	Water
TPC in GAE (mg/g)	165.95± 4.48	109.18 ± 4.94
TFC in QE (mg/g)	125± 3.55	69.44± 2.89

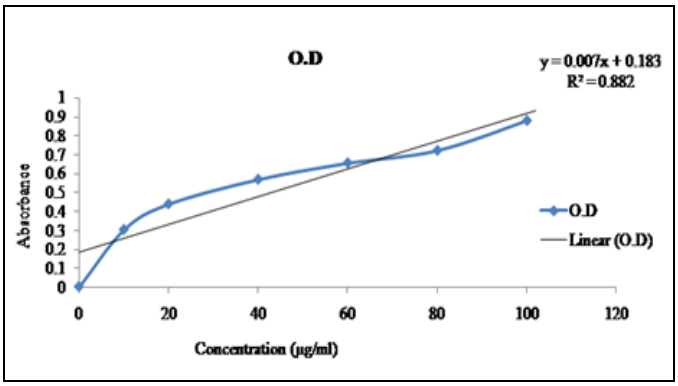

FIG. 9: STANDARD CURVE OF GALLIC ACID FOR PHENOL ESTIMATION

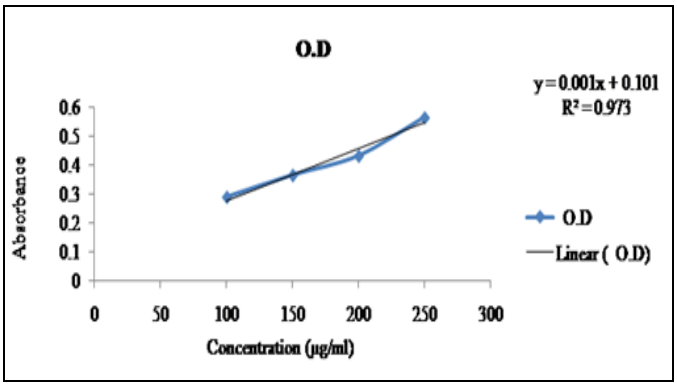

FIG. 10: STANDARD CURVE OF QUERCETIN FOR FLAVONOID ESTIMATION

Antioxidant Activity of Polyherbal Formulation: The antioxidant activity of methanolic and aqueous extracts was evaluated by DPPH and H_2O_2 methods. In both DPPH and H_2O_2 methods, methanol crude extracts shows IC_{50} values at 200 µg/ml and inhibition % was 54.10 ± 1.03 in DPPH and 53.12 ± 0.67 in H2O2 method. In case of aqueous extracts, it shows IC_{50} values at 250 µg/ml and inhibition % was 57.54 ± 0.56 in DPPH and 56.67 ± 1.05 in H_2O_2 method. These results indicated that both methanolic and aqueous extracts exhibited the highest ability to scavenge the DPPH free radical comparable to Hydrogen peroxide (H_2O_2). The results also indicated that methanol extracts show good antioxidant activity (IC_{50} values at 200 µg/ml) than water extracts (IC_{50} values at 250 µg/ml).

