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PHARMACOGNOSTICAL AND PHYTOCHEMICAL STUDIES OF POLYHERBS FROM INDIAN SYSTEM OF MEDICINE

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ABSTRACT

Polyherbals starts to gain its popularity recently worldwide, owing to the fact that it possesses some advantages which is not available in allopathic drugs. The polyherbs known to express high effectiveness in a vast number of diseases. As aforementioned, the therapeutic effects of herbal medicines are exerted due to the presence of different phytoconstituents and the effects are further potentiated when compatible herbals are formulated together in polyherbal preparations. Based on this the three medicinal herbs are selected . The leaves and stems of *Ipomoea staphylina* Roem. & Schult. belonging to the family convolulaceae occurs common on hedges and bushes in the forest and waste lands in Kurnool, Anantapur, Guntur, Cuddapah, Chittoor, and Visakhapatnam. *Ficus racemosa* linn belonging to the family Moraceae. The plant is scientifically proved for the glucose – lowering efficiency of a methanol extract of the stem bark of *Ficus racemosa* linn (Family Moraceae) both in normal and alloxan induced diabetic rats, based on this is plant has been selected. The third plant is leaves of *Araucaria bidwillii* belongs to the family Araucariaceae, is also reported for its antidiabetic activity. Combinations of these herbs are studied for its physicochemical and phytochemical analysis to identify and quantify the phytoconstituents present in it by TLC and HPTLC methods for standardization. The phytochemical constituents present in this facilitate the desirable therapeutic efficacy of medicinal formulation as whole in ailments and also helpful in underlying the mechanisms of pharmacological action.

Key words: *Ipomoea staphylina*, *Ficus racemosa* , *Araucaria bidwillii*, Phytochemicals and polyherbs.

INTRODUCTION

Medicinal plants are the major sources of medicines in Ayurvedha, Siddha, and Folk medicine systems. In India about 95% of all modern drugs are derived from medicinal plants and very likely most of these medicines are used by people to cure many ailments. The *Ayurvedic* literature *Sarangdhar Samhita*' highlighted the concept of polyherbalism to achieve greater therapeutic efficacy. The active phytochemical constituents of individual plants are insufficient to achieve the desirable therapeutic effects.

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When combining the multiple herbs in a particular ratio, it will give a better therapeutic effect and reduce the toxicity. Most of them are effective even at a low dose and safe at high dose, thus they have superior risk to benefit ratio. Based on this the present study deals with physicochemical , phytochemical studies such as and biochemical estimation of medicinal plant of combined mixture of polyherbs *Ipomoea staphylina. Ficus racemosa* , *Araucaria bidwillii* These plants possess analgesic, anti inflammatory, wound healing and anti diabetic, laxative and antibacterial and antifungal. Leaves and stem *Ipomoea staphylina* used for curing poisonous bites, including snake and scorpion bite, sexual diseases, jaundice, skin diseases, ulcer, dysentery, diabetes, common cold and fever (Parthasarathy, 2008). The stem latex is applied on to the cracked feet (fissures in foot) once in a day at bedtime for a week for healing the cracks. Literature shows all the three herbs poses anti diabetic

activity. Exploring of medicinal plants can only be achieved by evaluating and analyzing using sophisticated modern techniques of standardization.

MATERIALS AND METHODS

Selection of Indian medicinal plants

The leaves of *Ipomoea staphylina* Roem. & Schult. Belomging to the family convolulaceae Occurs Common on hedges and bushes in the forest and waste lands in Kurnool, Anantapur, Guntur, Cuddapah, Chittoor, and Visakhapatnam. Leaves and stem *Ipomoea staphylina* used for curing poisonous bites, including snake and scorpion bite, sexual diseases, jaundice, skin diseases, ulcer, dysentery, diabetes, common cold and fever.(Parthasarathy, 2008). The stem latex is applied on to the cracked feet (fissures in foot) once in a day at bedtime for a week for healing the cracks. (Anitha, 2008). Tamil palm leaf manuscript owned by a sidha practitioner report that the plant posses anti diabetic activity. It is also scientifically proved. Based on this plant is selected. *Ficus racemosa* linn belonging to the family Moraceae. Rao *et al.,* (2000) have reported the glucose – lowering efficiency of a methanol extract of the stem bark of *Ficus racemosa* linn (Family Moraceae) both in normal and alloxan induced diabetic rats, based on this is plant has been selected. The third plant is leaves of *Araucaria bidwillii* belongs the family Araucariaceae, is also reported for its antidiabetic activity.

