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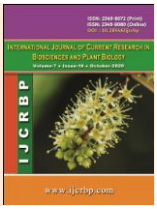

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Comparative evaluation of phytochemicals in leaf, stem bark and root bark of *Holarrhena antidysenterica*, *Wrightia tomentosa* and *Wrightia tinctoria*

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ABSTRACT

Various phytochemicals like alkaloids, tannins, flavonoids, carbohydrates, proteins, saponins, polyphenolic compounds and steroids etc. are found in different parts of plant. These are primary and secondary metabolites formed as byproduct of various biochemical pathways and play a major role in combat and cure of various diseases. A large number of diseases which are incurable through allopathic medicines show promising response to ayurvedic medicines. Prevention of these incurable diseases achieved through use of herbal medicines. It is necessary to identify, isolate and quantify such phytochemicals which are being used in the preparation of medicines or drugs formulations. The present study deals with comparative phytochemicals analysis in leaf, stem bark and root bark of *Holarrhena antidysenterica* (Roth) Wall ex. A. DC., *Wrightia tomentosa* Roem. et Schulta and *Wrightia tinctoria* R. Br.

Introduction

Plants are powerful resources of bio-chemicals and thus, good source of phytomedicines since ancient times. Natural constituents can be extracted from any part of the plant like leaves, bark, flowers, fruits, seeds, roots, etc. which contain bioactive compounds. The medicinal property of bioactive compounds is the result of the combinations of secondary metabolites present in the plant (Shwetha et al., 2011). Plants have the ability to synthesize aromatic products mainly secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, etc. of which about 12,000 secondary metabolic compounds have been isolated. These isolated secondary metabolites showed wide range of pharmacological properties such as *in-vitro* antibacterial and antifungal activities (Zahan et al., 2013). Their major role is in the plant defense system against herbivores, microorganisms and insects. Some are involved in plant pigmentation (tannins and quinines), odour (terpenoids) etc. and thus responsible for a definite physiological effect. It clearly indicates that the medicinal property of plants lie in the

bioactive phytochemical constituents (Patel et al., 2015). Many secondary metabolites of plants are commercially important and are being used in a number of pharmaceutical compounds (Meenakshi, 2015). Phytochemical investigation of bioactive compounds from plants has led to the discovery of many therapeutic drugs which effectively used in protection and treatment of various diseases including cancer (Sharma et al., 2017). With this background and abundant source of unique active components harbored in plants, the present study was conducted on leaf, stem bark and root bark of three medicinal plants viz., *Holarrhena antidysenterica* (collected from two locations); *Wrightia tomentosa* and *Wrightia tinctoria*. These plant species belong to the family Apocyanaceae.

H. antidysentrica is a small deciduous tree with cream white flowers and found throughout the dry forests of India. The different parts of the plant are used in the indigenous system of medicine. It is used in the treatment of dysentery, diarrhea, helminthic, astringent and haemostatic disorders (Ahirwar et al., 2019).

W. tomentosa is an endangered medicinal tree placed in the category I of Red Data (Nagalakshmi and Murthy, 2015) with many medicinal uses. Ethnomedically *W. tomentosa* is widely used to treat stomach ache, tooth ache, fever, hemorrhage, arthritis and snake bite (Srinivas et al., 2013; Ahirwar et al., 2019).

Wrightia tinctoria R. Br. is a small to medium size deciduous tree, which is widely distributed all over India. In South India, it is commonly called as "Jaundice curative tree" traditionally (Dixit et al., 2014). The common name of plant is Paalai and called “Sweet Indrajao”. The plant are effective in stomachic, in the treatment of abdominal pain, anti- diarrhoeal and antihaemorrhagic (Deventhiran et al., 2016). Wrightial, cycloartenone, cycloeucalenol, indigotin, indirubin, tryptanthrin, isatin, rutin, β-sitosterol, β-amyrin, wrigatiadione are the main isolated compounds from seeds, leaves and bark of this plant (Singh et al., 2015). Reviewed on phytochemical, pharmacological and pharmacognostical profile of *W. tinctoria* and noted its anti-cancer, anti-HIV, and anti-diabetic properties too (Srivastava, 2014). The aim of the present study was investigation of different phytochemicals in selected parts of *H. antidysenterica, W. tomentosa* and *W. tinctoria*.