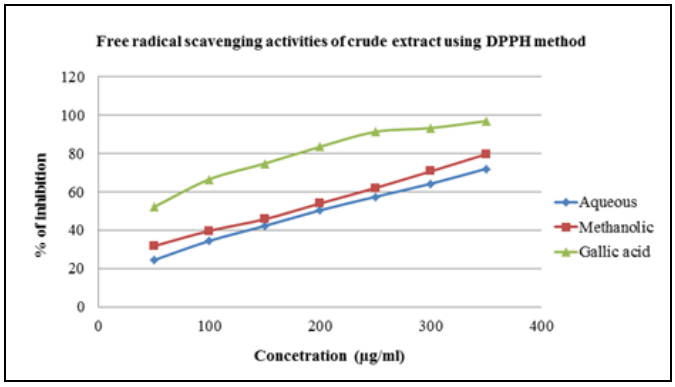

FIG. 11: FREE RADICAL SCAVENGING ACTIVITIES OF CRUDE EXTRACT (FORMULATION) OF DPPH METHOD

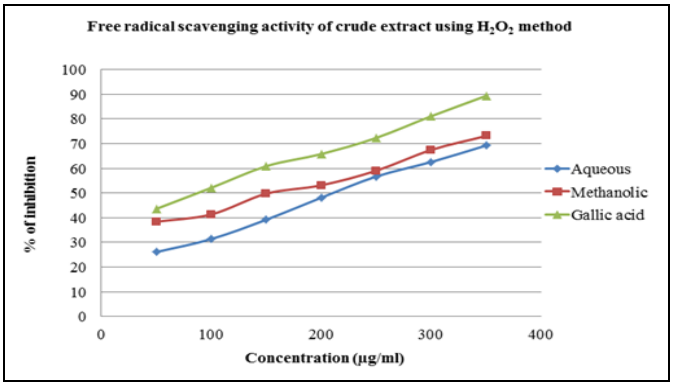

FIG. 12: FREE RADICAL SCAVENGING ACTIVITY OF CRUDE EXTRACT (FORMULATION) OF H_2O_2 METHOD

FTIR Analysis: Results of FTIR Spectra of the methanolic and aqueous crude extracts revealed the presence of different functional groups. The major absorption peaks in FTIR spectra of methanolic extract of polyherbal formulation located mainly at 3337.81 cm^{-1}, 2925.34 cm^{-1}, 1622.73 cm^{-1} and 1051.13 cm^{-1} and minor peaks at 2853.83 cm^{-1}, 1395.21 cm^{-1}, 1365.05 cm^{-1}, 1362.92 cm^{-1}, 1245.59 cm^{-1}, 915.88 cm^{-1}, 864.39 cm^{-1}, 814.87 cm^{-1} and 770.51 cm^{-1} and in aqueous extract, major

absorption peaks mainly located at 3335.63 cm^{-1}, 1584.74 cm^{-1}, 1394.97 cm^{-1} and 1062 cm^{-1} and in minor peaks located at 2943.84 cm^{-1}, 1309 cm^{-1}, 1259 cm^{-1} and 923.35 cm^{-1} in the region of 500 cm^{-1}, 4000 cm^{-1}. The FTIR Spectra of crude extracts *(i.e.* aqueous and methanolic extract) of polyherbal formulation revealed the presence of polyphenols due to O-H stretch at the 3335.63 cm^{-1} and 3337.81 cm^{-1}, terpenes were present with C-H (Stretch) at the 2943.84 cm^{-1}, 2925.34 cm^{-1} and 2853.83 cm^{-1}, Alkaloids are present due N=O stretch at the 1365.05 cm^{-1} and 1362.92 cm^{-1}, C- N stretch at the 1245.59 cm^{-1}, 1309 cm^{-1}, 1259 cm^{-1} and 1062 cm^{-1}, and N-H (bend) at 1584.74 cm^{-1}. Terpene was present with C=C (Stretch) at the 1622.73 cm^{-1} **Table 5**.