Collection and Authentication of selected plants

The fresh aerial parts of *Ipomoea staphylina* **,** stem bark of *Ficus racemosa* and leaves of *Araucaria bidwillii* Hook were collected from Tirupati hills and also from Chittoor, Andhra Pradesh, and authenticated Botanist Dr. P. Jayaraman, Director, Plant Anatomy Research Centre (PARC), Tambaram, Chennai, Tamil Nadu, A voucher specimen (SVCOP- 1-2014) of the all the three plant were of no: SVCOP 2014/022,023 and 024 has been deposited at the herbarium unit of the Department of Pharmacognosy, Sri Venkateswara College of Pharmacy, Chittoor.

Preparation of Extracts

All the three plants were cleaned, washed in order to free it from dust, soils and other unwanted materials that may adhere to it and air dried individually and after drying the plant material is individually powdered coarsely. The Coarsely powdered dried aerial part of *Ipomoea staphylina,* stem bark of *Ficus racemosa* and leaves of and *Araucaria bidwillii* bark were mixed in equal ratio 1:1:1 (5 Kg) were extracted in 50 % aq. Ethanol. The extract is vacuum filtered and air dried and preserved in air tight containers. The percentage yield is calculated as % w/w.

Physicochemical analysis

Physicochemical parameters such as total ash value, acid insoluble ash value, water soluble ash value and moisture content were determined as per method described by Pharmacoepia using powdered preparation of selected medicinal herbs.

Phytochemical screening

The Polyherbs after extracted with the 50% Aqueous alcohol by cold maceration method the extract is further fractioned with various solvents like hexane, chloroform, ethyl acetate and methanol and water to yield the respective fractions. All the fractions were collected filtered, and the solvent was evaporated to dryness under reduced pressure in a Rotary evaporator at 40º-45ºC. All the fractions were stored in a well closed air tight container and kept in desiccators and it is subjected to preliminary phytochemical analysis to identify various phytochemical constituents as per standard procedures (Harborne, 1973; Brinda *et al.,* 1981 and Lala, 1993).

Quantitative estimation

For biochemical analysis such as estimation of carbohydrate, protein, amino acid, total free phenols, tannins, and flavanoids were carried out using standard methods. The total carbohydrate was estimated by anthrone method of Sheifter *et al.,* (1950). Protein was estimated by Lowry's method (Lowry *et al.,* 1951). Amino acid was estimated by ninhydrin method as suggested by Rosen,(1957). The estimations of total free phenols by Folin-Ciocalteu's method by Sadasivam and Manickam (1992),

Estimation of total flavonoids

Total Flavonoids content in plant fractions was estimated by spectrometric method. (Kadifkova *et al.,* 2005). The ethyl acetate and methanolic fractions of powdered plant material was filtered and concentrated under vacuum up to a concentration of 1 gm/ ml of extract and dried over anhydrous sodium sulphate, They were further diluted with ethyl acetate to obtain 0.01 gm /ml solutions used in the experiments. About 10 ml of the solution was transferred into a 25 ml volumetric flask, 1 ml of 2% $AlCl_3$ was added and the solution was filled to volume with methanol-acetic acid and was kept aside for 30 min, the absorbance was measured at 390 nm against the same solution without $AlCl_3$ being blank. Luteolin was used to construct the calibration curve in the concentration range 1.0-10.0 µg/ml.

Thin layer chromatographic studies (TLC) of flavonoids

Pre coated silica gel GF_{254} Plate 15 cm×20 cm (E. Merck, Mumbai, India) was used as the stationary phase. Ethyl acetate and the methanolic fractions were

dissolved in ethanol. These fraction was applied by means of a Linomat IV sample applicator to the plates about 1 cm above the edge. The chromatogram was developed up to 10 cm with Toluene: Ethyl acetate: Formic acid as the solvent system in a CAMAG twin trough chamber. The developed TLC plate was observed under UV-light. From the thin layer chromatographic studies, the presence of various flavanoids was observed. with Rf values between 0.14 and 0.74.