Materials and methods

Collection of plant samples and powder preparations

Leaf and stem bark of *Holarrhena antidysenterica* was collected from Sati Anusuiya area and root bark was collected from Bagdara Ghati of Gupt Godavari area of Chitrakoot forest whereas, leaf, stem bark and root bark of *Wrightia tinctoria* was collected from Bagdara Ghati of Gupt Godavari area of Chitrakoot forest of Satna district of Madhya Pradesh. Samples of *Holarrhena antidysenterica* were also collected from Talawada area of Guna district of Madhya Pradesh. While the leaf, stem bark and root bark of *Wrightia tomentosa* were collected from the Holipur area of Budhni tehsil of Sehore district of Madhya Pradesh and identity was confirmed by Dr. R.L.S. Sikarwar, a taxonomist of Department of Ayurveda Sadan (Research Laboratory), Arogyadham, Deendayal Research Institute (DRI), Chitrakoot, Satna (M.P.) India. Collected plant samples were brought to the laboratory and washed with running tap water, dried under shade and prepared powder with the help of electric grinder. The powder was sieved (No. 43) and stored in air tight closed containers separately for protection from moisture and contamination.

Phytochemical screening

The powdered material weighing 2.0g was extracted in 100 ml ethanol by continuous extraction for 6 hours on rotatory flask shaker and left for 18 hours for maceration. The extract was filtered through Whatman filter paper no. 1 and tested for various classes of active chemical constituents by using standard methods described in Ayurvedic Pharmacopeia of India (API) and "Methods of Biochemical Analysis" (Anonymous 2010; Harborne 1984; Thimmaiah 1999).

Phytochemicals estimation

Determination of alkaloid, flavonoid, saponins and crude protein by gravimetric method (in %) and total carbohydrate, soluble protein and

tannin (mg/ml) were estimated in three replications by spectrophotometric methods in leaf, stem bark and root bark of three species. Alkaloid was estimated by both gravimetric and spectrophotometric methods.

Alkaloid- Harborne (1973) method was used for the determination of alkaloid.

Flavonoid- Bohm and Kocipai – Abyazan (1994) method was used for the determination of flavonoid.

Saponins- Obadani and Ochako (2001) method was used for the determination of saponins with modification of petroleum ether in place of diethyl ether.

Crude protein- Kel plus method was used for estimation of crude protein (Sadasivam and Manickam 1996).

Sample preparation: Taken 0.5g powder of samples in digestion tubes and added punch of digestion mixture and 10 ml of sulphuric acid in all trubes. Sample was digested until green or dark green colour appeared. After cooling of tubes, sample particles attached side of the tubes dissolved with the help of distilled water and total volume of sample maintained to 20ml. Samples were digested until sample residues dissolve completely and homogeneous sample forms. Samples were run in Kel – Plus machine.

Procedure: Added 30 ml of methylene red-methylene blue mixed indicator (20 ml of methylene red plus 10 ml methylene blue) in 4% boric acid (1970 ml of 4% boric acid + 30 ml of mixed indicator) and fit the gallon of 4% boric acid (total volume 2000 ml) and 40% sodium hydroxide solution (total volume 2000 ml) in tubes of acid and alkali solution. Tap water supplied for mixing of ammonia gas. Collected 25 ml of distillate sample in a conical flask and titrated against 0.1 N HCl standard acid solutions until green or blue colour end point reaches. Blank prepared by same procedure with distilled water. Calculated nitrogen (N) content by applying formula:

$$\% \text{ of } N = \frac{14.1\,(B-S) \times 0.1N\ HCl \times 100}{\text{Weight of sample taken} \times 1000}$$

Where, B and S represent titration value of blank and sample respectively.

Protein Nitrogen: Total nitrogen value multiplied with 6.25 for crude protein content which also includes non-protein nitrogen.

Total carbohydrate- Total carbohydrate or total soluble sugars estimated by Anthrone method (Thimmaiah, 1999).