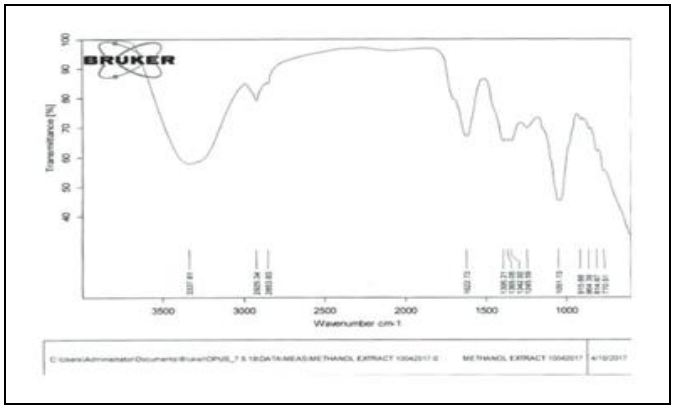

FIG. 13: FTIR ABSORPTION PEAK IN METHANOLIC EXTRACTS OF FORMULATION

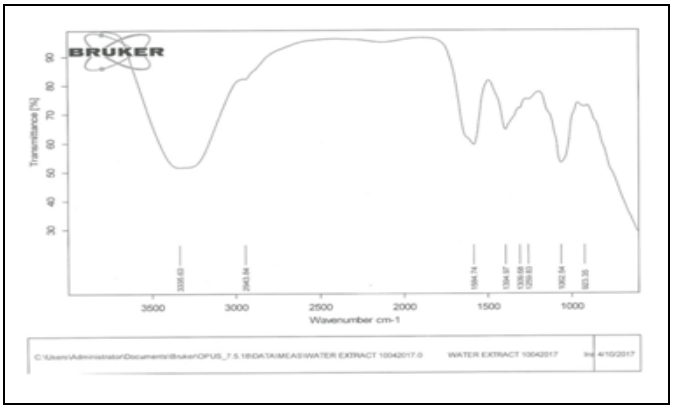

FIG. 14: FTIR ABSORPTION PEAK IN AQUEOUS EXTRACT OF FORMULATION

TABLE 5: FTIR SPECTRA ANALYSIS OF FORMULATION EXTRACTS

Functional groups	Components (Peaks)	Absorption spectra (Frequency cm^{-1})	
		Aqueous extract	Methanolic extract
Alcohols & Phenols	O – H (Bonded) (3400 – 3200 cm^{-1})	3335.63	3337.81
3º Alcohol	O –H (Bend) (1410- 1310 cm^{-1})	1394.97	1395.21
Alkane	C-H (Stretch) (3000-2850 cm^{-1})	2943.84	2925.34,2853.83
Alkene	C= C (Stretch) (1680 – 1600 cm^{-1})		1622.73
1º & 2º Amines and Amides	N-H (bend) (1640- 1550 cm^{-1})	1584.74	N. D
	C-N (Stretch) (1350- 1000 cm^{-1})	1309,1259 ,1062	1245.59, 1051.13
Aromatic Nitro compound	N=O (Symmetric Stretch) (1370 – 1330 cm^{-1})	N.D	1365.05, 1362.92
Aromatic phosphates	(P-O-C stretch) (995- 850 cm^{-1})	923.35	915.88, 864.39
Chloride (Aliphatic)	Chloride (Aliphatic)	N. D	770.51
Esters	Esters	N. D	864.39, 814.87

N. D. = Not Detected

Determination of Antibacterial Activity: The antibacterial activity of formulation extracts (methanolic and aqueous) was evaluated against two-gram positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* and three-gram negative bacteria *Escherichia coli*, *Enterobacter aerogenes* and *Klebsiella pneumoniae* in a concentration of 50 mg/ml. The methanolic extract exhibited a stronger antibacterial activity against all pathogenic bacterial strains, *Escherichia coli* (16 mm) followed by *Bacillus subtilis* (15 mm), *Klebsiella pneumoniae* (13 mm), *Enterobacter aerogenes* (11mm) and *Staphylococcus aureus* (12 mm). Whereas, 14 mm, 9 mm, 12 mm, 13 mm and 7 mm diameter zone of inhibition was seen in aqueous extract against above pathogenic bacteria. From the above results, it is revealed that the anti-microbial activity of methanolic extract showed significant results against all the bacterial strains, whereas, the inhibitory effect of aqueous extract is comparatively low against *Bacillus subtilis* and *Enterobacter aerogenes* **Table 6**.