High performance thin layer chromatography

High performance thin layer chromatography (HPTLC) is modern adaptation of TLC with improved versatility, separation efficiency and detection limit. HPTLC is useful for identification of plants and their extracts because each plant species produce a distinct chromatogram with unique marker compounds used for plant identification. It is used a quality control tool since comparison of chromatograms of different lots can demonstrate the similarities and differences between the test samples and standard chemical markers. HPTLC is a reliable method for quantitation of a nanogram level even when present in complexes formulation. HPTLC finger print analysis is used for rapid identity check, for monitoring purity of drugs, for a detection of adulterants for determining whether a material is derived from defined botanical species also to known whether the constituents are clearly characterized (Sethi, 1996).

Development of HPTLC fingerprint

- **Instrument**

CAMAG TLC Scanner 3 "Scanner 3 – 070408" S/N 070408 (1.41.21) was used for detection and CAMAG Linomat V sample applicator was used for the application of the tracks.

- **Sample**

The fractions used for taking HPTLC finger prints were ethyl acetate and methanolic fractions of plant extracts (10µl).

- **Stationary phase**

Aluminum sheets pre coated with silica gel Merck G $F_{254,}$ 0.2mm layer thickness were used as the stationary phase.

- **Mobile phase**

$CHCl_3$: MEOH: FA: GAA (7: 2: 1: 1) was used as the mobile phase for developing the chromatogram for ethyl acetate fraction. Ethyl acetate: Hexane (4:6) for methanolic fraction. The mobile phase was taken in a CAMAG twin trough glass chamber.

- **Detection wavelength**

The developed plates were examined at wavelength 254 and 366 nm.

Chromatographic condition

Sample : Ethyl Acetate fraction
Stationary phase : Silica gel GF_{254}
Mobile phase : $CHCl_3$: MEOH: FA: GAA (7: 2: 1: 1)
Scanning wavelength : 340nm
Applied volume : 5, 10, 15µl
Development mode : Ascending

The percentage recovery was calculated for each extracts analysis by comparing the values with standard.

Chromatographic condition

Sample : Methanolic fraction
Stationary phase : Silica gel GF_{254}
Mobile phase : Ethyl acetate: Hexane (4:6)
Scanning wavelength : 340nm
Applied volume : 5, 10, 15µl
Development mode : Ascending

The percentage recovery was calculated for each fractions analysis by comparing the values with standard.

Table 1. Physicochemical analysis of *Commenlina benghalensis* L. dried shoot and root system (n=3; means ± SE)

Parameter	Powdered plant material
Moisture content % (w/w)	(10.062±0.623%).
Total ash % (w/w)	11.23±0.115%,
Acid insoluble ash % (w/w)	1.126±0.347%
Water soluble ash % (w/w)	13.340±1.45%

Table 2. Preliminary phytochemical screening of fractions of polyherbs

Phytoconstituents	Solvents				
	Hexane	Chloroform	Ethyl acetate	Methanol	Water
Alkaloids I. Mayer's test	-	+	+	+	-
II.Wagner's test	-	-	-	+	-
Anthraquinones (Borntrager's test)	-	-	-	-	-
Catechin	-	-	-	+	-

Coumarin	-	-	+	+	-
Flavonoids	-	+	+	+	+
Phenols	-	-	+	+	+
Quinones	-	-	+	+	+
Saponin (Foam test)	-	-	-	+	+
Steroids	+	+	+	+	+
Sugar I. Benedict's test	-	-	+	+	+
II. Fehling's test	-	-	+	+	+
Glycosides I. Anthrone test	-	-	-	+	+
II. Borntrager's test	+	+	-	+	-
Amino acids Ninhydrin test	-	-	-	+	+
Xanthoprotein	-	-	+	+	+
Protein	-	-	+	+	+
Fats and oils	+	+	+	+	-
Gums and mucilage	-	-	-	+	-