Soluble protein- Lowry's method was used for the estimation of soluble protein or true protein (Lowry et al., 1951; Mani et al., 2019; Sadasivam and ManickaM, 1996).

Tannin- Folin-Denis method was used for the estimation of tannin (Sadasivam and ManickaM, 1996; Thimmaiah, 1999).

Alkaloids - Alkaloids was estimated spectrophotometrically by Dragendorff's reagent method (Nayeem et al., 2011).

Sample extract preparation- 10g coarsely powdered plant material was extracted with 25 ml 2% aqueous acetic acid at room temperature for 10 minute. The procedure was repeated 3 times. All three extracts were mixed and diluted to 100 ml with 2% aqueous acetic acid.

Procedure for estimation of alkaloids - A 5 ml amount of the extract was taken and pH maintained at 2.0 - 2.5 with dilute HCl. A 2 ml of DR was added to it, and the precipitate formed was centrifuged. The centrifugate was checked for complete precipitation by adding DR. After centrifugation, the centrifugate was decanted completely and meticulously. The precipitate was further washed with alcohol. The filtrate was discarded and the residue was than treated with 2 ml disodium sulfide solution. The brownish black ppt. formed was than centrifuged. Completion of ppt. was checked by adding two drops of disodium sulfide. The residue was dissolved in 2 ml conc. nitric acid. This solution was diluted to 10 ml in a standard flask with distilled water. 1 ml was then pipette out and 5 ml thiourea solution was added to it. The absorbance was measured at 435nm against the blank containing nitric acid and thiourea. The amount of bismuth present in the

solution was calculated by multiplying the absorbance values with the factor taking suitable dilution factor into consideration. The factor is obtained from the standard curve which is constant for different concentrations.

Factor = concentration/absorbance.

Results and discussion

Preliminary phytochemical screening revealed (Table 1) that resin was present in all samples while saponin and starch was absent in all samples. Alkaloids were observed in all samples except leaf of HALC and WTLC. Carbohydrate was present in all samples except in HALT. Proteins were not observed in HALC, HALT, HARBT, WTLH, WTRBH, WTLC and WTSBC. Flavonoid was found in HALC, WTSBH, and WTLC but absent in all other samples. Steroid was present only in HASBT, HARBT but absent in all other samples. Glycoside was absent in all samples except HALC and WTLC. Whereas, tannins were found present in HALC, HASBC, HARBT, WTLH, WTLC, WTSBC, WTRBC and absent in HARBC, HALT, HASBT, WTSBH and WTRBH. Similar study of leaf of *W. tinctoria* was conducted by Mahendra and Nityanand (2009) and Vedhanarayanan et al. (2013).

Table 1. Qualitative phytochemical screening of ethanolic extract of selected plants.

Name of samples	Name of phytoconstituents									
	Alkaloids	Carbohydrate	Proteins	Resins	Saponin	Starch	Flavonoid	Steroid	Glycoside	Tannins
HALC	-	+	-	+	-	-	+	+	+	+
HASBC	+	+	+	+	-	-	-	+	-	+
HARBC	+	+	+	+	-	-	-	+	-	-
HALT	+	-	-	+	-	-	-	+	-	-
HASBT	+	+	+	+	-	-	-	-	-	-
HARBT	+	+	-	+	-	-	-	-	-	+
WTLH	+	+	-	+	-	-	-	+	-	+
WTSBH	+	+	+	+	-	-	+	+	-	-
WTRBH	+	+	-	+	-	-	-	+	-	-
WTLC	-	+	-	+	-	-	+	+	+	+
WTSBC	+	+	-	+	-	-	-	+	-	+
WTRBC	+	+	+	+	-	-	-	+	-	+

*+ = Present; *- = Absent; *HALC= *H. antidysentrica* leaf Chitrakoot, *HASBC= *H. antidysentrica* stem bark Chitrakoot, *HARBC= *H. antidysentrica* root bark Chitrakoot; *HALT= *H. antidysentrica* leaf Talawada, *HASBT= *H. antidysentrica* stem bark Talawada, *HARBT= *H. antidysentrica* root bark Talawada; *WTLH= *W. tomentosa* leaf Holipur, *WTSBH= *W. tomentosa* stem bark Holipur, *WTRBH= *W. tomentosa* root bark Holipur; *WTLC= *W. tinctoria* leaf Chitrakoot, *WTSBC= *W. tinctoria* stem bark Chitrakoot, *WTRBC= *W. tinctoria* root bark Chitrakoot.