TABLE 6: ANTIBACTERIAL ACTIVITY OF FORMULATION EXTRACTS AGAINST PATHOGENIC BACTERIAL STRAINS

Tested bacteria	Zone of inhibition (diameter) mm		
	Aqueous 50 mg/ml	Methanolic 50 mg/ml	Ciprofloxacin 1 mg/ml
Escherichia coli	14	16	44
Klebsiella pneumonia	12	13	40
Staphylococcus aureus	13	12	36
Bacillus subtilis	09	15	38
Enterobacter aerogenes	07	11	32

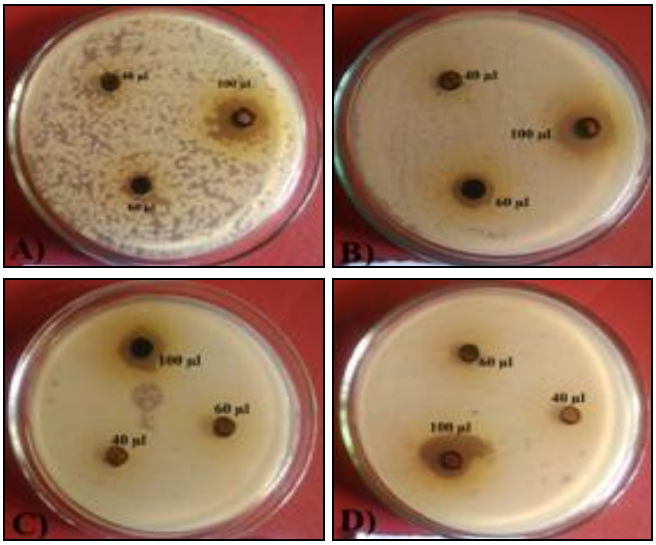

FIG. 15: ANTI-MICROBIAL ACTIVITY OF METHANOLIC EXTRACT; (A) *ESCHERICHIA COLI*, (B) *BACILLUS SUBTILIS*, (C) *KLEBSIELLA PNEUMONIA*, (D) *STAPHYLO-COCCUS AUREUS*

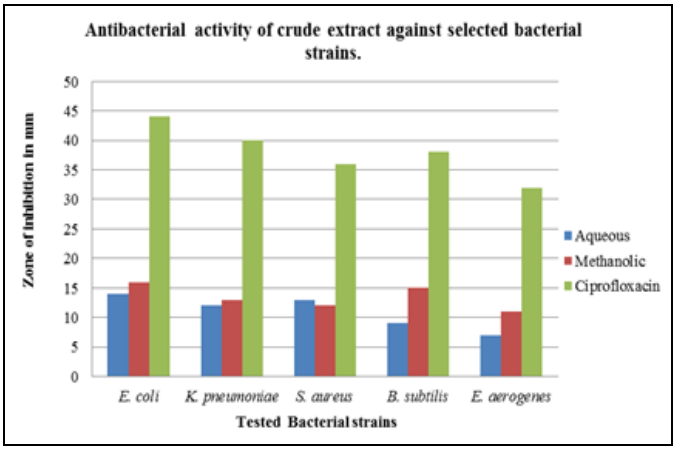

FIG. 16: ANTIBACTERIAL ACTIVITY OF CRUDE EXTRACT OF FORMULATION AGAINST SELECTED BACTERIAL STRAINS

Determination of Microbial Contamination (*E. coli* test): Determination of microbial contamination is one of the most important parameters for the standardization of herbal drugs. Because of the medicinal plant may associate with a broad variety of microbial contaminants, represented by bacteria, fungi and viruses. Stability test against microbial contaminants *(i.e.* test for *E. coli*) was performed according to WHO guidelines. The prepared formulation was kept in a tight container at room temperature for 45 days. After 45 days, the sample was tested for *E. coli*. According to WHO guidelines, the maximum limits of *E. coli* is 10^1/gram. No bacterial colony was seen on the MacConkey agar plates at different dilutions. From this result, it was revealed that the formulation was strong stable against contamination of *E. coli*.