('+' present, '-' absent)

Table 3. Biochemical composition of polyherbs

Parameter	Extracts of medicinal Plants.
Carbohydrate mg/g	0.462±0.012mg/g
Protein mg/g	13.29±0.244 mg/g
Amino Acid mg/g	2.224±0.160 mg/g
Total free Phenols (mg/g)	14.3±0.126 mg/g
Tannins (mg/g)	12.1±0.27mg/g
Total Flavonoids	28.89± 2.58mg/g

Protein as Amino Acids was determined by using HPLC System (LACHROM – 700)

Table 4. Amount of amino acids present in Poly herbs

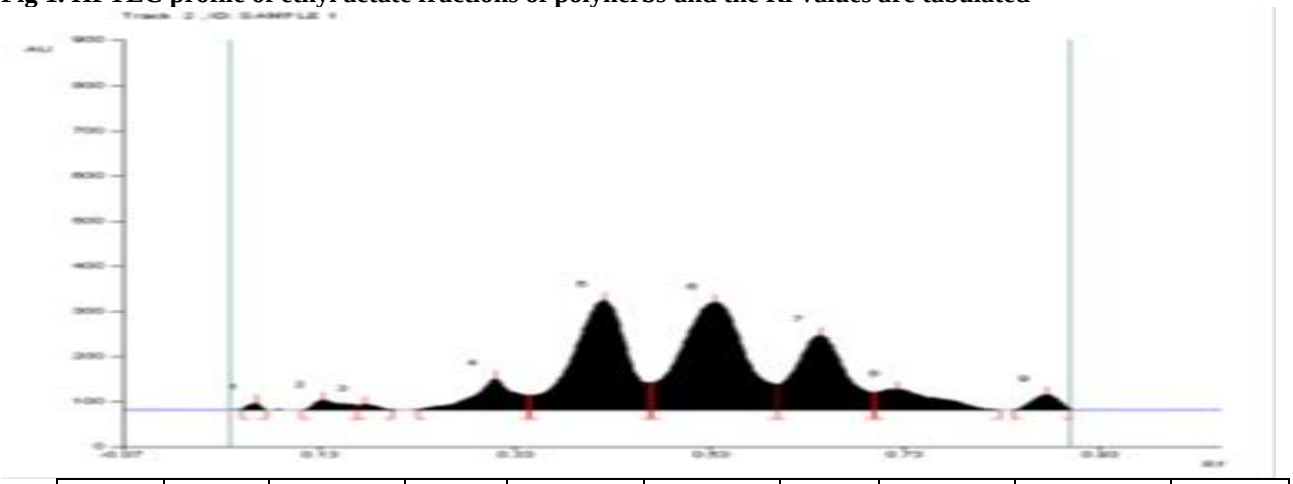
S.No	Name of the Amino Acid	Amount in mg
1.	Glutamic acid	0.089
2.	Asparagine	0.056
3.	Glutamine	0.034
4.	Arginine	0.317
5.	Alanine	0.125
6.	Threonine	0.206

HIGH PEFORMANCE THIN LAYER CHROMATOGRAPHY

Table 5. R_f values of ethyl acetate and methanolic fractions of Polyherb by HPTLC