Average yield of phytoconstituents *viz.,* alkaloid, flavonoid, saponin and crude protein in leaf, stem bark, root bark of *H. antidysentrica* of Chitrakoot and Talawada, *W. tomentosa* of Holipur and *W. tinctoria* of Chitrakoot determined in percentage (%) by gravimetric method (Table 2). Experimental findings exhibited maximum alkaloids in HALT followed by HASBT, WTLH, WTLC, HALC, WTSBC, WTRBC, HARBT, WTSBH, HARBC, HASBC and WTRBH. Flavonoids were in decreasing order in WTLH, HASBC, HALC, HASBT, HARBC, HALT, WTLC, HARBT, WTSBH, WTRBH, WTRBC and WTSBC. Highest content of saponin was found in HARBT followed by HALT, WTLC, HASBC, WTLH, WTRBH, HASBT, HALC, WTRBC, WTSBH, HARBC and WTSBC whereas, highest contents of crude protein was found in WTRBH followed by WTRBC, WTSBH, WTSBC, HALT, HASBC, WTLC, WTLH, HASBT, HARBT, HARBC and HALC respectively. Contrast result of alkaloid, saponin and tannin reported by Mahendra and Nityanand (2009). Sharma et al. (2017) also confirm the presence of alkaloids, flavonoids and terpenoids in four species of *Wrightia*.

Total carbohydrate estimation was done spectrophotometrically by Anthrone method. The linear equation (y = mx + c) and correlation coefficient (R^2) of carbohydrate from calibration curve was y = 0.127x + 0.258 and R^2 = 0.957 recorded. The content of soluble sugar was found highest in HASBT followed by WTRBH, HALT, WTRBC, HARBT, WTLH, HASBC, WTSBC, WTSBH, HARBC and HALC respectively (Table 3). Estimation of soluble protein was carried out by Lowry's method. The calibration curve of protein was equivalent to bovine serum albumin (Fraction

V). The linear equation, y = 0.431x + 0.125 and R^2 = 0.998 of protein was recorded. The soluble protein in all samples was recorded in decreasing order in HALT, HARBT, HALC, WTLH, HASBT, HARBC, WTLC, HASBC, WTRBC, WTSBC, WTSBH and WTRBH respectively (Table 3).

Table 2. The average percentage yield of some phytoconstituents by gravimetric method.

Name of samples	Name of phytoconstituents (n = 3 ± SD)			
	Alkaloids	Flavonoid	Saponin	Crude protein
HALC	4.83 ± 0.051	21.33 ± 0.358	10.29 ± 0.283	0.02819 ± 0.00099
HASBC	1.11 ± 0.069	21.47 ± 0.667	12.79 ± 0.326	3.2488 ± 0.07695
HARBC	1.14 ± 0.095	17.18 ± 0.131	6.86 ± 0.274	0.17553 ± 0.00305
HALT	7.9451± 0.23285	16.63267 ± 1.19639	19.51867 ± 0.9255	4.67254 ± 0.15053
HASBT	5.421 ± 0.524535	17.766 ± 0.33010	11.29467 ± 0.51908	0.52832 ± 0.00210
HARBT	3.41167 ± 0.31465	12.625±0.91995	20.58833±0.76044	0.3505 ± 0.01878
WTLH	5.06 ± 0.885	25.23 ± 1.265	12.37 ± 0.545	0.717 ± 0.04229
WTSBH	2.68 ± 0.162	10.19 ± 0.765	9.29 ± 0.438	10.84995 ± 0.28059
WTRBH	0.41 ± 0.026	9.51± 0.322	12.27 ± 0.913	12.98147 ± 0.61248
WTLC	4.885 ± 0.09913	14.2557 ± 0.1470	13.687 ± 0.34953	0.92077 ± 0.06726
WTSBC	4.555 ± 0.16423	7.47367 ± 0.44699	4.56303 ± 0.33350	8.24508 ± 0.41685
WTRBC	4.41 ± 0.29962	9.251 ± 0.28804	9.407 ± 0.43795	12.184987 ± 0.28676

*SD = standard deviation; *Mean; n=3 ± SD

Table 3. Total carbohydrate and soluble protein estimation in selected parts of plants.