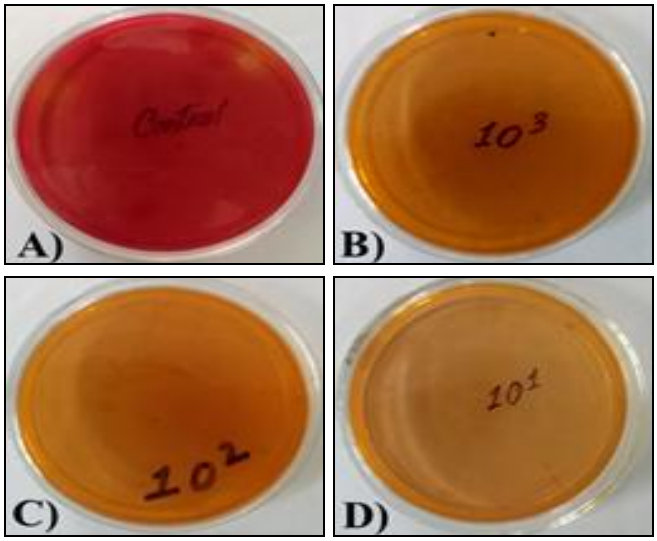

FIG. 17: THE INCUBATED SAMPLE SOLUTION ARE INOCULATED ON MACCONKEY AGAR AT (A) CONTROL; (B) 1000 mg/ml; (C) 100 mg/ml; D: 10 mg/ml

CONCLUSION: The proper botanical identification of plant samples used in gutika formulation for the treatment of hemorrhoids (piles) provides useful information to develop modern drugs. Microscopic observation is the first step in the identification and evaluation of plant samples. The study of the Transverse section (T.S), stomata, trichome types, and different types of intercellular deposition of the crude drug give authentic information about the plant. The physicochemical characteristics of formulation revealed the appropriate information about pH, ash value, extractive value and physical characteristics of the powdered drug. The preliminary phytochemical study and FTIR analysis of crude drugs indicated the presence of important bioactive compounds like alkaloid, glycoside, phenols, flavonoids, tannins tannin, saponin and carbohydrates. The crude extracts prepared from formulation showed good antioxidant and antibacterial activity.

The microbial contamination test of crude drug strongly evidenced that the drug sample was completely free from *E. coli*. Besides this, in an antibacterial assay, the methanolic extract showed the highest inhibition rate against *E. coli*. The present study thus concluded that, above physicochemical parameters and *in-vitro* bioassay provided quality control standards which may help in the authenticity of the drug and to compile suitable information as per the pharmacopoeial

monograph for the better utility and safe use of this formulation in the treatment of hemorrhoids (piles).

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REFERENCES:

1. World Health Organization. Geneva: WHO Traditional Medicine Strategy 2013; 2014-2023.
2. Gureje O, Nortje G, Makanjuola V, Oladeji B, Seedat S and Jenkins R: The role of global traditional and complementary systems of medicine in treating mental health problems. Lancet Psychiatry 2015; 2(2): 168-77.
3. WHO. Traditional Medicine Strategy 2002-2005. Geneva: World Health Organization 2005.
4. Prakash BN, Pradeepa M, Sowmya S, Nagarajan M and Subramanian PV: Standardization of a Traditional Polyherbal Malaria Prophylactic Formulation. Asian Journal of Biomedical and Pharmaceutical Sciences 2014; 4 (28): 13-19.
5. Palpu P, Rawat A, Rao C, Srivastava S and Kartik R: Herbal Formulation for the treatment of piles. US 20070172529A1 Patent 2007.
6. Agbo SP: Surgical management of hemorrhoids. J Surg Tech Case Rep2011; 3: 68-75.
7. Aggrawal K, Satija N, Dasgupta G, Dasgupta P, Nain P and Sahu AR: Efficacy of a standardized herbal preparation (Roidosanal) in the treatment of haemorrhoids: A randomized, controlled, open label multicentre study. Journal of Ayurveda and Integrative Medicine 2014; 5(2): 117-24.
8. http://www.theplantlist.org
9. Cybulski JS, Clements J and Prakash M: Foldscope: Origami-Based Paper Microscope. PLOS ONE 2014; 9(6): e98781.
10. Qureshi MS, Reddy AV, Kumar GS and Nousheen L: Pharmacognostic, Physicochemical Standardization and Phytochemical Analysis of leaves of *Hydrolea zeylanica* Vahl. (Hydrophyllaceae). Research Journal of Pharmacognosy and Phytochemistry 2017; 9(1): 01-07.
11. Jain N, Jain P, Sahu D, Parkhe G and Jain SK: Formulation and optimization of sustained release floating gastro retentive tablet of nifedipine using natural polymer. Asian Journal of Pharmaceutical Education and Research 2016; 5(4): 38-48.
12. Rather GJ, Hamiduddin, Ikram M, Fatima S and Naquibuddin MD: Physicochemical Standardization of Polyherbal Powder Formulation: Safoof-e-Makhana. Pharmacogn J 2018; 10(5): 899-06.
13. Mangal AK, Tewari D, Srikanth N, and Singh H: Formulation and Standardization of Shatsakar Churna: Quality Control Studies for Polyherbomineral Ayurvedic Formulation. International Journal of Pharmacognosy and Phytochemical Research 2015; 7(4); 832-36.
14. Chukwuemeka PA, Jimson OO and Augustine OO: Physicotechnical, spectroscopic and thermogravimetric properties of powdered cellulose and microcrystalline cellulose derived from groundnut shells. J Excipients and Food Chem 2012; 3 (3): 106-15.
15. Yadav RNS and Agarwala M: Phytochemical analysis of some medicinal plants. J of Phytology 2011; 3(12): 10-14.
16. Gul R, Jan US, Faridullah S, Sherani S and Jahan N: Preliminary phytochemical screening, quantitative analysis of alkaloids, and antioxidant activity of crude plant extracts from *Ephedra intermedia* indigenous to Balochistan. The Scientific World Journal, vol. 2017, Article ID 5873648, 7 pages, 2017; 1-7.
17. Siddiqui N, Rauf A, Latif A and Mahmood Z: Spectrophotometric determination of the total phenolic content,spectral and fluorescence study of the herbal Unani drug Gul-e-Zoofa (*Nepeta bracteata* Benth). Journal of Taibah University Medical Sciences 2017; 12(4): 360-63.
18. Mathur R and Vijayvergia R: Determination of total flavonoid and phenol content in *Mimusops elengi* Linn. Int J of PharmaSciences and Research 2017; 8(12): 5282-85.
19. Saikia M and Handique PJ: Antioxidant and antibacterial activity of leaf, bark, pulp and seed extracts of Seabuckthorn (*Hippophae salicifolia* D. Don) of Sikkim Himalayas. J Med Plant Res 2013; 7(19): 1330-38.
20. MENG GE, Tian YC, Yang Y and Shi J: Evaluation of DPPH Free Radical scavenging activity of various extracts of *Ligularia fischeri in-vitro*: a case study of Shaanxi Region. Indian J Pharm Sci 2016; 78(4): 436-42.
21. Moonmun D, Majumder R and Lopamudra A: Quantitative phytochemical estimation and evaluation of antioxidant and antibacterial activity of methanol and ethanol extracts of *H. rostrata*. Ind J Phar Sci 2017; 79(1): 79-90.
22. Banik B, Sarkar P, Sultana F, Saikia M and Dey A: *In-vitro* antimicrobial screening with phytochemical study of *Plumbago zeylanica* L. collected from two regions of Eastern Himalayas- A comparative study. International Journal of Phytopharmacy 2014; 4 (5): 120-23.
23. Balouiri M, Sadiki M and Ibnsouda SK: Methods for *in-vitro* evaluating antimicrobial activity: a review Journal of Pharmaceutical Analysis 2016; 6: 71-79.
24. Agrawal SS and Paridhavi M: Herbal Drug Technology. Universities Press (India) Pvt. Ltd., Second Edition 2012.

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