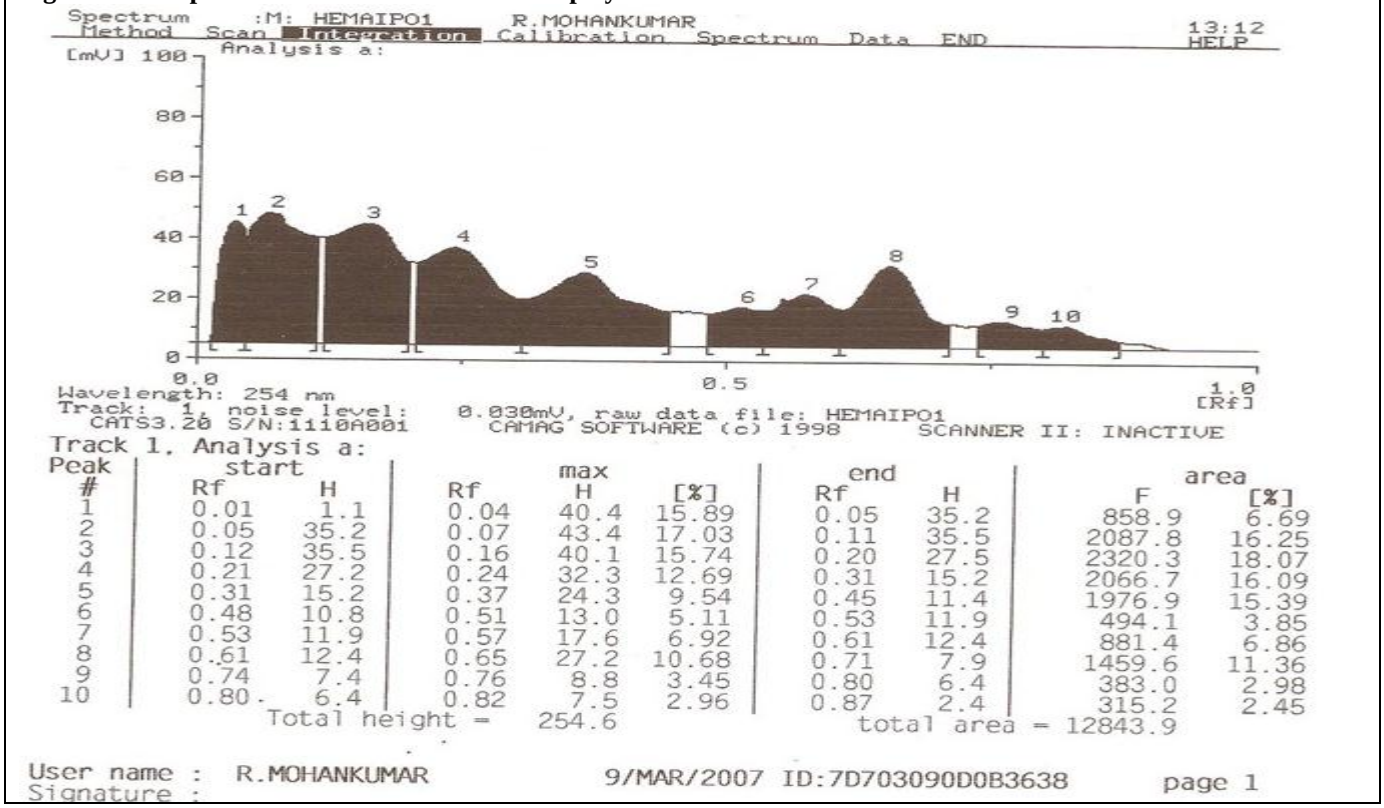
S. No.	Name of the fraction	Solvent system	Detection wavelength	No. of spots	R_f value
1	Ethyl acetate	chloroform: methanol: formic acid: glacial acetic acid (7: 2: 1: 1)	254 & 366	09	0.07, 0.13, 0.18, 0.31, 0.42, 0.54, 0.64, 0.72, 0.88
2	Methanol	ethyl acetate: hexane (4:6)	254	10	0.04, 0.07, 0.16, 0.24, 0.37, 0.51, 0.57, 0.65, 0.76, 0.82

Fig 1. HPTLC profile of ethyl aetate fractions of polyherbs and the Rf values are tabulated



Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %
1	0.05	2.4	0.07	16.0	1.88	0.08	0.2	170.3	0.46
2	0.11	0.1	0.13	22.9	2.69	0.17	11.7	558.5	1.49
3	0.17	11.8	0.18	12.6	1.49	0.21	1.0	201.3	0.54
4	0.23	1.0	0.31	69.6	8.18	0.35	32.5	2415.9	6.46
5	0.35	32.6	0.42	243.2	28.59	0.47	60.1	10991.9	29.40
6	0.47	60.1	0.54	238.7	28.06	0.60	57.7	12944.2	34.62
7	0.60	57.8	0.64	166.4	19.56	0.70	39.2	7007.2	18.74
8	0.70	39.3	0.72	46.7	5.49	0.83	0.0	2306.6	6.17
9	0.84	0.5	0.88	34.6	4.07	0.90	2.0	795.9	2.13