Phyto-constituent	Name of samples											
	HALC	HASBC	HARBC	HALT	HASBT	HARBT	WTLH	WTSBH	WTRBH	WTLC	WTSBC	WTRBC
Total carbohydrate (mg/ml)	0.98 ± 0.001	4.62 ± 4.617	1.85 ± 1.853	5.801 ± 0.001	6.822± 0.003	5.635 ± 0.003	4.99 ± 0.003	2.55 ± 0.003	6.66 ± 0.003	1.473 ± 0.0002	3.580 ± 0.003	5.717 ± 0.003
Soluble protein (mg/ml)	3.79 ± 0.002	2.66 ± 0.003	2.73 ± 0.004	5.801 ± 0.001	2.739 ± 0.003	5.635 ± 0.003	3.31 ± 0.002	1.07 ± 0.002	0.89 ± 0.003	2.705 ± 0.002	1.244 ± 0.004	2.337 ± 0.007

Table 4. Tannin and alkaloid estimation in selected parts of plants.

Phyto-constituent	Name of samples											
	HALC	HASBC	HARBC	HALT	HASBT	HARBT	WTLH	WTSBH	WTRBH	WTLC	WTSBC	WTRBC
Tannin (mg/ml)	0.78 ± 0.003	0.39 ± 0.001	1.30 ± 0.003	0.074± 0.003	0.155 ± 0.002	0.638 ± 0.005	0.38 ± 0.003	0.14 ± 0.001	ND	0.886 ± 0.002	0.139 ± 0.003	0.689 ± 0.003
Alkaloid (µg/ml)	0.9571 ± 0.005	63.486 ± 0.087	52.859 ± 0.005	2.702± 0.014	32.055 ±0.021	44.373 ± 0.002	65.843 ± 0.013	7.937 ± 0.046	36.172 ± 0.046	8.114 ± 0.039	33.643 ± 0.053	55.564 ± 0.062

ND = Not detected.

The calibration curve of tannin was equivalent to tannic acid. The linear equation, y = 0.4235x + 0.0855 and R^2 = 0.993 of tannic acid was recorded. The highest tannin content was found in HARBC, WTLC, HALC, WTRBC, HARBT, HASBC, WTLH, HASBT, WTSBH, WTSBC and HALT while, tannin content was not detected in WTRBH sample (Table 4). The calibration curve of alkaloid was equivalent to bismuth nitrate pentahydrate. The linear equation, y = 0.017x + 0.0184 and R^2 = 0.9636 was recorded. The decreasing order of alkaloids content recorded in WTLH, HASBC, WTRBC, HARBC, HARBT, WTRBH, WTSBC, HASBT, WTLC, WTSBH, HALT and HALC respectively (Table 4).

Conclusion

The comparative study of phytochemicals in leaf, stem bark and root bark of *H. antidysenterica* (Chitrkoot, Talawada), *W. tomentosa* (Holipur) and *W. tinctoria* (Chitrakoot) was carried out and results shows that the phytochemicals were observed in the order of HALC, HASBC, WTSBC, WTLC (same number) followed by HARBC, WTLH, WTRBC (same number) than HASBT, HARBT, WTRBH, WTSBC (same number) and lowest in HALT. Highest content of alkaloids, flavonoid, saponin and crude protein were found in HALT, WTLH, HARBT, WTRBH (gravimetric

method). While spectrophotometric method reveals highest content of total carbohydrate, soluble protein, tannin and alkaloids also in HASBT, HALT, HARBC and WTLH. These phytoconstituents are responsible for therapeutic and pharmacological properties of plants and thus makes the plant medicinally more valuable. Root bark and stem bark of these plants are useful in prevention and slowing the progress of various oxidative stress- related diseases or potentially applicable in both medicine as well as to develop nutritionally rich healthy food products by industries at commercial level.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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