Fig 2. HPTLC profile of methanolic fractions of polyherb



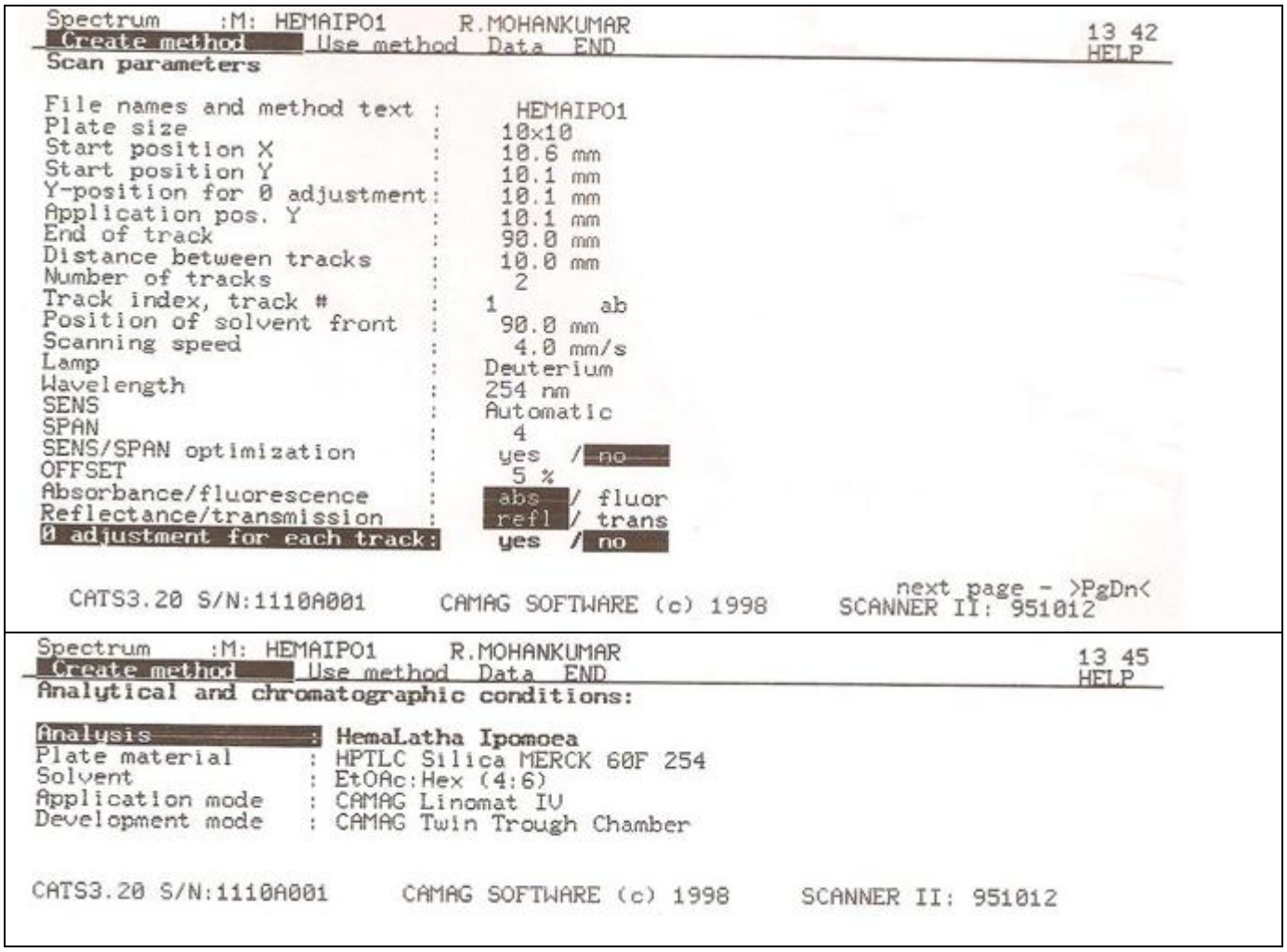
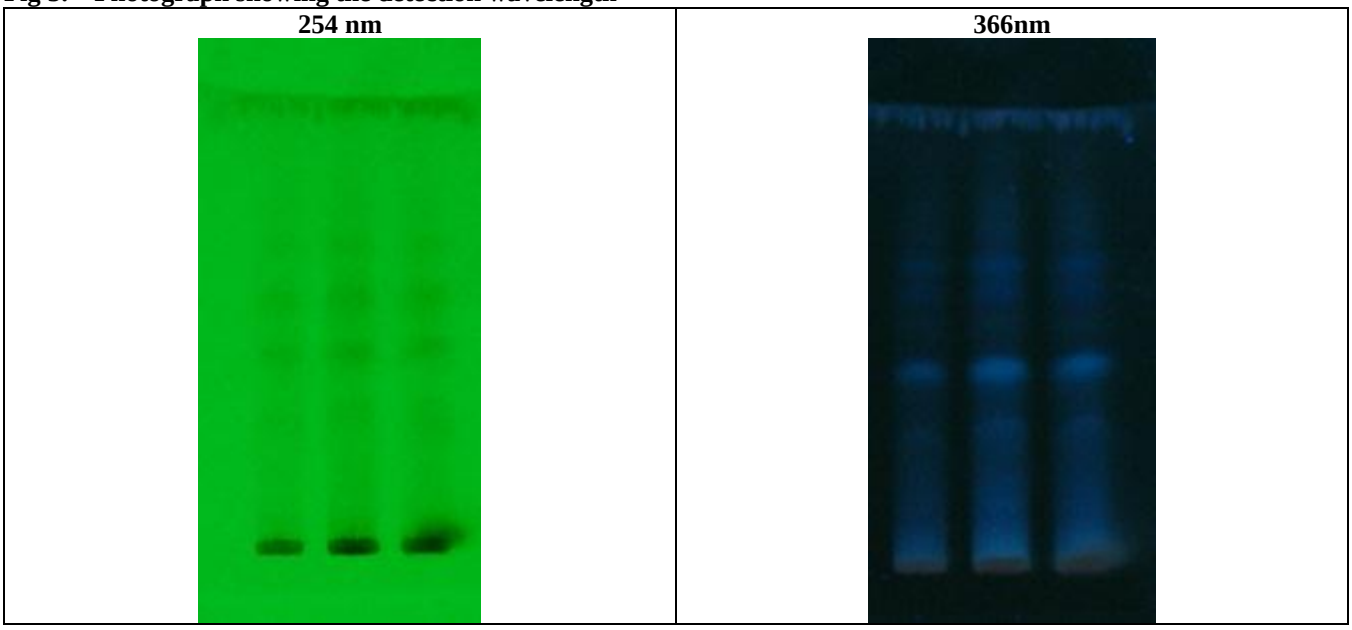


Fig 3. Photograph showing the detection wavelength



RESULTS AND DISCUSSION

Physicochemical analysis

The aerial parts of *Ipomoea staphylina* , stem bark of *Ficus racemosa* and leaves of *Araucaria bidwillii* were coarsely powdered mixed in the ratio of 1;1;1 and studied for physicochemical analysis. In this study moisture content, total ash value, acid insoluble ash value, and water soluble ash value were determined is presented in Table - 1. The moisture content of powder is (10.062±0.623%). Total ash, acid insoluble and water soluble ash value is 11.23±0.115%, 1.126±0.347%, and 13.340±1.45% were respectively noted. The amount of composition of ash remaining after combustion of plant material varies considerably according to the part of the plant, age, treatment etc. the constituents of the ash also vary with time and from organ to organ. Ash usually represents the inorganic part of the plant (Vermani Archaet al., 2010). Ash value is useful in determination authencity and purity of sample and also these values are of important qualitative standards. When the plant is consumed higher value of acid insoluble ash indicates the higher digestability. The amount of inorganic matter present in the sample and the acid insoluble ash almost within the limit which expresses low siliceous matter present in the sample

Phytochemical screening

The phytochemical screening of fractions of extracts was presented in Table-2. The bioactive compounds were identified in fractioned extracts. The hexane extract showed the presence of steroids, fats and oils. The chloroform extract showed the presence of steroids, alkaloids, glycosides. Ethyl acetate extract showed the positive test for steroids, alkaloids, glycosides and flavanoids. The methanolic extract showed the presence of sugars, amino acids, proteins, fats, alkaloids, glycosides, flavanoids, gums and mucilage. The presence of flavanoids in plant extracts are further confirmed by chromatographic technique by TLC and HPTLC. It was observed that the HPTLC finger print pattern of ethyl acetate fraction when examined at 254 nm showed nine peaks at R_f 0.17, 0.08, 0.21, 0.35, 0.47, 0.60, 0.70, 0.90 and 0.83 respectively. The major peaks were found at 0.47 and 0.60 with an abundance of 29.40 % and 34.62 % respectively. When methanolic fraction was examined at same 254nm showed the presence of 10 peaks at R_f 0.05, 0.11, 0.20, 0.31, 0.45, 0.53, 0.61, 0.71, 0.80 and 0.87 respectively. The major peaks were found at 0.11, 0.21, 0.3 and 0.71 with an abundance of 16.25, 18.07, 16.09 and 11.36%. The above The HPTLC finger prints of the various fractions studied above are useful in identification of the plant in future. The finger print profile can also be used as a quality control tool since comparisons of chromatograms of different batches can demonstrate the similarities and differences between the test samples. It will therefore, be useful for identity check, for monitoring purity of the drugs, for detection of adulterants, for determining whether a material is derived from a defined botanical species.

Phytochemical screening is one of the techniques to identify new sources of therapeutically and industrially important compounds present in the plant extract. The chemical constituents of plants are desirable in the synthesis of new bioactive compounds for treating the specific disease. phytochemical screening in various plants is reported by many workers. The presence of tannin shows that the plant is astringent as documented and suggests that it might have antiviral and antibacterial activity and can aid in wound healing which in turn cures ulcers in foot region. (Haslem, 1989; Ibrahim *et al.,* 2010). The secondary metabolites like total free phenols (14.3±0.126 mg/g) tannins in (12.1±0.27) and flavanoids (28.89± 2.58mg/g) were presented in fractions of medicinal plants respectively.

The flavonoids have various functions that make them an ideal candidate for a novel anti-diabetic treatment. Among diabetic animal models, flavonoids typically lead to reduced aldose reductase, regeneration of pancreatic beta cells, and increased insulin release. The authors term these "insulinomimmetic" activities of flavonoids, which are beneficial and desirable effects for diabetics. The trouble with isolating flavonoids for novel anti-diabetic treatment however, is that the compounds are poorly soluble and bioavailability would be lacking. Researchers hypothesize a nanoparticulate system for the purpose of flavonoid isolation in order to improve the solubility and enhance bioavailability and intestinal absorption. Development of such systems may result in creation of a novel class of anti-diabetic drug with less toxicity than, fewer side effects than, and potential for synergism with, many of those currently existing anti-diabetics on the market which also treats foot ulcers.

Biochemical Analysis

Biochemical constituents such as carbohydrate, protein, amino acid, were presented in Table 3. The primary metabolites of carbohydrate (0.462±0.012mg/g, protein (13.29±0.244 mg/g), and amino acid (2.224±0.160 mg/g) were presented in fractions of powdered plants. The presence of higher protein level in the plant towards their possible increase in food value and that a protein base bioactive compound could also be isolated in future (Thomsan *et al.,* 1991).

CONCLUSION

The use of *Polyherbs* has stood the test of time. Using the *Ayurvedic* concept of *Panchamahabhutas* and Tridoshas, Polyherbs provide treatment of diseases in a holistic approach. The scientific advancement carries with it the improvement in *Polyherbs* through the study of

various phytoconstituents and discovery of useful herbs combinations which work synergistically to produce desirable effect. The present study could be used to identify and standardize the bioactive substance.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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